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INSTITUTO BUTANTAN
SÃO PAULO, SP - BRASIL

Memórias do Instituto Butantan

Volume 68

December 2011

XIII Annual Scientific Meeting

São Paulo, SP - Brazil

ISSN 1982-3045

Support: Instituto Butantan and Fundação Butantan

Address: Biblioteca, Instituto Butantan
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Website: www.butantan.gov.br

Memórias do Instituto Butantan (Secretaria de Estado da Saúde).
São Paulo, SP, Brasil, 1918 - 1990, 52 (Supl. 1-3)
Em apenso, a partir de 1990, 52 (3): BOLETIM DE BIOTECNOLOGIA
1991, 53 (1) (Supl. 1, 2)
1992, 54 (1, 2)
1993, 55 (1, 2) (Supl. 1)
1994-1995, interrompido
1996, 56 bienal, nova forma
1998, 57 bienal
2001, 58 bienal
2002, 59 bienal
2003, 60 anual
2005, 61 anual
2005, 62 anual
2006, 63 anual
2007, 64 anual, CD-ROM
2008, 65 anual, CD-ROM
2009, 66 anual, CD-ROM
2010, 67 anual, CD-ROM
2011, 68 anual, CD-ROM

ISSN 1982-3045

EDITORIAL

The 68th volume of "Memórias do Instituto Butantan" presents the proceedings of the XIII Annual Scientific Meeting of Butantan Institute, organized by the Division for Scientific Development. The theme of the 13th edition of the Instituto Butantan´s meeting is "Butantan Institute, 110 years: a look to the future"

The scientific program is organized in three main sessions: Frontiers in Biosciences, Frontiers in Biotechnology and Frontiers in Science Dissemination.

Also, the meeting includes three poster sessions, Young Scientist Awards in four categories (scientific initiation, PAP program, master and doctoral degrees), one special session entitled "Memories of Instituto Butantan" with focus on the history of Instituto Butantan at the 80's and a tribute to scientific leaders, researchers and employees who participated in the renewal of Instituto Butantan at that time.

The 68th volume brings together all poster abstracts presented in the Poster Sessions of the following areas: Venoms and Envenomations; Biochemistry; Pharmacology; Immunology and Vaccines; Microorganisms; Biotechnology; Cellular Biology and Genetics; Animal Biology; History, Education and Science Dissemination; Others; PIBIC/PIBITI and PAP program. The impressive number of submitted posters indicates the involvement of our scientific community with the Annual Scientific Meeting. These abstracts are representative of the scientific research that was carried out at Instituto Butantan in the last year. Therefore, the "Memórias do Instituto Butantan" electronic issue is an important source of scientific information and knowledge dissemination.

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XIII Annual Scientific Meeting of Instituto Butantan

November 30th to December 2nd, 2011

Butantan Institute, 110 years: A look to the future

Scientific Program

Tuesday 11/29/2011

Satelite Event: Symposium of Instituto Butantan´s Graduate Program in Toxinology

Coordinator: Norma Yamanouye

14:00 – 14:20	**Oral communication** **Evaluation of crotoxin effects on pain and in the clinical signs of animals with experimental autoimmune encephalitis, an animal model of multiple sclerosis** Nathália Bernardes Teixeira – Master student
14:25 – 15:15	**Conference** **Studies in Toxinology: academic and scientific experiences and challeges of a unique biodiversity** Maria Elena de Lima Perez Garcia – Universidade Federal de Minas Gerais
15:15 – 15:35	**Coffee break**
15:35 – 15:55	**Oral Communication** **SVMP zymogens detection in venom and venom gland extracts from Bothrops jararaca** José Antonio Portes Junior Elbio Leiguez Junior – Ph.D. student

16:00 – 16:50	**Conference** **I think....therefore I insist on** Osvaldo Augusto Brazil Esteves Sant´Anna – Instituto nacional de Ciência e Tecnologia em Toxinas (INCTTOX), Instituto Butantan

Wednesday 11/30/2011

XIII Annual Scientific Meeting of Instituto Butantan - Butantan Institute, 110 years: A look to the future

09:00 - 09:30	**Opening Session**
09:30 - 12:30	**Thematic Session I: Frontiers in Bioscience** Coordinator: Jorge Elias Kalil Filho (Instituto Butantan)
09:30 - 10:10	**Frontiers in biodiversity studies: scientific production, biological collections and information manegement** – Hussam El Dine Zaher (Museu de Zoologia, USP)
10:10 - 10:50	**Systems Biology: a novel strategy for drug discovery and targets identification in toxinology** – Mário Sérgio Palma (UNESP - Rio Claro)
10:50 - 11:10	**Coffee break**
11:10 – 11:50	**Activities in computational genomics at Labinfo: applications in public health** – Ana Tereza Ribeiro Vasconcelos (Laboratório Nacional de Computação Científica)
11:50 – 13:00	**Lunch**
13:00 – 15:00	**Poster Session I** 1. Venoms and Envenomations

2. Biochemistry

3. Pharmacology

11. PIBIC/PIBITI

15:00 – 17:30	**Young Scientist Award - Scientific Initiation and PAP Program** **Award Committee:** Ana Paula Lepique (Instituto de Ciências Biomédicas/USP) Edison Barbieri (Instituto de Pesca) Mario Mariano (Universidade Paulista)
15:00 – 16:00	**Scientific Initiation Award** Coordinator: Denise Vilarinho Tambourgi (Instituto Butantan)
16:00 – 16:30	**Coffee break**
16:30 - 17:30	**PAP Program Award** Coordinator: Denise Silvina Piccini Horton (Instituto Butantan)

Thursday 12/01/2011

09:00 – 12:00	**Thematic Session II: Frontiers in Biotechnology** Coordenadora: Luciana Cezar de Cerqueira Leite (Instituto Butantan)
09:00 – 09:40	**Deep Sequencing, Immune Repertoires, and Antibody Discovery** – Gregory C. Ippolito (University of Texas)
09:40 – 10:20	**Systems Vaccinology: Learning to compute the behavior of vaccine induced immunity** – Helder I Nakaya (Emory Vaccine Center, USA)
10:20 – 10:40	**Coffee break**

10:40 – 11:20	**Snakebites as Neglected Tropical Diseases: Specific Antivenom Development and Supply** - Ana Padilla (World Health Organization)
11:20 – 13:00	**Lunch**
13:00 – 15:00	**Poster Session II** 7. Cellular Biology and Genetics 8. Animal Biology 9. History, Education and Science Dissemination 10. Others 12. PAP Program
15:00 – 17:30	**Young Scientist Award – Master and Doctoral Degrees** **Award Committee:** Carlos Arturo Navas (Instituto de Biociências, USP) Niels Olsen Saraiva Camara (Instituto de Ciências Biomédicas, USP) Ricardo Giordano (Instituto de Química, USP)
15:00 – 16:00	**Master Degree Award** Coordinator: Ana Maria Moura da Silva (Instituto Butantan)
16:00 – 16:30	**Coffee break**
16:30 – 17:30	**Doctoral Degree Award** Coordinator: Waldir Pereira Elias Jr (Instituto Butantan)

Friday 12/02/2011

09:00 – 11:20	**Thematic Session III: Frontiers in Science Dissemination** Coordenador: Fan Hui Wen – Instituto Butantan

09:00 – 9:40	**Cultural heritage and education: Border and crossings between science and art** – Claudinéli Moreira Ramos (Department of Culture, Sao Paulo State)
09:40 – 10:20	**Sharing biodiversity data: What does Brazil have to gain from the Global Biodiversity Information Facility?** – Tim Hirsch (Global Biodiversity Information Facility – GBIF)
10:20 – 10:40	**Coffee break**
10:40 – 11:20	**How to improve science journalism?** – Carlos Henrique Fioravante (Fapesp Magazine)
11:20 – 13:00	**Lunch**
13:00 – 15:00	**Poster Session III** 4. Immunology and Vaccines 5. Microorganisms 6. Biotechnology
15:00 – 15:30	**Instituto Butantan Flashbacks** **The 80's, Instituto Butantan and the sera crisis** Sávio Stefanini Sant´Anna - Instituto Butantan
15:30 – 15:50	**Special Session** **Tribute to Scientific Leaders, Researchers and Employees of Butantan Institute who contributed for the Institute renewal in the 80's** Wilmar Dias da Silva – Instituto Butantan
15:50 – 16:30	**Young Scientist Award**
16:30 – 17:00	**Closing Session**

1. Venoms and envenomations

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.01 New insights into matrix degradation induced by the hemorrhagic metalloproteinase HF3: characterization of the interaction and cleavage of proteoglycans by immunohistochemistry and surface plasmon resonance

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Introduction: Snake venom metalloproteinases (SVMPs) play a key role in envenomation causing local and systemic hemorrhage. The proteinase domain of hemorrhagic SVMPs is believed to function to degrade capillary basement membranes, endothelial cell surfaces, and the stromal matrix, ultimately causing extravasation of capillary contents into the surrounding stroma. Hemorrhagic factor 3 (HF3) is a glycosylated P-III SVMP isolated from *Bothrops jararaca* venom. HF3 is an extremely active toxin that shows a minimum hemorrhagic dose of 240 fmol on rabbit skin. Previous studies have shown that HF3 is able to degrade plasma and extracellular matrix (ECM) proteins such as fibronectin, vitronectin, fibrinogen, von Willebrand factor, and collagens IV and VI. However, the action of SVMPs on the ECM proteoglycans is unknown. **Objectives:** Considering the importance of proteoglycans in the physical properties of ECM, an analysis was performed in order to assess the effect of HF3 upon these proteins. **Methods:** Proteoglycans were incubated for 5 h at a 1:10 enzyme-to-substrate ratio with HF3 to analyze proteolytic cleavage *in vitro* by SDS-PAGE. The interaction of proteoglycans with HF3 was measured by surface plasmon resonance (SPR) using a BIAcore T100 system. HF3 was covalently immobilized on the CM5-sensor chip and proteoglycans were injected over the immobilized surface. The non-linear fitting of association and dissociation curves according to a 1:1 model was used for the calculation of kinetic constants. The effect of HF3 on proteoglycans *in vivo* was assessed by injecting HF3 (0.5 μg) intradermally on the dorsal area of Swiss mice. After 2 h the skin was removed and fixed to be embedded in paraffin. Tissue sections were processed for immunostaining using antibodies against proteoglycans. The distribution of proteoglycans was observed in the HF3-treated skin and compared with control skin. **Results and Discussion:** Decorin, biglycan, syndecan, glypican, and aggrecan were degraded by HF3; however, no cleavage of brevican was detected. In the SPR assays, biglycan, syndecan and glypican were demonstrated to bind to HF3 showing equilibrium dissociation constants compatible to a 1:1 binding model. The immunohistochemical analysis showed changes in the distribution of decorin and biglycan in the HF3-treated skin, while syndecan did not seem to be affected by HF3. Taken together, these results indicate that the proteolysis of some proteoglycans may contribute to the hemorrhage induced by HF3.

Supported by: FAPESP

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.02 Interaction of snake venom metalloproteinases from *Bothrops jararaca* with peptide and protein inhibitors

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Introduction: Snake venom metalloproteinases (SVMPs) participate in the venom hemorrhagic process by proteolytically degrading extracellular matrix and capillary basement membranes leading to hemorrhage and edema. Three SVMPs from *Bothrops jararaca* venom showed different hemorrhagic activities: HF3 (P-III class) is highly glycosylated and ~80 times more hemorrhagic than bothropasin (P-III class), which has a minor carbohydrate moiety and BJ-PI (P-I class) is not hemorrhagic but is highly proteolytic toward plasma and extracellular matrix proteins. **Objectives:** We evaluated the effect of some proteinase inhibitors on the activity of SVMPs: snake venom peptides, Z-PLG-hydroxamate, DM43, a metalloproteinase inhibitor from *Didelphis marsupialis*, and alpha2-macroglobulin. **Methods:** To evaluate the neutralization capacity of peptides pHpG1 and <EKW, Z-PLG-hydroxamate and DM43 on the hemorrhage induced by HF3, Swiss mice were injected intradermally on the dorsal region with HF3 pre-incubated with these molecules. After 2 h, the dorsal skin was sectioned and the underside was photographed. To analyze the effect on proteolytic activity, HF3, bothropasin and BJ-PI were pre-incubated with <ENWPHPQIPP (BPP-10c), <EKWAP (BPP-5a), <EKW, pHpG1 and Z-PLG-hydroxamate and further incubated with fibrinogen and collagen VI. Moreover, native and *N*-deglycosylated HF3, bothropasin and BJ-PI were pre-incubated with DM43 and alpha2-macroglobulin and then incubated with fibrinogen and collagen VI. Proteolysis results were analyzed by SDS-PAGE. **Results and Discussion:** <EKW inhibited the degradation of fibrinogen and collagen VI by HF3, bothropasin and BJ-PI; however, BPP-10c had no effect. pHpG1 and BPP-5a did not inhibit the degradation of fibrinogen and collagen VI by HF3, bothropasin and BJ-PI; however, different fibrinogen degradation patterns were observed compared to the control. Z-PLG-hydroxamate inhibited the fibrinogenolytic activity of the three SVMPs and the degradation of collagen VI by botropasin and BJ-PI. DM43 inhibited the degradation of fibrinogen and collagen VI by bothropasin and BJ-PI, but it had no effect on the ability of native and *N*-deglycosylated HF3 to hydrolyze these substrates. Interestingly, alpha2-macroglobulin did not inhibit the proteinases and was instead cleaved at different sites. These results suggest that although the SVMP active sites are similar they interact with peptide and protein inhibitors in different ways, probably due to their different tertiary structures.

Supported by: FAPESP

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.03 Diversity of metalloproteinases in *Bothrops neuwiedi* snake venom

Bernardoni JL[1], Sousa LF[1], Lima-dos-Santos I[1], Portes Jr JA[1], Lopes AS[2], Serrano SM[2], Prezoto BC[3], Moura-da-Silva AM[1]
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Introduction: Snake venom metalloproteinases (SVMPs) are abundant enzymes in venoms and responsible for most of the symptoms of envenoming, acting on different targets of hemostasis. This functional diversity is related to structural variations that exist within this complex family of proteins. **Objectives:** The aim of this study was to demonstrate the diversity of SVMPs in the venom of *B. neuwiedi* and to characterize the activity of the purified proteases on venom-induced hemostatic disturbances. **Methods:** *B. neuwiedi* venom was fractionated by HPLC in a C-18 column or by HiPrep Sephacryl followed by Mono-Q columns, using different buffer systems and gradients for elution. The fractions were analyzed for molecular mass and purity by 12.5% SDS-PAGE and SVMP identity by the reactivity with specific antibodies, anti-SVMPs class P-III (jararhagin) and class P-I (BnP1), by dot-blot. **Results and Discussion:** Fractionation of the venom by HPLC resulted in thirty fractions. Three different fractions showed strong reactivity with anti P-III antibodies and similar elution time of previously reported P-III SVMPs, and were considered such; one fraction previously identified as a P-I SVMP reacted moderately only with anti P-III; and one still unidentified fraction reacted weakly only with anti P-I. To isolate these SVMPs, we fractionated the venom on Hiprep column resulting in eight peaks that were further chromatographed on a Mono-Q column, resulting in two SVMPs of class P-I and two of class P-III. P-I SVMPs were homogeneous on SDS-PAGE, and corresponded to previously characterized BnP1 and BnP2. P-III SVMPs were eluted together in the same fraction. Bands were cut, submitted to mass spectrometry and characterized as jararhagin-like and HR1a-like class P-III SVMPs. All these samples reacted slightly with anti P-I, but fractions containing P-III SVMPs reacted strongly with anti P-III. To demonstrate the activity of the venom and isolated fractions on blood coagulation, initially, the crude venom was tested using thrombelastometric assay with the ROTEM® four-channel system. Significant decrease in clotting time was observed after the addition of 10 ng of crude venom. The action of each isolated SVMP is now under evaluation. Analyzing the data, we could see the high diversity of SVMPs in the venom of *B. neuwiedi,* which showed strong procoagulant activity. We demonstrated the presence of metalloproteinase from classes P-I and P-III. Our data suggest that different components of the coagulation system may be targeted by these toxins, thus accounting for the strong procoagulant activity of *B. neuwiedi* venom.

Supported by: FAPESP and CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.04 α-RgIIA – a novel peptide from *Conus regius* venom, antagonist of neuronal acetylcholine receptor

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Introduction: *Conus* venom is a rich source of biologically active peptides that act specifically on ion channels and/or neuromuscular receptors, efficiently paralyzing the prey. Each species of *Conus* has 500 to 200 uncharacterized bioactive peptides of pharmacological interest. *Conus regius* is a vermivorous species that inhabits Northeastern Brazilian tropical waters. **Objectives:** In this work, we characterized one peptide with activity on neuronal acetylcholine receptor (nAChR). **Methods:** Crude venom was purified by reverse-phase HPLC, and selected fractions were screened and sequenced by mass spectrometry, MALDI-ToF and ESI-Q-ToF, respectively. **Results and Discussion:** A new peptide was identified, bearing two disulfide bridges. The novel 2,701-Da sequenced peptide belongs to the CC-C-C cysteine pattern family. The biological activity of the purified peptide was tested by intracranial injection in mice, and high concentrations were found to induce hyperactivity in the animals, whereas lower doses caused breathing difficulty. The activity of this peptide was assayed in patch-clamp experiments, on nAchR-rich cells, in whole-cell configuration. The peptide selectively blocked neuronal receptors α3β4 and/or α3β4α5, with slow rise-time. According to the nomenclature, it was designated as α-RgIIA. This peptide may actively participate in the envenomation process, increasing the efficiency of the venom cocktail.

Supported by: FAPESP and CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.05 Purification and characterization of a metallopeptidase from *Tityus serrulatus* scorpion venom

Cajado DC[1], Kuniyoshi AK[1], Magnoli FC[1], Rioli V[2], Spadafora-Ferreira M[3], Tambourgi DV[1], Portaro FCV[1]
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Introduction: Scorpions are chelicerates widely distributed throughout the world, showing great evolutionary success. They are a main public health issue because they are the major cause of human poisoning by animals. In fact, the number of accidents involving humans has increased over the last years, and scorpion stings have become more frequent than snake and spider accidents. In Brazil, most fatalities result from stings of *Tityus serrulatus* scorpions, due to the increase in the number of animals, and to their potential to induce severe, even fatal, clinical manifestations, especially among children. In spite of this, little information about the proteolytic compounds present in the venom and their mechanism of action are available. **Objectives:** The aim of this study was to carry out a better biochemical characterization of the sole metallopeptidase in this venom, which was described up to now (antarease), and to correlate it with the poison's effects. **Methods:** In this first step, the *Tityus serrulatus* venom (TsV, 5 mg/mL) was fractionated using two ultrafiltration membranes with a molecular weight cut-off of 30 kDa and 10 kDa), resulting in 3 new fractions. These fractions (G, >30 kDa; M, 10-30 kDa and P, <10 kDa) were analyzed by SDS-PAGE and studied through the use of FRETs substrates in a spectrofluorimeter. The fractions that displayed peptidase activity (M and G) were subjected to HPLC purification using a C-4 RP column, and the peaks manually collected for further assays. **Results and Discussion:** Fractionation of the TsV was successfully performed, resulting in samples containing peptides or proteins, according to their molecular weight (P, M and G). The metallopeptidase was detected in fractions M and G by using the FRET substrate Abz-GGFLRRVQ-EDDnp. The tests indicated higher proteolytic activity in the G fraction, and therefore, this fraction was selected to be purified in the RP-HPLC system. The G fraction was separated in a HPLC C4 column, resulting in 10 peaks (G1-G10), which were collected and lyophilized. Of these 10 peaks, only two showed proteolytic activity (G2 and G3) upon the FRET substrate. This activity was abolished by 100 mM EDTA, probably due to the presence of antarease. The next steps will be to define ideal biochemical conditions for antarease, i.e., the effect of salts and pH on its proteolytic activity. In order to find a possible relationship of this metallopeptidase with the scorpion envenomation effects, biological peptides (such as dynorphin, angiotensin, bradykinin and neurotensin) will be used as putative substrates for antarease.

Supported by: CAPES and CNPq/INCTTOX

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.06 Main venomous arachnids in Brazil: methods to obtain and quantify venom

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Introduction: The principal venomous arachnids in Brazil are *Tityus serrulatus*, *Loxosceles* spp. and *Phoneutria nigriventer*. Accidents caused by scorpions are more common than by spiders. Among spiders, *P. nigriventer* shows the most records in Hospital Vital Brazil. Instituto Butantan is responsable for supplying 70% of polyvalent antiarachnid serum (*T.serrulatus*, *P. nigriventer* and *L. gaucho*) and antiscorpionic serum (*T. serrulatus*) for the Ministry of Health. During the 20th century and up to now, venom was obtained by macerating the spider and scorpion glands. However, this procedure did not result in a high-quality product, because impurities were found in the venom, and the arachnid was sacrificed in the process. In order to remedy these problems, Wolfgang Bücherl was the first researcher to apply an efficient mechanism of venom extraction with electrical stimulation at Instituto Butantan. Recently, in partnership with FATEC and PUC/SP, electrical stimulation equipment based on W. Büchel's research has been developed to minimize the collateral effects of electrical stimulation in arachnids and increase production efficiency. **Objectives:** We aimed to describe the method used to immobilize the arachnids during venom extraction and to quantify the venom production. **Methods:** To achieve the objectives, data were collected for the last six years. For manual handling of *L. gaucho,* the thumb and forefinger were used to immobilize the animal's legs. Scorpions were held by their metasoma with two tongs, so the telson was immobilized and transferred to the operator's hand and contained with the thumb and forefinger. For *P. nigriventer*, immobilization was done when the spider raises its legs as the attack position. The tongs must hold the spider by the cephalothorax. The immobilization was completed when the spider was transferred to the operator's hand and held by its legs. The spiders were placed in vertical position in front of the terminal to receive the electrical stimulation. With a precision pipette the venom was collected directly from the chelicerae. Electrical stimulation depends on the size of the arachnid. **Results and Discussion:** Since 2006, 13,747.4 mg of *T. serrulatus* venom were obtained, resulting in an average of 0.425 mg/individual, 3,573.2 mg for *L. gaucho*, resulting in 0.034 mg/individual, and 4,563.3 mg for *P. nigriventer*, resulting in 0.949 mg/individual. These methods have been shown to be the most efficient to date. Other methods were used in the past such as holding the arachnid with only one a pair of tongs without immobilizing it with the hand. Furthermore, when the operator feels safe, care for the animal and the procurement of venom increase.

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.07 Analysis of the individual variation of the venom proteome of *Bothrops jararaca* newborn specimens

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Introduction: The venom of *Bothrops jararaca* is one of the most explored in Brazil due to the large geographical distribution of this snake and the number of envenomation cases. Although a number of toxins have been isolated from this species, the use of powerful tools, such as proteomic and transcriptomic techniques, have improved the understanding of this venom as a whole. Snake venom proteome variability has long been appreciated by investigators, and it is a well-documented phenomenon. Previous studies have demonstrated individual, sex-based and ontogenetic variations in *Bothrops jararaca* venom. However, the individual variability among newborn specimens is poorly known. **Objectives:** The aim of this study was to analyze the individual proteomic profile of toxins in the venom of newborn specimens of *B. jararaca*. **Methods**: Venom from 27 newborn specimens of *B. jararaca* born in captivity at the Herpetology Laboratory of Instituto Butantan was milked and stored at -20°C until use. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out followed by specific staining for total proteins and glycoproteins. Proteolytic activity was assessed using dimethylated casein as substrate. Differential proteins detected in the SDS-PAGE analysis were subjected to *in-gel* trypsin digestion followed by liquid chromatography coupled to mass spectrometry in *tandem* (LC-MS/MS) and database search for protein assignment. **Results and Discussion:** SDS-PAGE analysis revealed clearly distinct profiles among the newborn venoms from sibling and non-sibling specimens. Differential proteins among the venoms were visualized in the molecular mass range of 90 kDa to 20 kDa. Staining with the fluorescent glycoprotein-specific reagent Pro-Q-Emerald showed a number of glycoprotein bands ranging from 70 kDa to 14 kDa in all venom samples; however, some subtle variation in the venom glycoproteomic profiles was detected. Proteolytic activity on casein showed variation among the samples and the specific activities ranged from 0.3 U/mg to 1.2 U/mg. The preliminary analysis of some protein bands by LC-MS/MS showed differential expression of metalloproteinases among the newborn venoms. These approaches should give rise to a more thorough understanding of the individual variability of newborn snake venoms and provide insights into the ontogenetic change of the *B. jararaca* venom profile.

Supported by: FAPESP

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1.08 Skeletal and visceral analysis of pups exposed to *Tityus bahiensis* scorpion venom during the gestational period

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Introduction: Scorpion envenoming is a public health problem. In Brazil, the scorpion *Tityus serrulatus* is considered the most dangerous, but a large number of exposures also occur with *Tityus bahiensis*. There are quite a few studies in the literature about the toxic effects of this venom. **Objectives:** The objective of this work was to determine the possible toxicological effects *Tityus bahiensis* venom on the skeletal and visceral development of rat pups, when the venom was administered to pregnant dams. **Methods:** A dose of 2.5 mg/kg of venom was injected subcutaneously. The experimental groups were injected with venom on the 5th (GD5; n=10) or on the 10th (GD10; n=10) gestational day. The control group (C; n=10) was injected with 1.46% NaCl (subcutaneous injection) on both days. On the 21st gestational day, the pups were taken out by laparotomy and divided into two groups that received specific treatments for skeletal and visceral analyses, respectively. **Results and Discussion:** No external or internal anomalies and malformations were observed in the offspring of both experimental groups. There was an alteration in heart weight (C=0.04±0.01; GD5=0.06±0.01*; GD10=0.05±0.01*) and in lung weight (C=0.14±0.01; GD5=0.15±0.01*; GD10=0.15±0.01*) in GD5 and GD10 and in liver weight (C=0.47±0.01*; GD10=0.58±0.01*) in GD10. Therefore, moderate envenomation by *Tityus bahiensis* scorpion venom does not induce external anomalies or malformations in the offspring of pregnant rats treated during the gestational period but does cause an increase in the weight of some organs.

Supported by: CAPES and Fundação Butantan

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1.09 Some aspects of inflammation induced by *Potamotrygon motoro* stingray venom in mice and its blockade by indomethacin

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Introduction: Stingray accidents cause an intense pain followed by edema, erythema and necrosis. There is no specific treatment so far, and therapy is based on the administration of analgesic, antipyretic and anti-inflammatory drugs. **Objectives:** The aim of this work was to study edema formation, leukocyte recruitment and release of inflammatory mediators after the injection of *Potamotrygon motoro* stingray venom into footpad of mice, and the modulation of inflammation using indomethacin. **Methods:** Samples of *P. motoro* venom (2, 8 and 32 µg/30 µL) were injected into the mouse footpad to evaluate edema formation, cell recruitment and release of inflammatory mediators. Edema formation was measured by plethysmography at 0.25, 0.5, 1, 4, 24 and 48 h after venom injection. In an independent assay, the right paw of mice injected with *P. motoro* venom was processed and the supernatant was used to assess the inflammatory mediators released (IL-6, MCP-1 and KC) using EIA kits, and cell pellets were recovered to perform cell counts. In order to assess pharmacological inhibition of inflammation, indomethacin (4 mg/kg) or PBS (control) were injected (500 µL) i.p. 30 min before injection of venom into the mouse footpad. **Results and Discussion:** Edema was observed as soon as 15 min after venom injection at all doses tested, reaching a peak in 0.5 h, and at 48 h it was still detected with the 8- and 32-µg doses. *P. motoro* venom stimulated leukocyte recruitment at all doses tested, especially neutrophils, to the injection site, for all time periods analyzed. After 24 h, an increase in monocytes/macrophages was also detected. Inflammatory mediators released (IL-6, MCP-1 and KC) were already detected at 1 h after venom injection and significantly increased at 4 h, in relation to the control group (PBS). Only MCP-1 levels were detected at 24 h with the 32-µg dose. No significant levels of these inflammatory mediators were detected at 48 h. Indomethacin partially reduced edematogenic activity (13 - 24 %) mainly at early times (0.5 - 4 h) with the 8- and 32-µg doses. At 24 h, this drug decreased edema in the group injected with 8 (47 %) and 32 µg (34 %) of venom. Indomethacin was also able to reduce leukocyte influx at 4 h with the 8- and 32-µg doses, reaching 20 and 36 % of inhibition, respectively. These results show that stingray accidents evoke a complex inflammatory reaction, with the involvement of eicosanoids, leukocyte recruitment and release of cytokines and chemokines. However, further studies are necessary to elucidate the mechanisms involved in stingray envenomation.

Supported by: FAPESP and CAPES

1.10 Inflammatory mediators generated in the local reaction induced by *Scolopendra viridicornis* centipede venom

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Introduction: Envenomation by centipedes is generally mild, and human victims usually manifest burning pain, paresthesia and edema, which may develop into superficial necrosis at the site of the bite. Treatment of stings is supportive and, when necessary, includes the administration of anti-inflammatory and analgesic drugs. Despite the abundance of these animals and frequency worldwide, centipede venoms are poorly characterized in the literature, and some authors have considered them as a neglected group of venomous animals. **Objectives:** The aim of this work was to evaluate edema formation, leukocyte infiltration and release of inflammatory mediators after injection of *S. viridicornis* centipede venom into mice. **Methods:** Swiss mice (n= 4-6) were i.d. injected in the right hind paw with PBS (control) or venom (3.8, 15 and 60 μg). At 0.5, 1, 4, 6, 24 and 48 h, the paws were processed to obtain cell pellets and supernatants, in order to perform cell counts and to assess the release of cytokines and chemokines by ELISA, respectively. Paw edema was determined by plethysmography at the same time periods described before. **Results and Discussion:** The highest edematogenic activity of venom was noticed 30 min after venom injection, reaching 35, 53 and 75 % of edema at doses of 3.8, 15 and 60 μg, respectively. At 24 h, edema was detected in animals that received 15 and 60 μg of venom. At 48 h, edema was observed only in animals injected with 60 μg of venom. In relation to leukocyte recruitment, *S. viridicornis* venom induced cell recruitment, mainly neutrophils and monocytes/macrophages, at all doses and time periods analyzed in comparison with PBS-injected mice. An increase in lymphocytes was detected especially between 1 and 24 h at 60 μg venom. Besides, eosinophil recruitment was observed mainly with 15- and 60-μg doses in early time periods (1 - 6 h). Moreover, *S. viridicornis* venom stimulated the release of IL-6 (1 - 6 h at all venom doses; 24 h only with 60-μg dose), MCP-1 (1 - 48 h, all venom doses), KC (1 - 6 h, all venom doses; 24 - 48 h only with 60 μg venom) after venom injection. An increase in IL- 1β levels was only observed at 4 (60-μg dose) and 6 h (15- and 60-μg doses) after venom injection in relation to control group. Conversely, *S. viridicornis* venom did not induce the release of detectable levels of TNF-α. We demonstrated that the edematogenic activity induced by *S. viridicornis* venom was of rapid onset, and the venom stimulated the secretion of proinflammatory mediators, which contribute to the inflammatory reaction induced by *S. viridicornis* venom in the mouse footpad.

Supported by: INCTTOX, FAPESP and CNPq

1.11 *Tityus serrulatus* venom induces a greater inflammatory response in the lung of genetically selected mice

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Introduction: *Tityus serrulatus* is the main cause of accidents involving poisonous animals in Brazil. Several toxins acting on ion channels have been described in the scorpion's venom. However, little is known about other components and their mechanisms of action. Some authors have demonstrated that *T. serrulatus* venom induces the release of inflammatory mediators by immune cells. It is known that the amount of venom injected and age, physical condition genetic factors regarding the victims influence the severity of symptoms reported by patients. **Objectives:** This study aimed to evaluate the action of *T. serrulatus* venom (TsV) and some of its components, in the formation of pulmonary edema and inflammatory infiltration in strains of mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response and BALB/c and to determine if genetic factors are involved in the response to the venom. **Methods:** AIRmax, AIRmin and BALB/c mice were inoculated with 0.75 μg/g TsV, and after different periods, the lungs were collected. The lungs were fixed and stained with H&E for histopathological analysis. Cells were extracted from the lung for phenotype characterization by flow cytometry. For indirect assessment of cellular infiltrate the activity of myeloperoxidase (MPO) was quantified in the lungs. The cytokines IL-1β, IL-6 and TNF-α were quantified in the lung. **Results and Discussion:** Lung histological analysis of TsV-treated AIRmax and AIRmin mice showed an increased perivascular infiltrate of mono- and polymorphonuclear cells at 60 min, when compared to control group. Quantification of MPO activity showed that the venom is able to induce a significant migration of neutrophils in the lung, of AIRmax when compared to AIRmin and BALB/c, 2h after venom inoculation. The phenotypic analysis of cell populations present in the lung of TsV-treated AIRmax mice showed a greater number of $Ly6G^+$ and $CD19^+$, cells, and TsV-treated AIRmin displayed more $Ly6G^+$, compared to their respective controls. In addition, AIRmax displayed a greater number of $Ly6G^+$ compared to treated AIRmin mice, 1 h after venom inoculation. However, 2 h after venom inoculation, there was an increase in $F4/80^+/CD11b^+$ cells in both strains. TsV-treated AIRmax mice showed higher levels of IL-1β, IL-6 and TNF-α in the lung, compared to AIRmin and BALB/c. Our results suggest that *T. serrulatus* scorpion venom is able to induce a significant inflammatory response in the lung, as well as inducing cytokine production. This inflammatory response is more pronounced in AIRmax animals, thus suggesting the importance of genetic factors in the inflammatory response to animal venoms.

Supported by: INCTTOX Program, CNPq and FAPESP

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1.12 Crotoxin induces a long-lasting inhibitory effect on phagocytosis by neutrophils by inhibiting tyrosine phosphorylation and actin polymerization

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Introduction: *Crotalus durissus terrificus* snake venom (CdtV) inhibits, in vitro and in vivo, the phagocytic activity of macrophages and neutrophils. Crotoxin (CTX), the main component of this venom, is responsible for this effect. The inhibitory effect observed in vivo is long-lasting, since a single dose of CdtV or CTX inhibits phagocytosis in macrophages or neutrophils for at least 14 days. In macrophages, in vitro CTX inhibits phosphotyrosine, but causes excessive actin polymerization. **Objectives:** Considering that the mechanisms involved in the long-lasting inhibitory effect of CTX on phagocytosis by neutrophils is still unknown, the aim of this study was to investigate the in vivo effect of CTX on tyrosine phosphorylation and actin polymerization on nascent phagosomes of neutrophils. **Methods:** Male Wistar rats (CEUAIB 705/10) were subcutaneously treated with CdtV (0.18 mg/kg) or CTX (0.1 mg/kg) 2 h or 4 or 14 days before the intraperitoneal administration of carrageenan (4.5 mg/kg). Neutrophils were obtained from the peritoneal cavity, 4 h after the injection of carrageenan, and analyzed for phagocytosis of opsonized zymosan for 5 min. The neutrophils were then fixed, permeabilized, post-fixed and rehydrated with non-immune goat serum. Incubation with primary antibody against phosphotyrosine was performed overnight. The cells were then incubated with the FITC-labeled secondary antibody and stained with rhodamine-phalloidin. Nuclei were stained with DAPI. Slides were mounted and observed by confocal microscopy. Quantification of fluorescence intensity in the samples was performed by the program ImageJ. **Results and Discussion:** The pretreatment of animals for 2 h or 4 or 14 days with CdtV or CTX induced a marked reduction in staining of phosphotyrosine (2 h – CdtV: 86%, CTX: 85%; 4 days – CdtV: 68%, CTX: 78%; 14 days – CdtV: 70%, CTX: 74%) and F-actin (2 h – CdtV: 83%, CTX: 90%; 4 days – CdtV: 84%, CTX: 86%; 14 days – CdtV: 49%, CTX: 49%) in neutrophils during phagocytosis, when compared to controls. Unlike what occurs in macrophages, our results demonstrate that CdtV and CTX inhibit tyrosine phosphorylation and consequently actin polymerization. The results presented herein contribute to explanation of the long-lasting inhibitory effect of CdtV, particularly of CTX, on phagocytosis by neutrophils, since a single dose of CdtV or CTX inhibits tyrosine phosphorylation and actin polymerization for 14 days. Furthermore, taking into account the importance of these phagocytes in inflammatory responses, these results contribute to the elucidation of the mechanisms involved in the anti-inflammatory effect of CTX that has been reported in the literature.

Supported by: FAPESP, CAPES and INCTTOX

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1.13 Accident caused by *Centruroides testaceus* (DeGeer, 1778) (Scorpiones, Buthidae), native of the Caribbean, in Brazilian airport

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Introduction: Envenomation associated with scorpion stings is an important public health problem in tropical and subtropical countries. Although there are approximately 1,500 scorpion species described, currently distributed in 18 families, about thirty of them are recognized as potentially dangerous for humans. Less than a dozen of them are responsible for serious envenomation or death. In the world, the annual number of scorpion stings exceeds 1.2 million leading to more than 3,250 deaths. Severe cases are seen especially among children. While scorpions of the genus *Tityus* are recognized as the main cause of scorpionism in Brazil, the genus *Centruroides* is considered important in southern United States, Mexico, Central America and the West Indies. Found in the Antilles, there are no cases of scorpionism caused by *Centruroides testaceus* (DeGeer, 1778) reported in the literature. **Objectives:** Our aim was to describe a case of a 6-year-old girl who was stung by a *Centruroides testaceus* in Brazil. **Methods:** Case report. **Results and Discussion:** A previously healthy 6-year-old girl was seen at Vital Brazil Hospital, Butantan Institute, one hour and fifteen minutes after being stung by a scorpion inside the Guarulhos International Airport, São Paulo, Brazil. She had disembarked from a flight coming from Bonaire, with stops in Curaçao and Bogota (Colombia). She was carrying her backpack on her shoulders when she felt sharp pain in her right arm and noted that there was a scorpion on her shirt, identified as *Centruroides testaceus*. Clinical examination on admission showed local pain with a small area of erythema in the anterior middle third of her right arm. Blood pressure was 95/65 mmHg, heart rate 61/min, and temperature 36.1 ºC. There were no signs of respiratory distress, and oxygen saturation measured by pulse oximetry was 98% on room air. Cardiac and pulmonary auscultations were normal. The patient was treated with dipyrone orally, hot water bottle at the site of the sting, and kept under observation with cardiac monitoring. About two hours later, she was totally asymptomatic and was discharged, not needing to receive antivenom, despite belonging to the pediatric age group, in which the most severe cases caused by *Centruroides* scorpions occur. Physicians who treat patients stung by scorpions should be alerted to the possibility that such accidents may have been caused by non-native species, especially those occurring nearby airports or ports.

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1.14 Preliminary analysis of the validity of *Bothropoides jararaca* venom produced in the Laboratório de Herpetologia, Instituto Butantan

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Introduction: Snake venoms are constituted by organic and inorganic components, mainly proteins, some being enzymes which have a certain degree of instability. The consequences of long-term storage are little known. The venom obtained at the Laboratório de Herpetologia of the Instituto Butantan are destined for research and specific production of antivenins, and after their processing they are stocked in a -20°C freezer. **Objectives:** The objective of the present study was to analyze possible alterations in the biological and biochemical activities of venoms of *Bothropoides jararaca* stored for years. **Methods:** Thirteen venom samples from *B. jararaca* were studied; they were stored between the years of 1959 and 2000 (1959, 1961, 1963, 1966, 1968, 1970, 1973, 1976, 1977 to 1988, 1993, 1997, 1999 and 2000). Dry in desiccator or lyophilizer and stored to room temperature or -20°C. They were analyzed for protein content, electrophoretic profile in SDS-PAGE, caseinolytic activity, coagulant activity on plasma and toxicity. **Results and Discussion:** The protein content was approximately 70% in all samples, showing thus a considerable loss of protein content, in spite of this, the electrophoretic profile showed preservative aspects for the majority of the samples, with components predominantly of low molecular weight, distributed among the molecular weights 67 and 15 kDa. Corroborating standardization data, the venoms showed proteolytic activity on casein with values between 90 and 136 U/mg except those produced in the years of 1959 and 1963. Clotting activity was altered in the samples of 1959, 1976, 1977 to 1988 and 1993, with values above 200 mg/L. All the samples presented decrease in their toxicity level when compared with venoms of the same species, produced and prepared more recently. The results suggest that in spite of the drying conditions and storage, considered inadequate, the venoms preserved part of their activities. Additional tests are in progress for confirmation of the validity of venoms stored for long periods.

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1.15 Stability of *Crotalus durissus terrificus* venom submitted to different storage conditions

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Introduction: In Brazil, about 7% of the snakebite accidents are caused by snakes of the genus *Crotalus*. The venom is constituted by a mixture of proteins and polypeptides with neurotoxic, myotoxic and clotting activity. It is known that these components, especially the ones with enzymatic activities, can undergo alterations depending on storage conditions to which they are submitted. **Objectives:** The objective of the present study was to test the stability of the venom of *Crotalus durissus terrificus* in the liquid and lyophilized states and submitted to different storage conditions, in stipulated periods. **Methods:** The venoms were analyzed for protein content, electrophoretic profile and toxicity (LD_{50}), immediately after the extraction from the animal and 30 days after liquid and lyophilized powder were stored at room temperature and in a refrigerator (approximately 4°C), -20°C freezer and -80°C freezer. **Results and Discussion:** The protein content of the recently extracted venom was approximately 180.0 mg/ml. After 30 days, a decrease in protein content was observed in the liquid venom stored at room temperature, while venom stored in a refrigerator, -20°C freezer and -80°C freezer retained the same amount protein concentration. The lyophilized samples showed 90 to 100% of proteins for all storage temperatures. The electrophoretic profile in SDS-PAGE showed bands between 67.0 kDa and below 15.0 kDa for the fresh venom. After 30 days, all venoms, both liquid and lyophilized powder, did not change significantly. The venom of *C. d. terrificus* did not show alterations in its toxicity when storage under various conditions and in different physical state, except in the liquid state at room temperature, where it was 3 times less poisonous. The preliminary data indicate that lyophilized venom samples stored at low temperatures maintain their characteristics, corroborating previous studies. Following the proposal of this work, the samples will be tested over one year for better evaluation of their stability.

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1.16 Lipid body formation in murine leukocytes, induced by crude venoms from *Bothrops jararaca* (*Bothropoides*), *Bothrops insularis* (*Bothropoides*), *Bothrops atrox* and *Bothrops moojeni*

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Introduction: Lipid bodies (LBs) are cytosolic inclusions present in most eukaryotic cells, containing neutral lipids surrounded by a single phospholipid membrane and specific proteins, such as the protein related to differentiation of adipocytes (ADRP). LBs are involved in lipid metabolism and generation of inflammatory mediators such as eicosanoids. Moreover, increased numbers of LBs has been demonstrated in inflammatory leukocytes. Venoms from *Bothrops* genus snakes induce a marked inflammatory local reaction characterized by leukocyte influx and release of a variety of inflammatory mediators. However, formation of LBs in leukocytes present at the site of *Bothrops* venom injection is still unknown. **Objectives:** In this study the ability of snake venoms from different species of *Bothrops genus,* namely *B. jararaca (*BjV), *B. insularis* (BiV), *B. moojeni* (BmV) and *B.atrox* (BatxV), to induce LB formation and upregulation of ADRP protein expression was comparatively evaluated in mouse peritoneal leukocytes. **Methods:** Male Swiss mice were used (Butantan Institute Ethical Committee ref. 729/10). These animals received intraperitoneal (i.p) injection of inflammatory doses of BjV (0.250 mg/g), BmV (0.250 mg/g), BiV (0.050 mg/g), BatxV (0,025 mg/g) or apyrogenic saline (control). At selected periods of time (1, 3, 6, 12, 24, 48 or 72 h) after each venom injection, the inflammatory exudates were harvested to determine total number of leukocytes in a Neubauer chamber and subtypes of leukocytes using Hema3 stained cell smears. LB formation was determined in macrophages stained with osmium tetroxide (1%) followed by counting under phase contrast microscopy. ADRP protein expression was evaluated by Western blotting. **Results and Discussion:** Results showed that i.p injection of venom from BjV, BiV, BmV or BatxV caused a significant increase in the numbers of LBs from 1 up to 24 h (200%) when compared to control cells (2.0 ± 0.5 LB/cell, n=4), with no statistical difference between the snake species studied. The highest LB numbers were detected at 12 h (300%) after venom injection in comparison to controls (2.4 ± 0.7 LB/cell, n=4). Moreover, all venoms studied caused a significant increase in expression levels of ADRP protein in leukocytes collected 12 h after their injection. The data indicate the ability of *Bothrops* venoms either from the mainland or island to induce lipid body formation and upregulate ADRP expression in leukocytes. Moreover, these data suggest that LBs have a role in the inflammatory reaction induced by snake venoms from the *Bothrops* genus.

Supported by: FAPESP and CAPES

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1.17 Potential anti-inflammatory properties of crotoxin: role of this toxin on cytokine secretion and adhesion molecule expression

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Introduction: *Crotalus durissus terrificus* snake venom (CdtV) modulates the inflammatory response, and shows a long-lasting anti-inflammatory effect. Recent data demonstrated that a single dose of crotoxin (CTX), the main toxin of CdtV, is responsible for this long-lasting anti-inflammatory effect inhibiting paw edema, leukocyte-endothelium interaction and consequently cell migration induced by carrageenan (Cg). **Objectives:** The aim of this study was to evaluate histologically the long-lasting anti-inflammatory effect of CTX on cell migration to the subcutaneous tissue and investigate if this effect involves the action of CTX on the secretion of cytokines (IL-1β and IL-6) and adhesion molecules (ICAM-1 and P-selectin) involved in inflammatory response induced by Cg. **Methods:** A single dose of CTX (0.89 μg/50 μL s.c.) or saline was administered 7 days or 1 h before Cg injection in male Swiss mice. For histopathological analysis, the footpad was removed 6 h after intraplantar injection of Cg (300 μg/50 μL) or saline. The tissue was processed for light microscopy. Sections were obtained from paraffin-embedded pieces and stained with hematoxylin and eosin (H/E). To evaluate the effect of CTX on cytokine secretion, 4 h after intraperitoneal injection of Cg (300 μg/200 μL) or saline, peritoneal exudate was collected and cytokines (IL-1β and IL-6) were determined by ELISA. To evaluate P-selectin and ICAM-1 expression, the footpad of the treated animals with CTX was removed 30 min or 1 h after intraplantar Cg injection and analyzed by immunohistochemistry. **Results and Discussion:** Histological analysis confirmed once again the potential inhibitory effect of CTX on cell migration. Tissue sections from animals treated with CTX 7 days or 1 h before intraplantar injection of Cg had significantly fewer areas of edema and consequent reduction of inflammatory infiltration (polymorphonuclear cells) compared to untreated animals (Saline+Cg). The exudates of animals pretreated with CTX showed a significant decrease in the secretion of IL-1β and IL-6 when compared to the control group. The same treatment decreased the expression of P-selectin and ICAM-1 in the mouse footpad. These results contribute to the characterization of the long-lasting anti-inflammatory properties of CTX, suggesting that the inhibitory effect involves the action of this toxin in the expression of important pro-inflammatory cytokines and adhesion molecules that have an important role during the inflammatory process. The data reinforce that CTX is a potential natural product in controlling inflammatory diseases.

Supported by: CAPES, FAPESP and INCTTOX

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.18 Characterization of a wild type and a mutant human scFv anti-crotoxin antibody obtained by site-directed mutagenesis based on molecular modeling

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Introduction: Single-chain variable fragments (scFv) contain the variable domain of heavy (VH) and light (VL) chains linked by a flexible polypeptide $(G_4S)_3$ and may be useful as auxiliary therapy for envenoming by snake bite. A human antibody against crotoxin (CTX) was previously isolated by phage display technology from a naive library of more than 10^{10} scFv clones with partial *in vivo* neutralizing activities against CTX and crude venom. Aiming to improve neutralization, a 3-D model of the scFv6 clone was constructed, by homology modeling. Computer-aided docking and energy minimization calculations of the antibody-CTX complex were also performed. From these simulations, three candidate binding sites were chosen to be mutated. **Objectives:** The aim of this study was to characterize biochemically and functionally the scFv6 anti-CTX wild type (wt) and a mutant obtained by site-directed mutagenesis. **Methods:** The desired mutation (S^{30} for A^{30}) was obtained using the QuikChange Multi Site-Directed Mutagenesis Kit. ScFv6 coding sequence was cloned into pET20b+ vector and the construction was used to transform C43 bacteria. The production of scFvs was accomplished using 0.5 mM IPTG and growth conditions at 30°C for 4 h. The mutated protein and wt of the scFv were all expressed in soluble form in C43 *Escherichia coli*. Periplasmic fraction of induced cultures was isolated by osmotic shock and further purified by Ni(2+)-immobilized metal affinity chromatography (IMAC). ELISA, SDS-PAGE and dot-blot were used to evaluate the purity and specificity of the sample. Purified proteins were analyzed for total yield. The capacity of scFv wt and mutant to inhibit CTX-induced rat mast cell line (RBL) degranulation was also evaluated. Degranulation intensity was determined by β-hexosaminidase release. **Results and Discussion:** The yield of scFv wt and mutant was similar. DNA sequencing of mutant scFv6 confirmed mutation of the wt reported sequence (Genbank: AJ132608). Preliminary results indicated that both scFvs were able to inhibit mast cell degranulation induced by CTX in 40%, indicating functional scFvs were produced, although mutation did not seem to have improved scFv, as tested so far. Neutralizing ability of these two scFvs and the other two mutants is yet to be tested and will be tested on different venom toxic activities. Wt and mutants scFvs will also be analyzed by circular dicroism spectropolarimetry and affinity to CTX in surface plasmon resonance (Biacore) assay.

Supported by: INCTTOX program of CNPq and FAPESP and CAPES

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.19 Molecular cloning, expression, function, structure and immunoreactivities of a sphingomyelinase D from *Loxosceles adelaida*, a Brazilian brown spider from karstic areas

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Introduction: Envenomation by spiders belonging to the genus *Loxosceles*, found in temperate and tropical regions of America, Africa and Europe, commonly results in impressive local necrotic skin lesions and can also cause systemic effects, including intravascular hemolysis. *Loxosceles* is the most poisonous spider in Brazil and three different synanthropic species of medical importance are known, namely *L. intermedia, L. gaucho, L. laeta*, and more than 5000 cases of envenomation by *L. intermedia* alone are reported each year. We have purified, characterized, cloned and expressed the toxins from *L. intermedia* and *L. laeta* venom that are responsible for all the local and systemic effects induced by the whole venom. Highly homologous proteins with sphingomyelinase activity (SMases D) were able to induce all the local and systemic effects induced by whole venom. *Loxosceles* species are present in several different habitats, including the karstic environment, and in Brazil it is the most common troglophile arachnid. **Objectives:** The aims of the present study were to clone and express SMases D from the spider gland of *L. adelaida*, captured in the caves of PETAR (Brazil), to compare the functional activities of the recombinant proteins to those of toxins from synanthropic species and to investigate the inter- and intraspecies cross-reactivities of antibodies raised against the purified recombinant proteins. **Methods:** The gene was amplified by RT-PCR and cloned into a prokaryotic expression vector pRSET*A*. The structure-function of the recombinant protein was characterized using *in vitro* and *in vivo* assays, and compared to that of the previously characterized SMase D, in terms of biological, biochemical and structural properties. **Results and Discussion:** We cloned, expressed and biochemically and structurally characterized a new sphingomyelinase D from *Loxosceles adelaida* spider and showed that it displays all the functional characteristics of whole venom. The recombinants toxins, representing different classes of SMase D molecules, will allow us to further characterize the functionally important domains of these proteins. The identification of the active site(s) would aid in the design and testing of suitable anti-sphingomyelinase compounds in the development of novel therapies to treat loxoscelism.

Supported by: FAPESP and CNPq (INCTTOX)

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.20 Comparative study of toxic activities of *Bothriechis schlegelii* snake venoms from Colombia and Costa Rica and neutralization by antibothropic serum

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Introduction: *Bothriechis schlegelii* is a snake found in Mexico, Central America and northwest of South America. Bites in humans are characterized by pain, edema and ecchymosis at the bite site, and slight defibrinating effect. **Objectives:** The aim of this work was to carry out a comparative study of toxic activities of *B. schlegelii* snake venoms from Colombia (BsCo) and Costa Rica (BsCR), and also to investigate the efficacy of antibothropic serum (ABS) produced against a mixture of *B. jararaca*, *B. alternatus*, *B. neuwiedi*, *B. jararacussu* and *B. moojeni* venoms to neutralize these toxic activities. **Methods:** Silver stained SDS-PAGE (12 %) was used to compare the protein profile of BsCo and BsCR venoms (5 μg). Cross-reactivity between BsCo and BsCR venoms was detected by ELISA and Western blotting (WB) using ABS. These venoms (0.5, 1, 2, 4 and 8 μg) were injected into the footpad of mice to evaluate edema development at 0.5, 1, 2, 4, 24, 48 and 72 h. Hemorrhagic activity was observed 2 h after injection (100 μL, i.d.) of BsCo or BsCR (4, 8, 16, 32 and 64 μg) venom in the mouse dorsum. ABS was previously incubated (37 °C, 1 h) with 8 μg or 2 MHD (minimum hemorrhagic dose) of BsCo or BsCR venom to evaluate the neutralization of edema or hemorrhagic activity, respectively. **Results and Discussion:** Many components with similar molecular masses between 250 - 20 kDa were observed in the two venoms, but some protein bands of 60 - 20 kDa were observed exclusively in BsCo venom. Intense cross-reactivity between BsCo and BsCR was detected by ELISA using ABS (titers 256,000). Many different components located around 250 and 15 kDa in BsCo and BsCR venoms were recognized using ABS by WB. The highest edematogenic activity of venoms was noticed at 30 min. However, BsCR venom was more edematogenic when compared to BsCo venom, since the edema persisted up to 48 h. Edema induced by both venoms was partiality neutralized by ABS in the first 4 h. ABS also neutralized the edema induced by BsCR venom up to 48 h. BsCR venom (MHD= 3.9 μg) was almost 4 times more hemorrhagic when compared to BsCo venom (MHD= 14.2 μg). ABS totally neutralized the hemorrhagic activity induced by both venoms. Our results demonstrate that geographic distribution can influence the composition and activity of *B. schlegelii* venom. High antigenic cross-reactivity was detected in BsCo and BSCR venoms using ABS. ABS was also effective in neutralizing some toxic activities from BsCo and BsCR venoms.

Supported by: FAPESP and CAPES

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1.21 Acute and long-term effects of three Geitlerinema spp extracts administrated to mice: histopathological aspects

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Introduction: Cyanobacteria are commonly found in drinking water supplies, and are responsible for numerous cases of human intoxications. *Geitlerinema* is a genus described as unable to form blooms; however, it is very frequent in Sao Paulo´s reservoirs. During the search for bioactive substances from strains maintained in the Cyanobacteria Culture Collection of the Institute of Botany, Sao Paulo, three strains of *Geitlerinema* spp. (SPC920, SPC1044 – *G. amphibium*, SPC939 – *G. splendidum*) showed toxicity in a mouse bioassay (i.p.). **Objectives:** We studied the histopathological aspects of the vital organs of mice used in the toxicity bioassay, treated with the extracts of the three above mentioned *Geitlerinema* spp strains, aiming do determine the cause of death and intoxication. **Methods:** Extract preparation: the cultured cyanobacteria cells were filtered through a AP-20 filter, freeze-dried and then extracted with 0.1M acetic acid (4x) or MeOH/H_2O (75:25, v/v) (5x) by ultrasonication and centrifugation. The supernatant was concentrated, and the extracts were kept at -20°C until use. The toxicity tests (i.p.) were performed in male Swiss-Webster mice (19-21 g). The mouse symptoms were observed up to 7 days after administration. After death by acute toxicity or by euthanasia, necropsy was performed and tissue samples were taken from liver, kidneys and lungs, fixed and used for histological analysis. **Results and Discussion:** The methanol extract (*ME*) of SPC920 showed a MLD_{100} of 500 $mg.kg^{-1}$ b. w. Deaths occurred within 50 min – 48 h. Histological analysis showed hemorrhagic foci in the lungs and erythrocytes within some alveolar sacs. The kidneys had fluid accumulation in the tubules, and the liver, small hemorrhagic foci. SPC1044 acetic acid extract (*AE*) and *ME* did not cause death at 1 $g.kg^{-1}$. Histological analysis of mice euthanized after 8 days showed that *ME* induced an increase in the thickness of inter-alveolar walls, but no alterations in liver and kidneys tissues. SPC939 *AE* caused death within 2 h, while *ME* induced no symptoms in mice (1 $g.kg^{-1}$). After one week, the euthanized animals showed signs of hemorrhage in the lungs. Histological analysis of tissue samples is ongoing. The symptoms observed in this bioassay were different from those displayed by animals poisoned with the already known cyanotoxins. In such cases, histological analysis of vital organs is important to determine the cause of death and intoxication. Isolation and characterization of the toxins is in progress in our laboratories.

Supported by: CNPq and FAPESP

1.22 Anticoagulant activity of crotalic venoms on avian whole blood samples

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Introduction: Snake venoms are a complex mixture of proteins, enzymes and other chemicals. Its composition is highly polymorphic. The crotalic venom shows procoagulant activity in mammalian plasma due to gyroxin and convulxin toxins, which are glycoproteins that act on different coagulation factors. Differences in clotting activities can occur because of variation in venom composition or substrate. Gyroxin is a serine protease with thrombin-like activity. Hieda and Oguiura (2010), using Western blotting, observed three patterns of gyroxin in *Crotalus durissus* venom: two bands with 32.5 and 35.5 kDa, one strong band of 32.5 kDa or one weak band of 32.5 kDa. **Objectives:** The aim of this study was to test the coagulant activity of *Crotalus durissus* venoms with different gyroxin profiles in avian whole blood (WB) samples. **Methods:** Three pools: VP1 (two bands), VP2 (one weak band) and VP3 (one strong band) were preincubated with citrated chicken WB samples before recalcification. The coagulation was monitored by thromboelastometry (TEM) using the ROTEM® four-channel system (Pentapharm, Munich, Germany). **Results and Discussion:** The TEM assay showed the delay of clotting time of avian WB samples by the three pools of crotalic venom at concentrations of at least 30 ng/ml. This anticoagulant activity was dose-dependent. The delay of clotting time of avian WB was also observed in almost 40 % of patients bitten by rattlesnakes. The TEM assay in citrated WB samples seems to be one suitable method to meet our objectives, since it evaluates the effects of venoms on cellular and plasma components of the hemostatic process.

Supported by: FAPESP

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1.23 *Bothrops jararaca* high molecular mass kininogen (BjHK) inhibits changes in leukocyte-endothelial interactions induced by *B. jararaca* snake venom metalloproteinases

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Introduction: *Bothrops jararaca* (*Bj*) snake plasma is rich in protease inhibitors, some of which have inhibitory activity on toxins from its own venom. One of these, which has a molecular weight of 110 kDa, is a potent inhibitor of cysteine proteases and releases a peptide that induces contraction of homologous smooth muscles. Because of these characteristics, this protein, named BjHK (***Bothrops jararaca*** **H**igh molecular weight **K**ininogen), was correlated to mammalian high molecular weight kininogens. Severe local inflammatory symptoms are common in envenoming induced by *B. jararaca* snakebites, and It is known that metalloproteases are the main class of toxins involved in the inflammatory activity of this venom. **Objectives:** Our goal in this study was to test the effect of the BjHK on changes in the leukocyte-endothelial interactions induced by Bj venom or by an isolated *Bj* metalloproteinase, *in vivo*, using intravital microscopy. **Methods:** *Bj* venom (1 μg) or jararhagin (JAR) (0.5μg), a hemorrhagic P3 metalloproteinase isolated from *Bj* venom, incubated (30 min, 37 ºC) or not with BjHK (2 μg), was injected (100 μL) into the scrotal bag of Swiss mice (25 to 28g body weight, n= 5/ group). The microcirculation of cremaster muscle was analyzed 2 or 24 h after the injections. We analyzed a segment (100 μm) of a post-capillary venule for 5 min and counted the number of adhered and emigrated leukocytes, statistically comparing to that observed in the control group injected with sterile buffered saline (significant when $p < 0.05$). **Results and Discussion:** Adhesion 2 h: control= 1.4±0.6; BjHk= 1.8±0.6; VBj= 12.2±0.6; JAR= 15.2±1.0; VBj+BjHK= 4.0±0.6; JAR+BjHK= 4.0±0.9. Emigration 24 h: control= 1.2±0.4; BjHK= 0.6±0.4; VBj= 14.4±0.6; JAR= 16.8±0.6; VBj+BjHK= 2.3±0.5; JAR+BjHK= 3.25±0.5. The results showed that BjHK did not induce changes in leukocyte-endothelial interactions, when compared to the control group. In groups injected with *Bj* venom or with JAR, adhered and emigrated leukocytes were greatly increased at all times studied. When incubated with BjHK, neither *Bj* venom nor JAR induced significant changes in leukocyte-endothelial interactions in microcirculation when compared to the control group. This effect was similar to that observed when *Bj* venom and JAR were treated with o-phenanthroline, despite the mechanism of action of BjHK in the catalytic activity of JAR not being like that of a chelating agent. We conclude that BjHK can inhibit this inflammatory activity of *Bj* venom probably by inhibiting the activity of its metalloproteinases.

Supported by: FAPESP, CAPES, CNPq, and INCTTOX

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.24 Hyperalgesic and inflammatory effects of an isolated molecule from sea urchin (*Echinometra lucunter*) spines

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Introduction: *Echinometra lucunter* is the most common sea urchin in Brazil, and can be found along all the Western Central Atlantic waters. Its body is covered by calcified spines that are mainly involved in the defense of the animal, making this urchin responsible for about 50% of the accidents caused by marine animals in Brazil. Victims have been treated mostly for the mechanical consequences of the spines penetrating the skin. The symptoms, on the other hand, are usually beyond trauma and may be pathologically varied and lasting differently, ranging from spontaneous healing in a few days to painful consequences lasting weeks. We have shown that there are toxins in the *E. lucunter* spines that cause inflammation and nociception in animal models. **Objectives:** The aim of the study was to isolate the molecule responsible fpr the physiopathological effects observed in the extract of *E. lucunter* spines. **Methods:** An aqueous extract of *E. lucunter* spines was fractionated by solid phase extraction (SPE), with elutions of ACN. The fractions were purified by RP-HPLC, in a C18 column. The fractions and the peaks obtained by HPLC were tested to identify the active molecule. Samples (10 μg) were injected in mice (CEUAIB 438/07), and after 2 h, the alterations in the microcirculation (rolling, adhered and migrated cells) were observed. Rat paw edema was evaluated by a plethysmometer 1, 2 and 4 h after injection. In order to verify the hyperalgesic effect, the pain threshold, in rats, was measured before and after administration, at 1, 2, 4 and 8 h, using a pressure apparatus. Spectroscopic analysis of NMR and IR was performed to characterize the active molecule. **Results and Discussion:** The effects observed with the crude extract could be related to the SPE 25% ACN. This fraction was purified, and one peak was found to be responsible for the effects on microcirculation, edema and hyperalgesia. In microcirculation, a significant increase in the number of adhered and migrated cells was observed. This molecule also caused edema, although not intense. The pain threshold significantly diminished one hour after the injection of the molecule and only eight hours after the administration, the pain threshold value had returned to the initial level. Spectroscopic analyses showed that the molecule is aliphatic, with CH_2 and CH_3, OH and amine groups. Complementary analyses are currently ongoing to elucidate the structure of the molecule. Our data show a single molecule from the *E. lucunter* spines that causes inflammation (edema and alterations in the microcirculation) and hyperalgesic effect.

Supported by: FAPESP and CNPq

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.25 Parotoid gland secretion contents: comparison between toads of Rhinella and Rhaebo genera

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Introduction: The common toads, that until recently were all grouped in the genus Bufo, have lately been divided into two genera: i) Bufo: basically restricted to species in Europe, Asia and Africa; and ii) Rhinella: South American species. Basal species, such as the previously known *Bufo guttatus*, are now allocated in Rhaebo, which comprises nine species, distributed from Honduras to Colombia, going through Ecuador, Venezuela, Guianas, Peru, Bolivia and Brazil. Although these genera (Rhinella and Rhaebo) are related, some species may share profound differences. *Rhinella jimi* inhabits the caatinga, a semi-arid biome of Northeast Brazil. *Rhinella major*, on the other hand, inhabits the Amazon Rainforest floor. The toxic substances present in the animals' skin glands (either parotoid or granular) may be used by the animal as chemical defense against predators and/or microbial infection, and are very diverse in their chemical composition, including proteins, peptides, biogenic amines, toxic steroidal bufadienolides, toxic samandarine alkaloids, and the indolic pseudophrynamine alkaloids, depending on the species. **Objectives:** The objective of the study was to perform a biochemical characterization of the major components of the venom of four *Rhinella* species (two from the caatinga and two from the rainforest) and one *Rhaebo* (representing a very basal species) in order to determine whether differences between (and within) Rhinellas and Rhaebo (habitat, diet and behavior) may also be present (or reflected) in the venom composition, which may display characteristics that are associated with the environment and/or defense strategies, as well as evolutionary features. **Methods:** Amphibian skin was compared by means of RP-HPLC with either UV or MS detection and by SDS-PAGE. **Results and Discussion:** Although some components were common between the secretions, we were able to identify molecules exclusive to some animals. Habitat and/or diet (and not only evolution) seemed to directly influence the skin secretion composition, since closely related animals living in different habitats secreted different molecules into the skin.

Supported by: FAPESP/CNPq/INCTTOX

1.26 Pulmonary effects induced by *Tityus obscurus* scorpion venom in rats

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Introduction: Scorpions of medical importance in the Brazilian Amazon belong to the *Tityus* genus, and *Tityus obscurus* is the species responsible for the greater number of accidents in the northern area of the country. **Objectives:** This work aimed to determine if *Tityus obscurus* scorpion venom induces pulmonary edema as observed with other *Tityus* species. **Methods:** The scorpions were captured in the west region of Pará State and submitted to electrical stimulation to obtain their venom. A dose of 10 mg/kg diluted in 1.46% NaCl was used. Male Wistar rats (230-260 g) were separated into 3 experimental groups injected with scorpion venom and euthanized 1, 4 or 6 h after injection, and 1 control group injected with 1.46% NaCl (1 ml/kg) and euthanized 1 h later. Five animals were utilized per group injected intraperitoneally. The lungs of animals were removed, free from trachea, and weighed. The severity of the pulmonary edema was estimated by the lung/body index (lung weight X 100/ body weight). Student's t-test was used for statistical analysis, $p<0.05$. **Results and Discussion:** The venom caused respiratory distress and hemorrhagic points. However, pulmonary edema was not observed in any group (C 0.516 ± 0.041; E1h 0.490 ± 0.404; E4h 0.479 ± 0.048 and E6h 0.459 ± 0.526). The *Tityus obscurus* scorpion venom evoked some pulmonary effects without pulmonary edema. This kind of effect is different from that induced by the venoms of other *Tityus* species, which cause pulmonary edema with smaller doses and less extensive hemorrhage.

Supported by: CAPES and INCTTOX

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.27 Digestive carboxypeptidases and the development of *Plasmodium* in *Anopheles aquasalis* and *Aedes aegypti*

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Introduction: Malaria affects 300 million people worldwide every year. The main malaria vectors in Brazil are *An. darling and An. aquasalis*. Infection by *Plasmodium* occurs through the digestive system of the insect host making the digestive enzymes effectors of the parasite's development. Carboxypeptidases remove amino acids from the C-terminal end of proteins by hydrolysis. Midgut carboxypeptidase and antibodies against carboxypeptidase of *An. gambie* affect *P. falciparum* development. **Objectives:** This study aimed to characterize the effect of the infection of *An. aquasalis* and *Ae. aegypti* by *Plasmodium* sp through the expression of midgut carboxypeptidase by real-time PCR, recombinant carboxypeptidases from *Ae. aegypti* and *An. aquasalis* and the effect of antibodies in mosquitoes diet. **Methods:** Sequence coding for midgut carboxypeptidase of *Ae. aegypti* (CarboxyA) and *An. aquasalis* was obtained in Vectorbase or by Race 3´ (CarboxyQ), respectively. CarboxyA and the CarboxyQ were cloned into the pAE expression vector (pAE-Carboxy-Anopheles and pAE-Carboxy-Aedes), which were used to transform the OrigamiB (DE3) strain. Expression in the transformed cells harboring pAE-CarboxyA or pAE-CarboxyQ was induced with 1 mM IPTG. After 0 and 4 h inductions, 1 ml of the bacterial culture was centrifuged and resuspended in loading buffer. Total cellular proteins were separated by 12% SDS-PAGE. Alternatively, the transformed cells were treated with 1 mM IPTG for 5 h (37°C or 30°C, 250 rpm) or 15 h (20°C, 250 rpm.). Cells were centrifuged at 3000 rpm for 10 min at 4°C, treated, disrupted by sonication and centrifuged at 10,000g for 30 min at 4°C in order to determine expressed protein solubility which was visualized by SDS-PAGE. Quantitative expression of CarboxyA in *Ae. aegypti* midgut after ingestion of *P. gallinaceum* was determined by real-time RT-PCR at different time points (3 h, 24 h and 48 h) and normalized to the expression of the ribosomal protein gene s6. **Results and Discussion:** The analysis of total cell proteins from IPTG-induced Origami DE3 pAE-CarboxyQ by SDS-PAGE exhibited a predominant protein band of 46 kDa. However, the expressed protein was in inclusion bodies. The same results were obtained with *Ae. aegypti* carboxypeptidase. Expression of CarboxyQ differed in mosquitoes fed *P. gallinaceum* and mosquitoes fed non-infected blood. The strong effect was observed at 24 h post-blood meal, when carboxypeptidase expression decreased twofold, compared to the level detected in mosquitoes fed non-infected blood. The modulation of transcription by the parasite could increase its survival and development in the insect, since early insect forms of *Plasmodium* are susceptible to protease digestion.

Supported by: FAPESP and CNPq

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1.28 GST-INS activity (a recombinant disintegrin from *Bothrops insularis* venom): coagulation parameters and platelet aggregation inhibition *in vitro* and *in vivo* models

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Introduction: Disintegrins are soluble small, non-enzymatic proteins from snake venom, which inhibit platelet aggregation via the occupancy of fibrinogen-binding receptors. Like other disintegrins, insularin was isolated from *Bothrops insularis* venom and identified based on its antagonism of GPIIbIIIa and strong inhibitory activity against ADP-induced platelet aggregation. Both native (INS) and recombinant insularin (GST-INS) inhibit aggregation of human platelets in a dose-dependent manner as other non-peptide drugs (GPIIbIIIa antagonist) used in human clinical practice (tirofiban). **Objectives:** The aims of the study were 1) to determine GST-INS, INS, tirofiban (Tir) activity in an in vitro coagulation parameters model by tromboelastography and 2) to analyze GST-INS activity in the inhibition of platelet aggregation in vivo. **Methods:** Human whole blood from healthy donors and volunteers was collected in 3.8% citrate buffer and centrifuged at 800 rpm for 20 min to obtain platelet-rich plasma (PRP). A volume of 400 µl of PRP was incubated with PBS, GST, GST-INS, INS and Tir at a concentration that was shown to inhibit 100% of platelet aggregation induced by 10 µM ADP. The samples were subjected to thromboelastometry to analyze the effect of these proteins on coagulation parameters such as clotting time (CT), time to clot formation (CFT), and maximum clot firmness (MCF). GST-INS (375µg/kg) was injected intravenously in rabbits properly anesthetized and their blood was collected at the following times: 30, 60, 120 and 180 min. The blood was centrifuged to obtain PRP. The kinetic time for inhibition of platelet aggregation was determined after challenge with 10 µM ADP. **Results and Discussion:** There were no differences between platelet antagonists themselves and the controls (PBS or GST) by the thromboelastometry technique. GST-INS showed efficiency in inhibiting the in vivo platelet aggregation model. The maximum time of GST-INS activity was detected at 120 min post-administration of GST-INS, and no increase in clotting time or local or systemic bleeding was detected at any of the times after the administration of GST-INS. The overall results obtained in vitro and in vivo show that insularin is a selective antagonist of platelet aggregation with no coagulation disturbance and GST-INS is a potential tool in the development of therapeutic agents in thrombosis.

Supported by: FAPESP

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

1.29 Comparison of proteins sets present in the crude venom and in the microvesicles of *Crotalus durissus terrificus* snake venom

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Introduction: *Crotalus durissus terrificus* venom is composed of toxins that are responsible for the clinical complications caused by envenoming. The toxins are not the only components present in the venom; numerous electron-dense microvesicles (40 – 80 nm in diameter) are observed in the venom gland and also in the venom. These structures have intramembranous particles on the cytoplasmic leaftet, suggesting the presence of transmembrane proteins. We have shown that the isolation of the microvesicles by ultracentrifugation did not change in morphology, and we also detected the presence of 18 specific proteins in the microvesicles. **Objectives:** The aim of this study was to compare the proteins present in the crude venom and in the microvesicles in order to confirm previous data. **Methods:** The venom used was manually extracted from *Crotalus durissus terrificus* maintained in the Laboratory of Herpetology at Instituto Butantan. A volume of 7.5 ml of venom was diluted with cold PBS (1:4) and centrifuged at 150 g for 15 min at 4°C to eliminate cell debris. The supernatant (S1) was ultracentrifuged at 200,000 g for 60 min at 4°C. Supernatant (S2) was used as a control venom without microvesicles. The pellet was resuspended in 10 ml of cold PBS and ultracentrifuged again under the same conditions. The resulting pellet was processed for two-dimensional electrophoresis (2-DE). The protein spots in the 2-DE images were analyzed by ImageMaster Platinum 7.0 software. Fresh crude venom was also used as a control. Western blotting was performed to determine whether *Crotalus* antivenom produced by Instituto Butantan can recognize the proteins of the microvesicles. **Results and Discussion:** When fresh crude venom was used as a control, besides the 18 spots detected before, 10 more spots were detected in S2 and microvesicle extract, which were not present in the crude venom (spots of 75 kDa and PI around 10 and spots of 36 kDa and PI ranging from 5 to 7 and from 9 to 10). Western blotting analysis showed that the *Crotalus* antivenom did not recognize the microvesicle proteins. In conclusion, our data showed that although the morphology of most of microvesicles is preserved after ultracentrifugations, some microvesicles seem to be lysed since 10 spots were found only in S2 and microvesicle extract, but not in the crude venom. Besides, specific proteins in microvesicles were not recognized by antivenom suggesting that these proteins could have an important role such as regulating the activity of toxins from the venom, or even having a biological activity that contributes to the pathology of envenoming.

Supported by: FAPESP, CAPES and CNPq

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1.30 Prolonged multiple paralysis with ophthalmoplegia after *Crotalus durissus terrificus* bite

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Introduction: Accidents involving rattlesnakes are responsible for approximately 9% of accidents caused by venomous snakes in Brazil. In the year 2009, 2,211 cases were reported to the Ministry of Health. The venom has neurotoxic and myotoxic activity, causing cranial nerve involvement, paralysis of skeletal muscles, rhabdomyolysis, and renal failure progressing to acute tubular necrosis.**Objectives:** The aim of this study was to report a rare case of *Crotalus d. terrificus* snakebite with prolonged multiple paralysis of several cranial nerves and related muscle groups.**Methods:** Review of clinical record of Hospital Vital Brazil. **Results and Discussion:** On April, 12, 2009, a 42-year-old male from Santana de Parnaíba, SP, Brazil, was admitted to Vital Brazil Hospital, Butantan Institute (São Paulo, SP, Brazil) 1 h 20 min after being bitten by an adult of *Crotalus durissus terrificus*, according to the Herpetology Laboratory of this Institute. The patient was drunk and while trying to capture the snake, was bitten on both hands. The bite sites showed pain, bleeding, ecchymosis and swelling. He was conscious and hypotensive (75/40 mm Hg), with blood pressure normalized after volume resuscitation. The accident was classified as moderate, and ten vials of anti-crotalic antivenom were prescribed. Five hours after admission, he developed myalgia associated with change of visual acuity, and bilateral ptosis, besides normal urine color. Blood was incoagulable, and a peripheral blood film revealed only neutrophilia with 90% segmented, CK 1,095 U/l (normal: 38-174U/l) and fibrinogen less than 50 mg/dl (normal: 175-400 mg/dl).On the third day of hospitalization, the patient remained with blurred vision, bilateral ptosis, complete ophthalmoplegia with mild anisocoria, tearing, conjunctival hyperemia, hiccups that only improved after the use of chlorpromazine hydrochloride, salivation, difficulty chewing and swallowing solid food, reported bitter taste, decreased olfaction and difficulty walking. He developed non dialytic acute renal failure and outcome with remission of myalgia. Bilateral ptosis with ophthalmoplegia, mild anisocoria and tearing persisted for two more months after the snakebite. Reports of many compromised cranial nerves and muscle groups after snakebites with pre-synaptic neurotoxic activity are very unusual.

1.31 Venomous pet: a case of *Bothrops atrox* bite in São Paulo City, southeastern Brazil

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Introduction: The intensification of air traffic between different regions of the world and the progressive increase in people with animals in their homes, whether domestic or wild, has been described with increasing frequency of accidents by mammals, birds, reptiles, fishes, spiders and scorpions. Despite the prohibition of importing exotic snakes and trading native ones, they are still found as pets in São Paulo City (SPC), including venomous species. **Objectives:** The aim of this study was to report a case of a bite by a non-native pitviper from SPC, *Bothrops atrox*. **Methods:** Review of clinical record to Hospital Vital Brazil.**Results and Discussion:** Patient, 26-year-old male, event promoter, reported having been bitten by his pet snake at 23:00 h on 4/22/2011 while feeding the animal in SPC. The site of the bite was on the second finger of the left hand. He was admitted to Hospital Vital Brazil, Instituto Butantan, SPC, 30 min after the accident. The patient reported pain, showing mild edema and erythema at the bite site. Sweating, headache, and nausea were also reported. There was previous history of dry snake bite without use of antivenom. Physical examination: Good general condition, site of the bite with punctiform injury, bruising, swelling and redness in the distal phalanx. The accident was classified as mild, and the patient received four ampules of antivenom. The patient developed a small blister at the site of approximately 1 cm in diameter, edema progressed to the back of the hand and the presence of bruising on the anterior forearm. Coagulation test showed incoagulable blood, and a peripheral blood film revealed the following: fibrinogen less than 60 mg/dl (normal: 175-400 mg/dl); D dimer >10,000 (< 500) platelets 285,000/ (150,000-400,000/mm^3); hemoglobin 16.4 g/dl (14-17.4 g/dl); hematocrit 51% (41.5-50.4%); white blood cells 10,100/mm^3 (4,400-11,300/mm^3) with 59% segmented (1-5%); 33% lymphocytes (18-40%); 2% eosinophils (1-4%); 5% monocytes (2-9%), urea 31 mg/dl (10-50 mg/dl); creatinine 1.1 mg/dl (0.4-1.3 mg/dl), creatine kinase 1,095 U/l (38-174 U/l); and lactate dehydrogenase 448 U/l (100 U/l). Eighteen days later, the bite evolved to necrosis at the bite site and was treated with debridement, without sequelae. Despite prohibition in Brazil, snakes are kept as pet animals, including venomous ones, from other geographical regions within the country, and even from other continents.

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1.32 Accident by *Potamotrygon motoro* (stingray): soft tissue infection caused by *Aeromonas caviae*

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Introduction: Although rarely reported, accidents caused by aquatic venomous animals are common on the Atlantic coast and Brazilian rivers.The injuries caused by freshwater stingrays often cause severe pain and local inflammatory lesions that frequently evolve to necrosis and secondary infection. **Objectives:** The aim of this study was to report a *Potamotrygon motoro* (stingray) accident and secondary infection. **Methods:** A serious accident caused by a freshwater stingray (*Potamotrygon motoro)* with secondary infection by *Aeromonas caviae* was described after reviewing clinical records of Hospital Vital Brazil, Butantan Institute, São Paulo, Brazil. **Results and Discussion:** On January 6, 2011, a 25-year-old female biologist, diving in an exhibition aquarium and stepped on a stingray. After being stung, the woman immediately developed severe pain in the lower third of leg left with a wound of 3 cm. She received medical care and the lesion was sutured. Seven days later she returned to the clinic with fever, diarrhea, local cellulitis with purulent hemorrhage up to the left knee, and blistering. The sutures were removed and the lesion drained; intravenous ceftriaxone and clindamycin were introduced and the patient initially improved. After the third day, the fever returned, with worsening edema and erythema. Abnormal results of laboratory tests: mild leukocytosis with neutrophilia. Laboratory tests showed normal renal and hepatic function. Oxacillin and levofloxacin were administered, and surgical debridement was performed and again four days later because she showed exposure of tendons and necrotic tissue. Culture of secretions from a second approach revealed growth of *Aeromonas caviae* sensitive to third generation cephalosporin, imipenem and piperacillin/tazobactam. On January 27, she underwent microsurgical flaps of the *rectus abdominis* combined with skin graft on her left ankle region, which was successful. The patient showed good evolution and regression of the infectious process, resolution of local inflammatory process and began physical therapy at the time of discharge. The genus *Aeromonas* is responsible for significant number of diarrhea cases in people who drank contaminated water. Moreover, accidents caused by freshwater fishes can develop secondary infection.

1.33 Activation of human complement system by a serine protease found in the *Premolis semirufa* caterpillar venom

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Introduction: *Premolis semirufa* catterpillar, called PARARAMA, is native to Amazonian rubber tree plantations. *Pararamose* is an occupational arthropathy, which may evolve bone alteration and immobilization of the affected articulations, after accidental penetration of PARARAMA caterpillar bristles. Despite the public health hazard of *Premolis semirufa* caterpillar poisoning, little is known about the nature of the toxic components involved in the induction of the pathology. We have recently demonstrated that the extract of *Premolis semirufa's* bristles is able to activate the human complement system, generating anaphylatoxins, and to induce cleavage of C3 and C4. Moreover, high serine protease activity was detected in the bristle extract. **Objectives:** Aiming to isolate the component responsible for C-activation, *Premolis semirufa's* bristles extract was submitted to chromatography and the fractions tested. **Methods:** For this purpose, samples of normal human serum were incubated with the fractions and the residual complement lytic activity was assessed in hemolytic assays, using conditions to develop alternative and classical pathways. Furthermore, activation of the lectin pathway was also determined on microtiter plates coated with mannan (10 µg/mL) and the generation of anaphylatoxins C3a/C3a desArg, C4a/C4a desArg, and C5a/C5a desArg was determined. In addition, direct proteolytic activity of the fractions on components of the complement system, such as C3 and C4, was also evaluated. **Results and Discussion:** A fraction containing an 82.0-kDa serine protease, designated Ps82, induced a complete ablation of the alternative pathway lytic activity and inhibited, partially, the lectin pathway. No effect on the classical pathway was observed. The three anaphylatoxins were generated in human serum treated with the Ps82, not only through C-activation, but also through direct cleavage, as determined using purified C3 and C4. These data suggest that a serine protease present in the extract pf *Premolis semirufa* bristles is responsible for complement activation, which can play an important role in the inflammatory process seen in humans after envenomation.

Supported by: FAPESP, CNPq and INCTTOX

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1.34 Contribution of metalloproteinases, serine proteinases and botrocetin to the genesis of systemic hemostatic disturbances in *Bothrops jararaca* envenomation

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Introduction: Bites by *Bothrops jararaca* (*Bj*) snakes evoke various clinical manifestations, and hemostatic disturbances are the most prevalent signs among systemic manifestations caused by envenomation. The pathophysiology of such hemostatic disturbances in *Bothorps* envenomation is known to be complex, and several mechanisms may contribute to bleeding tendency therein. Metalloproteinases (MP), serine proteinases (SP) and C-type lectins (botrocetin) found in *Bj* venom with anti-hemostatic activity may trigger hemostatic disorders in patients. **Objectives:** Taking into account that thrombocytopenia is one of the most frequent hemostatic disturbances observed in snakebite victims; the main goal of this study was to investigate experimentally which mechanisms induce hemostatic disturbances, especially thrombocytopenia, in bites inflicted by *Bj* snakes. **Methods:** The main protease families (SP and MP) and botrocetin present in *Bj* venom were specifically inhibited by incubation of crude *Bj* venom with 4 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF), 13 mM Na_2-EDTA or anti-botrocetin IgG, respectively, and injected s.c. into male Wistar rats, and hemostatic parameters were evaluated 3 h later. Rats administered vehicle-treated *Bj* venom, non-immune IgG from non-immunized rabbits and saline were used as controls. **Results and Discussion:** Mean platelet counts decreased in rats administered AEBSF (*ca.* 82%, p=0.002), EDTA (*ca.* 33%, p=0.091), anti-botrocetin IgG (*ca.* 79%, p=0.015), vehicle-treated *Bj* venom (*ca.* 82%, p=0.002) and control IgG (*ca.* 84%, p=0.001), in comparison with saline-treated rats. However, no statistically significant difference was noticed when comparison was carried out between vehicle-treated *Bj* venom and venom treated with AEBSF, EDTA, anti-botrocetin IgG or control IgG. These findings showed that EDTA treatment partially protected animals, but isolated inhibition of SP, MP and C-type lectins could not totally abrogate platelet consumption. Altogether, these data demonstrate that MP may have a more important role *in vivo* in comparison with other venom components in inducing thrombocytopenia, but it cannot be ruled out that SP and C-type lectins are not associated with the genesis of thrombocytopenia.

Supported by: FAPESP

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1.35 Experimental envenomation induced by *Bothrops jararacussu* snake venom: efficacy of different antivenin treatments

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Introduction: Snakebites are a public health problem in Brazil and twenty thousand bites by venomous snakes are reported each year, with approximately 90% of them being inflicted by the genus *Bothrops*. The most effective treatment for snakebites is serum therapy, and the effectiveness of antibotropic serum for the neutralization of *Bothrops jararacussu* venom (VBju) has been discussed by many groups. **Objectives:** The aim of this study was to compare the efficacy of different antivenin treatments in the experimental envenomation induced by VBju. **Methods:** Groups of mice (n=5) were injected *i.v.* with 2 x minimum defibrinogenating dose (0.50 mg/kg) of VBju, or saline (envenoming control), and treated with botropic (SAB), crotalic (SAC) and botropic/crotalic (SABC) antivenin, or saline (treatment control), 1 hour (h) after venom inoculation (*i.v.*). Blood samples were collected at 3, 6, and 12 h after the treatment, from the orbital plexus. The plasma fibrinogen levels (PFL) were determined and thrombelastography (TEG) was carried out on whole blood samples with the ROTEM® coagulation analyzer. The NATEM® test was used to assess native whole blood clot formation. The test parameters clotting time (CT, s), clot formation time (CFT, s), alpha-angle (°) and maximum clot firmness (MCF, mm) were acquired for 1 h. Statistical analysis were accessed by one-way ANOVA and Tukey's test. **Results and Discussion:** Regarding PFL at 3 h, the animals envenomed and treated with different antivenom showed great variation in the groups. At 12 h, there were no statistical differences between the PFL of groups treated with saline and the group treated with antivenin, indicating that fibrinogen was spontaneously normalized independent of the treatment. On the other hand, at 6 h, the animals treated with SABC showed significantly ($p<0.05$) higher levels (189.09±18.27 mg/dL), when compared to those treated with SAB (139.02±6.87 mg/dL), SAC (93.66±11.42 mg/dL) or saline (117.59±8.31 mg/dL). PFL of control groups injected with saline and treated with SABC, SAB, SAC and saline were respectively: 261.16±13.36 mg/dL, 311.48±22.93 mg/dL, 227.73±22.41 mg/dL and 317.36±32.91. Results of TEG are in agreement with PFL obtained from the different groups. Thus, at 3 h, most samples remained unclottable, while at 12 h, animals injected with VBju or saline showed similar results. On the other hand, at 6 h, the TEG graph results demonstrated that animals treated with SABC had a good recovery, showing lower CFT (176.75±28.45 s), when compared to those of SAB (350.66±83.38 s), SAC (unclottable) and saline (434.25±159.01 s). In conclusion, SABC treatment is better than SAB in the Bju experimental envenoming in mice.

Supported by: INCTTOX, CNPq and CAPES

2. Biochemistry

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2.01 Cryptic peptides obtained by the action of serine proteases fraction from *Bothrops jararaca* venom on myoglobin

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Introduction: Snake venoms contain a great variety of molecules that affect vital physiological systems. Among these molecules, serine proteases and metalloproteases are the main *B. jararaca* venom enzymes acting on the victim's tissues and proteins. As a result of their direct actions on tissue proteins, these proteases could generate peptides with specific actions in cells or other mechanisms. The most common sources for bioactive peptides are natural precursor proteins. Recent studies have shown that a new class of proteins not designed as precursors, the crypteins, may under some conditions originate bioactive peptides, or cryptides. **Objectives:** The aim of this study was to isolate and identify cryptic peptides generated by the action of the venom serine proteases fraction (SPF) and by commercial trypsin (CT) on a myoglobin (Myo) with different incubation times. **Methods:** The venom SPF was separated from the whole venom using an HPLC molecular exclusion column, verifying the activity of the fractions on Myo. This substrate was incubated with the venom SPF as well as with CT, at incubation times of 0 h, 30 min, 1 h, 2 h, 3 h, 18 h and 36 h. The resulting peptides were isolated by fractionation by HPLC (C_{18}-RP), and the fractions were tested on cell cultures. Active fractions were re-chromatographed in order to obtain the pure cryptides. After the activity was confirmed, the peptides were sequenced and synthesized. **Results and Discussion:** CT activity on myoglobin generated the peptides ALELFR, TGHPETLEK and GLSDGEWQQVLNVWGK, showing proliferative activity in FN-1 and HUVEC cells. 3D modeling of Myo, using the Cn3D software, showed that the three peptides are located on the surface of the protein. Digestion of Myo with the venom SPF generated an HPLC profile similar to the one obtained with CT. To confirm the similarity of the peptides from both profiles, they were sequenced by Edman degradation, which showed homology between them. Some of the cryptides generated by SPF after as little as 30 min were still produced after 36 h of incubation. Other generated cryptides were also sequenced for more information about the generation of cryptides over time. This study suggests that CT and SPF generate cryptides, some of which at significant concentrations even after only 1 h of incubation. This study also suggests that the venom SPF may generate cryptides with relevant effects and which would not be neutralized by serum therapy, suggesting new aspects in the process of envenomation.

Supported by: FAPESP, CNPQ, INCTTOX and CAPES

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2.02 Effects of mastoparan and casoparan in the B16F10 murine melanoma cells

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Introduction: Mastoparans are α-helical and amphipathic toxic tetradecapeptides first isolated from the venom of the wasp *Vespula lewissi.* These peptides exhibit biological effects including antimicrobial activity, histamine release from mast cells, stimulation of various phospholipases and a potent mitochondrial permeability transition. **Objectives:** The aim of this study was to evaluate the activity of mastoparan (MP) (INLKALAALAKKIL-NH_2) and casoparan (CP) (INKKI), in B16F10 murine melanoma cell cultures. **Methods:** B16F10 cells were cultured in RPMI-1640 at 37°C and 5% CO_2. About $1x10^4$ cells were added to a 96-well plate and treated with concentrations of MP and CP ranging from 6 μM to 100 μM and 15 μM to 500 μM, respectively, maintained for 24 h at 37°C and 5% CO_2. Cell viability was measured by MTT or by exclusion with trypan blue. For cell morphology analysis, cells were treated with 100 μM MP, 500 μM CP, and maintained at 37 °C and 4 °C. The cells were photomicrographed and morphological aspects were observed for 0 to 6 h and 24 h. The Annexin V-FITC/PI apoptosis detection kit (BD® Bioscience) was used to detect the effects of MP and CP in the death pathway. The statistical analysis was performed by Student's t test and one-way ANOVA using the program Instat (version 2.0), with significance at $p< 0.05$. **Results and Discussion:** The results showed that MP was cytotoxic against B16F10 cells at concentrations from 12 to 100 μM (IC50% 33 μM). B16F10 cells treated with 500 μM CP displayed 68% decrease in viability (IC50% 386 μM). The morphological aspects of B16F10 maintained at 37 °C and 4 °C and treated with MP, at concentrations 25 to 100 μM, displayed changes in cell morphology with the generation of pores in the cell membrane. Below 25 μM, cells did not exhibit necrotic features, suggesting that at low concentrations another process of death may be involved. These data were confirmed by detection of apoptotic cells by flow cytometry with annexin V and PI staining. MP at 20 μM induced an increase in the population of later apoptotic cells. The morphological aspects of B16F10 after 2 h incubation, cells maintained 37 °C, showed a change in morphology with the presence of rounded cells, suggesting a cytostatic activity, while the cells maintained 4 °C showed no morphological differences with different incubation times. This suggests that the pathway of action of this peptide depends on energy to trigger signaling. Therefore, the results showed that MP was cytotoxic to B16F10 cells and that CP has mainly cytostatic activity.

Supported by: FAPESP, INCTTOX and CAPES

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2.03 Venous thrombosis: effect of plant *Bauhinia bauhinioides* inhibitors (BbKI)

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Introduction: *Bauhinia bauhinioides* kallikrein inhibitor (BbKI) is a serine proteinase inhibitor similar to plant Kunitz-type inhibitors, but it is devoid of disulfide bridges. BbKI inhibits kallikrein and prolongs aPTT in human and rat plasma. **Objectives**: The aim of this study was to evaluate the antithrombotic action of BbKI in a model of venous thrombosis, **Methods**: Wistar rats were anesthetized, randomly allocated into three treatment groups and received, 30 min before the thrombus induction, native BbKI (2.0 mg/kg or 0.12 mM), 7.5 mM HEPES buffer, pH 7.4, positive control or unfractionated heparin (250 U/kg or 2.5 mg/kg). Ten minutes after drug administration, bleeding time (BT) was measured, the abdomen was opened, a ligature was placed in the vena cava just below the left renal vein and the abdominal incision was closed. Three and a half hours later, the animals were reanesthetized and the abdomen reopened. The inferior vena cava was isolated, and the thrombus, if present, harvested and weighed. Activated partial thromboplastin time (aPTT) was determined. **Results and Discussion**: BbKI reduced the thrombus formation in this model by 65% in comparison with the HEPES group and significantly decreased the thrombus weight (2.09 ± 0.69 mg *vs* 6.02 ± 1.70 mg). In the heparin group no thrombus was observed. No difference in aPTT was observed between BbKI and HEPES (67.78 ± 8.60 s *vs* 56.03 ± 8.06 s). For heparin, aPTT was > 300 s. No change in BT was observed in the different groups. These data show that BbKI was effective in preventing venous thrombosis in rats, justifying further studies to clarify the mechanisms of this effect.

Supported by: CAPES, FAPESP, FADA/FAP, MCT/CNPq-CTBIOTECNOLOGY and IEP Hospital Sírio Libanês

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2.04 HSP70 induction by cadmium in *Biomphalaria glabrata*

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Introduction: HSP70 (70 kDa heat shock protein) is involved in the regulation of protein homeostasis. Due to evidence that exposure to a wide variety of stressors can induce the heat shock response, many studies are being performed to establish this family as biomarkers of environmental stress. **Objectives:** The aim of this work was to investigate the induction of HSP70 by cadmium, an important environmental pollutant, in *Biomphalaria glabrata*, a freshwater snail recognized as a suitable organism for experimental research. **Methods:** We evaluated the HSP70 expression in the digestive gland of snails by Western blotting. A group of 70 snails were exposed for 96 h to 0.09 ppm to 0.7 ppm $CdCl_2$. Surviving animals were dissected to investigate induction of HSP70 as a possible mechanism of protection to toxic effects of cadmium. Proteins were extracted with RIPA buffer from digestive gland tissues, fractionated in dodecylsufate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, and detected with a HSP70-specific antibody. **Results and Discussion:** We observed that the highest doses induced higher mortality and determined an LC_{50} of 0.37 ppm. Western blotting of digestive gland extracts showed that the induction of HSP70 in surviving exposed snails was clearly higher than in control ones, which strongly suggests that HSP70 played a protective role against the lethal effects of metal, as already observed in other species. Our results reinforce the proposal of HSPs as a potential tool for environmental monitoring, providing further evidence to the establishment of *B. glabrata* as a bioindicator in ecotoxicological studies.

Supported by: CAPES

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2.05 Oligovetin: novel ultrashort antibacterial peptide from the eggs of the spider *Phoneutria nigriventer* (Ctenidae)

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Introduction: The proliferation of infectious diseases is one of the most important human health problems. Inappropriate use of antibiotics contributes to the appearance of bacterial strains resistant to conventional drugs. Therefore, the study of antimicrobial peptides may be advantageous for human use due to its faster action in comparison to conventional antibiotics. They are ubiquitously produced throughout the phylogenetic tree, suggesting that they play a key role in innate immunity. Many studies have identified antimicrobial peptides in several species, from invertebrates to frogs and mammals. Microplusin, for example, is produced by the cattle tick *Boophilus microplus* and has been identified in hemolymph and also in eggs. Although many tissues and toxins from spiders have been widely studied, no research has reported antimicrobial activity from spider eggs. **Objectives:** We aimed to characterize the antimicrobial peptide oligovetin, previously isolated from *Phoneutria nigriventer* spider eggs. **Methods:** We used reversed phase high pressure liquid chromatography (RP-HPLC) to purify proteins and peptides to homogeneity, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF/MS) to characterize them and Edman degradation for sequencing. Chemical synthesis was performed using fmoc technology. **Results and Discussion:** We detected antifungal and antibacterial activities in the *Phoneutria nigriventer* eggs. A novel peptide with antimicrobial activity against Gram-positive bacteria was identified, which we proposed to call oligoventin-1. The amino acid sequence determined by Edman degradation was QPFSLERW, with a molecular mass of 1062.4 Da. We also synthesized an analog of oligovetin-1, which displays a pyroglutamic acid at the N-terminus. Oligovetin-1 has shown minimal inhibitory concetration in the micromolar range against *S. aureus* and *S. epidermides.* It is the smallest natural peptide from arachnids found to date, with only eight residues. To our knowledge, this is the first report of antimicrobial peptides from spider eggs.

Supported by: FAPESP and CNPq

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2.06 Digestive lipases from mosquito *Aedes aegypti* in adult and larval stage

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Introduction: The mosquito *Ae. aegypti* is the vector of dengue and yellow fever in Brazil. Lipases (EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols (TAG) resulting in the release of non-essential and essential fatty acids. Little is known about lipid digestion in *Ae. aegypti* and the importance of this enzyme in vector-parasite interactions. **Objectives:** The aim of this study was to characterize lipid digestion in the different feeding development stages of *Ae. aegypti*: larvae and adult females. This study analyzed differences of specificity related to substrate by enzyme kinetics and the expression patterns of distinct lipase genes in the midgut from *Ae. aegypti* larva and adult by RT-PCR amplification. **Methods:** Midguts from *Ae. aegypti* adult and larva were isolated and homogenized in MilliQ water in a Potter-Elvehjem homogenizer. Lipase activity was measured using 4-methylumbelliferyl oleate (MUO), 4-MU-butyrate (MUB), 4-MU-heptanoate (MUH) and 4-MU-stearate (MUS) as substrate. The effect of different substrate concentration on lipase activity was determined by the measure of activity using a set of at least 12 different substrate concentrations to both samples. Km and Vmax were determined by Enzfitter. Chromatographic separation in an anion exchange column was used in order to compare larval and adult digestive lipases. Bioinformatic analyses allowed the selection of 8 possible digestive lipases from *Ae. aegypti* genome. Specific primers were synthesized and used for RT-PCR amplifications. **Results and Discussion:** Digestive lipase from larvae hydrolyzed MUH>MUB>MUO>MUS and from adult female MUB>MUH>MUO>MUS, indicating differences of specificity of lipases expressed in these development stages. This result was corroborated by Km values determined using different substrates with partially isolated lipases. The values of Km in lipase from *Ae. aegypti* larva were 112 μM (MUO), 506 μM (MUB), 48 μM (MUH) and 329 μM (MUS); Kms for *Ae. aegypti* adult lipase were: 121 μM (MUO), 92 μM (MUB), 86 μM (MUH). MUS was not hydrolyzed by partially purified enzyme. Chromatographic resolution also indicates that enzymes involved in lipid digestion are different in *Ae aegypti* larvae and adult. RT-PCR results allowed the identification of two truly digestive lipases, one (AAE007051-Llip) exclusively expressed in larval midgut and another (AAE 007055-Alip) expressed only in the midgut of fed adult female. Alip was expressed in female fed on saccharose or on blood. Lipases are important enzymes involved in the digestive process in both feeding developmental stages: larva and adult. However, enzymes involved in this process are products of different genes and show different specificities.

Supported by: CNPq and CAPES

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2.07 Cloning and biochemical characterization of digestive cathepsins L-like proteinases in *Nephilengys cruentata*

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Introduction: Spiders are able to capture and consume preys bigger than themselves and digestion process starts externally with regurgitation of midgut and midgut diverticula (MMD) secretion, the digestive juice, into prey. Previous studies indicated that MMD have cathepsin L-like cysteine peptidase as the most important endopeptidase activity. Cathepsin-L is widely spread in living organisms and is associated with protein degradation in lysosomes, but some groups of Arthropoda (Hemiptera, Heteroptera, Coleoptera and Acari) show this enzyme related to digestion of proteins ingested. For the first time this kind of enzyme was found in spiders in both MMD and digestive juice. **Objectives:** The aim of this study was the molecular cloning of peptidases present in MMD as well as biochemical characterization of the peptidases found in digestive juice. **Methods:** Adult females of *Nephilengys cruentata* were submitted to two weeks of fasting and then fed. When the feeding process was finished 1) digestive juice was collected by mechanical stimulation of the abdomen or 2) MMD was isolated by dissection. Digestive juice was used as enzyme source and assayed with different peptidase substrates in the presence or absence of peptidase inhibitors. MMD were homogenized in Trizol reagent and the total RNA isolated. The cDNA was synthesized using the kit Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen) and the amplification was performed using degenerate primers for cathepsin L based on conserved regions. **Results and Discussion:** Two cathepsin L-like cysteine peptidases were cloned from *Nephilengys cruentata* midgut and diverticula and they were called catL-N1 and catL-N2. Both enzymes have the pro-peptide inhibitory region and the four residues of the active site (Cys 158, His 292, Gln 152 and Asn 308-papain numbering) common to enzymes of the papain family, which includes cathepsin L enzymes. Inhibitory assays in regurgitated material were performed after ion-exchange chromatography using the peptidase inhibitors E-64 (cysteine peptidase), pepstatin A (aspartic peptidase) and PMSF (serine peptidase). E-64 showed 100% inhibition and pepstatin 85%, whereas PMSF did not cause any effect. Activity against Z-FR-MCA was 275 times higher than on Z-RR-MCA, which together with the inhibition assay observed for E-64 is strong evidence that the major endopeptidases from regurgitate are cathepsin L-like enzymes. These enzymes also showed activation under acid conditions. Thus, molecular cloning and biochemical characterization showed that cathepsin L-like cysteine peptidases are the main endopeptidases responsible for digestion in *Nephilengys cruentata*.

Supported by: CNPq and CAPES

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2.08 Production and characterization of a new platelet aggregation inhibitor from the leech *Haementeria depressa*

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Introduction: Hematophagous animals have developed during evolution a great variety of substances that help feeding and digestion of blood, keeping it fluid during these processes. Compounds were identified and the profile of transcripts and proteins from *H. depressa* leech salivary complexes was determined by biochemical, transcriptomic and proteomic analyses. In this tissue some clones were detected that were similar to an inhibitor of platelet aggregation from the *H. officinallis* leech, called LAPP. LAPP inhibits platelet aggregation through collagen. **Objectives:** The aims of this work were to obtain the recombinant protein (45% similar to LAPP) by cloning, expression and purification and to characterize its biologic activity and action on the platelet pathway. **Methods:** The transcript H06A09_pGEM11Zf from the *H. depressa* salivary complex cDNA library was amplified by PCR and cloned in pPIC9K vector. The clone was transformed in Pichia pastoris (GS115) by electroporation and expressed under different conditions. The recombinant protein expressed was submitted to some different purification methods (ultra filtration, gel filtration, anionic exchange and reverse phase). The N-terminal sequence was determined by Edman degradation. The recombinant protein activity was determined on platelet aggregation using some agonists such as collagen, ristocetin, ADP, arachidonic acid and thrombin in PRP and/or washed platelets. The assay of interaction with different platelet receptors was determined by flow cytometry (Guava PCA cytometer) both in PRP and in washed platelets. **Results and Discussion:** The H06A09_pGEM11Zf was subcloned in pPIC9K and transformed in Pichia pastoris. The best expression method was standardized in BMGY culture medium at 28°C, 260 rpm using 96 h of induction by 0.5% methanol feeding/24 h. The best method of purification was to submit the culture supernatant initially to dialysis and concentration by ultrafiltration (Amicon 5 kDa) followed by gel filtration in Superdex 75. The N-terminal sequence of protein was confirmed. The aggregation platelet tests showed that the recombinant protein is a specific inhibitor of platelet aggregation by collagen both in PRP and in washed platelets with an IC(50) of 3.3 and 118 nM, respectively. Assays using flow cytometry showed that this inhibitor blocks the GPIb-IX-V complex, specifically by the Ibα subunit, which is responsible for binding of FvW to platelets.

Supported by: FAPESP/INCTTOX

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2.09 Activation of snake venom gland: a proteomic approach

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Introduction: Venom gland of viperid snakes has a central lumen where the venom produced by secretory cells is stored. When the venom is lost from lumen, secretory cells are activated and new venom is produced. We have shown that the protein composition of venom gland changes during the venom production cycle. Noradrenaline released just after venom removal regulates the activation of transcription factors and consequently regulates the synthesis of proteins in the venom gland that is important to activate venom gland for venom production. **Objectives:** The aim of this study was to analyze proteins of venom gland during venom production cycle and proteins whose expression is regulated by alpha and beta adrenoceptors stimulation. **Methods:** Venom glands were obtained from female *Bothrops jararaca* snakes in quiescent stage and in activated stages (4 days and 7 days after milking). Some snakes were treated with reserpine (20 mg/kg, sc, 24 h before the first milking, followed by daily injections of 5 mg/kg, for 4 days), and some reserpine-treated snakes received phenylephrine and isoprenaline (100 mg/kg, sc), just after milking (N=3 for each group). Extracts of glands were prepared and the proteins were analyzed by 2-dimensional gel electrophoresis (2-DE). Gels were stained with Coomassie Blue G and the density of spots was quantified using ImageMaster 2D Platinum 7. Proteins were identified by mass spectrometry MALDI-TOF/TOF after trypsin digestion. **Results and Discussion:** Comparison between groups pointed out that different proteins are expressed. Specific spots were submitted to mass spectrometry and the analysis of protein identified showed that expression of proteins of the endoplasmic reticulum, cytoskeleton, nucleus and mitochondria were upregulated and proteins of the cytoplasm and membrane were downregulated in the activated stage. Treatment with reserpine showed that the expression of cytoskeleton proteins is upregulated by sympathetic outflow. Although the expression of total secreted proteins was not changed, metalloproteinases were upregulated and serine proteinases and glycoprotein IB-binding protein (GPIb-BP) were downregulated by sympathetic outflow. It is interesting to note that the expression of GPIb-BP and serine proteinases is higher in quiescent venom gland and metalloproteinases and L-amino oxidase are higher in activated venom gland. The data showed that a great variation in the expression of proteins occurs during the venom production cycle. Besides, sympathetic outflow regulates the expression of some proteins. The identification of these proteins allows us to understand the mechanism of venom gland activation and venom production.

Supported by: FAPESP and CNPq

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2.10 Cloning, expression in *Pichia pastoris* system and purification strategies of new antistasin-type molecules from *Haementeria depressa* leeches

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Introduction: The compounds from salivary complexes of the *Haementeria depressa* leech have been studied by our group. Recently, through proteomics and transcriptomic analysis of this tissue, we determined the profile of transcripts and proteins produced by it. Among the most abundant transcripts detected were some transcripts (H05D10_pGEM11Zf and H01C09_pGEM11Zf) with 67 and 55% similarity to therostasin, respectively (FARIA et al, 2005), an antistasin-type inhibitor of FXa from the leech *Theromyzon tessulatum* (CHOPIN et al, 2000) which has conserved cysteines and even an arginine in the P1' region of the reactive site. These transcripts were previously expressed in prokaryotic system (*E.coli* – BL21DE3) unsuccessfully, and we already started the expression of one of them in a eukaryotic system (*Pichia pastoris*) **Objectives:** The aim of this study was to clone the H01C09 transcript in pPIC9K vector, to determine the expression in the *P. pastoris* system of these transcripts, to improve methods of purification and to obtain recombinant molecules with activity. **Methods:** The transcript (H01C09_pGEM11Zf) was subcloned in pGEM-Teasy system to finally be cloned in pPIC9K plasmid between Eco *RI* and Not*I* sites, the sequence was confirmed, and then the new vector was linearized by Sac I digestion, followed by transformation in the yeast *P. pastoris* GS115. The other transcript (H05D10_ pPIC9K) was expressed in *P.pastoris* to improve the methodology of the purification. The expression was done in BMG (Buffered Minimal Methanol Medium) at 28ºC. The purification was done using several methods, including dialysis, gel filtration chromatography and heparin-Sepharose affinity chromatography under different conditions (elution NaCl concentrations, high and low pressure, etc). The different steps of expression and purification were monitored by SDS-PAGE and the inhibitory activity was assayed on FXa using a specific chromogenic substrate (S2765). **Results and Discussion:** The methods of the new transcript cloning were successfully carried out, and its expression is being standardized, and for H05D10_pPIC9K as well. Until now the heparin-sepharose chromatography was the most efficient method for the purification of the recombinant protein, which was isolated in 550 mM NaCl and it showed a size of about 16 kDa in SDS-PAGE. As expected, the molecule was able to inhibit FXa. In further steps we intend to complete the expression of the H01C09_pPIC9K clone, to obtain the proteins in their pure form and to characterize them with regard to the FXa specificity. These new antistasin-family members are potential anticoagulants to be explored in further investigations.

Supported by: CAPES and INCTTOX

2.11 Analysis of outer membrane protein profile of atypical enteropathogenic *E.coli* (EPEC)

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Introduction: *Escherichia coli* (*E. coli*) is a member of the human intestinal microbiota, but in some cases may be pathogenic. Enteropathogenic *E. coli* has been identified as a main causative agent of acute diarrhea in developing country populations. Diarrhea is still one of the most significant causes of global child mortality between 0 and 5 years old. **Objectives:** The focus of this study was to characterize and identify the outer membrane proteins (OMPs) of extracts derived from one strain of EPEC. **Methods:** Strain BA320 (serotype 055:H7) was selected for this study. OMPs were analyzed by two-dimensional electrophoresis (2-DE), the first dimension by isofocusing on 13-cm pH 4-7 strips (IPGphor III, GE Healthcare) and the second by SDS-PAGE using 15% SDS-polyacrylamide gels (SE 600 Ruby, GE Healthcare). The identification was done by removing the spots from the gels and digestion with trypsin followed by mass spectrometric analysis on ESI QTOF Ultima – Waters. The resulting data were analyzed with a non-redundant protein database (NCBInr) using Mascot v3.0 engine (Matrix Science, www.matrixscience.com). **Results and Discussion:** Thirty-three spots were identified with high scores, allowing the characterization of twenty different proteins. All proteins were localized in the membrane (with the exception of one, 50S ribosome protein), indicating the efficiency of the extraction method. Seven proteins were OMPs or porins (Omp A, Omp W, Omp X, nucleoside channel receptor of phage T6 and colicin K, maltoporin, outer membrane channel specific tolerance to colicin E1 and TolC). Two transporters were identified (Tsx and fatty acid transporters). Two enzymes were detected (LysM domain protein and FOFI ATP-synthase). Moreover, one chaperonin (Groel GROES ADP7), one flagellin, one elongation factor, one protection protein (DPS), one inhibitor of C-type lysozyme (Ivy), one lipoprotein, one ribosomal protein (50S) and one scaffolding protein were identified. Omp A was one of the most abundant components. The preliminary data indicate that the majority of proteins identified have important roles in membrane permeability and at least three of them, Omp A, Omp X and flagellin are involved in the adhesion of the pathogen to host cells.

Supported by: FAPESP

2.12 Anti-inflammatory properties of *Bothrops jararaca* snake antithrombin and proteomic analysis of inflammatory exudates for identification of biomarkers

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Introduction: Antithrombin is an important anticoagulant molecule involved in the inhibition of thrombin and factor Xa. Recent studies showed that human antithrombin also has anti-inflammatory activity. **Objectives:** In this study, we investigated the effects of *Bothrops jararaca* antithrombin on acute inflammation induced by carrageenan in mice, and we are performing peritoneal exudate proteomic profiling for identification of biomarkers. **Methods:** Antithrombin was purified from *Bothrops jararaca* snake plasma by affinity chromatography using a HiTrap Heparin HP column. To evaluate edema formation, antithrombin (20 μg) was endovenously administered 1 j before subplantar injection of carrageenan (15 mg/kg) into the mouse hind paw. To analyze leukocyte influx, antithrombin (20 μg) was endovenously administered 1 h before intraperitoneal carrageenan injection (15 mg/kg^{-1}). IEF of peritoneal exudate proteins was undertaken using pre-cast Immobiline DryStrip gels, pH 3-10 gradient, followed by SDS-PAGE using 10% resolving gels. Protein bands were visualized using Coomassie blue R-350. Image acquisition was performed using the ImageScaner III densitometer (GE Healthcare) and the gels were analyzed using ImageMaster Platinum 7.0 software (GE Healthcare). **Results and Discussion:** Pretreatment with *Bothrops jararaca* antithrombin inhibited paw edema formation and migration of polymorphonuclear cells. However, these anti-inflammatory effects were completely abolished by pretreatment with indomethacin (4 mg/kg), suggesting the involvement of prostacyclin. Proteomic analysis is under evaluation. Our results suggest that *Bothrops jararaca* antithrombin induces an anti-inflammatory effect both on paw edema and cell migration induced by carrageenan. Proteomic analysis will allow the identification of the proteins involved in this mechanism.

Supported by: FAPESP and CNPq

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2.13 Detection of SVMP zymogens in venom and venom gland extracts of *Bothrops jararaca*

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Introduction: Snake venom metalloproteinases (SVMPs) are abundant enzymes in *Bothrops* venoms and are responsible for local and systemic symptoms of human poisoning. SVMPs are synthesized as zymogens and the enzyme activation is regulated by removing the pro-domain, as occurs in MMPs. There is evidence that this process occurs in the lumen of the venom gland; however, the presence of pro-domain has already been seen in some samples of venom. **Objectives:** In this work we aimed to trace the SVMP processing route by detecting the zymogen molecule or cleaved pro-domain in *Bothrops jararaca* venom glands using anti-pro-domain antibodies. **Methods:** The sequence of the pro-domain was amplified by PCR from cDNA extracted from venom glands of *B. jararaca.* The gene obtained was cloned into the pAE vector and the selected clone was sequenced. The recombinant protein (PD-Jar) was produced in *E. coli* and purified with immobilized metal affinity chromatography (IMAC). PD-Jar was inoculated into mice to produce polyclonal antibodies, which were tested by ELISA and Western blotting (WB) in venom and venom gland extracts collected at 7, 14, 21 and 40 days after milking. **Results and Discussion:** The pro-domain sequence amplified by PCR generated a single band with estimated size of 507 bp and this product was cloned into pAE vector. *E. coli* were transformed with this clone and expression induced by IPTG resulted in a major protein with molecular mass of 21.2 kDa. After immunization, anti-PD-Jar serum showed an ELISA titer of 1:200,000 against the recombinant protein. In WB analysis, the antiserum was able to recognize bands of approximately 22 and 47 kDa predominantly in venom samples collected 7 days after milking, either in presence or absence of proteinase inhibitors. These molecular masses may represent free pro-domain or unprocessed SVMPs class P-I, respectively, suggesting that the pro-domain is present in the venom in the processed and unprocessed forms. In the extracts of venom glands, antiserum was able to recognize bands of approximately 47 and 76 kDa in all samples collected. The molecular mass of these bands correspond to P-I and P-III SVMPs, respectively, in their unprocessed form. Presence of pro-domains on positive bands is currently being confirmed by mass spectrometry. This information supports the notion that SVMPs are found mainly as zymogens within the venom-secreting cells and processing is very likely to occur in the lumen of the venom gland. This hypothesis will be further confirmed by immunohistochemistry of gland tissues.

Supported by: FAPESP and CNPq

2.14 Study of Lopap (*Lonomia obliqua* prothrombin activator protease) and derived peptide activities in the modulation of HUVEC survival

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Introduction: Lopap, the most abundant protein found in the bristles of *Lonomia obliqua* caterpillars, was characterized as a prothrombin activator serine protease which belongs to the lipocalin family. The members of this family show a wide functional diversity, including the modulation of cell growth, proliferation and survival, where Lopap is considered the only member of this family to possess proteolytic activity. Through a conserved domain of the lipocalin family present in the Lopap sequence, a peptide with 11 amino acids was obtained, called P4. **Objectives:** The aim of this study was to obtain a recombinant Lopap (rLopap) and to study the cytoprotective effects of this protein and derived peptide (P4) in cultures of HUVECs (human umbilical vein endothelial cells). **Methods:** The recombinant protein was expressed with a histidine tag using the expression vector pAE, which was transformed in *E. coli* BL21-DE3 by heat shock. The expression was done in 2xYT medium and induced with IPTG (0.5 mM) for 3 h at 37ºC. Afterwards, the recombinant protein was separated in a Ni-Sepharose column and eluted with imidazole buffer (150 mM), followed by desalting in a G-25 column to remove the imidazole salt. Steps of expression and purification were monitored by SDS-PAGE and the concentration of rLopap was quantified by the Bradford assay. The synthetic peptide (P4) was obtained by chemical synthesis in solid phase according to a conserved domain of lipocalin family found in the Lopap sequence. The HUVECs were cultivated following the method described by Jaffe et al. (1973), kept on deprived fetal bovine serum medium (RPMI + 1% FBS) and treated with rLopap (10µg/ml, 5µg/ml and 2.5µg/ml) and P4 (0.9µg/ml, 0.3µg/ml, 0.1µg/ml, 0.03µg/ml) (n=3) for 48 and 72 h. Cultures kept with 10% and 1% of serum and no treatment were used as control, and the cell viability measured by the MTT assay. **Results and Discussion:** The Lopap was obtained in the recombinant form with a yield of 2 mg/L. When tested on HUVECs for 48 h and 7 2h, compared with the control, 1% FBS, rLopap (10 µg/mL) caused an increase of 20% ($p<0.05$) in cell viability. Cells treated for 48 h with P4 (0.1µg/mL) showed an increase in viability of 20% ($p<0.05$) compared with the control, 1% FBS, and after 72 h, in the same concentration, induced a viability increase of 9%, which was not significant. Our results indicate that rLopap, as well as P4, have potential properties on modulation of cell survival, which is independent of the proteolytic activity, as predicted for this molecule.

Supported by: FAPESP

2.15 Isolation and partial characterization of a biomolecule from *Passiflora edulis* with anticoagulant activities

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Introduction: *Passiflora edulis* is popularly known for its sedative and calming properties, belongs to the family *Passifloraceae* and originated from tropical and subtropical regions of the Americas. Objectives: Based on some cases of incoagulability associated with excessive consumption of passion fruit juice (*Passiflora edulis*) in anticoagulated patients, we proposed to investigate the presence of blood clotting inhibitors *in vitro* in *Passiflora edulis*. Methods: The isolation procedure comprised extraction, precipitation with acetone 83% and size exclusion chromatographies on Sephacryl S-200 and Sephadex G-25. The fraction obtained in the Sephacryl S-200 was then applied on a μ-Bondapack C 18 column (reverse phase HPLC). All the fractions obtained were measured the inhibitory activity to trypsin. The assay was performed by measuring hydrolysis of the substrate L-BAPNA (1.0 mM) in 0.1 M Tris-HCl buffer, pH 8.0, containing 0.02 % calcium, by trypsin (37 nM). The inhibitor activity was not observed when the extract was dialyzed extensively, suggesting a possible low-molecular weight of the inhibitor. Heat stability of the precipitated fraction was determined by incubation at 100°C for 10 min. PT and aPTT assays were determined in a semi-automated coagulometer BFT II (D. Behring). The total plasma was obtained by centrifugation of several human blood samples at 1,726 xg, for 15 min (25°C). The PT assay control was performed with 25 μL of water and 25 μL of plasma, incubating for 60 s, with subsequent addition of 100 μL of reagent (Thromborel S-Dade Behring) and aPTT assay control was carried out with 25 μL of water, 25 μL of plasma and 50 μL of aPTT reagent (Dade actin activated cephaloplastin-Dade Behring), incubating for 120 s with subsequent addition of 50 μL of 0.025 M calcium chloride. The fractions isolated in increasing concentrations, after pH neutralization, were subjected to blood cogulation time assays, prolonging the PT and aPTT. The tests were done in duplicate and the results were expressed as average of each sample determination. Results and Discussion: These results suggest that the biomolecule isolated is a peptide that can affect the activity of coagulation factors, especially those of the common pathway of the coagulation cascade, such as human factor Xa and thrombin, and provides preliminary evidence of the effect of *Passiflora edulis* on coagulation parameters, important to the studies related to thrombosis and hemorrhagic diseases.

Supported by: CAPES, FAPESP and CNPq

2.16 Evaluation of deglycosylation of Amblyomin-X produced in shaker

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Introduction: Amblyomin-X, a recombinant protein characterized as a Kunitz type inhibitor, was expressed in *Pichia pastoris* yeast system using the pPIC9K vector inhibits FXa and causes tumor remission in the implanted mouse with melanoma cells, without, however, affecting the normal cells. **Objectives:** The aim of this work was to evaluate the glycosylation of Amblyomin-X in producing in system yeast *Pichia pastoris,* due to possible potential of the glycosylation of the protein and your great pharmacological interest. **Methods:** This system offers the advantage to produce an inhibitor to be up-streamed, 10.8 liters of inhibitor had been produced in a shaker. The recombinant protein was separated from the culture medium by different processes: membrane clarification (S6-4106-22 CFP-1-E-4X2MA), concentration (UFP-5-C-4X2MA) and purification by an ion-exchange chromatography in a Source Q, heparin-Sepharose and gel filtration (System ÄKTA purifier - GE). Analysis of glycosylation was done using (Kit PNGase F - Glycerol Free) and (Kit Endo Hf), both purchased from Biolabs, New England. The purifications steps were analyzed by 12.5% SDS-PAGE and Western blotting. Amblyomin-X was tested for FXa amidolytic activity using chromogenic substrate S-2765. **Results and Discussion:** Amblyomin-X produced without deglycosylation showed about 35 kDa by SDS-PAGE, and when deglycosylated with Endo Hf it showed 15 kDa. Both forms were recognized by anti-Amblyomin-X, inhibited FXa amidolytic activity using the chromogenic substrate S-2765 (28%), and showed cytotoxic activity against cell lines Mia-PaCa-2 and Sk-Mel-28. Based on these findings, Amblyomin-X can become a drug to prevent thrombosis and intravascular coagulation in cancer patients.

Supported by: FAPESP, CNPq and INCTTOX

2.17 Modulation of pro-inflammatory molecules by Amblyomin-X in metastatic melanoma model

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Introduction: Amblyomin-X is a recombinant protein obtained from a cDNA library of the *Amblyomma cajennense* salivary glands. This molecule induces tumor regression of B16F10 (murine melanoma) through an apoptotic mechanism. Proteasome inhibition and changes in cell cycle gene expression were also observed. The role of cytokines produced in the tumor microenvironment is well known to be important mediators in cancer pathogenesis. **Objectives:** The aim of this study was to evaluate the effect of Amblyomin-X on the production and secretion of molecules related to the inflammatory process and metastasis using a metastatic model (*in vivo*) and in a co-culture of peritoneal cells and B16F10 (*in vitro*). **Methods:** The murine melanoma tumor line B16F10 was selected for its ability to form pulmonary tumor nodules. This was accomplished by injecting tumor cells iv in C57BL/6 mice. At 24 h after injection, the animals were treated daily for 14 days with Amblyomin-X or PBS. Plasma samples were collected from the animals, and the levels of cytokines were measured by ELISA. Peritoneal cells were collected and cultured for 48 h and their supernatants were collected to measure NO and cytokine levels. For the *in vitro* model, peritoneal cells were collected from naïve animals and incubated for 2 h with Amblyomin-X. After washing, peritoneal cells were co-cultured with the B16F10 cells for 24 and 48 h. The H_2O_2 level was measured using 24 h co-culture cells. Cellular metabolism (MTT), cytokines and NO were measured using 48 h co-culture cells and supernatant, respectively. **Results and Discussion:** Our results demonstrated an increase in IL10 and IL1β levels and decrease or non detection of IL6, IL12, TNFα in plasma. On the other hand, when the same cytokines were measured in the supernatant of peritoneal cells of the in vivo model, IL6, IL12 were increased and IL10 decreased. In the co-culture assay increase of H_2O_2, IL-12, IL-6, TNFα, IFNγ and decrease of NO levels were observed. It is well documented in the literature that the immune system can suppress or promote cancer development, but with no links, up to now, with this present molecule. Our results suggest that Amblyomin-X can modify the cytokine profile and also that it can modulate oxidative stress through levels of H_2O_2 increasing and NO decreasing. Besides that, the cellular metabolism was altered inducing cell death. Altogether, our results suggest that Amblyomin-X can modulate pro-inflammatory molecules involved with tumor cell progression.

Supported by: FAPESP, União Química Farmacêutica, CNPq and INCTTOX

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

2.18 Optimization of phosphoprotein identification and enrichment using mitosis-blocked Y1 cells

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Introduction: The fibroblast growth factor 2 (FGF2) is associated with proliferation and carcinogenesis; however, it shows anti-proliferative and tumor suppressive functions depending on the cellular context. In Y1 murine adrenocortical carcinoma cell line, FGF2 promotes G0 → G1 transition but delays S-phase and permanently blocks cells in G2/M. It is known that reversible phosphorylation of serine, threonine and tyrosine is a key step in cell signaling controlling many processes, including most cell cycle checkpoint transitions. Phosphorylation is a widespread modification; however, as the phosphoprotein concentration is very low, the study of phosphoproteome can be very tricky. To better understand the molecular mechanism induced by FGF2 in the cell cycle, we decided to study the phosphoproteome of Y1 cells. **Objectives:** Mitosis contains high phosphorylation site occupancy and therefore is a valuable source of phosphorylated proteins. Thus, here we used mitotis-blocked cells to optimize the enrichment and identification of Y1 phosphorylated peptides by proteomics. **Methods:** Y1 cells were grown in DMEM medium supplemented with 10% fetal bovine serum at 37ºC in the presence or absence of 2 μM colchicine for 24 h. Total protein was extracted and digested with trypsin followed by desalting. The peptides were analyzed by ESI-QTOF (Waters) mass spectrometry coupled online with nanoflow HPLC system. The MS/MS spectra were searched against SwissProt (*Mus musculus*) database using MASCOT. Alternatively, total protein extracts were analyzed by 2D-gel electrophoresis using a precast IPG strips for the first dimension separation and a 12% SDS-polyacrylamide gels for the second dimension. Gels were stained for phosphoprotein with Pro-Q Diamond and for total protein with silver. **Results and Discussion:** As expected, colchicine-treated cells were enriched in mitosis when compared with exponential cells as shown by fluorescence-activated cell sorting (FACS) analyses. By mass spectrometry, 91 proteins were identified in colchicine extracts with 34% of them phosphorylated, including histones (H3 and H2B), eIF-5A and HSP90. One hundred and twelve proteins were identified in exponential growing cells, while 26.7% contained at least one phosphorylated site. Moreover, when exponentially and colchicine-treated cells where compared, 13 proteins were differentially expressed. In preliminary results of 2D gel electrophoresis more than 16 phosphorylated proteins were detected in mitotic cells. We are currently performing the identification of these phosphorylated proteins by MS/MS as well as the enrichment procedures using TiO2 columns to obtain a more robust extract of phosphorylated proteins.

Supported by: FAPESP

2.19 Tempol ameliorates murine viral encephalomyelitis by preserving the blood–brain barrier, reducing viral load, and lessening inflammation

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Introduction: Multiple sclerosis (MS) is a progressive inflammatory and/or demyelinating disease of the human central nervous system (CNS). Although the triggers of MS remain under scrutiny, it is widely accepted that an immune-mediated inflammatory response induced by lymphocytes, macrophages and microglia in the CNS participates in the pathogenesis of the disease. The resulting inflammatory environment favors high rates of superoxide anion and nitric oxide formation. **Objectives:** Here, we infected female C57BL/6 mice with a neurotropic strain of the mouse hepatitis virus (MHV-59A) to evaluate whether treatment with the antioxidant tempol (4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy) affects the ensuing encephalomyelitis. **Methods:** Mice were inoculated with MHV-A59 virus (500 PFU) or PBS (control mice) and treated with tempol (24 mg/kg, i.p., 2 doses on the first day and daily doses for 7days plus 2 mM tempol in the drinking water *ad libitum*). Disease evolution was scored by disease symptoms and histology. Viral titers in the CNS tissues were assayed by plaque assay. Permeability of the blood-brain barrier was assayed by fluorescein uptake. Relative quantification of inflammation markers in the CNS were measured by semiquantitative microdensitometric/morphometric image analysis of immunoreactive areas for Mac-2, iNOS and 3-nitrotyrosine in the brain of the animals. Relative quantification of CD4 and CD8 transcripts in the spinal cords and the spleen were measured by densitometry of the corresponding PCR bands. **Results and Discussion:** In untreated animals, neurological symptoms developed quickly: 90% of infected mice died 10 days after virus inoculation. Treatment with tempol profoundly altered the disease outcome: neurological symptoms were attenuated, mouse survival increased up to 70%, and half of the survivors behaved as normal mice. Not surprisingly, tempol substantially preserved the integrity of the CNS, including the blood–brain barrier. Furthermore, treatment with tempol decreased CNS viral titers, macrophage and T lymphocyte infiltration, and levels of inflammation markers, such as expression of iNOS, TNF-α, and IFN-γ, and protein nitration. The results indicate that tempol ameliorates murine viral encephalomyelitis by altering the redox status of the infectious environment that contributes to an attenuated CNS inflammatory response. Overall, our study supports the development of therapeutic strategies based on nitroxides to manage neuroinflammatory diseases.

Supported by: FAPESP and CNPq/Redoxoma

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

2.20 Evaluation of the effects of alkaloids isolated from the skin secretion of *Rhinella Jimi* and *R. icterica* in the penetration of rabies virus in mammalian cells mediated by nicotinic acetylcholine receptors

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Introduction: Rabies is an acute infectious disease caused by a virus that affects the central nervous system. The virus replicates at the inoculation site and it is believed that it uses the nicotinic acetylcholine receptors, located in the neuromuscular junctions to reach the nerve endings. Many alkaloids have been isolated from a large number of amphibians and used in various forms of therapy. **Objectives:** The aim of this study was to assay alkaloids extracted from the skin of amphibians as possible interfering agents in the process of infection of the rabies virus in mammalian cells. **Methods:** Amphibian skin secretions were collected by mechanical stimulation and filtered through a size-excluding membrane. A liquid-liquid partition was made and the solutions were purified by RP-HPLC, in a C18 column. For the structural determination, mass spectrometry and nuclear magnetic resonance were performed. For the cytotoxic tests of the isolated compounds, BHK-21 cell line and murine neuroblastoma (N2A) were used. Briefly, 96-well microtiter plates containing the cells were incubated for 24 and 48 h, respectively, in the media containing different dilutions of the purified molecules. For the virologic test, fixed strain PV (Pasteur virus) and CVS (challenge virus standard) were used based on simplified fluorescence inhibition (SFIMT) and the quick test fluorescent focus inhibition test (RFFIT), with modifications. Initial virologic assays were performed with the simultaneous treatment of the cells with both the virus and the fractions. **Results and Discussion:** A total of 16 fractions were obtained by HPLC and the mass spectrometry analyses showed that most of these fractions are pure molecules, ranging from 180 to 800 Da. Toxicity tests were performed, and 9 fractions were toxic to BHK-21cells. Preliminary virologic tests indicate that Fraction 2 was able to diminish the viral infection by 15% in the BHK-21 cells. This fraction was also not cytotoxic and found to contain one major component of 417 Da. The initial results are promising. Fraction 2 will be further purified into individual components and the molecules will be retested biological for activity. Moreover, the virologic assays will be performed in different ways, namely: i) cells will be treated with the molecules prior to virologic infection, and ii) previously infected cells will be treated with the molecules in order to evaluate possible post-infection effects.

Supported by: Instituto Butantan, Instituto Pasteur, FAPESP and CNPq

3. Pharmacology

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.01 PGE2 potetiates crotalphine-induced endogenous opioid peptides and cGMP release in cultured DRG neurons

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Introduction: Crotalphine, a peptide first identified and isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus*, induces potent antinociception, mediated by activation of opioid and cannabinoid receptors. Previous studies showed that the high efficacy and long-lasting activity of this peptide is only observed in the presence of tissue lesion/inflammation. The molecular mechanism underlying this effect is under investigation. **Objectives:** The aim of our study was to investigate the effect of crotalphine on rat dorsal root ganglia (DRG) neurons, determining the effect of this peptide on the levels of intracellular cAMP and cGMP during sensitization by PGE2 as well as the release of endogenous opioid peptides. In addition, we aimed to see if the release of endogenous opioids from DRG is dependent on the activation of cannabinoid (CB2) and opioid (kappa) receptors. **Methods:** Wistar rat DRG neurons were cultured in 24-well plates. Two days after plating, the culture medium (DMEM) was replaced by Hanks' balanced salt solution containing PGE2 (1 μM) for 15 min followed by 15 min with crotalphine, morphine (1 μM), CB2 (AM1241, 1 μM) or kappa (U50488, 1 μM) agonists. The supernatant was collected to measure the levels of dynorphin A, beta-endorphin, met-enkephalin and CGRP. DRG neurons were harvested for cAMP and cGMP quantification. The quantification was performed using ELISA kits. **Results and Discussion:** PGE2 significantly increased CGRP release from DRG neurones, whereas crotalphine (1 and 10 μM) reduced the effect of PGE2 on CGRP release. PGE2 increased crotalphine (1 and 10 μM) and CB2 agonist (1 μM) release of dynorphin A and beta-endorphin and also the release of met-enkephalin by crotalphine. The CB2 agonist potentiated crotalphine (10 μM) release of beta-endorphin, met-enkephalin and the crotalphine inhibitory effect on CGRP release. PGE2 also increased the inhibitory effect of crotalphine (0.001, 1 and 10 μM) and morphine on cAMP release and potentiated the release of cGMP induced by crotalphine (1 μM) and kappa agonist. The present findings suggest that the release of endogenous opioid peptides and cGMP from DRG neurons constitute part of the molecular mechanisms involved in the action of crotalphine. In addition, activation of the cannabinoid system potentiates crotalphine effects. The results obtained in this study further contribute to the understanding of the mechanisms involved in the action of crotalphine.

Supported by: CAT/CEPID/FAPESP, INCTTOX/FAPESP/CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.02 Crotoxin stimulates glucose metabolism in peritoneal macrophages during tumor progression

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Introduction: Crotoxin (CTX), the major toxin of *Crotalus durissus terrificus* venom induces an inhibitory effect on tumor growth and modulates, particularly, the functions of macrophages (MØ), essential cells in providing a defense mechanism against tumor cells. In early tumor progression, MØ are avidly phagocytic (*M1)* releasing reactive nitrogen intermediates-RNI/ROI and cytokines TNF-α, IL-1β and IL-6. However, when the tumor is established, tumor-associated MØ (*M2*) show a decrease in these abilities. Our group demonstrates that CTX stimulates the H_2O_2 release and NO production and the secretion of the cytokines by peritoneal MØ obtained from both Walker 256 tumor-bearing rats (*M1* and *M2*) and non-tumor-bearing rats. It has been demonstrated that glucose metabolism is related to the function of these cells in inflammatory and immune responses. **Objectives:** The aim of this work was to evaluate the mechanisms involved in MØ polarization (*M1*/*M2*) during tumor establishment and progression, investigating the effects of CTX on glucose metabolism of peritoneal MØ obtained from Walker 256 tumor-bearing rats in two different protocols: 1- CTX injected during the establishment of the tumor (action of CTX on *M1* MØ) and 2- CTX injected on the 4th day after inoculation of rats with Walker 256 tumor cells (action of CTX on *M2* MØ). **Methods:** Male Wistar rats were inoculated s.c. in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells and immediately treated with CTX (18 μg in 300 μL per rat), or saline was administered s.c., using the same volume (control). In other groups, rats were inoculated s.c. in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells and treated on the 4th day with CTX (18 μg in 300 μL per rat) or saline administered s.c., using the same volume (control). Animals from control groups were inoculated s.c. in the right flank with 1 mL of PBS, treated with CTX or saline. Peritoneal MØ were obtained on the 14th day after tumor cell inoculation and evaluated with respect to activity levels of key enzymes: hexokinase, glucose-6-phosphate dehydrogenase and citrate synthase. **Results and Discussion:** In both protocols, CTX stimulated the activities of enzymes of glucose metabolism of peritoneal MØ obtained from both Walker 256 tumor-bearing rats and non-tumor-bearing rats. For the first time, it was shown that CTX modulates, *in vivo*, the metabolism of peritoneal MØ, using a single dose of CTX. These results contribute to the elucidation of mechanisms involved in the antitumor effect of CTX, generating new prospects for the development of a new substance with therapeutic properties.

Supported by: FAPESP, CAPES and INCTTOX

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.03 Peripheral sensitization increases the antinociceptive effect of crotalphine in TRP model of overt pain

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Introduction: Crotalphine (CRP), a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, induces a long-lasting (2-5 days) analgesic effect mediated by activation of peripheral δ- and κ-opioid receptors. Interestingly, the high effectiveness and long-lasting action of CRP is observed only in the presence of inflammation or tissue lesion, indicating that tissue sensitization is an important phenomenon for the expression of CRP effect. Despite these data, the effect of this peptide in experimental models of nociception that involve direct activation of receptors coupled to ion channels has not yet been evaluated. Data from the literature have demonstrated that transient receptor potential (TRP) channels are involved in nociception and that their activity can be modulated by peripheral opioid receptor agonists. **Objectives:** The aim of this work was to investigate the analgesic effect of crotalphine in models of overt pain mediated by the activation of TRPs and the role of previous sensitization to the action of this peptide. **Methods:** Male Swiss mice received an intraplantar injection of formalin (2%) or capsaicin (CPS; 1 nmol/paw) or the corresponding vehicles. In the experiments of previous sensitization, mice received an i.pl. injection of PGE_2 (0.01 nmol/paw) 1 h before CPS (0.03 nmol/paw., sub-threshold dose) administration. Immediately after treatments, overt nociception [licking time (s)] was recorded for 5 min. Three hours after capsaicin or formalin injection, the development of allodynia was also evaluated, in the same animals, using von Frey filaments (VFF). In this experiment, a 0.6 g VFF was applied to the plantar surface of the hind paw of the mice and the percentage of withdrawal response determined. **Results and Discussion:** The intraplantar administration of CPS or formalin induced overt nociception and mechanical allodynia, as compared to controls. CRP (50-2000 μg/kg,), administered p.o. 1 h before CPS (1 nmol/paw) or formalin (2%), reduced mechanical allodynia, without interfering with overt nociception. PGE_2 (0,01nmol/paw), injected 60 min before CPS (0,03 nmol/paw), significantly increased overt nociception and mechanical allodynia caused by CPS. This PGE_2-induced increase in CPS nociception was reduced by CRP (200 μg/kg, p.o.) and this antinociceptive effect of CRP was blocked by naloxone (s.c., 5 mg/kg), a nonselective antagonist of opioid receptors. These data corroborate previous findings from our group, which demonstrated that the antinociceptive effect of CRP depends on sensitization/inflammation/lesion, and confirm the involvement of opioid receptors.

Supported by: FAPESP and INCTTOX

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3.04 PKC and PI3K are involved in lipid body formation induced by CB, a phospholipase A_2 isolated from *Caudisona durissa terrificus*

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Introduction: The phospholipase A_2 CB is a subunit of crotoxin, a major component of *Caudisona durissa terrificus* venom. This phospholipase A_2 is neurotoxic and myotoxic and affects different defense functions of macrophages, key cells in host defense. Upon activation macrophages display several inflammatory activities, including increase in the number of lipid bodies (LBs), which are relevant organelles in lipid metabolism and the synthesis and release of inflammatory mediators. **Objectives:** The aim of this study was to evaluate the effect of CB on lipid body formation in isolated macrophages, and the participation of kinases (PKC, PI3K e $p38^{MAPK}$) in this effect. **Methods:** Thioglycolate-elicited macrophages from male Swiss mice were used. These cells ($2x10^5$cells/mL) were incubated for 1 h with either culture medium (control) or non-cytotoxic concentrations of CB (1.5, 3.15, 6.3 and 12.6 μg/mL). Lipid bodies were quantified by staining with osmium tetroxide (1%), followed by analysis under phase-contrast microscopy. In order to assess the kinetics of the CB-induced effect, macrophages were incubated with CB (6.5 μg/mL) or culture medium (control) for 1 to 12 h. Participation of signaling proteins was evaluated by treating cells with specific inhibitors before stimulation with CB (6.5 μg/mL) for 3 h. **Results and Discussion:** Incubation of cells with CB at concentrations of 3.15 to 12.6, but not 1.5 mg / mL, for 1 h, caused an increase in the number of LBs in comparison to control. Moreover, CB-induced increase in LB numbers was observed for all time periods evaluated (1-12 h), with a maximum at 12 h. Pre-treatment of macrophages with H7 (6 μM), a PKC inhibitor, or with LY294002 (1 μM), a PI3K inhibitor, significantly reduced CB–induced LB formation, with percentage increases of around 43.62% and 37.96%, respectively. Treatment of cells with SB202190, a $P38^{MAPK}$ inhibitor, did not modify LB formation induced by CB. The data obtained showed the ability of CB to induce LB formation in macrophages. This effect was time- but not concentration-dependent. Moreover, pharmacological treatment of cells indicated that CB-induced LB formation is dependent on PKC and PI3K but not $p38^{MAPK}$ pathways.

Supported by: CAPES, INCTTOX and CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.05 Crotalphine induces a potent and long-lasting analgesic effect in bone cancer pain

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Introduction: Crotalphine (CRP), a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, produces an analgesic effect. **Objectives:** The aim of this study was to characterize the analgesic effect of crotalphine in a new model of bone cancer pain induced by injection of Walker 256 cells into the rat femoral cavity. **Methods:** Histological analyses showed alterations in bone tumor implantation site, and metastasis was observed in the femur, lung, spleen, kidney on day 21. Bone metabolic alterations were determined by scintigraphy, using ^{99m}Tc-MDP. Femoral images were obtained before and 7, 14 and 21 days after tumor cell injection. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, using the rat paw pressure test or von Frey filaments. **Results and Discussion:** Photomicrographs analyzed 21 days after injection of tumor cells, demonstrated the presence of tumor cells in the femur of animals. Incorporation ^{99m}Tc-MDP was significant at 7, 14 and 21 days, suggesting the development of tumor in the femoral cavity. Histopathological analysis also demonstrated the presence of tumor cells in the lung and spleen, but not in the liver and kidneys. The results indicate that cells inoculated into femoral bone marrow can spread to some organs, including lymphoid organs. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after tumor cell injection. Interestingly, the paw withdrawal threshold in the von Frey test was reduced not only in the ipsilateral hind paw inoculated with the tumor, but also in the contralateral one, demonstrating the existence of bilateral allodynia-mirror-image pain. To evaluate the involvement of prostanoids, indomethacin, a cyclooxygenase inhibitor, was administered 3, 7, 14 and 21 days after tumor cell injection. Indomethacin partially inhibited hyperalgesia and allodynia induced by bone cancer, indicating the involvement of prostanoids in bone cancer pain The contribution of protanoids was more significant within the first 3 days after cell injection, since on day 7 and 14 the inhibitory effect of indomethacin was significantly lower when compared to days 1 and 3. CRP administered on day 21, blocked hyperalgesia, allodynia and mirror image pain. The analgesic effect was detected up to 2 days after peptide administration and was inhibited totally by κ and partially by δ opioid antagonists. Morphine partially inhibited allodynia and hyperalgesia. The results indicate that the injection of tumor cells causes bone cancer and pain. CRP induces a potent and long-lasting antinociception in this model, with higher efficacy as compared to standard analgesic drugs.

Supported by: FAPESP, CAT and INCTTOX

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.06 Sympathetic outflow and protein expression in 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 the key activator of the venom gland. However, data in the literature show that sympathetic outflow only has a role in stimulating synthesis and secretion of the saliva proteins in mammals. The new function of the sympathetic innervation was discovered just because the venom gland can assume different quiescent and activated stages, in contrast to the salivary gland which is constantly in an activated stage. **Objectives:** The aim of this study was to determine whether sympathetic innervation is also important to keep the submandibular gland in an activated stage. **Methods:** Adult Swiss male mice (25-30g) were divided into 3 groups: 1) control - treated with vehicle (n=3); 2) treated with reserpine (n=3) for 6 days (0.5 mg/kg - i.p.); 3) treated with reserpine for 6 days (0.5 mg/kg - i.p.) and phenylephrine (PHEN) plus isoprenaline (ISO) (20 mg/kg – i.p.) in the last day of treatment (n=3). Extracts of submandibular glands were prepared and proteins were analyzed by two-dimensional gel electrophoresis (2-DE). Gels were run in triplicate, stained with Coomassie Blue G, and the quantification of density of the spots and comparison between groups were done using ImageMaster 2D Platinum 7. The difference in spot density was analyzed by ANOVA and the probability of less than 0.05 was considered statistically significant. **Results and Discussion:** The 2-DE images of submandibular gland extracts showed stained spots with PI ranging from 3 to 10 and molecular mass ranging from 216 to 7 kDa in all groups. The analysis of these gels showed that in a total of 703 spots of protein, only 75 spots were expressed in all groups. Specific spots were found in each group (146 spots in control group, 188 spots in reserpine-treated group and 169 spots in reserpine and PHE plus ISO-treated group), indicating that different proteins are expressed in all groups. In addition, 54 spots appeared in both control groups and reserpine and PHEN plus ISO-treated group, and 71 spots appeared in both reserpine-treated group and reserpine and PHEN plus ISO-treated group, while no common spot was observed between control group and reserpine-treated group. Our results obtained from 2-DE analysis showed that the impairment of sympathetic innervation caused by reserpine promotes drastic changes in protein profile of submandibular gland and that the stimulation of both α- and β-adrenoceptors partially restores the reserpine effect in accordance with our previous results using one-dimensional electrophoresis. These results are very similar to those observed in Viperidae snake venom gland, suggesting that sympathetic innervation could be important to keep the mouse submandibular gland in an activated stage.

Supported by: FAPESP

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

3.07 Investigation of the regulation of striatal GABA release and consequences of the Parkinsonian lesion

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Introduction: Parkinson's disease, a progressive neurodegenerative condition, is related to the death of neurons located in the substantia nigra pars compacta (SNpc), a basal ganglia component. By the death of nigral dopaminergic neurons, this inhibitory pathway is lost, leading to a stimulatory hyperactivity of the subthalamic nucleus (STN) over lateral and medial globus pallidus (LGP and MGP), also increasing GABAergic efference on the indirect pathway (mediated by D2 dopaminergic receptors to LGP), as well as GABAergic inhibition of SNr (substantia nigra pars reticulata) over thalamus. The latter, in turn, decreases the cortical activity resulting in the classical symptoms of the disorder (bradykinesia, akinesia, shaking). These findings let to a recent alternative therapy in which the hyperactive STN would be injured and the symptoms would thereby be minimized. **Objectives:** The aim of the present study was to evaluate GABA release by normal striatal tissue and after unilateral 6-OHDA lesion in rats, an important model of Parkinson. An extensive investigation of the second messengers and neurotransmitters on this tissue was undertaken. **Methods:** Neurotransmitter release is evaluated by *in vitro* superfusion and release of pre-loaded radio-labeled GABA. The effects of a variety of drugs were assessed to pinpoint the role of different stemp in transmitter release. Nigral lesion was produced by stereotactic surgery and 6-OHDA microinjection to the medial forebrain bundle. **Results and Discussion:** The results obtained in tests with Ca^{2+}-free solutions demonstrated that GABA release corresponds to vesicular exocytosis. When the calmodulin inhibitor W-7 was used, it was found that the release of GABA was also greatly diminished, indicating that Ca^{2+} is indeed essential for this process. With cypermethrin (a calcineurin-PP2b inhibitor) a decrease was also observed. Tests with NMDA (glutamate receptor agonist) showed that, unlike what happens with acetylcholine and dopamine, there is no change in GABA release, which leads us to believe that the dopaminergic bundles originating in the substantia nigra do not project over GABAergic cells.

Supported by: FAPESP and CNPq

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3.08 Macrophages treated with crotoxin stimulate H_2O_2 release by neutrophils in co-culture model

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Introduction: Crotoxin (CTX), the major toxin of *Crotalus durissus terrificus* venom produces anti-inflammatory and immunomodulatory effects. Despite the inhibitory actions on neutrophil migration and the phagocytic activity of these cells, this toxin does not alter the microbicidal activity of neutrophils activated by PMA. On the other hand, CTX enhances the generation of reactive oxygen intermediates and nitrogen by macrophages, cells central to innate defense mechanisms. Recent studies have shown the importance of testing in co-culture, showing the interaction between macrophages and neutrophils and the importance of this interaction in the functional and secretory activity these cells in the control of the inflammatory response. Considering these stimulatory actions of CTX on macrophages and the importance of cooperation between macrophages and neutrophils in adaptive antimicrobial defense, it is important to investigate the actions of crotoxin on the antimicrobial activity of neutrophils co-cultured with macrophages. This study contributes to the elucidation of the mechanisms involved in the modulatory actions of CTX on the inflammatory response. **Objectives:** The aim of this work was to evaluate the effect of macrophages treated with crotoxin on H_2O_2 release by neutrophils in a co-culture model. **Methods:** Macrophages were obtained from the peritoneal cavity of male Wistar rats, and cells ($4x10^6$) were incubated with CTX (0.3 μg/mL) for 2 h and cultured for 24 h at 37°C in 24-well culture dishes. Afterwards, neutrophils were obtained from the peritoneal cavity of male Wistar rats 4 h after the i.p. administration of carrageenan (4.5 mg/kg), and cells ($4x10^6$) were co-cultivated with macrophages in 24-well culture dishes for 1 h at 37°C. After this period, neutrophils were obtained and H_2O_2 release by neutrophils was determined. Control monocultures of macrophages and neutrophils were treated with CTX or medium culture for 1 h at 37°C. **Results and Discussion:** The results showed that macrophages previously incubated in the presence of CTX generated a greater quantity of H_2O_2 (51%) than did control cells. This toxin did not alter H_2O_2 release by neutrohils activated by PMA. On the other hand, macrophages treated with CTX stimulated H_2O_2 release by neutrophils (28%), in cell-to-cell contact. These results demonstrate that the CTX can contribute to a system with two phagocytic cells involved in the resolution of the infection-associated inflammation.

Supported by: FAPESP and INCTTOX

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3.09 Role of the calcium and the phospholipase C signaling pathway in the vasoconstriction response induced by the atypical angiotensin II (Ang II) receptor of the rattlesnake *Crotalus durissus terrificus*

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Introduction: Ang II produces its vascular effects in mammalian species through interaction with angiotensin receptors, AT_1 and AT_2, and in some vertebrates such as *Crotalus durissus terrificus* (Cdt), with a pharmacologically atypical Ang II receptor subtype. AT_1 receptor stimulation results in vasoconstriction through the activation of intracellular signaling molecules, such as intracellular calcium, phospholipase C (PLC), D, A_2 and protein kinase C (PKC). We have previously demonstrated that PLC and adenylyl cyclase pathways are not involved with Ang II response in *Bothrops jararaca* (Bj), but intracellular calcium and PKC are important molecules in Ang II signal transduction in this snake. **Objectives:** The aim of this study was to evaluate the role of cytosolic calcium and the PLC/PKC pathway in the vasoconstriction response induced by Ang II in the aorta of Cdt. **Methods:** Isometric tension induced by cumulative concentration-effect curves to Ang II (10^{-10} – 10^{-6}M) in the absence or presence of U73122 (PLC inhibitor), chelerythrine and GF109203X (PKC inhibitors) and SKF96365 [store-operated calcium channel (SOC) inhibitor] was obtained in aorta rings from Cdt. The contribution of the extracellular calcium to the vasoconstriction effect of Ang II was analyzed by its concentration (10^{-7}M) in Ca^{+2} – free physiological solution plus EGTA (1 mM - depletes external Ca^{+2}). **Results and Discussion:** U73122 (10 μM, n=5) and chelerythrine (10 μM, n=6) did not modify Ang II-induced contraction, while GF109203X (10 μM, n=4) reduced the maximum response (E_{max}) by 31%. Our results also showed that depletion of the extracellular calcium strongly inhibited Ang II-induced contraction (23% residual response), and its reintroduction almost restored Ang II contraction (78%). Moreover, blocking the store-operated calcium channel with SKF96365 (30 μM, n=7) modified the Ang II cumulative curve and reduced E_{max} (40%). These data indicate that PLC pathway are not involved with Ang II-induced contraction in the aorta of Cdt, but suggest that PKC could contribute to this response, based on the GF109203X inhibition. Another signal transducer pathway must be involved in the PKC activation. Furthermore, extracellular calcium has an important role in the contractile process, and SOC seems to participate in this mechanism. Like Bj, PKC and Ca^{+2} are also second messengers in the intracellular signal process activated by Ang II receptor in the aorta of Cdt. Although these results contribute to the knowledge of the signal transducer mechanism of the atypical Ang II receptor in these snakes, other intracellular signal pathways should be investigated.

Supported by: FAPESP

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3.10 PPAR-γ and –β receptors are involvement in lipid body formation induced by a phospholipase A_2 isolated from *Bothrops* snake venom

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Introduction: Lipid bodies (LBs) are lipid inclusions formed by neutral lipids involved in both lipid metabolism and the inflammation process. The nuclear receptors known as peroxisome proliferator-activated receptors (PPAR-γ and -β) are lipid-activated transcription factors that have emerged as key regulators of lipid metabolism and inflammation. Recently, we demonstrated that MT-III, a secreted phospholipase A_2 ($sPLA_2$) isolated from *Bothrops asper* snake venom, induces the formation of LBs in macrophages. However, the involvement of PPARs in this MT-III- induced effect was not investigated. **Objectives:** The aim of this study was to evaluate the participation of both PPAR-γ and PPAR–β in LB formation and protein expression of these receptors induced by MT-III in macrophages. **Methods:** Thioglycolate-elicited macrophages from male Swiss mice (Butantan Institute Ethical Committee ref. 744/10) were incubated with MT-III (0.4 μM) or culture medium (control) for 1 to 24 h, and protein expression of PPAR-γ and –β were determined by Western blotting. Participation of PPAR-γ and –β in LB formation was evaluated by treating macrophages with the specific inhibitors GW9662 and GSK660 (10 μM), respectively, 1 h before stimulation with MT-III (0.4 μM) for 12 h. LBs were quantified by staining cells with osmium tetroxide (1%), followed by analysis by phase contrast microscopy. **Results and Discussion:** Incubation of macrophages with MT-III significantly increased PPAR-γ protein expression by 45% and PPAR-β protein expression by 74% for 1 to 24 h in comparison with control cells (average AU: 0.373± 0.06, n=4), with no statistical difference between the time periods evaluated. Incubation of macrophages with MT-III induced a marked increase in LB numbers (7.62±0.3 LBs/cell; basal: 2.2±0.21, n=4). Pretreatment of cells with either GW9662 or GSK660 compounds (10 μM) reduced MT-III-induced LBs formation by 74.44 % and 92.96 %, respectively. In conclusion, these results demonstrate that LB formation induced by a secreted PLA_2 (MT-III) is dependent on PPAR receptors (γ and β). Upregulation of expression of both PPAR subtypes may be an important mechanism for LB formation induced by this $sPLA_2$.

Supported by: FAPESP, CAPES, CNPq and INCTTOX

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3.11 The antinociceptive effect of crotalphine, an opioid-like analgesic peptide, involves stimulation of peripheral cannabinoid receptors and endogenous opioid release

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Introduction: In the last decades, several studies have been carried out with the aim to develop new analgesic drugs. Despite the advances in this field, medications providing effective treatment of some types of pain are still scarce. In addition, many patients still suffer with adverse effects caused by some of the drugs clinically used, demonstrating the need for new analgesic substances. In this regard, crotalphine (CRP), a peptide first identified and isolated from *Crotalus durissus terrificus* snake venom produces a long-lasting analgesic effect, which is mediated by activation of peripheral δ- and κ-opioid receptors. However, despite showing opioid activity, preliminary results obtained in binding experiments indicate that crotalphine does not directly activate opioid receptors. Behavioral and molecular studies have demonstrated a strong interaction between opioid and cannabinoid systems and also that cannabinoids may induce the release of endogenous opioids in the same manner that opioids may induce the release of endocannabinoids. **Objectives:** The aim of this work was to characterize the mechanisms involved in the antinociceptive effect of CRP, evaluating the participation of cannabinoid receptors in this effect as well as the possible interaction of these receptors with the opioid system. **Methods:** Male Wistar rats were used. Hyperalgesia was induced by prostaglandin E_2 (PGE_2, 100 ng/paw). The antinociceptive effect of CRP (1 μg/kg, p.o.) was determined using the paw pressure test. The involvement of cannabinoid receptors was investigated using selective antagonists of these receptors (CB_1, AM251, 80 μg/paw and CB_2, AM630, 50 μg/paw). The contribution of endogenous opioid system to antinociception induced by CRP was investigated using antibodies anti-β-endorphin (5 μg/paw), anti-enkephalin (50 μg/paw) and anti-dynorphin A (1 μg/paw). The involvement of endogenous opioids was confirmed by measurement of β-endorphin, met-enkephalin and dynorphin A release from skin tissue by enzyme immunoassays. **Results and Discussion:** Our results demonstrated that both CB_1 and CB_2 receptor antagonists and antibody anti-dynorphin A inhibited the antinociceptive effect of crotalphine. *In vitro* enzyme immunoassay confirmed that CRP induces the local release of dynorphin-A and that this effect is blocked by CB_2 receptor antagonist. These results indicate that both peripheral CB_1 and CB_2 receptors are involved in antinociception induced by crotalphine, where this effect is dependent on endogenous opioid release, particularly dynorphin-A.

Supported by: FAPESP and INCTTOX

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3.12 Prostanoid production in rat microvascular endothelial cells induced by PLA_2 subunit of crotoxin from *Crotalus durissus terrificus* snake venom

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Introduction: Crotoxin (CTX), the major component of *Crotalus durissus terrificus* snake venom (CdtV) contains two subunits: crotapotin (CA subunit) and phospholipase A_2 (CB subunit). The CB subunit exerts neurotoxic and myotoxic effects and alters macrophages functions. The phospholipase A_2 (PLA_2) enzymes catalyze the cleavage of arachidonic acid (AA) from the *sn*-2 position of phospholipids with subsequent conversion of free AA into prostaglandin H_2 by cyclooxygenases (COXs); newly formed PGH_2 is then converted into different prostaglandins by terminal synthases. The endothelial cells (EC) produce several prostanoids that act in an endocrine, paracrine, or autocrine fashion to modulate vascular physiology. Prostaglandins of the series I (PGI_2) and J (PGJ_2) play immunomodulatory roles in vascular endothelium. **Objectives:** The aim of this study was to determine the effect of CB on release of both prostaglandin I_2 and J_2 by rat microvascular endothelial cells was evaluated. **Methods:** Primary microvascular EC were obtained from rat cremaster muscle and maintained under culture conditions until confluence. Next, cells were placed in 96-well microplates and allowed to grow. After reaching confluence, monolayers were incubated with CB subunit (0.4 μM) or culture medium (control) for selected periods of time (1, 3, 6, 12 and 24 h). PGI_2 production was measured by enzyme immunoassay (EIA). **Results and Discussion:** Incubation of EC monolayers with a non-toxic concentration of CB (0.4 μM) induced a significant increase in PGI_2 levels from 3 (877 % increase) up to 24 h (105% increase) in comparison with controls, where levels of PGJ_2 were not modified. These data indicate that the phospholipase-subunit of crotoxin stimulates PGI_2 but not PGJ_2 production in microvascular endothelial cells *in vitro*. Considering that increased levels of PGI_2, the major prostanoid produced by EC, lead to hypotension and blood coagulation disorders, which are commonly observed during *Crotalus durissus terrificus* envenomation, the present data suggest that PGI_2 is a relevant mediator of venom-induced disturbances in vascular homeostasis. Moreover, the phospholipase A_2 subunit of crotoxin, by inducing PGI_2 release, appears to be a key element for systemic disturbances induced by the whole venom.

Supported by: FAPESP and CNPq

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3.13 Effects of mammalian and venom secretory phospholipases A_2 ($sPLA_2$s) on the expression of cyclooxygenase-2 (COX-2) by isolated macrophages (Møs)

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Introduction: $sPLA_2$s are expressed in inflammatory processes and are released upon cell activation. Several $sPLA_2$s have been suggested to control the induction of COX-2 through mechanisms that involve catalytic action or no catalytic mechanisms including heparan sulfate proteoglycan (HSPG) or binding to M-receptor or activation of cytosolic PLA_2 ($cPLA_2$). One of the major sources and targets of $sPLA_2$s at the inflammatory site are the Møs. However, no comparative study has been carried out with different $sPLA_2$s on isolated Møs whose activation promotes the production of proinflammatory mediators such as the prostaglandins (PGs). **Objectives:** Considering that COX is a key enzyme for the synthesis of PGs and that $sPLA_2$s induce the release of these mediators, the aim of this study was to investigate the mechanisms of COX-2 induction by several $sPLA_2$s-treated mouse resident peritoneal Møs. **Methods:** Cultured peritoneal Møs ($1x10^6$cells/mL), established from C57BL/6 mice, were incubated with mouse recombinant $sPLA_2$s of types X (mGX) or V (mGV) or IIA (mGIIA) (400 nM) for 2, 4 and 6 h. Møs were also treated with $sPLA_2$s type IIA isolated from *Bothrops asper* venom: myotoxin-III (MT-III), with catalytic activity and myotoxin-II (MT-II), which lacks enzymatic activity. The importance of the catalytic activity of $sPLA_2$s in COX-2 expression was evaluated with the inhibitor Ly329722 (5 μM) and an inactive mutant mGXH48Q; for the involvement of HSPG and $cPLA_2$ in $sPLA_2$-induced COX-2, heparinase III (HIII) (200 mU/mL) and pyrrolin-1(PY-1) (2.5 μM) were used, respectively. The protein expression of COX-2 and type M receptor were determined by Western blotting. **Results and Discussion:** $sPLA_2$s but not mGXH48Q induced COX-2 expression from 3 up to 6 h of incubation by Møs ($p<0.05$). Preincubation of these cells with Ly329722 or HIII decreased COX-2 expression induced by MT-III, mGIIA and mGX or by MT-II, MT-III and mGV, respectively ($p<0.05$). PY-1 reduced COX-2 expression induced by MT-II, mGIIA, mGX and mGV ($p<0.05$). Beside these results, peritoneal Møs did not show protein expression of type M $sPLA_2$s receptor. The data indicate that the catalytic activity of mGIIA and mGX as well as the interaction with HSPG by MT-II and mGV are critical in the induction of COX-2 in Møs. However, both mechanisms are important for an effect on MT-III-induced COX-2 expression. $cPLA_2$ participates in $sPLA_2$-induced upregulation of COX-2, except in MT-III-activated Mø. In conclusion, these findings show different mechanisms for COX-2 protein induction by $sPLA_2$s in Møs, and such effects are independent of activation of type M $sPLA_2$ receptor.

Supported by: CNPq

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3.14 Participation of S100A9 protein and neutrophils in glycogen-induced antinociception

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Introduction: Glycogen-induced peritonitis has been shown to induce antinociception in mice submitted to the abdominal contortion test, and that secretion by neutrophils of calcium-binding protein S100A9 mediates this effect. **Objectives:** This study aimed to assess the involvement of neutrophils in nociception control after glycogen injection in rats submitted to the paw pressure test, and to investigate S100A9 protein expression during glycogen-induced antinociception. **Methods:** Male Wistar rats were injected intraplantar (i.pl.) with 5% glycogen (Gly) solution and evaluated in the nociceptive test between 1 and 24 h after treatment. Animals were injected by the intravenous route (i.v.) with fucoidan (5 mg/kg), a selectin inhibitor, 15 min before i.pl. injection of Gly or saline, and the paw pressure test was performed after different periods of time. In addition, the same procedures were used to obtain the plantar tissue for histological analysis. To verify the participation of opioid peptides in Gly effect, naloxone (1 mg/kg) was (a) subcutaneously administered 15 min before the nociceptive evaluation in animals previously injected with Gly or (b) i.pl. administered 15 min before Gly injection in the hind paw; the nociceptive test was evaluated 2, 3 and 4 h after Gly administration. S100A9 expression in samples obtained from rat paws injected with Gly or saline for different periods of time was evaluated by immunoblotting. **Results and Discussion:** Gly induced antinociception at 2, 4, 6, 8 and 12 h after its injection in rats, and the pre-treatment with fucoidan reversed this effect between 2 and 8 h; in addition, fucoidan also induced hyperalgesia between 2 and 6 h after Gly injection. Histological analysis demonstrated increased migration of neutrophils between 2 and 8 h after Gly administration, which was inhibited in rats pre-treated with fucoidan. Naloxone did not interfere with the Gly antinociceptive effect. In regard to S100A9 expression, it was observed that samples obtained from footpads injected with Gly, between 2 and 12 h, had a band with a molecular weight of 14 kDa, corresponding to the relative molecular mass of S100A9. Relative quantification of bands reacting with anti-S100A9 antibody in the time periods between 2 and 12 h showed a significant increase in S100A9 expression in samples obtained from paws treated with Gly, compared to those treated with saline. These data suggest that antinociception induced by Gly in rats submitted to the paw pressure is dependent on neutrophil migration. Moreover, this effect is not related to the release of opioid peptides, but possibly to S100A9 secretion by neutrophils.

Supported by: FAPESP and Fundação Butantan

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3.15 Effects of ovariectomy and 17β-estradiol replacement on mitochondrial proteins involved with apoptosis in the rat hippocampus

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Introduction: 17β-estradiol plays a potent neurotrophic and neuroprotective role in the brain, and several candidate targets have been identified. Recent studies from our laboratory have shown that 17β-estradiol may help maintain long-term neuronal viability in the rat hippocampus by regulating the expression of members of the BCL-2 family. Furthermore, the duration of the treatment with 17β-estradiol after ovariectomy is a key factor in restoring the expression of BCL-2 and BAX to control levels. Along with these proteins, other mitochondrial proteins, such as apoptosis-inducing factor (AIF), cytochrome *C* and endonuclease-G, are known to play a key role in mammalian cell apoptosis. AIF and endonuclease-G are released from mitochondria into the cytosol, translocate to the nucleus, and cause chromatin condensation and fragmentation of DNA when cells are exposed to an apoptosis-inducing stimulus. Cytochrome *C* is also released from mitochondria into the cytosol and activates caspase 9, a cysteine protease.
Objectives: In the present study, we examined the effects of different periods of ovariectomy and the regulatory effects of the administration of 17β-estradiol for different periods on the expression of AIF, cytochrome *C* and endonuclease-G in mitochondrial and cytosolic fractions obtained from adult female rat hippocampus.
Methods: Hippocampi were obtained from rats in proestrus (control), ovariectomized rats after 15, 21 and 36 days, ovariectomized rats after 15 days and then treated with 17β-estradiol benzoate (50 μg/kg, sc, every other day) for 7 or 21 days, and rats ovariectomized and immediately treated with 17β-estradiol for 21 days. AIF, cytochrome *C* and endonuclease-G expression were determined by Western blotting.
Results and Discussion: Both ovariectomy and 17β-estradiol replacement did not affect AIF and cytochrome C expression in the mitochondrial and cytosolic fractions of the hippocampus. On the other hand, endonuclease-G expression decreased in the mitochondrial fraction and increased in the cytosolic fraction of the hippocampus obtained from ovariectomized rats. The treatment with 17β-estradiol for different periods was able to prevent the effect of ovariectomy, suggesting that 17β-estradiol is involved in the regulation of endonuclease-G. 17β-estradiol may help maintain long-term neuronal viability by regulating the expression of members of the BCL-2 family. Regulation of endonuclease-G released from mitochondria, but not of AIF and cytochrome *C*, also seems to be involved in the neuroprotective actions of 17β-estradiol. These data provide new understanding in the mechanisms involved in the neuroprotective role of estrogen.

Supported by: FAPESP and CNPq

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3.16 Internalization of *CF-[Gln[1]]-crotalphine* in dorsal root ganglion (DRG) neurons

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Introduction: Crotalphine (*CRP*) is a 14-mer peptide isolated from the venom of *C. durissus terrificus,* which triggers long-lasting antinociception in animal experimental models of acute and chronic pain. This effect is mediated by activation of peripheral κ and/or δ-opioid receptors. Despite this knowledge, the molecular targets of this peptide have not yet been characterized. Fluorescent analogues have been used to study processes triggered by ligand-receptor interaction with the aim to identify their binding sites. *CF-[Gln[1]]-CRP*, a functionally active carboxyfluorescein (CF) labeled analogue of *CRP* has shown similar antinociceptive activity as native or synthetic *CRP*, allowing the use of this analogue to characterize the molecular mechanisms of action of *CRP*. **Objectives:** The aims of this work were to characterize the antinociceptive activity of *CRP* and *CRP*-fluorescent analogues in the prostaglandin E_2 (PGE_2)- or bradykinin (BK)-induced hyperalgesia model and to investigate the effect of *CF-[Gln[1]]-CRP* on DRG neuron cultures. **Methods:** All procedures were approved by CEUAIB (protocol: 623/09) and performed in accordance with the guidelines for the ethical use of conscious animals in pain research published by IASP. Antinociceptive activity was evaluated by the rat paw pressure test, in animals injected by the intraplantar (i.pl.) route with PGE_2- or BK and treated (i.pl.) with *CRP* or *CRP*-fluorescent analogues. The effect of *CF-[Gln[1]]-CRP* on DRG neurons was evaluated by confocal microscopy in cell cultures pre-treated with 10 μM BK for 30 min and by double-label immunocytochemistry using anti-opioid and anti-cannabinoid receptor antibodies. **Results and Discussion:** The *i.pl.* injection of PGE_2 and BK caused a significant decrease in pain threshold, characterizing the hyperalgesia phenomenon. The *i.pl.* injection of *CRP, [Glu[1]]-CRP, CF-[Gln[1]]-CRP* and *CF-[Glu[1]]-CRP* (0.6 ng/paw) caused inhibition of PGE_2- and BK-induced hyperalgesia (antihyperalgesic effect). In addition, *CRP, CF-[Gln[1]]-CRP* and *CF-[Glu[1]]-CRP* increased the pain threshold to values above the baseline values (antinociceptive effect), where the fluorescent analogues were more potent than *CRP* in the BK-induced hyperalgesia model. These results suggest that the large fluorescent moiety positively affects the pharmacophore structure required to trigger antinociception. *CF-[Gln[1]]-CRP* internalizes in intermediate (30 μm) and small (10 μm) diameter neurons pretreated with BK, in a concentration-dependent manner and do not co-localize with either opioid (κ and δ) or cannabinoid (CB_1 and CB_2) receptors, indicating that this process is not mediated by these receptors.

Supported by: INCTTOX/CNPq and FAPESP

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3.17 Central effects of intrahippocampal injection of TsTX-I isolated from *Tityus serrulatus* scorpion venom in rats

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Introduction: TsTX-I, a β-toxin extracted from *Tityus serrulatus* scorpion venom, is able to bind to site 4 of the sodium channel and change the ionic permeability of nervous cells, changing the release of neurotransmitters. Despite that this toxin is widely studied, little is known about its role on the central nervous system after direct injection in this area. **Objectives:** The aim of this study was to evaluate the effects of TsTX-I toxin on the levels of the neurotransmitters glycine, glutamate and GABA in the hippocampus and to verify the integrity of neuronal cells. **Methods:** Male Wistar rats (230 to 250g) were submitted to stereotaxic surgery for implantation of guide cannula. Eluates were collected hourly by microdialysis (total of 8 samples). After the collection of 3 samples, the animals were injected with saline (1 µl) or with the toxin (125 ng/µl) and the collection was continued. For histological examination, CA1, CA3 and CA4 hippocampal areas, ipsi- and contralateral to injection (CA1i, CA1c; CA3i, CA3c; CA4i, CA4c), were analyzed with a light microscope with a magnification of 40 times. **Results and Discussion:** The toxin induced behavioral changes such as seizures, myoclonus, prostration and respiratory distress. However, it was not able to promote significant changes in the amino acid levels (glutamate: 2.79±0.75 µg/µl – baseline; 3.09±0.93 µg/µl - after injection; glycine: 1.16±1.16 µg/µl – baseline; 1.15±1.15 µg/µl - after injection; GABA: 1.4±0.45 µg/µl – baseline; 1.41±0.53 µg/µl - after injection) and did not promote changes in the integrity and number of hippocampal cells (control group: CA1i 55.97 ± 5.11, CA3i 34.2 ± 5.5 and CA4i 34.57 ± 3.2; CA1c 55.28 ± 4.78, CA3c 35.57 ± 3.35 and CA4c 35.45 ± 3.82; experimental group: CA1i 50.76 ± 11.78, CA3i 37.14 ± 8.7, CA4i 32.73 ± 6.54; CA1c= 51.85 ± 15.17, CA3c= 32.42 ± 4.24, CA4c 31.62 ± 3.9). Data are presented as mean±SD and analyzed by ANOVA followed by Tukey's test, $p<0.05$. These results show that the intrahippocampal injection of TsTX-I produced effects different from that observed after the injection of other toxins present in the same venom. These differences indicate that if the toxins bind at different sites of sodium channels, different physiological responses are evoked. This study shows the importance of toxins as pharmacological tools to better understand the involvement of ionic channels in events in the central nervous system.

Supported by: FAPESP, Fundação Butantan INCTTOX and CAPES

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3.18 Effects of phospholipases isolated from the venom of micrurus lemniscatus on astrocytes culture

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Introduction: The predominant clinical manifestations of Elapid snake bite are related to the neurotoxic and myotoxic actions of the venom, causing blockade of peripheral nervous transmission. The phospholipase A2 neurotoxins are the principal components of the venom and induce cell death in hippocampal neurons. Glial cells, astrocytes in particular, exert a profound effect on neuronal development providing trophic support. Besides, their role in synaptic activity has been demonstrated, where the synapse is referred to as tripartite because it includes astrocytes and pre-synaptic and pos-synaptic neurons. **Objectives:** The aim of this work was to investigate the effects of Mlx-8 and Mlx-9 phospholipase A2 toxins isolated from Micrurus lemniscatus venom on the viability of astrocytes. **Methods:** Male Wistar rats with 3 months of age (230/250g) were used. The astrocytes were obtained by papain dissociation of the pineal glands. Astrocytes were maintained for 48 h in DMEM medium, 37 °C, 5% CO_2 and then incubated, under the same conditions, for 24 h with the toxins Mlx-8 or Mlx-9 (1, 10, 100, 1000 ng/ml) with or without atropine, competitive muscarinic cholinergic receptor antagonist (10-5M), or BB1101, a potent TNF-α processing inhibitor (10-5M). The MTT test was used for evaluation of cell viability. **Results and Discussion:** There was a significant reduction in viability when the cells were treated with Mlx-8 or Mlx-9 at all concentrations used (viability in relation to control group: 1 ng/ml: 56.5%, 10 ng/ml: 68.7%, 100 ng/ml: 69%, 1000 ng/ml: 88.1%; ANOVA: $p<0.05$). Atropine reversed the reduction of cell viability induced by Mlx-9 toxin (viability in relation to control group: 1 ng/ml: 91.3%, 10 ng/ml: 113.8%, 100 ng/ml: 110.8%; ANOVA: $p<0.05$) but not by Mlx-8. BB1101 did not reverse the effects of Mlx-9 or Mlx-8 toxins on cell viability The Mlx-8 and Mlx-9 phospholipase A2 neurotoxins isolated from the venom of Micrurus lemniscatus are lethal to astrocytes in culture. The effect of neurotoxin Mlx-9 seems to involve the muscarinic cholinergic system, whereas the effect of neurotoxin Mlx-8 does not. The release of cytokine TNF-α does not seem to be an important mechanism of death induction by both Mlx-8 and Mlx-9 toxins in astrocyte culture.

Supported by: CNPq

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3.19 Crotoxin interferes with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis

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Introduction: Multiple sclerosis (MS) is a central nervous system inflammatory demyelinating disease where the primary symptoms, losses of sensory and motor functions and chronic pain, are a major concern, affecting 50% to 80% of patients. MS has no cure, where therapeutic approaches focus on stopping disease progression and cumulative neurological disability. To date, however, few studies have investigated the mechanisms of chronic pain in animal models of MS since locomotor impairments make pain evaluation difficult. Recently, it was demonstrated that in the MOG_{35-55}-induced EAE, an animal model of MS, hypernociception appears before the onset of motor disability, allowing the study of these two phenomena separately. **Objectives:** The aim of this study was to evaluate the effect of crotoxin, a neurotoxin isolated from *Crotalus durissus terrificus* snake venom, which displays antinociceptive, anti-inflammatory and immunomodulatory effects, in the pain and progression of symptoms of EAE. **Methods:** Procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (protocol number 757/10). EAE was induced by s.c. immunization of C57BL/6J mice (18-20g) with 200 µl of an emulsion containing 150 µg of MOG_{35-55} peptide and 400 µg of *Mycobacterium tuberculosis* extract in incomplete Freund's adjuvant oil. In addition, the animals received 150 ng of pertussis toxin i.p. on day 0 and day 2. Pain threshold was determined using an electronic pressure-meter test. Motor activity was assessed using the rota rod test. Clinical signs of EAE were assessed according to the following score: 0, no signs of disease; 1, loss of tone in the tail; 2, hindlimb paresis; 3, hindlimb paralysis; 4, tetraplegia; 5, moribund. **Results and Discussion:** The pain threshold of the animals decreased at day 4, while the first sign of disease appeared at days 11–12, coinciding with the onset of motor abnormalities. Crotoxin (40 µg/kg, s.c.) administered 5 days after immunization (1 day after onset of pain threshold alteration) was able to induce antinociception without interfering with clinical signs of EAE. Interestingly, when crotoxin was injected for 5 consecutive days, it induced analgesia and also reduced EAE progression. The antinociceptive effect of crotoxin was blocked by the pretreatment of the animals with Boc2 (10 µg, i.p.), a selective antagonist of formyl peptide receptors. These results indicate that crotoxin interferes with the pain and progression of EAE. The antinociceptive effect involves the participation of formyl peptide receptors.

Supported by: FAPESP and INCTTOX

3.20 Stimulatory effects of BaP1 on rat B type synoviocytes in culture

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Introduction: Matrix metalloproteinases (MMPs), a family of Zn-dependent neutral proteolytic enzymes, are known to play a critical role in the erosion of cartilage during arthritis. Moreover, levels of these enzymes are increased in inflamed articular joints. MMPs and snake venom metalloproteinases (SVMPs) share common domain organization and exhibit an identical Zn-binding motif. Studies on SVMPs may thus provide insights into the functions of MMPs. **Objectives:** In this study we analyzed the effects of BaP1 on isolated synoviocytes, the main articular cells involved in production of inflammatory mediators, evaluating: a) release of prostaglandin E_2 (PGE_2); b) participation of NF-kB in COX-2 protein expression and PGE_2 release and c) expression of the EP4 receptor of PGE2. **Methods:** B type synoviocytes isolated from rat knee joint synovial membranes were used. Levels of PGE_2 were measured by EIA, and protein expression of both COX-2 and EP4 receptor determined by Western blotting. **Results and Discussion:** Stimulation of B type synoviocytes with BaP1 induced release of PGE_2 after 1, 3 and 6 h, but not 30 min incubation, in comparison with control cells incubated with RPMI alone. BaP1 induced COX-2 protein expression by synoviocytes (30 min – 3 h) without modification of COX-1 expression. Inhibition of NF-kB either by TPCK or SN50 significantly decreased BaP1-induced COX-2 protein expression (average of 43% reduction). Treatment of cells with SN50 but not TPCK markedly reduced BaP1-induced PGE_2 release (98%). Moreover, increased levels of EP4 receptor expression (52 and 65 kDa) were detected in synoviocytes stimulated by BaP1 during 3 h in comparison with control cells. The present data indicate that BaP1 can directly stimulate synoviocytes for synthesis of PGE_2 and expression of COX-2. These effects are mediated by NF-kB. In addition, BaP1 is able to increase expression of EP4, which may contribute to the amplification of PGE_2-induced effects. These findings suggest novel regulatory mechanisms for metaloproteinases in B type synoviocytes.

Supported by: FAPESP and CNPq

4. Immunology and Vaccines

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4.01 Crotoxin from *Crotalus durissus terrificus* down-modulates the TNF-α and IL-17 in experimental model of acute intestinal inflammation in mice

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Introduction: Among the components isolated from the *Crotalus durissus terrificus* rattlesnake venom, the crotoxin (CTX) is able to modulate the immune response to unrelated antigens. Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBDs) possibly due to an abnormal activation of the immune response against constituents of the luminal flora. Experimental models, such as Trinitrobenzene sulfonic acid (TNBS)-induced colitis, are helpful for understanding the IBDs. **Objectives:** In this work we investigated the effects of the CTX and the immune mechanisms involved in the TNBS experimental model of acute intestinal inflammation in mice, focusing on the TNF-α and IL-17 production and the presence of $CD4^+CD25^+FOXP3^+$ T cells. **Methods:** Male BALB/c mice (n=20) were anesthetized and 0.1 mL of TNBS (100mg/Kg in 45% ethanol) was administered intrarretal to acute colitis induction. The control mice received only 0.1mL of 45% ethanol. After 18 hours of the induction, mice were injected with 0.3mL CTX (2.4μg/mL i.p.) or phosphate buffered saline (PBS) by intraperitoneal route. At day 4 post induction, the mice were sacrificed, rapidly dissected, and the entire colon was quickly removed and gently cleared of feces for measurement of mieloperoxidase (MPO) enzyme activity and cytokine levels by ELISA. Mesenteric lymph nodes and Peyer's patches were obtained for the analysis of $CD4^+CD25^+FOXP3^+$ positive T cells by flow cytometry. **Results and Discussion:** MPO activity was significantly higher in the TNBS-group than those in TNBS that received CTX. This evidences the reduction of the index tissue inflammation after the toxin administration. High secretion of IL-17, IL-6, IL-1 and TNF-α was verified in cell supernatants from TNBS-mice. In contrast, these cytokines secretion was lower in mice that received CTX. Furthermore, the administration of CTX also stimulated an enhancement of the IL-10 and TGF-β secretion and, in preliminary experiments; CTX was able to induce higher percentage of $CD4^+CD25^+FOXP3^+$ T cells when compared with the TNBS-group. These results contribute to the understanding of the immunomodulatory role of CTX in acute intestinal inflammation induced by TNBS administration.

Supported by: CAPES, CNPq and FAPESP

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4.02 Immune response induced by a conjugate vaccine against diarrheagenic *Escherichia coli* belonging to serogroup O111

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Introduction: *E. coli* belonging to serogroup O111 is responsible for cases of acute diarrhea, outbreaks of hemorrhagic diarrhea and uremic hemolytic syndrome in underdeveloped countries. This serogroup is found in the following categories of E. coli: enteropathogenic (EPEC), enterohemorrhagic (EHEC) and enteroaggregative (EAEC). A way of preventing diarrhea induced by these pathogens is by vaccination, with the ideal form of administration being oral. For oral immunization the use of an adjuvant capable of breaking oral tolerance is necessary, as well as a molecular transporter that protects the antigen during its passage through the digestive system. **Objectives:** The aim of this study was to verify the capacity of the conjugate administered orally to induce a systemic humoral immune response and to generate mucosal IgA and IgG antibodies against the O111 polysaccharide. **Methods:** In this study, the recombinant B subunit of the LT toxin (LTB) of an ETEC with oral adjuvant capacity was conjugated to the O111 polysaccharide and incorporated into the carrier, Vaxcine. Mice were immunized three times with the conjugate incorporated in Vaxcine while mice receiving subcutaneous injection were used as a control. Feces and blood samples were collected before the first immunization and ten days after the last, to determine the level of antibodies by ELISA. The capacity of the antibodies to recognize live EPEC, EHEC and EAEC was determined by a flocculation technique. **Results and Discussion:** The results showed that, independent of the route of administration, the conjugate, incorporated or otherwise in Vaxcine, was capable of inducing IgG and IgA antibodies against the O111 polysaccharide in feces and in blood. However, a higher level of antibodies of both classes was observed in mice immunized with the antigen in Vaxcine. It was also shown that these antibodies were capable of recognizing the three categories of *E. coli* belonging to serogroup O111. These results indicate that the O111-LTB conjugate on Vaxcine has the potential to be used orally for the prevention of *E. coli* belonging to serogroup O111.

Supported by: CNPq

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4.03 Macrophage activation by tioglycollate inflammation and BCG infection in mice bearing distinct functional *Slc11a1* alleles

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Introduction: Mouse lines genetically selected for maximal (AIRmax) and minimal (AIRmin) acute inflammatory response (AIR) are respectively resistant and susceptible to *S. Typhimurium* infection. The *Slc11a1* gene is involved in the resistance to this infection and in the control of *M. bovis* BCG and *L. donovani* infection. Mouse lines homozygous for resistance and susceptibility *Slc11a1* alleles ($AIRmax^{RR}$, $AIRmax^{SS}$, $AIRmin^{RR}$ and $AIRmin^{SS}$) were produced in our laboratory. These lines show differences in inflammatory capacity, where the phenotype influenced by macrophage (Mϕ) activation. **Objectives:** The aim of this study was to evaluate the effect of *Slc11a1* gene polymorphism in Mϕ activation induced by thioglycollate (TG) or by BCG infection in these mice. **Methods:** Adherent peritoneal cells harvested at 96 h after thioglycollate ip injection or 14 days after BCG ip infection were cultured for 48 h with and without LPS. NO was measured with the Griess reagent, and cytokines were determined in culture supernatants by ELISA. H_2O_2 production was analyzed in total peritoneal cells stimulated with PMA using phenol red solution. Spleen and lung were homogenized and cultured in specific BCG medium and the bacterial colonies were counted. **Results and Discussion:** TG promoted cell migration and induced low secretion of NO by MΦ in all lines. However, when LPS was added, higher NO secretion was observed in $AIRmax^{RR}$ followed by $AIRmin^{RR}$, $AIRmax^{SS}$, and $AIRmin^{SS}$. The same profile was observed for IL-10 secretion. High levels of H_2O_2 were observed in all lines. $AIRmax^{RR}$ secreted higher levels of IL1β and IL-12p40 than did other lines. On the other hand, IL-18 and IL-4 were produced at baseline levels. When mice were infected with BCG, Mφ from AIRmax mice bearing the *Slc11a1* R allele produced high levels of IL-1β, IL-12, IL-6 and TNFα, whereas low levels of these cytokines were found in the other mouse lines. However, when LPS was added there was overexpression of these cytokines in all lines. On the other hand, NO and H_2O_2 production was higher in $AIRmin^{SS}$, which, according to *Slc11a1* genotype, is the most susceptible strain to BCG infection, evaluated by bacterial growth in the spleen and lung. Our results show that Mϕ of mice $AIRmax^{RR}$ were activated efficiently to kill the bacteria, secrete cytokines, and they indicate that NO and H_2O_2 play a limited role in the protection against BCG infection. In this model, the genetic background relevant to acute inflammatory response regulation and the polymorphism of *Slc11a1* interferes differently in cytokine, H_2O_2 or NO production by TG- or BCG-induced peritoneal macrophages.

Supported by: CAPES, CNPq and FAPESP

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4.04 Biochemical characterization of PAS-1, an immunomodulatory protein secreted by *Ascaris suum*

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Introduction: Helminths secrete several molecules that can modulate the host immune responses, acting at different points such as cell proliferation and activation, antibody production and regulatory response stimulation. In previous reports, we described the immunosuppressive activity of an *Ascaris suum*-derived immunomodulatory molecule, called PAS-1. PAS-1 was isolated from adult worm extract and highly suppresses antibody production, acute LPS-induced inflammation and lung allergic inflammation, due to the stimulation of the release of regulatory cytokines by T reg cells. **Objectives:** The aim of the present study was to determine the N-terminal of the PAS-1 obtained by *in vitro* culture of earlier larval stages of *A. suum*. **Methods:** *Ascaris suum* eggs isolated from worm uteri were embryonated *in vitro* and cultured in DMEM medium for two weeks. After *in vitro* culture of larval stages, the protein was purified by affinity chromatography (using a monoclonal antibody which recognizes PAS-1 coupled to Sepharose 4B) followed by reverse-phase chromatography (C-18 column). To determine the N-terminal sequence, the purified protein was submitted to the Edman degradation method performed with an automated protein sequencer (Shimatzu, PPSQ-21A). **Results and Discussion:** The amino-terminal of the PAS-1 is composed of the amino acids His-His-Phe-Thr-Leu-Glu-Ser-Ser-Leu-Asp-Thr. Our results demonstrated that PAS-1 is homologous to the ABA-1 allergen (a protein from *Ascaris lumbricoides*) and to a polyprotein ABA-1 (from *Ascaris suum)*. Moreover, PAS-1 shows 90% similarity with Ladder protein from *Toxocara canis*. It is interesting that although PAS-1 is homologous to ABA-1, these proteins have different biological activities. ABA-1 has allergen properties inducing IgE antibody production and allergic responses, while PAS-1 is a potent immunosuppressive protein.

Supported by: CNPq

4.05 Potency analysis of rabies vaccine by NIH and ABT tests

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Introduction: The NIH test is the most widely method used to evaluate the efficacy of inactivated rabies vaccines. Although the World Health Organization (WHO) recommends this test to determine the potency of each final lot of vaccine, there are concerns about the method, such as the high variability, the difficulty of obtaining valid results in individual tests, the large number of animals required and the long duration of the test (28 days). During the production phases, antigen content may be determined by other methods, such as single radial diffusion (SRD), enzyme immunoassay (EIA) or antibody binding test (ABT). WHO also encourage the support of the data generated by NIH test by the antigen content determination in order to ensure production consistency. **Objectives:** In this study, the potency of 24 lots of rabies vaccine suspension, either concentrated or formulated product, produced at Instituto Butantan, Brazil, were analyzed by two methods: NIH and ABT. **Methods:** For NIH tests, groups of 18 mice were immunized twice ip with 0.5 mL of serial dilutions of a standard or test vaccine on days 0 and 7. On day 14, the animals were injected ic with 30 μL of a CVS rabies virus. The number of mice that died between the 5th and 14th day after challenge was recorded. For ABT, serial dilutions of a standard or test vaccine were incubated with a defined concentration of rabies virus-neutralizing antibodies. The antigen not neutralized in these mixtures was indicated by addition of a defined concentration of CVS rabies virus and, after a time interval, the mixtures were added to a BHK-21 cell suspension in a microtiter plate. The growth of the indicator virus (CVS) was observed by staining with fluorescent antibodies. For both methods, the results were used to calculate the 50% end-point dilution of the test vaccine. **Results and Discussion:** There was a correlation coefficient of 0.82 between the two methods, which is considered good for biological assays. Moreover, the ABT showed high repeatability, without test rejection, while 20 of the 62 NIH tests performed were rejected by non-linearity, parallelism or regression, by unsatisfactory values of the viral titer or the reference vaccine's DE_{50} or due to the large difference between the replicates' results. We concluded that ABT is appropriate to determine the potency of rabies vaccines during production phases.

Supported by: Fundação Butantan

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4.06 Conjugation of *Haemophilus influenzae* type b polysaccharide (Hib) and tetanus toxoid protein (TT) activated by DMT-MM. Novel vaccine candidate of Butantan Institute

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Introduction: *Haemophilus influenzae* serotype b (Hib) is a gram-negative encapsulated bacterium that can cause systemic sepsis, pneumonia and meningitis. The polysaccharide can be converted into a T-cell-dependent antigen by chemical conjugation to a carrier protein improving the immunogenicity of Hib vaccines in young children. **Objectives:** The goal of this study was the development of a conjugate vaccine between capsular polysaccharide polyribosyl ribitol phosphate (PRP) and tetanus toxoid (TT) using DMT-MM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) as activating molecule. **Methods:** The synthetic sequence of this conjugate vaccine is composed of three steps. The first one was the oxidation of polysaccharide by $NaIO_4$.The reaction mixture was prepared mixing PRP (10 mg/mL), $NaIO_4$ (5 mM) in 10 mM phosphate buffer, pH 7.5, for 15 min and stopped with glycerol (10 eq). The next step was the reaction of PRP-Oxi (6 mg/mL) with ADH (20 eq), in 10 mM phosphate buffer, pH 7.5, for 24 h, followed by $NaBH_4$ (20 eq), resulting in PRP-NH_2. The last step was the conjugation of PRP-NH_2, i.e., PRP-ADH (20 mg) with TT (30 mg/mL) mediated by DMT-MM (0.1 M) for 24 h. All products were purified by chromatography or ultrafiltration system. Once the PRP-TT Butantan vaccine was synthesized, BALB/c mice were immunized s.c. with it in three-dose scheme at fortnight intervals, and the sera were collected on the 13th, 27th and 41st day by retro-orbital bleeding. Saline, PRP and TT co-administrated and commercial vaccine were used as controls. The anti-PRP IgG levels were measured by ELISA as well as anti-IgG avidity index. **Results and Discussion:** The oxidation step reduced the polysaccharide size and introduced aldehyde reactive group resulting in 98% yield and MW of 30 kDa. The reaction of PRP-Oxi with ADH resulted in 90% yield and the conjugation step in 46% yield. The DMT-MM is used to activate the carboxyl groups present in the protein, forming a triazinyl ester as intermediate, which is susceptible to nucleophilic attack of PRP-NH_2. This final yield is remarkable, since studies show ranges from 20 to 30%. Anti-PRP IgG level for Butantan vaccine measured by ELISA was 5.1 ± 1.3 μg/ml, and studies in humans show a post-vaccination level ≥1 μg/ml, predicting long-term protection. Our finding was not statistically different from commercial vaccine. The anti-IgG avidity index was 50%. In conclusion, this approach using DMT-MM as activating molecule seems to be useful for acquiring a conjugate vaccine. Further analysis must be done, as well as studies of new conjugates with different PRP/TT ratios.

Supported by: FAPESP

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4.07 Development of an ELISA for the titration of mouse IgG1 and IgG2a specific for rabies virus glycoprotein

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Introduction: The recombinant expression of rabies virus glycoprotein (RVGP) has been performed by two different systems: a non-replicative viral vector SFV and a *Drosophila melanogaster* S2 cell-based system. The RVGP produced by these two systems has been administered to mice in immunization studies. The amounts of anti-RVGP antibodies have been precisely determined. Among the anti-RVGP antibodies, the predominance of a subtype IgG1 or IgG2a is considered to be indicative of the shift of the immune response towards a Th2 or Th1 pattern, respectively. The complete study of the immune response to a recombinant antigen is mandatory for the analysis of its possible use as a new vaccine. **Objectives:** The aim of this study was to develop and standardize an ELISA for titration of IgG1 and IgG2a anti-RVGP antibodies. **Methods:** For the establishment of an ELISA protocol, 96-well plates were coated overnight with different amounts of purified RVGP at 4ºC. The next day, plates received serial dilutions of sera from mice immunized with rabies vaccine and then were incubated at 37ºC for one hour. After washing, plates received different dilutions of HRP-rat-anti-mouse IgG1 or HRP-rat-anti-mouse IgG2a and incubated at 37ºC for 30 min. Plates were revealed with hydrogen peroxide and OPD. The best conditions were applied for several samples of serum from mice immunized with different preparations for validating the protocol. **Results and Discussion:** We successfully established the protocols for anti-RVGP antibody subtype titration. Differences in the anti-mouse antibodies required the utilization of equal dilutions prior to incubation (1:1000), but different amounts of hydrogen peroxide enzyme substrate and OPD chromogen (1:5000 or 1:2500 for IgG1 or IgG2a, respectively). The absence of a standard curve was circumvented by determining titers as the inverse of sample dilution in which absorbance reached threefold the negative control value. Utilizing these protocols we could determine the IgG1/IgG2a ratio of anti-RVGP antibodies produced in mice immunized with S2 or SFV derived RVGP.

Supported by: FAPESP

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4.08 Study of different resin concentrations in rabies virus purification

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Introduction: Rabies is a viral disease that infects mainly domestic and wild animals and humans. Rabies vaccine is the only preventive medicine for rabies disease. Rabies vaccines are produced with rabies virus suspensions. The chromatography is a good strategy for purification and reducing residual cellular DNA of these suspensions. **Objectives:** The aim of this study was to use the ion exchange resin (DEAE-cellulose) in different concentrations in the purification of the rabies virus suspensions obtained from Vero cells cultivated in serum-free medium in a bioreactor. **Methods:** In this study, rabies virus suspensions obtained from Vero cells maintained in serum-free medium in a bioreactor of 150 L and infected with PV rabies virus were used. The virus suspensions after concentration by tangential filtration (13x) were submitted to a purification process using DEAE-cellulose at different concentrations. Virus suspensions with $10^{5.2}$ FFD_{50}/ml and 39.93 pg of cellular DNA were divided into four parts and each one of them was mixed with 5, 10, 20 and 30% DEAE-cellulose. After 15 min at 4°C, the supernatants were harvested and filtered, and samples were taken to determine the residual cellular DNA and virus titers. The virus titers were determined by the RFFIT test, and results were expressed in FFD_{50}/ml. **Results and Discussion:** The virus titers obtained in the purifications with 5, 10, 20 and 30% DEAE-Cellulose were $10^{5.3}$, $10^{5.0}$ and $10^{5.2}$ FFD_{50}/ml, respectively. The cellular DNA concentrations were 38.57 pg/ml (5% resin), 15.16 pg/ml (10% resin), 15.11 pg/ml (20% resin), and 14.10 pg/ml (30% resin). The results showed that values of residual cellular DNA and viral titers were similar to purification with 10, 20 and 30% of DEAE-Cellulose. Five percent DEAE-Cellulose is not good because the capacity of resin was less than the necessary quantity. The use of DEAE-Cellulose in the purification process directly affects the reduction of the cost of the vaccine, because the methodology is easier, less expensive and more efficient. Thus, the best concentration of DEAE-cellulose is 10%.

Supported by: Fundação Butantan

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4.09 Rotavirus (serotype G1) produced in Vero cell cultures

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Introduction: Rotavirus is responsible for severe diarrhea in children less than five years. The best strategy to prevent this disease is the vaccination of the children with a good rotavirus vaccine. There are different rotavirus serotypes in many countries. Thus, the rotavirus vaccines were formulated with serotypes predominant in the country that the vaccine will be used. The serotype G1 is common in many countries and it is used in some vaccine formulations. **Objectives:** The aim of this study was to produce the rotavirus serotype G1 in Vero cell culture for rotavirus vaccine formulation. **Methods:** Vero cell cultures maintained in 20 T-flasks with serum-free-medium were used in this study. These cultures were washed with DPBS and Eagle's MEM, and 18 T-flasks were infected (MOI= 0.2) with reassortant rotavirus serotype G1 (strain from NIH) activated with irradiated trypsin (10 μg/ml) and two T-flasks only medium (Control). After inoculation the cultures were incubated at 37°C for one hour and Eagle's MEM medium was added with 2 μg/ml trypsin. The T-flasks were incubated again at the same temperature. After 55, 70 and 73 h of incubation, when the cultures showed 80, 90 and 100% CPE, respectively, the flasks were stored at -80°C (6 flasks/time of incubation). The cultures were thawed, the harvests were made, and the samples were taken to determine the viral titers by PFA (plaque-forming assay). **Results and Discussion:** The rotavirus titers found in the samples were $10^{6.7}$, $10^{6.4}$ and $10^{6.4}$ for harvests made at 55, 70 and 73 h, respectively. The values found at 70 and 73 h were the same, while the titer obtained at 55 h was higher than the others. The results showed that the optimal time of the harvest was after 55 h of incubation, because the time is less, with a good rotavirus titer.

Supported by: FAPESP, BNDES and Fundação Butantan

4.10 The influence of kefir on the humoral immune response induced by the B subunit of cholera toxin and by BSA in the presence of $Al(OH)_3$

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Introduction: It has been observed that the adjuvant capacity of aluminum hydroxide is related to the activation of NALP3, while the adjuvant property of the cholera toxin is related to its molecular bond to gangliosides through its B subunit and cyclic AMP activation by its A subunit. Kefir, a beverage derived from milk fermentation, when administered orally prior to intraperitoneal immunization with adsorbed ovalbumin to $Al(OH)_3$, inhibits the generation of IgE and IgG1 against the antigen. **Objectives:** In the present work, we determined the influence of kefir on humoral immune responses generated in immunized animals with CTB (B subunit of cholera toxin with traces of the A subunit) or BSA in the presence of $Al(OH)_3$. **Methods:** BALB/c mice were immunized by subcutaneous injection with CTB in PBS, or with BSA adsorbed to $Al(OH)_3$ in the presence and absence of kefir. Serum was collected 30 days after immunization to determine the level of antibodies IgG1 and IgG2a against the antigen, by ELISA. The groups that received BSA in $Al(OH)_3$ with or without kefir, were immunized a second time and the proliferation of T and B lymphocytes was determined by flow cytometric analysis. Results showed that that the response of antibodies IgG1 and IgG2a anti-BSA was reduced in immunized animals in the presence of kefir. However, kefir was not capable of influencing the antibody response in mice immunized with CTB. We also found that there was no significant difference in the quantity of T and B lymphocytes in the spleen of mice immunized with BSA adsorbed on $Al(OH)_3$, in the presence or absence of kefir. **Results and Discussion:** These results show that kefir is capable of reducing the adjuvant response induced by $Al(OH)_3$, but does not influence the humoral immune response induced by CTB, indicating that kefir´s influence on humoral immune responses depends on the adjuvant's mechanism of action. In addition, the results also indicate that the amount of T and B lymphocytes found in the spleen of immunized mice in the presence of kefir is not related to the reduction of the antibodies found.

4.11 Early inflammatory events during ear tissue regeneration in mice

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Introduction: The biological response to skin injury can be subdivided into two distinct categories, regenerative and non-regenerative types of wound healing. Homozygous AIRmax and AIRmin sublines for *Slc11a1 S* alleles, produced by genotype-assisted breeding, differ in ability to completely repair ear holes. AIRmax^{SS} mice showed fast ear tissue regeneration while AIRmin^{SS} mice did not show regeneration after ear punch. **Objectives:** Our aim in this work was to evaluate the influence of the early inflammatory response in the determination of these different phenotypes. **Methods:** Two-millimeter ear holes were punched in mice of each subline and the inflammatory reaction was characterized by histomorphometric analysis and MPO activity. Global gene expression analysis was used to identify sets of differentially-expressed genes during the inflammatory stage of regeneration, and quantitative PCR experiments were performed to validate the microarray results. **Results and Discussion:** The local inflammatory response was more intense in AIRmin^{SS} than AIRmax^{SS} mice at 24 and 48 h after ear punch, which was demonstrated by histomorphometric analysis and MPO levels. Global gene expression analysis demonstrated over-represented distinct biological themes between AIRmax^{SS} and AIRmin^{SS} control mice. Inflammatory response biological category was observed only in AIRmin^{SS}. At 24 h after punch, both AIRmax^{SS} and AIRmin^{SS} mice showed significant ($P<0.001$) up-regulated genes related to inflammation. However, angiogenesis, regulation of myeloid cell differentiation and epidermal growth factor receptor signaling pathway related genes were expressed only in AIRmax^{SS}. Microarray results were validated by quantitative PCR. The higher initial inflammatory response in the inflamed ear tissue combined with elevated RNA expression of genes involved in inflammation could inhibit epimorphic regeneration in AIRmin^{SS} mice. These results suggest that the extent of inflammatory response in the early events after injury modulates the quality of regeneration or wound healing.

Supported by: FAPESP and CNPq

4.12 Improvement of anti-diphtheria serum produced in horses

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Introduction: Diphtheria is a respiratory and cutaneous illness caused by *Corynebacterium diphtheriae*. Prophylaxis of this infection is attained through vaccination with the inactivated diphtheria toxoid [DTx], and the illness itself is treated by the application of a hyperimmune horse anti-diphtheria serum produced at Butantan Institute. During the last years, some difficulties were identified in the production of this serum, probably due to the inappropriate immunization of animals. **Objectives:** The aim of this study was to establish an adequate immunization protocol in order to improve the anti-DTx serum production for therapeutic use. **Methods:** Initially, the immunization schedule included several doses with high amounts to the DTx that may induce tolerance. An experimental study was done immunizing mouse lines such as outbred NIH or the genetically selected high responder mice (H_{III} line) with different adjuvants [the nanostructured SBA-15 silica or the $Al(OH)_3$] aiming to characterize the immunogenicity of two DTx batches. Afterwards, two groups of horses were immunized as follows: 16 horses, that were not immunized for 1 year, received subcutaneously 10 mg of DTx adsorbed on $Al(OH)_3$; 14 horses that remained without immunizations for 6 months were immunized with 10 mg of DTx encapsulated/adsorbed in SBA-15 [n=7] or 10 mg of DTx adsorbed on $Al(OH)_3$. **Results and Discussion:** The sera from immunized animals were analyzed by ELISA and ToBI and clearly showed that the horses that remained for 1 year without immunization showed higher specific antibody titers [11 $\log_2$ - ToBI test and 16 $\log_2$ – ELISA] while the other group had lower levels of specific antibodies [8 $\log_2$ - ToBI assay and 14 $\log_2$ – ELISA]. With these new protocols, the preliminary results indicate the efficient serum-neutralizing activities to diphtheria. The kinetics of specific antibody responsiveness will be compared to the results obtained *in vivo*, which expressed higher titers than those obtained by ToBI, searching for a correction factor. This work demonstrates that the cooperation between basic and applied research is essential for the comprehension of the immunological parameters involved in the improvement of serum qualities.

Supported by: Cristália Pharmaceutics and CNPq

4.13 Inflammation and hepatic and lung cancer in mouse lines selected for high or low acute inflammatory response

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Introduction: Chronic infections and inflammation are major risk factors for various types of cancer, and the approach involving prevention or therapy is still in development. The hepatocellular carcinoma (HCC), for example, is a cancer closely related to sex and inflammation, where it is three times more prevalent in men than in women and related to infectious disease such as hepatitis. Lung cancer is another type of cancer related to inflammation, mainly to inhalation of toxic substances, such as those present in cigarette smoke. Several studies show the significant increase in some cytokines, especially TNFα and IL-6 in susceptible mice at an early tumor stage, just hours after injection of the tumor-specific drug. To obtain liver and lung carcinogenesis in mice, two drugs are well described, DEN - diethylnitrosamine and urethane (ethyl carbamate), respectively. **Objectives:** The aim of this study was to determine the genetic changes related to cancer and inflammation by comparative analysis of susceptibility to liver and lung tumors induced by chemical carcinogens DEN and urethane in strains AIRmax and AIRmin, genetically selected for maximum and minimum inflammatory response. **Methods:** AIRmax and AIRmin mice were injected with DEN (25 mg/kg) ip at 15 days of life for long term tumor development analysis, adult mice were injected with a higher dose of DEN (100mg/kg) for acute cytokine response analysis, and another group was injected at seven days of life with urethane (300 mg/kg) sc. **Results and Discussion:** At 14 h after high-dose DEN, there was a peak of TNFα mRNA expression in the liver of both strains, which was higher in AIRmax (27-fold) than in AIRmin (4-fold) compared to untreated controls. There was no change in IL-6 mRNA expression in the liver of all groups. Two and four months after DEN injection, no animals had visible liver disorders. At 8 months, most AIRmax males were affected by HCC, with many lesions, some of them bigger than 10 mm. AIRmax females were also affected, but they had fewer and smaller lesions. AIRmin were more resistant to liver cancer, affecting few males and no females. The contrary was observed in animals injected with urethane: AIRmax were resistant to lung cancer and AIRmin were susceptible. Unlike hepatocarcinogenesis, the incidence of lung tumor was not related to sex. We conclude that the prevalence of liver cancer was much higher in AIRmax compared to AIRmin and that the opposite was true for the lungs, where AIRmin was more susceptible. These results demonstrate that a group of genes controls the inflammatory response and susceptibility or resistance to different types of cancers and also highlight the specific role of local cells in the control of immunity to tumor development.

Supported by: CNPq

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4.14 Peritoneal inflammation induced by BnP1, a P-I metalloproteinase from *Bothrops neuwiedi* venom

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Introduction: BnP1, a novel P-I snake venom metalloproteinase (SVMPs) from *Bothrops neuwiedi* venom has been isolated and partially characterized as hydrolyzing clotting factors and inducing apoptosis in endothelial cells. However, whether it is able to induce a leukocyte influx and cytokine release *in vivo*, as has been shown for BaP1, remains to be investigated. **Objectives:** The aim of this study was to evaluate the inflammatory reaction elicited by BnP1 in the mouse peritoneal cavity. **Methods:** BaP1 or BnP1 (5 μg /animal) dissolved in sterile saline, was injected by the intraperitoneal route in BALB/c mice (n=5). Groups of mice were also injected with sterile saline or lipopolysaccharide (LPS-0.1 μg) as negative and positive controls, respectively. After 6 h, the animals were sacrificed and peritoneal exudate cells (PEC) were obtained through peritoneal lavage with 2 mL of phosphate-buffered saline (PBS). The samples were used to determine the total and differential cell counts, hydrogen peroxide (H_2O_2) production using phenol red reduction and the secretion of cytokines by ELISA. **Results and Discussion:** We found an increase in the total cell counts in PEC samples obtained from mice injected with LPS (33%), BaP1 (107%) and BnP1 (174%) when compared to the PEC- from PBS-injected mice. The differential cell counts showed a predominance of neutrophils (65-70%) in the groups of mice injected with LPS, BaP1 and BnP1 in contrast to the prevalence of macrophages (83%) in PEC in the PBS group. High production of H_2O_2 (49.2, 37.0 and 41.0 μM) was observed by cells from mice that received LPS, BaP1 and BnP1, respectively, when compared to PBS-injected mice (10.4 μM). The secretion of IL-6 and IL-1β was also enhanced in cultures of PEC from mice injected with LPS (591 and 454 pg/mL, respectively) or BnP1 (336 and 442 pg/mL, respectively) compared to those observed in cultures of PEC from mice injected with PBS (192 and 204 pg/mL). Finally, BnP1 (2.5 ng/mL) induced an increase in IL-6 (416 pg/mL) and IL-1β (378 pg/mL) production in cultures of PEC from non-treated mice in comparison to non-stimulated cells (193 and 200 pg/mL). Our data showed that BnP1 was able to elicit a marked leukocyte influx in the peritoneal cavity, mainly of neutrophils with high secretion of H_2O_2 and pro-inflammatory cytokines at levels comparable to those with BaP1, suggesting that BnP1 also plays an important role in the local inflammatory reaction observed in *Bothrops neuwiedi* envenomation.

Supported by: CAPES, CNPq and INCTTOX

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4.15 Early inflammatory events modulate pristane-induced arthritis in mice bearing distinct *Slc11a1* alleles

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Introduction: Rheumatoid arthritis causes chronic inflammation of synovial joints, erosion of bone, joint destruction, pain, disability, and a reduced life expectancy. The etiology of autoimmune disease has not been established. Mice selected for maximal acute inflammatory reaction (AIRmax) show high susceptibility to pristane-induced arthritis (PIA). The *solute carrier family 11a member 1 (Slc11a1)* gene interacts with AIRmax genetic background and modulates this susceptibility. This gene is involved in the ion transport at the endosomes in macrophages and neutrophils, interfering in their activation. **Objectives:** The aim of this study was to investigate the gene expression levels of peritoneal macrophages from AIRmax^{RR} and AIRmax^{SS} during early phase of PIA. **Methods:** Mice received 0.5 mL i.p. pristane injection and the peritoneal macrophages were isolated at 2, 7 and 14 days. Macrophage mRNA transcript levels of several inflammatory related genes (*Il1, Il6, Tnf, Cxcl2*) and *Tgfb1* were measured by quantitative real-time PCR. Nitric oxide (NO) production was detected in culture supernatants at 48 h after LPS stimulation in different groups after PIA. **Results and Discussion:** Results showed that pristane treatment significantly decreased the total cell number in the AIRmax^{RR} and AIRmax^{SS} peritoneal cavity at 7 and 14 days ($p<0.05$). Different *Tnf*, *Il1b*, *Il6* and *Cxcl2* gene expression levels between AIRmax^{RR} and AIRmax^{SS} macrophages were observed. Basal inflammatory cytokines were expressed higher in AIRmax^{RR} than AIRmax^{SS} macrophages. *Tnf* was expressed higher in AIRmax^{RR} than AIRmax^{SS} macrophages at 2, 7 and 14 days. On the other hand, *Il1* was significantly increased in AIRmax^{SS} at 14 days. *Cxcl2* and *Il6* RNA expressions were lower in AIRmax^{RR} after pristane treatment at 7 and 14 days. *Tgfb1* did not show significant differences among all the groups. Significantly different ($p<0.001$) NO production was observed in AIRmax^{SS} at 2 days after PIA and all lineages after LPS stimulation *in vitro*. These results suggest that early inflammatory events are critical for susceptibility to pristane-induced arthritis in AIRmax mice.

Supported by: FAPESP and CNPq

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4.16 Characterization of antibodies and cross-reactivity in serological tests against botulinum toxins types A, B and E

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Introduction: Botulinum toxins (BT) are considered the most deadly neurotoxin known. Human botulism is caused by neurotoxins types A (BT-A), B (BT-B) and E (BT-E). Currently, no commercial vaccines are available and the treatment consists of antitoxic therapy prepared by hyperimmunized horses.**Objectives:** The aim of this study was to compare the antibody production against BT types A, B and E in horse serum producer, including their neutralization capacity and avidity, and to examine the serologic cross-reactivity among BT antibodies. **Methods:** The serum obtained by horses was purified and concentrated. Both bivalent (type A and B) and a monovalent (type E) sera were used for the experiment. The total quantity of specific antibodies and their subclass was performed by ELISA. Antibody potency and cross-reactivity were measured by serum neutralization assay in mice. Cross-reactivity was evaluated using the standard antitoxin and specific sera (produced by the Butantan Institute) diluted to 1 IU/mL. To improve results immunodiffusion experiments were performed to determine cross-reactivity. **Results and Discussion:** The specific antibody titers on bivalent AB serum were similar to type A and B (1/1,024,000). However, the titer of monovalent E serum was significantly different (1/256,000). The potency of serum type B (28,660 ± 1337 IU/mL) was significantly higher than serum type A (414.7 ± 38.2 IU/mL) and type E (155.9 ± 45.7 IU/mL). The IgG subclass pattern was similar among the types, with predominance of IgGT (1/256,000 to type A, 1/512,000 to type B and 1/128,000 to type E), followed by IgGA (1/64,000 to types A and B and 1/16000 to type E). In the first cross-reactivity test performed, a strong cross-reaction occurred when type A standard antitoxin was tested against type B toxin. A randomized double-blind trial of cross-reactivity was then conducted using all types of toxins, standard antitoxins and specific sera. Type E and type B antibodies confer protection only against their specific toxin. However, type A antibodies, both standard antitoxin and specific serum, confer protection against type A and type B toxins. The strong cross-reactivity revealed that protection conferred by type A antibodies against type B toxin was greater than that conferred by type B antibody itself. Apparently, the BT-A and BT-B have the same capacity to stimulate antibody production and induce the same IgG subclass pattern. Thus, the higher potency of type B antibody in bivalent AB serum is not only related to a higher protection, but also to the strong cross-reactivity that occurs between type A antibody and type B toxin.

Supported by: Fundação Butantan

4.17 Involvement of c-type lectin receptors in the recognition of high molecular weight components of *Ascaris suum* extract by dendritic cells

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Introduction: Distinct receptors, such as toll-like (TLRs) and C-type lectin (CLRs), are involved in the recognition of pathogens as well their compounds by dendritic cells (DCs), which are strongly involved in the induction of the specific cellular response. We previously described that high MW components (PI) from *Ascaris suum* extract have the ability to suppress the immune response to unrelated antigen and down-modulate the capacity of APCs to activate OVA-specific T cells. **Objectives:** The aim of this study was to analyze the presence of glycosylated components in PI and the binding to CLRs in DCs, and to evaluate the ability of DCs incubated with PI to activate OVA-specific T cells. **Methods:** The content of glycosylated components in PI was evaluated by affinity chromatography in a ConA-Sepharose column. PI and BSA were subjected to ConA-Sepharose column and the glycosylated components eluted with sugar competitor. The binding of PI to CLRs on DCs was analyzed by flow cytometry. Thus, bone-marrow derived DCs from BALB/c mice were incubated with PI-FITC (3 μg) or BSA-FITC (6 μg) as control. DCs pre-incubated with mannan (1 μg) or anti-DC-SIGN MoAb (8 μg) were stained with PI-FITC (3 μg). T cell proliferation was evaluated in co-cultures of DCs purified from OVA-primed mice and pulsed with OVA (200 μg/mL), OVA+PI (200+200 μg/mL), OVA+mannan (200+50 μg/mL) or OVA+PI+mannan (200+200+50 μg/mL). **Results and Discussion:** The chromatographic profile showed a high content of glycosylated components on PI. There was a high binding rate of PI-FITC on DCs (78%) compared to BSA-incubated cells (4%). In contrast, this PI-binding on DCs was inhibited when the cells were pre-incubated with mannan (41%) or α-DC-SIGN (30%). Low T cell proliferative response was induced by OVA+PI-pulsed DCs when compared to those promoted by OVA-pulsed DCs. Furthermore, T cell proliferative response was partially restored when DCs were pulsed with mannan and incubated with OVA+PI. The results suggest that CLRs on DCs can be involved in this modulatory activity of the PI.

Supported by: CNPq and FAPESP

4.18 Distinct inflammatory profiles in the exudates and subcutaneous tissues caused by biogel in mice selected for maximal and minimal inflammatory response

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Introduction: AIRmax and AIRmin mice differ quantitatively in cellular influx and protein concentration after 24 h injection of polyacrylamide beads (Biogel) in the subcutaneous dorsal region. In order to investigate the long-lasting inflammatory process in these lines, we studied the 30-day kinetics of cellular infiltration and cytokine concentration in the exudates, as well as tissue histology and gene expression. **Objectives:** The aim of this study was to detect differences in the cellular and molecular profiles of the subcutaneous tissues and exudates of AIRmax and AIRmin mice after Biogel injection. **Methods:** AIRmax and AIRmin mice were injected with Biogel-P100 in the subcutaneous dorsal region, and the exudates were recovered for cell counts and for cytokine measurements by ELISA after 2, 4, 7, 15 and 30 days. Subcutaneous tissues were excised for histological study and mRNA extraction for real-time PCR analysis. **Results and Discussion:** AIRmax mice had a greater cellular influx in the whole analyzed period with significantly ($P<0.05$) higher expression of the inflammatory genes *Il6*, *Tnfα*, *Cxcl2*, *Ccl2* and *Mmp9* compared to AIRmin mice. The cytokine levels in the exudates were also higher in AIRmax mice in the early time points ($P<0.05$), as seen for IL-6, IL-1β and Ccl2. Histological study agreed with these results, showing a strong cellular infiltration in the AIRmax mouse tissues 2 days after Biogel injection, while a moderate infiltration in AIRmin mice after 4 days was observed. These results suggest that the higher cellular infiltration in AIRmax mice may be due to an over-expression of inflammatory cytokines in the subcutaneous tissue and exudates. Moreover, AIRmin mice showed delayed inflammatory response to the Biogel stimulus, when compared to the AIRmax line.

Supported by: FAPESP and CNPq

4.19 Skin transcriptome shows different gene expression profiles in loci regulating high or low inflammation intensity

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Introduction: Two mouse lines were phenotype-selected for maximum (AIRmax) or minimum (AIRmin) acute inflammation responses to polyacrylamide bead (Biogel) subcutaneous injection. These lines differ in terms of bone marrow granulopoiesis, neutrophil resistance to apoptosis, and inflammatory cytokine production. Genome wide scan using SNP markers detected two significant quantitative trait loci (QTL) on chromosomes 5 and 7 ($p < 10^{-6}$), as well as suggestive ones on chromosomes 2, 4, 6 11, 12 and 17 ($p < 10^{-3}$). **Objectives:** We compared gene expression profiles of epidermal tissues of AIRmax and AIRmin mice submitted to acute inflammatory reactions, in order to correlate with QTL found. **Methods:** Epidermal tissues were recovered 48 h after Biogel subcutaneous injections. Global gene expression analysis was performed on Affymetrix bioarrays (27k genes) using individual RNA from both control and Biogel-treated AIRmax and AIRmin mice. **Results and Discussion:** Differentially-expressed genes were statistically established and the over-represented gene ontology biological process categories were identified. Up-regulations of about 479 and 121 genes were observed in Biogel-treated AIRmax and AIRmin mice, respectively, but only 1 gene was found to be down-regulated in AIRmin, as compared to 167 genes in AIRmax mice. The over-represented biological themes of the differently expressed genes between AIRmax and AIRmin mice represent inflammatory response, signal transduction and cell adhesion. We were able to demonstrate a distinct gene usage between both lines during acute inflammation, and a significant differential expression of genes co-localized with mapped regions on chromosomes 2, 4, 5, 6, 7, 11, 12 and 17.

Supported by: FAPESP and CNPq

4.20 An alternative method for purifying and detoxifying Diphtheria toxin

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Introduction: Infections caused by *Corynebacterium diphtheriae* frequently induce situations in which very small doses of antigens injected intradermally can cause strong inflammatory reactions. This bacterium secretes the diphtheria toxin (DT), a virulence factor that can be lethal to the human organism at doses below 0.1 µg/kg body weight. **Objectives:** The present work proposes alternative methods of DT purification using affinity chromatography and of DT detoxification through conjugation with the polymer methoxypolyethylene glycol activated (mPEG). **Methods:** Tests were performed to evaluate: the formation of edemas and the presence of dermonecrotic activity, *in vitro* cytotoxicity to Vero cells, the neutralizing activity of serum from guinea pigs immunized with the diphtheria toxoid inactivated with mPEG, and the immunogenic activity of the purified and modified toxin. **Results and Discussion:** The results indicated that purification with Blue Sepharose was an efficient method, yielding antigen purity equivalent to 2600 Lf/mg of protein nitrogen. The modification of the Purified Toxin with mPEG did not result in the formation of edema or necrosis although it was immunogenic and stimulated the formation of antibodies that could neutralize the Purified Toxin. The toxoid obtained from the purified toxin maintained its immunogenic characteristics, inducing antibodies with neutralizing activity; however, edema and necrosis were still observed.

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4.21 Stability study of pentavalent rotavirus vaccine produced at Instituto Butantan

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Introduction: Rotavirus is responsible for approximately 600,000 deaths per year in children under five years of age, mainly in Africa, Asia and Americas. This virus causes a severe diarrhea in children. To prevent the disease, children less than 5 years old were vaccinated with attenuated rotavirus vaccine. A new attenuated pentavalent rotavirus vaccine in lyophilized form with 4 doses per vial was developed at Instituto Butantan. This vaccine was formulated with G1, G2, G3, G4 and G9 rotavirus serotypes obtained from Vero cells infected with the reassortant strains received from the NIH (National Institutes of Health). **Objectives:** The aim of this study was to evaluate the stability of the pentavalent rotavirus vaccine at 2-8 °C. **Methods:** Six lots of pentavalent rotavirus vaccine were evaluated in this study, three lots produced in 2008 and another in 2009 in our laboratory. These lots with an initial potency of $10^{6.3}$, $10^{6.5}$and $10^{6.2}$ PFU/dose (lots of 2008) and $10^{6.6}$, $10^{6.7}$and $10^{6.6}$ PFU/dose (lots of 2009) were stored at 2-8°C for 36 and 27 months, respectively. The samples of the vials were taken every three months, reconstituted in citrate-phosphate diluent and used to determine the potency, pH and sterility. The potency was determined by PFA (plaque-forming assay) and the results were expressed in PFU/ml. **Results and Discussion:** The results of pH and sterility were satisfactory for all the samples. After 36 months at 2-8 °C, the potency of the lots produced in 2008 was $10^{6.2}$, $10^{6.0}$ and $10^{6.0}$ PFU/dose. The lots of 2009 showed a potency of $10^{6.3}$, $10^{6.6}$ and $10^{6.7}$ after 27 months of storage at the same temperature. Statistical analysis of the data showed that there were no significant differences between the potencies of these lots before and after storage. The results observed in this study with the lots of pentavalent rotavirus vaccine showed that this product has a good stability (3 years) when stored at 2-8°C.

Supported by: FAPESP, BNDES and Fundação Butantan

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4.22 Stability study of new tetravalent dengue vaccine developed at Instituto Butantan

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Introduction: Dengue is a common viral disease of humans that has become a major international public health concern. About 3.5 billion people live in dengue-endemic countries. This disease is an important public health problem in Brazil, mainly hemorrhagic dengue. Nowadays the major challenge to researchers is the development of a good vaccine to prevent this disease. A new attenuated tetravalent dengue vaccine was developed at Instituto Butantan. This vaccine was formulated with attenuated dengue virus 1, 2, 3 and 4 obtained from Vero cells infected with strains from the NIH (National Institutes of Health). This vaccine is provided in lyophilized form, with ten doses per vial. After production of a new vaccine many quality control tests were performed mainly to evaluate the safety and stability of the product. **Objectives:** The aim of this study was to evaluate the stability of tetravalent dengue vaccine after reconstitution in appropriate diluent. **Methods:** Three vials of three lots of tetravalent dengue vaccine were reconstituted with diluent. Afterwards, the vaccine was stored at 2 -8°C and samples were taken every hour for 8 h to determine the dengue titers of DENV-1, DENV-2, DENV-3 and DENV-4. The viral titers were determined by PFA (plaque-forming assay) and the results were expressed in PFU/ml. A virus titer $\geq 10^{2.5}$PFU/dose was considered satisfactory. **Results and Discussion:** The geometric mean of the titers obtained in the samples of each tetravalent dengue vaccine lot tested showed that after reconstitution the virus titers of DENV-1, DENV-2, DENV-3 and DENV-4 were satisfactory for 6, 5, 4 and 6 h, respectively. Considering the results found in DENV-3 (4 h), we can conclude that the stability of this vaccine after reconstitution is four hours.

Supported by: FAPESP, BNDES and Fundação Butantan

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4.23 Construction of a hybrid protein containing the N-terminal region of PspA and PdT

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Introduction: *S. pneumoniae* is a major human pathogen causing a number of potentially fatal diseases. The currently available anti-pneumococcal vaccines are based on the capsular polysaccharides, which are highly variable serologically and provide limited coverage. Formulations based on the more conserved pneumococcal proteins have been investigated for many years, with promising results. Among the vaccine candidates are the surface protein PspA and pneumolysin toxin (Ply), which are necessary for pneumococcal virulence in the host. The N-terminal region of PspA is recognized by most of the protective antibodies, and interacts with complement components and lactoferrin, preventing the bacterium from being phagocytized. Therefore, this region was selected for inclusion in the protein-based anti-pneumococcal vaccines. Ply or its non-toxic derivatives PDs bind TLR4, the Fc region of antibodies, complement proteins and cholesterol, inducing a strong inflammatory response. Both PspA and PDs have been shown to be immunogenic and protective in different animal studies. Furthermore, the combination of these proteins was able to increase protection against pneumococcal sepsis in mice. In previous studies we selected two PspAs from family 1 able to induce a high cross-reactivity; here, we used the gene fragment of one of these PspAs to construct a hybrid protein containing the PspA fused to PdT (a detoxified form of Ply). **Objectives:** The aim of this study was to produce a hybrid protein PspA-PdT and evaluate the immune response induced. **Methods:** The gene fragment of PspA was fused with the gene fragment of PdT and inserted into pQE-30 expression vector. This construction was expressed in *E. coli* M15 and the recombinant hybrid protein (PspA-PdT) purified by affinity chromatography. The expression of these two fused proteins was tested by immunoblotting using sera against both proteins. PspA and PdT were expressed in *E. coli* BL21 and *E. coli* M15 using previous constructions and purified by the same methods. BALB/c mice were immunized with three doses of PspA, PdT, PspA+PdT or the hybrid PspA-PdT protein at 14-day intervals. The antibody production was evaluated by ELISA. **Results and Discussion**: The immunoblot of the hybrid protein using antibodies against PspA or PdT confirmed the expression and purification of the protein in fusion. ELISA revealed that similar amounts of antibodies were induced by PspA and PdT when they were administered as the hybrid protein, alone or co-administered. Future studies will evaluate the ability of the antibodies to enhance complement deposition on pneumococci and to promote opsonophagocytosis.

Supported by: FAPESP and Fundação Butantan

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4.24 Comparison of two methods for assessment of antirabies immunoglobulin potency: rabies virus neutralization in mice and in BHK-21 cells

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Introduction: Nowadays, the method used for potency assessment of highly purified F(ab')2 equine antirabies serum in Brazil is the mouse seroneutralization assay (MNT), described in the Brazilian Pharmacopoeia. Animal-using tests are the object of criticism, either because of concern for animal suffering or the high variability of the results obtained. In many laboratories, the 3R policy (reduction, refinement, replacement) is being widely used, which stimulates the replacement of *in vivo* techniques by *in vitro* alternatives, providing more reproducible and reliable results. In 2008, a validation study of a virus neutralization potency test in BHK-21 cells was carried out in Brazil, at Instituto Butantan and INCQS (National Control), showing that this method is reliable and suitable to evaluate rabies immunoglobulin potency. **Objectives:** In this study, the method previously validated and MNT were used to evaluate the potency of 40 lots of rabies equine therapeutic immunoglobulin produced at Instituto Butantan, either concentrated or formulated products. **Methods:** For both methods, serial dilutions of a reference or a test immunoglobulin were incubated with a fixed amount of rabies virus for 90 min at 37 °C. The residual virus infectivity was then determined by inoculating Swiss mice or BHK-21 cells. The 50% end-point dilution of the sera was calculated by the number of mice that survived after 14 days or microscopic fields containing no infected cells after staining with fluorescent antibodies. **Results and Discussion:** The results obtained were normalized for virus lethal dose (LD_{50}) or focus-forming dose (FFD_{50}) and reference effective dose (ED_{50}). Data normalized for the *in vivo* and *ex vivo* tests showed a correlation of 93% between the two methods. In conclusion, the virus neutralization potency test in BHK-21 cells can safely replace MNT.

Supported by: Fundação Butantan

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4.25 Recognition of venom from different *Crotalus* snakes by the antiserum manufactured by Instituto Butantan

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Introduction: The genus *Crotalus* is represented in Brazil by one species, *Crotalus durissus terrificus*, and six subspecies: the highly distributed *C. d. terrificus* (South and Southeast regions), *C. d. collilineatus* (Southeast and Center-West regions), *C. d. cascavella* (Northeast regions), and the endemic *C. d. marajoensis*, *C. d. ruruima* and *C. d. trigonicus*. Despite this diversity, the anti-serum production at Instituto Butantan relies on the venom of two subspecies for their immunization process, *C. d. terrificus* and *C. d. collilineatus*. Considering that the anti-serum distribution works at the national level, it is necessary for the anti-serum to show protective activity against all major Brazilian rattlesnakes. Otherwise, the immunization process would have to be revised to account for regional differences. **Objectives:** The aim of this study was to verify the activity of the anti-serum against different crotalic venom and fractions. **Methods:** Six samples of commercial crotalic anti-serum, from different lots manufactured in the year 2010 by Instituto Butantan, were tested. The samples were submitted to ELISA analysis against the following antigens: *C. d. terrificus* venom, *C. d. collilineatus* venom, *C. d. cascavella* venom, *C. d. marajoensis* venom, purified crotoxin and purified PLA_2. Their titers were calculated in U-E/ml and compared by the Kruskall-Wallis test followed by Dunn's method. **Results and Discussion:** All six samples had high antibody titers, and the titers obtained against *C. d. collilineatus*, *C. d. cascavella* and *C. d. marajoensis* venom were significantly different from the titers against the purified fraction crotoxin and PLA_2 ($p<0.05$). The results indicate that the antibodies formed against *C. d. terrificus* and *C. d. collilineatus* venom show a high cross-reactivity with other subspecies' venom, thus making the commercial anti-serum produced by Instituto Butantan a reliable protective agent against all the major Brazilian *Crotalus*.

Supported by: CAPES

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4.26 New strategies for the production of crotalic antiserum

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Introduction: Crotalic venom is a complex mix of proteins, from which we can point out four major fractions: crotoxin, phospolipase A_2, crotamin and gyroxin. From those, only crotoxin and PLA_2 are related to serious accidents and death. The traditional approach to anti-serum production is based on immunization with crude crotalic venom, which results in antibodies against the medically important fractions as well as irrelevant proteins present in the venom. The immunization with purified fractions would demand less venom to be injected in the animal, and could result in an anti-serum with higher specific activity against the important fractions and a lower protein concentration. **Objectives:** The aim of this study was to test the hypothesis presented above, and obtain a anti-serum with higher activity against crotalic venom using the purified crotoxin and PLA_2 fractions for the immunization. **Methods:** For the immunization process, six horses (400-450 kg) were divided in three groups. The animals in group 1 (n=2) were injected with 500 µg/animal of crude *Crotalus durissus terrificus* venom, the animals in group 2 (n=2) were injected with 200 µg/animal of purified crotoxin, and the animals in group 3 (n=2) were injected with 100 µg/animal of purified PLA_2. Each group received 4 injections, with a 15-day interval between each, and 15 days after the last injection, 8 ml of blood were drawn from each animal. The plasma was separated and submitted to ELISA analysis against *C. d. terrificus* venom, crotoxin and PLA_2. The titers were calculated in U-E/ml for each group, and compared between groups and with a sample from Instituto Butantan's anti-crotalic serum. **Results and Discussion:** The experimental plasmas from the different immunization processes resulted in high titers. Although there was no statistically significantly difference between them, it is also important to note that there was also no significant difference from the experimental plasmas and the anti-serum manufactured by Instituto Butantan. This indicates that the immunization with the purified fractions is at least as efficient as crude crotalic venom in producing high antibody titers, and that the use of a lower protein concentration during the immunization can result in a high antibody production while being less damaging to the animal immunized.

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4.27 Influence of final sterilizing filtration in the haemagglutinin content of split influenza vaccines produced for the 2011 influenza season

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Introduction: Influenza vaccine is a sterile, aqueous suspension of strains of influenza virus, type A and B, grown individually in embryonated hen's eggs, inactivated and treated so that the virus particles are disrupted (split vaccine). It contains predominantly hemagglutinin and neuraminidase antigens. The monovalents of each relevant strain are mixed and diluted and at the end of the vaccine manufacturing process, there is a final sterilizing filtration in the final bulk. As recommended by the World Health Organization for the southern hemisphere, the 2011 seasonal trivalent influenza vaccines used in this study, contain the following influenza virus strains: an A/California/7/2009 (H1N1)-like virus; an A/Perth/16/2009 (H3N2)-like virus and a B/Brisbane/60/2008-like virus (A/Wisconsin/15/2009 and A/Victoria/210/2009 are A/Perth/16/2009-like viruses). The potency of influenza vaccines is determined by a immunodiffusion method using reference antigen and specific anti-hemagglutinin serum, suitable for use in the assay of the HA content of each component of inactivated vaccines and, according to the legislation, these vaccines must contain at least 15 µg of hemagglutinin per strain per dose. **Objectives:** The aim of the present study was to evaluate the influence of sterilizing filtration on the hemagglutinin content of the trivalent influenza vaccines produced in Butantan Institute for the 2011 season. **Methods:** Samples were collected from each vaccine lot produced in the season, just before and after the sterilizing filtration process. The hemagglutinin contents of the samples were determined by single radial immunodiffusion (SRID). In the SRID test the samples were analyzed in triplicate and the titers were determined by comparison with an influenza reference hemagglutinin antigen reagent. Calculations were made using slope ratio model in the Combistats program. References were acquired from the National Institute for Biological Standards and Control (NIBSC, UK). **Results and Discussion:** All the vaccine samples contained at least 15 µg of hemagglutinin of each strain. The preliminary results demonstrate that after the sterilizing filtration process there is a decrease in the hemagglutinin content and for the A/California/7/2009 strain, this difference was statistically significant. Regarding these results, it is necessary to take into consideration this possible decrease in hemagglutinin content in the process of influenza vaccine production in order to avoid product QC failure.

Supported by: Fundação Butantan

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4.28 Myelotoxic effects of 7,12-Dimethylbenz[A]anthracene on bone marrow cells from mice genetically selected for inflammatory reactivity

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Introduction: Polycyclic aromatic hydrocarbons (PAHs), such as DMBA, induce a decrease in the number of bone marrow cells (BMC) and hematological alterations resulting in an immunosuppressive state. DMBA metabolism depends on the activation of the aryl hydrocarbon receptor (AhR). Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response to s.c. injection of Biogel P100 showed a complete segregation of Ahr alleles endowed with low (Ahr^{d}) or high (Ahr^{b1}) affinity to PAHs, respectively. Accordingly, AIRmax are more resistant than AIRmin to DMBA induced skin and lung carcinogenesis. **Objectives:** We investigated the effect of DMBA treatment on BMC of AIR selected mice and its possible impact on acute inflammatory response. **Methods:** AIRmax and AIRmin mice were treated with a single i.p. dose of 50 mg/kg DMBA in olive oil. Flow cytometric analysis was used to determine hematopoietic stem cells (HSC) ($Lin^{-}/Sca\text{-}1^{+}/c\text{-}Kit^{+}$) and neutrophils ($Gr\text{-}1^{hi}/CD11b^{hi}$). Proliferation index of BMC was determined in response to GM-CSF stimulus. Acute inflammation response was also evaluated after 24 h of Biogel P100 subcutaneous (s.c.) injection. **Results and Discussion:** DMBA treatment resulted in a significant ($p<0.01$) decrease in neutrophil population and increase in HSC in bone marrow in AIRmin mice only. Blast cells from DMBA-treated AIRmin showed a dysplastic nucleus, which is one of the distinguishing features of preleukemia, and myeloid cells showed low proliferation capacity after *in vitro* GM-CSF stimulation. These effects on myeloid BMC reflect on an impaired cellular migration to the inflammatory site 24 h after Biogel injection. This investigation demonstrates that AIRmax mice are protected and AIRmin are prone to acute bone marrow cytotoxic and presumable preleukemic effects of DMBA. The complete segregation of alleles at the *Ahr* locus found in AIR mice may contribute to their differential inflammatory responses and to the phenotypes of susceptibility and resistance of BMC to DMBA-induced effects.

Supported by: CAPES and CNPq

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4.29 Correlation between *Clostridium tetani* growth kinetics monitored by in line sensor for turbidity and determination of optical density by sampling

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Introduction: Tetanus toxin is produced by growth of *Clostridium tetani* in culture. After detoxification and purification it is used for formulation of associated vaccines (DTP, DTP+hib, DTP+HB+hib, DT and dT). The bacteria are inoculated in parallel in two fermentors containing 360 L and 420 L of IB culture medium, respectively, for industrial production at Butantan Institute. The toxin released in the culture is separated from biomass by tangential flow filtration, concentrated by molecular ultrafiltration and detoxified by formaldehyde addition. The growth rate of *Clostridium tetani* is related to the amount of toxin produced in the fermentation process. The monitoring of bacterial growth during the cultivation is a tool to provide an indication of yield in term of toxin production. Usually, samples are taken out during culture in order to determine the growth by optical density (OD 590 nm), but with this method there is a risk of contamination due to manipulation and therefore not suitable for routine production. **Objectives:** The aim of this study was to evaluate cell growth in line on real-time monitoring through absorption probe directly in culture establishing a correlation with the results of OD 590 nm determination. **Methods:** *C. tetani* growth during culture was measured by turbidity absorption probe near infrared range (730-970 nm) Optek AS16N installed directly into the fermentor. In the same cultures, samples were taken every 12 h for determination of optical density (590 nm) and the toxin titer by flocculation limit (Lf/mL). The analysis was performed in four batches of tetanus toxin production. **Results and Discussion:** In all batches analyzed, tetanus toxin production was detected at around 48 h and showed peak release at 88 h. The maximum bacterial growth was observed at 40-48 h of culture using both measurements of optical density and turbidity. After this time, there was a decline in cell growth and increased release of tetanus toxin. The linear regression plots of four batches analyzed showed high correlation coefficients between turbidity and optical density ($r2 = 0.97, 0.90, 0.89$ and 0.95) showing that the in line real time monitoring turbidity absorption assay is suitable for determining the growth rate of *Clostridium tetani*, where it is more appropriate because it allows the measurement of bacterial growth directly in the culture without sampling.

Supported by: Fundação Butantan

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4.30 Study of microcarrier concentration in rabies virus production in bioreactor

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Introduction: In rabies vaccine preparation, virus suspensions are obtained from Vero cells cultured with Cytodex 1, infected with rabies virus and cultivated in serum-free medium in a bioreactor. Scale-up of this process involves challenges such as overcoming the problem of mass transfer and homogeneous mixing of the system without mechanical damage to the cells; providing high surface area for attached cells; eliminating growth-limiting factors, such as nutrient limitation and toxic metabolite accumulation. **Objectives:** The aim of this study was to evaluate the influence of different microcarrier concentrations on cell growth and rabies virus replication in Vero cell culture maintained in a bioreactor of 150 L. **Methods:** Vero cells from American Type Cell Collection (ATCC-CCL-81) and serum-free medium (VP-SFM AGT, Invitrogen) were utilized in the bioreactor cycles. The production cycles were performed and we evaluated: initial (8.3×10^9 to 2.4×10^{10}cells) and final cell concentration; microcarriers concentration (2.0, 2.5 and 3.0 mg/ml); initial no. cells/bead (9, 13, 14, 15 and 19) and viral titers. The viral titers were determined against BHK-21 and the results were expressed in FFD_{50}/ml. **Results and Discussion:** Five harvests of the supernatant were carried out, the first after 3 days and other every 24 h. After seven days of culture, the geometric means of the cell concentration in the bioreactor cycle were 3.6×10^{10}, 5.3×10^{10} and 5.2×10^{10} for the 2.0, 2.5 and 3.0 microcarrier concentrations, respectively. The geometric means of the titers in the harvested samples after this period were $10^{4.1}$, $10^{4.5}$, $10^{4.7}$, $10^{4.5}$ and $10^{4.3}$ FFD_{50}/ml for cultures with initial no. cells/beads of 9, 13, 14, 15 and 19 respectively. The results showed that the cycles with initial cell concentration of 1.2, 1.4 and 1.9×10^{10} (13, 14 and 15 cells/bead) produced a higher titer than others and that the optimal microcarrier concentration was 2.0 mg/ml. The shear stress caused by hydrodynamic effects of the high microcarrier concentration could be responsible for cell growth limitation.

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4.31 Characterization of the mucosal immune responses against *Streptococcus pneumoniae* respiratory infection in mice elicited by a vaccine composed of PspA and whole cell pertussis as adjuvant

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is an important agent of acute respiratory infections, also responsible for cases of otitis media, pneumonia and meningitis. Deaths of pneumococcal diseases reach 1 million cases per year, worldwide. Vaccines composed of pneumococcal surface protein A (PspA) and the whole cell pertussis low vaccine (wP_{low}) - a new cellular pertussis vaccine containing low levels of LPS, produced by Instituto Butantan) have been previously shown to confer 100% survival of mice against a pneumococcal respiratory challenge. **Objectives:** In the present work, we aimed to characterize the mucosal immune responses induced by this vaccine. **Methods:** BALB/c mice were immunized with PspA by the nasal route using wP_{low} as adjuvant (PspA-wP_{low}). Immunized mice were submitted to a lethal respiratory challenge with the pneumococcal ATCC6303 strain. Immune responses were characterized in lungs at different periods. **Results and Discussion:** Significantly higher levels of anti-PspA5 IgG and IgA were observed in broncheoalveolar lavage fluids (BALF) from mice immunized with PspA-wP_{low} before and 12 h after the challenge. These levels remained high until the 10^{th} day after challenge in surviving mice. Both anti-PspA IgA and IgG present in BALF were able to bind to pneumococcal surface as observed by flow cytometry analysis. In addition, the BALF samples from mice immunized with PspA-wP_{low} induced the deposition of the complement C3 component on pneumococcal surface. Analysis of the lymphocytes population induced by the vaccine showed that before the challenge, higher levels of $CD4^+$ and $CD8^+$ T lymphocytes as well as B lymphocytes were detected in lungs. However, at the peak of infection, 12 h after challenge, only higher levels of $CD4^+$ T and B cells were observed. Moreover, in convalescent mice, 10 days after infection, only the B cells remained significantly higher in mice vaccinated with PspA-wP_{low} when compared to mice vaccinated with PspA alone. The results showed that the activation of B lymphocytes with the induction of specific antibodies by the PspA-wP_{low} vaccine are important events related to protection. Further studies to conclude the role of $CD4^+$ T and B cells in this model are in progress.

Supported by: FAPESP, CNPq and Fundação Butantan

4.32 Standardization of cell culture conditions to evaluate the role of jararhagin-C, a disintegrin-like-ECD, in endothelial cell proliferation

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Introduction: The disintegrins isolated from snake venoms can be classified as *classic disintegrins*, containing only the disintegrin domain with the RGD (Arg-Gly- Asp) sequence on its binding site, or as *disintegrins-like*, with the sequence SECD (Ser-Glu-Cys-Asp) on its disintegrin domain and the cysteine-rich domain. Molecules such as disintegrins-like are involved in various cellular actions since its binding to an integrin receptor may interfere with the processing of information necessary for cell migration, differentiation, survival and proliferation. **Objectives:** The aim of this study was to determine the effects of jararhagin-C, a disintegrin-like toxin isolated from *Bothrops jararaca* venom, on human umbilical vein endothelial cells (HUVECs), analyzing their proliferation on different substrates, and the role of growth factors added to the cell culture medium. **Methods:** Initially, we used 10% Triton X-114 to remove LPS contamination from jararhagin-C samples. The effect of jararhagin-C on cell proliferation was evaluated using HUVECs cultured on gelatin or collagen coated-plates, in the presence or absence of growth factors (bFGF and EGF). For this experiment, the cells were incubated with 200, 100, 50 or 5 nM jararhagin-C or cell culture medium as a control, and the number of viable cells was measured by the MTT method after 72 h. **Results and Discussion:** Preliminary results showed that cells cultured on collagen I proliferate more when compared to cells grown on gelatin-coated plates, indicating that collagen is a more appropriate substrate for cell proliferation studies. We also verified that the presence of growth factors in the culture medium induced a higher cell proliferation, compared to cells grown in the absence of such factors. However, jararhagin-C did not increase cell proliferation at any doses tested, while the doses of 200 and 100 nM showed slight toxicity for HUVECs cultured on collagen, while on gelatin as substrate, high doses of jararhagin-C did not interfere significantly with cell viability. In order to achieve a dose of jararhagin-C able to induce cell proliferation, we intend to use lower doses of this disintegrin-like toxin. The collagen I as substrate is more efficient for cell proliferation compared to gelatin. However, the cells seem to be more sensitive to jararhagin-C, considering the toxicity. In the future experiments we also intend to compare the protein binding capacity of jararhagin-C on endothelial cells surface and the expression of genes related to cell survival on these two substrates.

Supported by: FAPESP

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4.33 APAS-3, an *Ascaris suum* component, is a secreted protein with allergenic proprieties

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Introduction: The potent allergenicity of helminthes has long been recognized by many investigators. In our previous studies, we found that although the *Ascaris suum* adult worm extract has immunosuppressive activity, it contains a 29-kDa immunogenic component, named APAS-3. In our laboratory, we produced a specific monoclonal antibody against APAS-3, which is an important tool to further characterize the modulation of host immune system by *Ascaris suum* infection. **Objectives:** The purpose of the present study was to investigate the presence of APAS-3 in earlier larval stages, adult worm body fluid and in the supernatant of *Ascaris suum* culture *in vitro*. We also analyzed the allergenic proprieties of APAS-3 by the ability to induce anaphylactic antibody production. **Methods:** *Ascaris suum* embryonated eggs were cultured in DMEM medium, and two weeks later, the supernatant was recovered and extracts of earlier larval stages were prepared. The body fluid was collected from adult worms obtained from FISA abattoir. The presence of APAS-3 in these different samples of *Ascaris suum* was tested by ELISA, using the monoclonal antibody MAC-3. APAS-3 was purified by affinity chromatography and 50 μg per animal were injected in 4-week-old BALB/c mice with Al(OH)3 as adjuvant, by intraperitoneal route. Blood samples were obtained weekly for a month and the antibody levels were determined by PCA (passive cutaneous anaphylaxis). **Results and Discussion:** The results showed that MAC-3 recognizes proteins present in all samples tested, indicating the presence of APAS-3 in earlier larval stages and adult worms. It is interesting to note that APAS-3 was also found in adult worm body fluid and in the supernatant of *Ascaris suum* embryonated eggs. It was also shown that the protein APAS-3 is able to induce the production of anaphylactic IgG1 and IgE antibodies. Taken together, our results demonstrated that APAS-3 is a secreted product of *Ascaris suum*, present in different larval stages and fluids. On the other hand, APAS-3 has allergenic properties, inducing the production of anaphylactic antibodies. These results are relevant due the potential effect on the host immune system.

Supported by: CNPq

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4.34 Is basic aminopeptidase a biomarker in collagen-induced arthritis?

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Introduction: Rheumatoid arthritis (RA) is an inflammatory, chronic, systemic and autoimmune disease of unknown etiology and difficult diagnosis. **Objectives:** The aim of this study was to identify new therapeutic and/or diagnostic targets for RA, this study investigated the catalytic activity of basic aminopeptidase (APB) and its association with periarticular edema and circulating tumor necrosis factor (TNF)-α and type II collagen (CII) antibodies (AACII) in a rat model of RA induced by CII (CIA). **Methods:** Adult male Wistar rats, 150-160 g, were anesthetized and then subjected to induction of RA by administration of C-II from chicken in acetic acid and adjuvant via a single intradermal dose of 0.4 mg/0.2 mL/animal (induced animals), or with 0.9% saline with the same scheme of administration (sham induction = Control). On day 41 after treatment the induced animals were separated into Arthritic (hind paw thickness > 7 mm) or Resistant (hind paw thickness similar to Control). Blood was withdrawn to obtain plasma, serum and peripheral blood mononuclear cells (PBMCs). Synovial fluid and soluble (SF) and solubilized membrane-bound (MF) fractions of the synovial tissue were also obtained. Protein (colorimetric) and AAC-II (ELISA) were measured in plasma, and TNF-α (ELISA) was measured in serum. APB activity was fluorometrically measured. **Results and Discussion:** Edema did not occur in part of CII-treated, even when AACII was higher than in control. TNF-α was detectable only in edematous CII-treated. APB in synovial tissue was predominantly a MF activity also present in SF and with higher activity in edematous than in non-edematous CII-treated or control. Synovial fluid and blood plasma had lower APB in non-edematous than in edematous CII-treated or control. In PBMCs, the highest levels of APB were found in SF of control and in MF of non-edematous CII-treated. CII treatment distinguished two categories of rats: one with arthritic edema, high AACII, detectable TNF-α, high SF and MF APB in synovial tissue and low APB in SF of PBMCs, and another without edema and with high AACII, undetectable TNF-α, low APB in the synovial fluid and blood plasma and high APB in MF of PBMCs. The data suggest that APB activity can be a new therapeutic target and a marker of diagnosis and/or prognosis of RA.

Supported by: FAPESP, CAPES and CNPq

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4.35 Different rabies vaccine immunization schedules and the use of nanostructured silica adjuvant influence antibody responsiveness

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Introduction: An efficient immune response is the result of interaction between antigen and a network of immunologically competent cells. Besides the aspects that constitute genetic immunization coverage, the qualitative and quantitative nature of the immune response is determined by many environmental factors such as the nature of the antigen and its concentration, route of administration and regimen used, characteristics that profoundly influence the outcome of the immune response. **Objectives:** In this study, five schedules of pre-exposure immunization with different concentrations, routes of inoculation and interval between doses were evaluated in the isogenic BALB/c mice with purified and inactivated rabies virus produced in Vero cells at Instituto Butantan. **Methods:** In Group I, mice received the WHO recommended scheme [three doses on days 0, 7 and 28 by the intraperitoneal route (IP)]; Group II, two IP doses on days 0 and 60; Group III, the 1st IP diluted 1/10 on day 0 and the 2nd on day 60; Group IV, the 1st by oral route and 60 days after the 2nd; Group V, the 1st orally with the antigen adsorbed/encapsulated in the new adjuvant nanostructured mesoporous silica SBA-15 and the 2nd IP, on day 60. **Results and Discussion:** All animals immunized with schemes I, II or III displayed satisfactory neutralizing antibody titers [NAs], although protocols II and III reached higher levels than scheme I, demonstrating that the application of smaller concentrations of antigen induces efficient response and memory. No mouse in Group IV showed NAs, even after a 2nd IP dose, suggesting suppression. The oral administration of antigen adsorbed/encapsulated in SBA-15 silica probably prevents antigen degradation by the gastrointestinal tract, since all mice showed NAs.

Supported by: CNPq

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4.36 Reactivity of serum and secretory IgA antibodies from healthy Brazilian parturients to vaccine antigens

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Introduction: Secretory IgA (SIgA) antibodies are crucial for the immune response in mucosal sites through a process known as immune exclusion. There is evidence that serum monomeric IgA fulfills an anti-inflammatory role in serum, down-regulating IgG-mediated response. The study of SIgA specificity combined with the determination of serum IgA reactivity with different antigens, is not only an immunological study, but also provides important epidemiologic data about our population. **Objectives:** The aim of this study was to evaluate serum and colostrum IgA antibody reactivity with tetanus and diphtheria toxoids, rotavirus G9 and whole cell pertussis. **Methods:** Specific IgA levels were evaluated in serum and colostrum samples from 54 parturients by ELISA. **Results and Discussion:** Significantly lower specific IgA titers were found in serum than in colostrum. The lowest IgA titers found in serum and colostrum samples were reactive with rotavirus and the highest were directed at tetanus and diphtheria toxoids. Significant correlation indices were observed between serum and colostrum IgA directed at tetanus, diphtheria and pertussis antigens. These results demonstrate that serum and colostrum IgA levels reflect the immunological experience of each mother, whether by vaccination or direct stimulation on mucosal surfaces, which will protect the infant against the prevalent pathogens in its environment.

Supported by: CNPq

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4.37 Comparison of *Bordetella pertussis* growth for inoculum production

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Introduction: Pertussis vaccine, one of the components of DTP (diphtheria, tetanus and pertussis) and DTP + Hib (DTP and Haemophilus influenzae type b) vaccines, has been part of national childhood immunization programs for prevention of pertussis (whooping cough). Butantan Institute is the sole Brazilian producer of pertussis vaccine and produces it using batch fermentations of *B. pertussis* 137 strain carried out in bioreactors containing the IB medium for production of *B. pertussis* suspension. The fermentation batches start with a seed culture, followed by inoculum preparation and finally the production culture batch. The inoculum is important to get an optimum fermentation process that reflects in highs levels of production. **Objectives:** The aim of this study was to compare *B. pertussis* growth for the inoculum preparation using the bioreactor "Wave" and the traditional system using shaker. **Methods:** The "Wave" is a GE single use bioreactor that has horizontal agitation, temperature and air flow control; the system used in inoculum production for industrial production nowadays consists of a glass flask with rotary agitation in a shaker and has passive oxygen transfer. Simulating the inoculum preparation at a small scale, *B. pertussis* 137 strain was grown in IB medium for production of *B. pertussis* suspension in a glass flask, incubated under rotary agitation as used at an industrial scale, and the same cell concentration was injected into a "Wave" bioreactor with horizontal agitation; both cultures were maintained for 34 h at 37°C. During fermentation, we evaluated pH, opacity and optical density (D.O.), performed at 590 nm. **Results and Discussion:** The initial O.D. of the two cultivation methods were the same, and at 9 h of fermentation, a higher cell concentration was observed in "Wave" compared to the flask, 1.65 and 0.59, respectively. After 20 h of fermentation this difference increased to 3.76 and 1.3, and at the end of fermentation, 34 h, the absorbance was 6.03 and 1.85, representing an increase of 226 % in "Wave" compared to the flask. The opacity level after 20 h of fermentations was 45 UO/mL in "Wave" and 20 UO/mL using a flask. At the end of 34 h of fermentation, the opacity units increased 300% in "Wave" compared to the traditional system. The pH after 20 h in "Wave" exceeded the ideal for *B. pertussis* culture, indicating that is not good to exceed 20 h of cultivation in the "Wave" bioreactor. The results indicate that the "Wave" method allows an increase in *B. pertussis* growth; moreover, the "Wave" method uses disposable bags and requires no cleaning or sterilization, providing easy operation and protection against cross contamination.

Supported by: Fundação Butantan

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4.38 Pneumococcal surface protein A as an active carrier protein in conjugates using polysaccharide serotype 6B of *Streptococcus pneumoniae*

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Introduction: Due to an increase in the incidence of serotypes not included in commercial pneumococcal conjugate vaccines (PCV), interest in new vaccine strategies has increased. In this sense, conjugation of polysaccharides with an active carrier protein could be a promising alternative. In this study, pneumococcal surface protein A (PspA), a protein expressed by all pneumococcal strains and that has been shown to induce protection in mice, was used as carrier of polysaccharide serotype 6B of *Streptococcus pneumoniae* (6B PS). **Objectives:** The aim of this study was to evaluate the immunogenicity to 6B PS and to PspA induced by two different conjugates and to analyze the influence of conjugation on the secondary structure of PspA. **Methods:** Conjugates were obtained by two different conjugation methods: 1) reductive amination, lacking a spacer molecule between PS and PspA, and 2) formation of carboxamides mediated by DMT-MM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride), using a spacer of 8 carbons between the molecules. The immune response against 6B PS and PspA induced by the conjugates was analyzed regarding IgG titer, IgG isotype, complement deposition and opsonophagocytic activity. The effect of conjugation in the PspA structure was assessed by circular dichroism (CD). **Results and Discussion:** Although CD analysis has demonstrated that the conjugation process changes the secondary structure of PspA, these changes do not seem to influence its immunogenicity. According to our study, the PspA capacity to induce protection was not only maintained but also improved when compared to the unconjugated PspA. The presence of a spacer molecule in the conjugate did not influence the induction of immune response for 6B PS either. Despite the low antibody titers to 6B PS induced by the conjugates, these antibodies were able to opsonize pneumococcus and favor phagocytosis by macrophages. Hence, our results point to a favorable use of PspA as an active carrier protein, since it can improve the response to 6B PS and since its ability to induce protection is maintained after the conjugation process. Therefore, PspA employment may enhance serotype coverage by conjugate vaccines.

Supported by: FAPESP

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4.39 Strategies for the preservation of the potency of rabies vaccine using lyophilization, for veterinary purposes

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Introduction: Rabies is considered one of the most important zoonoses in public health, not only because of its dramatic and lethal evolution, but also because of its high social and economic cost. It is estimated that bovine rabies in Latin America causes annual losses of hundreds of millions of dollars, caused by the death of thousands of head of cattle, in addition to indirect costs that may occur with the vaccination of millions of cattle and numerous post-exposure treatments of people who had contact with suspect animals. A lyophilized vaccine, compared to a liquid form, has many advantages, such as improvement in stability of the product to changes in temperature by increasing its shelf life, allowing better logistics for product to locations where access to refrigeration is difficult to achieve. **Objectives:** The strategies of the process for the preservation of the potency of rabies vaccine for veterinary use by lyophilization were investigated. **Methods:** Rabies inactivated vaccine (RIV), virus PV (Pasteur virus) produced in culture of BHK-21 cells (Baby Hamster Kidney) had the potency by the NIH test of 6.0 IU/mL. We tested 13 excipients in three different concentrations (RIV+excipients) to select the best formulation which had a good antigenicity by ELISA. Some microplates suitable for cell culture were adapted to freeze-dry different vaccine formulations with excipients and their concentrations; this technique was able to repeat the test several times to confirm the result and yet spend little sample. Lyophilization was performed in an FTS Systems freeze dryer, model TDS-00209-A. **Results and Discussion:** Statistical analysis was performed using SPSS® v.17, applying regression for categorical data analysis. The addition of 0.5% arginine, 0.5% PEG 3350, 2% sucrose, 1% maltose and 1% trehalose gave better results by increasing antigenicity. Other formulations and concentrations gave the same antigenicity of non lyophilized RIV or led to loss of antigenicity after freeze drying.

Supported by: FAPESP

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4.40 Preparation of RVGP and analysis of immune response in immunized mice

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Introduction: The rabies virus glycoprotein is the antigen responsible for the induction of neutralizing antibodies by the host immune system, protecting it against viral infection. We have expressed a recombinant rabies virus glycoprotein (RVGP) in *Drosophila melanogaster* S2 cells. An optimized immunofluorescence protocol was established for RVGP detection, allowing a fast semi-quantitative determination of expression during the cultivation period. Now, we are studying its use for immunization of mice by evaluating their immune response. **Objectives:** The aim of this study was to obtain RVGP from S2MtRVGP-H cell cultures and to study the immune response in mice immunized with this preparation. **Methods:** S2MtRVGP-H cells were grown in Schott flasks in a shaker incubator. RVGP expression was later induced with $CuSO_4$ (500 μM). For RVGP detection and quantification, cell samples from 24 h and 48 h post induction were taken to perform ELISA and immunofluorescence test. For immunofluorescence different fixation protocols were tested, varying acetone, methanol or paraformaldehyde concentrations for detection and visualization of RVGP expression on induced cell membrane. ELISA was performed according to the manufacturer's instructions. Mice were immunized with a total of 1.2 μg of RVGP in a vaccination schedule of prime and two booster doses at 7 and 14 days after the first dose. The immune response was evaluated by the titration of anti-RVGP antibodies by ELISA or by the titration of neutralizing antibodies by the RIFFIT method. **Results and Discussion:** S2MtRVGP-H inoculated at $1x10^6$ achieved high cell densities and expressed RVGP in good quality and quantity (0.84 μg/10^7-1.54 μg/10^7 cells). The three fixation methods used prior to immunofluorescence allowed the recognition of RVGP by labeled antibodies. The degree of cell permeabilization varied, as assessed by the use of a cytoplasmic dye (Evans blue), showing that although fixation was important to the technique, the less the external membrane was changed, the better was the labeling. Among all reagents tested, 4% paraformaldehyde and 30% acetone showed better results. Mice immunized with RVGP showed sufficient seroconversion levels after three doses. The titers of neutralizing antibodies clearly showed the high quality of RVGP as an antigen for immunization against rabies virus.

Supported by: FAPESP

4.41 Modulation of lung inflammation by Schistosomula

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Introduction: Schistosomas, aiming for their survival, have developed strategies to modulate the host immune response throughout the infection course: it induces a Th1 profile in the first weeks, during parasite migration; after worm maturation and mating, oviposition starts and it changes to a Th2 profile; finally, in the chronic phase of the infection, this Th2 immune response is down-modulated and the granulomas become smaller. This modulation helps parasite survival, but also decreases the pathology. Compared to egg regulation, we have little information on the immunemodulation of the parasite larval stages and these studies focus on the cercariae secretions or skin schitosomula interactions. **Objectives:** We developed a model to study the effect of lung inflammation on schistosomula during their migration, since this is a key step during its maturation. **Methods:** Four groups of mice were analyzed: control, cercariae challenged only, LPS-treated only and cercariae challenged and LPS-treated. Mice were anesthetized and submitted to challenge with 120 cercariae by skin penetration; 5 days later, 1 μg LPS was administered intranasally to induce lung inflammation. We evaluated the lung inflammation at three times after LPS treatment (24, 48 and 72 h), analyzing the total and differential cell counts and measuring IFN-γ, IL-12, IL-4, IL-5 and IL-10 levels in the bronchoalveolar lavage. We also evaluated the effect of LPS-induced lung inflammation on the maturation and viability of the worms, so two groups of mice were challenged with cercariae and one of them was treated 5 days later with LPS. Next, 45 days after the cercariae challenge, we assessed the worm burden and oviposition. **Results and Discussion:** Mice that were challenged with cercariae and received LPS treatment displayed ~40% reduction in the total cell number at 72 h after the LPS treatment. This reduction is due to the number of monocytes and neutrophils. We did not observe any difference in the number of BAL cells of the animals that did not receive the LPS treatment. We did not detect IL-5 or IFN-γ, and concerning the other cytokines, no remarkable results were obtained. Despite induction of a high level of inflammation in the lungs, the LPS treatment was not able to reduce the worm burden or oviposition. We developed a lung inflammation model that is a promising tool to investigate modulation of the host response by the parasite. These results support the hypothesis that lung schistosomula somehow modulate the host immune response, possibly creating a microenvironment that allows it to be hidden or not affected by the immune system. The next step to better characterize the model is to analyze other cytokines by qPCR and use other inflammatory agents.

Supported by: FAPESP

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4.42 Industrial scale production of yeast-derived recombinant hepatitis B vaccine containing S, pre-S1 and pre-S2 antigens

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Introduction: Hepatitis B is a major public health problem, affecting 2 billion people worldwide. In Brazil, generally 1 to 3% of the population are chronic carriers. The vaccine against the hepatitis B virus is the most effective measure in preventing infection, and is currently produced through recombinant DNA technology. The producer strain *Hansenula polymorpha* expresses the major virus surface antigen (S), monomers of which associate to form a virus-like particle. The promoter of the recombinant gene is under methanol control. Our vaccine gives seroconversion from 85% to 90% of immunizations. However, up to 5% of the healthy population do not respond adequately. In order to reach this population, Instituto Butantan has developed a new hepatitis B vaccine that includes the pre-S1 and pre-S2 antigens. The production of this variant was recently performed on pilot scale, and the criteria established in this phase were used as guidelines for the scale-up manufacturing process. **Objectives:** The aim of this study was to standardize the industrial scale production of hepatitis B vaccine containing S/pre-S1/pre-S2 antigens. **Methods:** The vaccine production consists of the *H. polymorpha* cultivation, followed by purification of the vaccine antigens. The fermentation process is initiated with the growth of the recombinant strain, from three inoculum steps till a 500-L methanol-fed cultivation, during which the vaccine antigens are expressed. Fermentation steps are monitored for biomass growth, absence of contamination and expression of the vaccine antigens. After cell harvest, biomass is disintegrated and processed in various purification steps. Downstream procedures are monitored for protein (OD_{280}) and antigen (passive hemagglutination assay) contents. Fractions from final purification steps are analyzed for purity, with SDS-PAGE. The quality of the final product is ensured by bacterial and fungal sterility tests, purity evaluation, endotoxin assay, immunogenicity assay, and determination of residual DNA and antigen contents. **Results and Discussion:** Three consecutive fermentation batches were performed. The results for the monitoring tests were satisfactory and very similar between batches. During the cultivation step, the growth pattern indicated a slightly slower methanol metabolism in the new strain, compared to the currently used one. Downstream purification monitoring data were within the acceptable range.

Supported by: Fundação Butantan

4.43 Early changes in peritoneal cell populations induced by mineral oil pristane

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Introduction: HIII mice are completely resistant to pristane-induced arthritis (PIA), while LIII mice show 100% incidence of severe lesions. Previous data suggest that the differences between HIII and LIII mice are expressed in the early phase of induction, influencing the late-phase of arthritis development. However, cellular involvement remains unclear. It is known that immune responses are normally initiated as a result of dendritic cell migration into secondary lymphoid organs, although this may also take place in compartments other than lymphoid organs in pathological conditions. **Objectives:** Herein, we evaluated whether peritoneal cell changes may be associated with PIA susceptibility/resistance in LIII and HIII mice. **Methods:** Three-month old LIII and HIII mice were i.p injected with 0.5 mL pristane. Seven days later, spleen, mesenteric lymph nodes (MLN) and peritoneal cells were collected, counted and stained for flow cytometric analysis using antibodies specific for CD3, CD8, CD4, CD19, CD5, CD23, CD11b, Gr-1 and CD11c. Ten thousand events were acquired in FACScantoII and data were analyzed on FlowJo software. ANOVA was used for statistical analysis. **Results and Discussion:** Peritoneal populations in control HIII mice were similar for T and B cells. However, $CD11c^+$ cell numbers were much lower in LIII control mice than in HIII control animals. Pristane treatment caused a striking increase in the $CD11c^+$ population in LIII mice and a strong reduction of these cells in the peritoneum of HIII mice. $CD11c^+$ numbers in MLN and spleen were not altered, suggesting that their decrease in HIII mice after pristane could be due to apoptotic cell death. In both strains, T cells were increased after pristane treatment, while B1 and B2 cell numbers were diminished. These results suggest that $CD11c^+$-expressing cells could initiate and maintain chronic inflammation in the peritoneal cavity after pristane injection in PIA-susceptible LIII mice, while their depletion in HIII mice may be related to resistance.

Supported by: CNPq, CAPES and FAPESP

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4.44 Bovine papillomavirus recombinant L2 protein as a potential vaccine for Simmental cattle

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Introduction: Papillomatosis is an infectious disease characterized by warts, located on the skin or mucosa, which affects several species of mammals, including humans. In cattle, this disease is responsible for appreciable economic losses, and the animals show, among other consequences, reduced milk production and weight loss. Papillomaviruses infect the basal cells of epithelia, inducing the formation of tumors. The tumors are commonly benign, regressing spontaneously. However, they can progress to malignancy. The bovine papillomaviruses 2 and 4 (BPV-2 and BPV-4) are associated with cutaneous fibropapillomas, mucosal papillomas and cancers of the bladder and upper digestive tract. Cloning and expression of minor (L2) viral capsid protein in a bacterial system is a viable approach for the production of immune inputs, such as diagnostic tests or vaccines. **Objectives:** The aim of this study was to evaluate the potential of recombinant L2 BPV protein immunization as an inducer of humoral response in a group of selected, non-BPV infected calves. **Methods:** The N-terminal portions of the proteins L2-BPV-2 / BPV-4, previously cloned, were purified by an affinity chromatography system for glutathione S-transferase (GST). The animals were divided into groups (L2-2, L2-4, and negative control). Protein concentration, as well as adjuvant, present in immunizations, was 330 μg and the interval between the two immunizations was up to 30 days. Antibody levels were analyzed in an ELISA reader, using microplates prepared with 1.0 μg of each protein. **Results and Discussion:** The immune response obtained by vaccination of calves was satisfactory, indicating the feasibility of this approach for implementing an anti-BPV vaccine program in the national herd. Antibody levels remained high when compared to pre-immunization even after 180 days. It is noteworthy; however, that quantification of IgG levels measured here is still a preliminary result and requires further analysis for specific antibodies directed against the L2 protein of bovine papillomavirus type 2 and 4.

Supported by: CAPES, CNPq and FAPESP

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4.45 Comparison of *Corynebacterium diphtheriae* growth for inoculum production

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Introduction: Butantan Institute produces diphtheria toxin by growing toxigenic *Corynebacterium diphtheriae* Park-Williams 8 strain on an industrial scale. Diphtheria toxin is inactivated using formaldehyde in order to obtain diphtheria toxoid. This product is used in associated vaccines such as DTP (diphtheria, tetanus and pertussis), DTP plus *Haemophilus influenzae* type b, DT (diphtheria - tetanus children's use), dT (diphtheria – tetanus, adult use) as well to immunize horses for antiserum production. Diphtheria toxin production starts with a working seed in a solid medium, followed by culture seed in liquid medium and then production of the inoculum, a critical point to obtain a optimum diphtheria toxin yield. The inoculum is aseptically introduced into a 500-L bioreactor for *C. diphtheriae* fermentation. **Objectives:** The aim of this study was to compare *Corynebacterium diphtheriae* growth for inoculum production in two different methods, using the bioreactor "Wave" (GE Healthcare) and the traditional system using shaker flask. **Methods:** The inoculum of *C. diphtheriae* using a shaker flask, with rotary agitation was prepared as in large-scale production. In the other method, *C.diphtheriae* was cultivated in a "Wave" bioreactor (10L - GE Heathcare) which consists of cellbag resting on a specifically designed rocking platform that induces waves in the culture, providing mixing and oxygen transfer. Both cultures were maintained at 36ºC±1ºC for 24 h. Samples were taken during the fermentation to measure the optical density (O.D.) at 530 nm and to determine the flocculation limit (Lf/mL). **Results and Discussion:** The average O.D. after 24 h of fermentation was 0.813 at a 1:30 dilution in the "Wave" bioreactor and 0.615 at a 1:10 dilution using the shaker flask representing an increase of 296% in O.D. The results of flocculation limit in "Wave" bioreactor were 20 Lf/mL, while in the flask there was no observed flocculation. These preliminary results indicate that the "Wave" method allows the increase in *C.diphtheriae* growth, and furthermore, the "Wave" method uses disposable bags, which represents a big advantage because we can eliminate the sterilization cycle and cleaning validation. This study will continue to test the inoculum prepared with the "Wave" method on a large scale, according to WHO guidelines for vaccine production.

Supported by: Fundação Butantan

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4.46 Detoxified BpMPLA as promising adjuvant component of the recombinant Butantan hepatitis B vaccine

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Introduction: Global vaccine production is limited by its established capacity and during adverse situations such as pandemic events, where the vaccine supply cannot reach developing countries whose budgets cannot afford high prices. The use of adjuvant is a rational solution to increase established vaccine production, and all vaccine manufacturers are investigating new adjuvants. **Objectives:** The aim of this study was to evaluate the microbial derivative adjuvant from *Bordetella pertussis* LPS with the recombinant hepatitis B vaccine. **Methods:** Mice were inoculated with five different formulations of monophosphoryl lipid A of *Bordetella pertussis* (BpMPLA) with the recombinant hepatitis B vaccine, the antigen Butang®, obtained through genetic engineering techniques, using *Hansenulla polymorpha* yeast. After 30 days, the animals were bled to determine antibody titers by ELISA. **Results and Discussion:** Tetraacylated BpMPLA (BpMPLA/4) was effective in reducing the influenza vaccine dose 4-fold. Now, we are showing the results using another monophosphoryl adjuvant variation mainly composed of penta- and tetraacylated forms (BpMPLA/4+5), the acid hydrolysis of Bp LPS was controlled for that purpose, combined with rHBsAg. Its efficacy was proved for any age group by seroconversion rates over 90% with 25 μg antigen concentration formulated in aluminum hydroxide. Our data showed that it was possible to get the same reference vaccine titer (173.4 mIU/mL, Group rHBsAg+$AL(OH)_3$) using half antigen concentration combined with 10 μg/mL of BpMPLA/4+5 plus alumen (164.9 mIU/mL, group rHBsAg+$AL(OH)_3$+BpMPLA).

Supported by: Fundação Butantan

4.47 Hepatitis B nanovaccine: the use of SBA-15 silica as an oral adjuvant

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Introduction: Despite the availability of efficient prophylactic vaccines, human hepatitis B virus (HBV) infection, along with its associated diseases, is still a major public health problem. Disease prevention by vaccination is a unique and effective strategy. **Objectives:** We aimed to prove the applicability of SBA-15 as an adjuvant in oral immunizations with the recombinant proteins HBsAg, the main component of hepatitis B vaccine. **Methods:** Groups of 8-week-old female BALB/c mice received subcutaneously (s.c.) or orally, 0.5 μg of HBsAg encapsulated/adsorbed on SBA-15 or adsorbed on $Al(OH)_3$ in a final volume of 0.25 mL PBS. A s.c. booster was administered in all experimental groups with the antigen adsorbed on SBA-15 or $Al(OH)_3$ 30 days after first immunization. Serum and fecal samples were collected for specific antibody titration. **Results and Discussion:** After the first dose, at 7, 14 and 30 days, no specific serum antibodies were detected in all experimental groups. IgG titers, after the second s.c. dose, were higher in both SBA-15 (5.2 $\log_2$, 7.8 $\log_2$ and 9 $\log_2$) and $Al(OH)_3$ groups (11 $\log_2$, 11 $\log_2$ and 9.6 $\log_2$). Anti-HBsAg IgG1 titers were similarly high in both groups (10.2 $\log_2$, 12.5 $\log_2$ and 12 $\log_2$ in mice immunized with $Al(OH)_3$ and 7.4 $\log_2$, 10.4 $\log_2$ and 9 $\log_2$ in HBsAg:SBA-15 immunized mice). Specific IgG2a titers detected post booster, were 8 $\log_2$, 8.5 $\log_2$ and 7.8 $\log_2$, respectively, in the $Al(OH)_3$ group and 5.8 $\log_2$, 6.6 $\log_2$, 6.8 $\log_2$ in the silica group. After oral immunization, IgG response in the group HBsAg:SBA-15 was 9.2 $\log_2$, 10.6 $\log_2$ and 10 $\log_2$ at days 7, 14 and 30 post booster. Anti-HBsAg IgG1 titers were 9 $\log_2$, 10.2 $\log_2$, 10 $\log_2$; while IgG2a titers were 6 $\log_2$, 6.2 $\log_2$, 7.4 $\log_2$. Mice immunized without silica did not respond. Specific serum IgA titers were only detected in the group HBsAg:SBA-15, 10 days after s.c. booster (4.5 $\log_2$). Analysis of specific secretory IgA (s-IgA) showed that, ten days after the first immunization, s-IgA titers were 3 $\log_2$ in the HBsAg:SBA-15 group and remained stable after s.c. booster. Our results support the idea of the promising use of SBA-15 silica as an adjuvant, even for oral immunizations, due to the physical protection of antigens. Compared to the HBV vaccines used nowadays, SBA-15 silica could anticipate and enhance the humoral immune response after the combined oral and subcutaneous immunizations.

Supported by: CNPq, FAPESP, INCTTOX and Cristália Pharmaceutics

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4.48 Surface leptospiral protein LcpA induces partial protection in hamsters and reduces renal colonization

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Introduction: Leptospirosis is a zoonosis caused by pathogenic bacteria from the genus *Leptospira*. The disease represents a serious public health problem in tropical developing countries. Over the past few years, our group has focused on the identification of immune evasion mechanisms shown by pathogenic leptospires. In this context, functional characterization of leptospiral outer membrane proteins, which represent the main targets for interaction with host molecules, is of great relevance. One strategy adopted by pathogenic *Leptospira* to resist hosts' innate immunity is their potential to acquire fluid phase complement regulators to their surfaces, particularly those of the alternative and the classical complement pathways. Recently, we have shown that a leptospiral surface protein named LcpA interacts with factor H and C4b-binding protein. Bound to the *Leptospira*'s surface, these molecules retain cofactor activity, thus indicating that acquisition of complement regulators may contribute to leptospiral serum resistance. **Objectives:** In this study, we evaluated the protective immunity of LcpA against lethal challenge with *L. interrogans* in hamsters. **Methods:** Recombinant LcpA expressed in *E. coli* was purified by nickel affinity chromatography. Hamsters were immunized subcutaneously with 50 µg of LcpA or LigAC (used as a positive control), and also with a commercial vaccine or PBS (negative control). Aluminum hydroxide was used as an adjuvant. Fifteen days following the second immunization, animals were given an administration of 2×10^7 low-passage *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 (first assay) or 2×10^5 low-passage *L. interrogans* serovar Pomona strain Fromm (second assay). Animals were followed for three weeks before euthanasia. **Results and Discussion:** Immunization of hamsters with LcpA conferred an immunoprotection of 50% in two independent assays. Animals immunized with LigAC conferred full protection against the homologous challenge (first assay) but only 40% protection against the heterologous challenge (second assay). Interestingly, animals immunized with LcpA showed a reduced bacterial load in kidneys compared to those immunized with LigAC. We propose that a combination of these two leptospiral antigens, LcpA and LigAC, may be a promising formulation for the development of an effective subunit vaccine against leptospirosis.

Supported by: FAPESP

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4.49 *N*-linked glycans and sialic residues are involved in IgE ability to induce anaphylactic reaction in mice

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Introduction: It is known that glycans attached to the Fc domain of immunoglobulins affect their functional activities. In this context, we previously described that the ability of murine IgG1 antibodies to mediate anaphylactic reaction is directly dependent on the sialic acid content in the *N*-linked carbohydrate chain of these antibodies. However, it is not clear whether the carbohydrate chain mainly the sialic acid residues are required for the activity of IgE antibodies. **Objectives**: The aim of this study was to elucidate the role of the *N*-glycan chain and the contribution of sialic acid residues in the ability of IgE to elicit anaphylaxis reaction. **Methods**: Lectin-binding assay was used to analyze the *N*-linked oligosaccharide chain of the mouse IgE anti-DNP monoclonal antibody. Samples containing IgE antibodies were submitted to enzymatic treatments for deglycosylation or removal of the sialic acid residues and then tested in PCA reactions, affinity chromatography to *Sambucus nigra*-lectin, antigen-binding and mast cell degranulation assays to analyze the role of the glycans in IgE activity. **Results and Discussion:** The binding of the IgE antibody to distinct lectins was similar to those observed with the anaphylactic IgG1 monoclonal antibody. We also observed that monoclonal IgE has high affinity for *Sambucus nigra* lectin as well the anaphylactic IgG1 antibodies. In addition, the enzymatically deglycosylated or desialylated IgE antibodies retain their ability to recognize the antigen in ELISA assay but lose the capacity to elicit anaphylactic reaction *in vivo* and to induce mast cell degranulation *in vitro*. The sialic acid residues attached to carbohydrate chains are essential for the anaphylactic activity of IgE as observed for murine monoclonal IgG1 antibodies.

Supported by: FAPESP and CNPq

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4.50 Cloning, expression and immunogenic potential of an outer membrane protein of *Leptospira interrogans* (LIC11087)

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Introduction: Leptospirosis is a worldwide disease caused by pathogenic spirochetes of genus *Leptospira*. When the most severe form, Weil's syndrome, is developed, mortality reaches 40%. Prophylactic interventions include vaccine preparation employing killed whole leptospires, but this strategy has many drawbacks such as low efficacy, requirement of annual immunizations, and failure to confer cross-protection immunity against different serovars. Outer membrane proteins (OMPs) have been studied due to their potential to elicit hosts´ immune system. **Objectives:** This work was aimed at the cloning and expression of rLIC11087 in *E. coli* and the evaluation of its immunogenic activity in mice. **Methods:** The LIC11087 coding sequence was amplified by PCR from genomic DNA by the use of specific primers, and cloned into the expression vector pAE. Recombinant clones were confirmed by complete DNA sequencing. Heterologous expression was conducted in transformed *E. coli* BL21 DE3, by addition of 0.25 mM IPTG. The recombinant protein (30 KDa) was expressed as inclusion bodies and purified by metal-chelating chromatography. Structural integrity of the purified protein was assessed by circular dichroism spectroscopy. Conservation of LIC11087 in leptospira strains was evaluated by PCR. The immunogenicity of the protein was assessed by subcutaneous immunization of BALB/c mice followed by ELISA. Recognition of the protein by sera of infected hamsters was also analyzed. **Results and Discussion:** The coding sequence LIC 11087 was successfully cloned into *E. coli* expression vector pAE and after the purification process, presented secondary structure. rLIC11087 proved to be an interesting antigen since high titer of antibodies was obtained after mouse immunization (1:200.000). In addition, this protein showed 40% reactivity with sera from experimentally infected hamsters. The gene is conserved in all pathogenic *Leptospira* strains analyzed and absent in the saprophytic species, *L. biflexa*. rLIC11087 appears to be a promising antigen, and other experimental approaches are necessary to characterize immune responses triggered by this antigen.

Supported by: FAPESP, CNPq and Fundação Butantan

4.51 Association between lung inflammation and gut bacterial translocation after ischemia and reperfusion in mice

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Introduction: Gut inflammation and loss of gut barrier function as a consequence of intestinal ischemia and reperfusion (I/R) has been implicated as the initial triggering events that contribute to the development of systemic inflammatory response, acute lung inflammation (ALI) and multiple organ dysfunction syndrome (MODS). However, the exact mechanism by which gut I/R leads to systemic inflammatory response remains unclear. Recently the role of *Hypoxia-inducible factor 1 alpha* (*HIF-1α*) in the regulation of bacterial translocation, apoptosis and gut inflammatory response was described. We observed in mouse strains selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response (AIR) a higher expression of *HIF-1α* in AIRmax mice subjected to intestinal I/R. **Objectives:** The aim of this study was to evaluate the role of bacterial translocation in acute lung inflammation after I/R in AIRmax and AIRmin mice genetically selected for AIR. **Methods:** The mice were subjected to superior mesenteric artery ischemia for 45 minutes and 4 hours of reperfusion. After I/R the mesenteric lymph node (MLN) was harvested and bacterial translocation level was quantified in blood and MacConkey agar plates. Lung inflammation was evaluated by myeloperoxidases (MPO) activity and lung gene expression was performed by real-time PCR. **Results and Discussion:** The percentage of bacterial translocation was higher in ischemic AIRmax mice (62%) than ischemic AIRmin mice (25%), besides the number of bacterial colonies in MLN of the I/R-AIRmax was 3-fold higher than I/R-AIRmin mice. Corroborating these results, MPO activity in lungs was higher in I/R-AIRmax showing an absorbance (A_{450nm}) of 0.54± 0.1 compared to I/R-AIRmin (0.36 ± 0.02), and *HIF-1α* gene expression was 7-fold higher in I/R-AIRmax lung. Given the association between bacterial translocation and lung inflammation in AIR mice, our hypothesis is that under adverse oxygen conditions, AIRmax mice express specific genes that favor the loss of gut barrier function and consequently lead to bacterial translocation resulting in systemic inflammation.

Supported by: CAPES and CNPq

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4.52 Dengue virus replication in Vero cells maintained in serum-free medium

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Introduction: Dengue virus (DENVs), family *Flaviviridae*, genus *Flavivirirus* include four serotypes (DENV-1 to DENV-4) that cause the most important arthropod-borne viral disease of humans and approximately 100 million cases and 25,000 deaths annually in the world. This virus is transmitted to humans primarily by *Aedes aegypti* mosquitoes, and infection leads to a spectrum of disease ranging from unapparent infection to classic dengue (breakbone) fever and to more severe and sometimes fatal dengue hemorrhagic fever. In tropical and subtropical regions across the world, these symptoms are considered one of the greatest threats to public health. **Objectives:** The aim of this study was to study dengue virus replications in Vero cells maintained in serum-free medium. **Methods:** Vero cells maintained in serum-free medium (225cm^2 T-flasks) were infected with DENV-1, DENV-2, DENV-3 and DENV-4 (strains from NIH) with MOI of 0.01 and 0.05 (DENV-3) and incubated at 37ºC for 14 days. Samples were taken each day to determine the virus titers by PFA (plaque-forming assay) and the results were expressed in PFU/mL. **Results and Discussion:** The higher virus titers obtained in the samples of cultures infected with the dengue serotypes 1, 2 and 4 were on days 8, 9, 10 and 11 and the titers found were $10^{6.6} - 10^{7.5}$, $10^{6.6} - 10^{7.5}$ and $10^{6.6} - 10^{7.5}$ PFU/mL for DENV-1, DENV-2 and DENV-4, respectively. For DENV-3, the best results were obtained on days 10, 11, 12, 13 and 14th with titers of $10^{6.1}$-$10^{6.4}$. The results showed that the virus replication peak of each serotype was at different times. These data are very important to determine the optimal days for harvesting dengue suspensions in dengue virus production for vaccine formulation. The poorer results obtained with DENV-3 indicate that it is necessary to optimize the production of this serotype.

Supported by: FAPESP, BNDES and Fundação Butantan

4.53 Development of *Mycobacteria bovis* (BCG) and *Mycobacteria smegmatis* strains expressing virulent factors of enteropathogenic *Escherichia coli* (EPEC)

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Introduction: EPEC is an important causative agent of diarrhea in infants mainly in developing countries. BfpA and intimin are the most important virulence factors of EPEC. Specific vaccines are the most important preventive measures for infectious diseases. Unfortunately, there is no EPEC vaccine yet. Expected recommended EPEC vaccine, besides showing specificity, must continually express their immunogens, be free of endogen toxicity, and be of low cost. **Objectives:** The aim of this study was to develop a recombinant vaccine capable of expressing full immunogenic and stable BfpA and intimin factors of EPEC. **Methods:** These factors were cloned in expression plasmid pMIP12, and inserted into BCG and *M. smegmatis*. The recombinant bacteria (rSmeg and rBCG) were used as immunogens, either p.o. or i.p. The presence of specific antibodies was evaluated in serum and stool by immunochemical methods. Concomitant assays using spleen cells from immunized mice were performed to determine the cytokine profile. The effectiveness of these antibodies to block host cell infection was assessed by inhibition assays of EPEC adhesion to Hep-2 cells. The nature of immune response was evaluated by the cytokines. **Results and Discussion:** The plasmid pMIP12-*bfpA* and pMIP12-*intimin* genes were successfully cloned in both. BCG and M. *smegmatic* as indicated by the specific DNA sequences, PCR amplification, and validated by of gene sequencing. The expression of recombinant proteins was stable, and retaining their immunogenic properties as assessed by the in vivo production of specific IgG in serum and IgA in serum and stool antibodies. These antibodies were capable of blocking over 90% of EPEC adhesion to Hep-2 cells. Previous contact of EPEC with the generated antibodies did not kill the bacteria. The cytokine profile observed was characterized by TNF-α and IFN-γ, IgG2a isotype predominance, typical of a Th1 response. In conclusion, BCG or *M. smegmatic* harboring *bfp*A and *intimin* genes are promising candidates for an anti-EPEC vaccine.

Supported by: FAPESP and CAPES

5. Microorganisms

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5.01 Microbiota of Brazilian species of amphibians kept in captivity

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Introduction: The microbiota of the skin of amphibians is directly associated with the adaptation of these animals to their habitats. Changes in this microbiota result in an increased susceptibility to infection by different pathogens. Despite the current interest in amphibians in terms of biodiversity, little is known about the microorganisms that colonize the skin of these animals. **Objectives:** Bacterial samples from the skin of various amphibian species were isolated, identified and analyzed. **Methods:** Samples were collected from the following: *Rhinella icterica* and *Trachycephalus mesophaeus* (Atlantic Forest); *Rhinella schneideri* (Cerrado) *Rhinella jimi*, *Phylomedusa nordestina*, *Leptodactylus labirynthicus*, *Corythomantis grenningi*, *Dermatonotus*, *Pleuroderma* and *Scinax* (Caatinga); and *Pipa pipa* (Amazon Forest). The isolation and identification of samples were performed using standard methods of cultivation on blood and MacConkey agar plates, biochemical assays, and Api20E/Api20NE kits. **Results and Discussion:** *Citrobacter freundii* (57%), *Klebsiella pneumoniae* (33%) and *Escherichia coli* (33%) occurred more frequently among the animals studied. Among these species, *C. freundii* and *E. coli* were isolated from animals in all regions surveyed. Other species less frequently found included *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Citrobacter youngae*, *C. Braak* and *C. diversus*, *Enterobacter aerogenes* and *E. cloacae*, *Hafnia alvei*, *Morganella morgani*, *Proteus vulgaris* and *P. mirabilis*, *Providencia rettgeri*, *Pseudomonas aeruginosa*, *Shigella sp* and *Serratia sp*. Two bacterial species associated with septicemia ("red-leg syndrome") in reptiles and amphibians, *C. freundii* and *A. hydrophila*, were isolated from diseased animals, which showed deep ulcerations in the skin, among other features. These bacterial species were also isolated from healthy animals. The animals studied showed a diversified microbiota, with bacterial species not previously reported. The fact that some bacterial species have been isolated from both healthy and diseased animals suggests that changes in captivity may negatively influence the bacteria/amphibian skin balance. On the other hand, it is quite possible that there is involvement of other microorganisms, such as fungi, in the establishment of these pathologies.

Supported by: FAPESP

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5.02 Atypical enteropathogenic *Escherichia coli* expressing Pic: a serine protease autotransporter of Enterobacteriaceae

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Introduction: In a study conducted in our laboratory that analyzed the prevalence of virulence factors of other pathotypes of diarrheagenic *Escherichia coli* in atypical enteropathogenic *E. coli* (aEPEC), we found one strain (BA589) carrying a DNA fragment corresponding to part of the passenger domain of protein involved in colonization (Pic). Pic was originally identified in cultures of enteroaggregative *E. coli* (EAEC) and, like other serine protease autotransporters of the Enterobacteriaceae (SPATE), shows proteolytic activity on mucin, which may have an important role in mucosal colonization by EAEC. Moreover, Pic has hemagglutination function and degrades components of the complement system. **Objectives:** This study aimed to determine the *pic* gene sequence, its localization and expression in one aEPEC strain. **Methods:** The aEPEC strain selected was isolated from a child with acute diarrhea. The plasmid extraction was analyzed by agarose gel electrophoresis (0.7%), followed by Southern blotting and hybridization reaction with a gene probe corresponding to the *pic* gene of the prototype EAEC strain 042. Nine sets of different primers, covering the entire *pic* gene sequence, were designed based on the *pic* sequence of EAEC strain 042. All fragments were sequenced and a contig sequence was obtained from start to stop codon. The deduced amino acid sequence was aligned with the Pic sequence of EAEC 042 (GenBank accession number: FN554766.1). Expression analysis of Pic was performed by immunoblotting with specific polyclonal antiserum. **Results and Discussion:** Electrophoresis of plasmid extraction showed the presence of eight plasmids ranging from 105 to 1.2 kb. Southern blot analysis showed that *pic* in BA589 is present in a plasmid of high molecular weight (~105 Kb), unlike what is observed in uropathogenic *E. coli* (UPEC), EAEC and *Shigella flexneri*, where it is located on the chromosome. The DNA sequence showed that *pic* of BA589 shows 99% identity with *pic* of EAEC 042. Immunoblotting showed high expression of Pic in this strain. Thus, this is the first description of aEPEC expressing Pic of EAEC. Studies to characterize the phenotypic features of Pic in strain BA589 are underway.

Supported by: FAPESP

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5.03 Prevalence of genes from the *pheU* pathogenicity island of enteroaggregative *Escherichia coli* among diarrheagenic and intestinal microbiota *E. coli* strains

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Introduction: Enteroaggregative *Escherichia coli* (EAEC), an emerging agent of acute and persistent diarrhea, is characterized by the expression of the aggregative adherence (AA) pattern in epithelial cells. The gold standard diagnostic test for EAEC is the observation of the AA pattern in HeLa or HEp-2 cells, which is expensive and limited to reference laboratories. PCR assays, employing more than one EAEC genetic marker, have been proposed as alternatives. Although none of these assays are suitable for the detection of both typical (*aggR*-positive) and atypical (*aggR*-negative) EAEC strains. A pathogenicity island (PAI) was identified on the chromosome of the prototypical EAEC strain 042. In this PAI inserted in *pheU* are located the genes of the *aaiA-Y* operon, which could be used in association with other genes in a multiplex PCR for EAEC diagnosis. However, the distribution of this *pheU* PAI of EAEC in other groups of *E. coli* is not known. **Objectives:** This study aimed to evaluate the prevalence of the four genes of the *pheU* PAI of EAEC in strains of the different groups of *E. coli* and other enterobacteria. **Methods:** The presence of *aaiA, aaiC, aaiG* and *aaiU* genes was investigated by PCR using specific primers based on their respective sequences described in GenBank (accession numbers: FN554766). Three hundred and four bacterial strains were selected for this study. These strains belonged to the following groups: typical EAEC (n= 54), atypical EAEC (n= 49), enteropathogenic *E. coli* (EPEC) (n= 20), diffusely adherent *E. coli* (n= 20), enterotoxigenic *E. coli* (ETEC) (n= 20), enteroinvasive *E. coli* (n= 17), Shiga toxin-producing *E. coli* (n= 20), intestinal microbiota *E. coli* (n= 50) and Enterobacteriaceae other than *E. coli* (n= 34). **Results and Discussion:** Among the EAEC strains, the searched genes were found in the following prevalence: *aaiA* was detected in 50 (48.5%) strains [36 (66%) typical and 14 (28.5%) atypical]; *aaiC* was detected in 31 (30.1%) strains [20 (37%) typical and 11 (22%) atypical]; *aaiG* was detected in 48 (46.6%) strains [32 (59%) typical and 16 (32%) atypical]; and *aaiU* was detected in 20 (66%) strains [16 (32%) typical and 4 (8%) atypical]. Regarding the other bacterial groups, these genes were identified in the following prevalence: *aaiA* was found in 2 (4%) strains of *E. coli* of the intestinal microbiota; *aaiC* was found in 1 (5%) ETEC and 1 (5%) of EPEC; *aaiG* was found in 2 (5%) EPEC; and *aaiU* was not detected. In conclusion, the genes of this *pheU* PAI of EAEC are less prevalent in fecal bacteria not classified as EAEC, indicating that they may be suitable for the molecular diagnosis of EAEC.

Supported by: FAPESP and CAPES

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5.04 Cloning expression and characterization of protein ecs1997 and investigation of prevalence of the gene ecs1997 in pathogenic and non-pathogenic enterobacteria

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is one of six *E. coli* diarrheagenic (DEC) pathovars, which produce a chromosomally encoded adherence factor, intimin (*eae* gene), located within the locus for enterocyte effacement (LEE) pathogenicity island. This pathovar is divided into typical and atypical EPEC, and the principal difference is the presence of the EPEC adherence factor (EAF) plasmid in tEPEC, which encodes the bundle-forming pili. In order to characterize novel adhesins, 4 isolates of the aEPEC:BA320 (displaying localized adherence [LA]-like pattern), BA4013 (not adherent); EC292/84 (aggregative adhesion) and 9100-83 (diffuse adhesion), were evaluated by proteomics analysis for identification of proteins present in their fimbrial extracts. The hypothetical protein Ecs1997 was identified in 3 isolates of aEPEC, and apparently the protein shows a filamentous characteristic. **Objectives:** The aim of this work was to determine the prevalence of the gene ecs1997 in strains of diarrheagenic *E. coli*, microbiota intestinal and the following enterobacteria: *Salmonella* spp, *Shigellla sonnei*, *Morganella morgani*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The same gene was cloned in the expression vector pAE to obtain the recombinant protein for later characterization. **Methods:** The primers were designed with the Gene Runner program, and the PCR was performed using Taq polymerase. The gene ecs1997 was cloned in pGEMT-Easy Vector and subcloned in pAE expression vector, and the confirmation of cloning was performed by restriction analysis and sequencing. The plasmid pAE_ecs1997 was transformed in BL21 DE3 pLysS for expression and later purification by affinity chromatography. **Results and Discussion:** The prevalence of the gene ecs1997 was analyzed by PCR in 72 strains of aEPEC, 37 strains of tEPEC, 46 strains STEC, 10 strains of ETEC, 6 strains of intestinal microbiota and 6 strains of enterobacteria above. The gene ecs1997 was found in 60% (43/72%) of isolates of aEPEC, 100% (37/37) tEPEC, 95.6% (44/46) STEC, 91% (10/11) ETEC, 100% (10/10) EAEC, 100% (6/6) isolates of intestinal microbiota and 33% (2/6) of others enterobacteria. The gene ecs1997 was cloned in expression vector pAE, the recombinant protein was produced in the soluble fraction and purified by nickel affinity chromatography. The presence of this gene in pathogenic enterobacteria and non-pathogenic bacteria demonstrate the ubiquity of the protein, and probably there is an implication in a metabolic process or physiological functions. The induction of protein *in vitro* enables subsequent characterization, which should contribute to defining their function in aEPEC strains.

Supported by: FAPESP and CAPES

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5.05 Morphological characterization of tissue damage in organs of mice strains infected with pathogenic *Leptospira*

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Introduction: Leptospirosis is a worldwide zoonosis caused by the highly invasive spirochaete *Leptospira*. Currently known are more than 250 *Leptospira* pathogenic serovars that can potentially infect humans. Rodents infected by *Leptospira* become carriers and shed bacteria through their urine into water or soil, promoting infection of human beings via skin contact. The bacteria disseminate through the systemic circulation to colonize target organs. The pathogenesis in humans is mainly observed in lungs, liver and kidneys. Damage to tissues of different mouse strains experimentally infected with *Leptospira* is being evaluated. **Objectives:** We examined the damage in kidneys, liver and lungs of mice from leptospira resistant and susceptible strains, C3H/HeJ, C3H/HePas and BALB/c. **Methods:** Animals of each mouse strain were infected ip with 1×10^7 spirochaetes of the virulent strain of *Leptospira interrogans* serovar Copenhageni and development of the disease was followed. The infection was confirmed by the presence of leptospiral DNA in the organs of the animals, demonstrated by PCR. Histological analyses were performed by HE stain in kidneys, liver and lungs collected from infected mice 7 days after inoculation. Control samples were prepared from non-inoculated animals. **Results and Discussion:** In this study, the mortality of C3H/HeJ mice was observed while its parental, C3H/HePas, showed jaundice and BALB/c mice remained asymptomatic. In the organs of infected mice the most characteristic observation in kidneys from C3H/HeJ was extensive tubular epithelial cell necrosis. There were acute inflammatory cells in the kidney of the C3H/HePas strain. In BALB/c, conspicuous edema and vasodilatation were observed. In the liver of BALB/c and C3H/HePas, we observed dilatation of hepatic sinusoids and Kupffer cell hypertrophy. In addition, C3H/HePas showed blood ectasia in the portal vein and necrosis of cells surrounding the central lobular vein. In the liver of C3H/HeJ, we observed extensive necrosis mainly in the portal triad with blood ectasia and disruption of hepatic cells. In the lung, the most characteristic modification was observed in the C3H/HeJ strain, displaying extensive bleeding and swelling of the alveoli and formation of a hyaline plate. The morphological differences observed between the three analyzed mouse strains could reflect the differences in the progression of the diseases. In fact, infected C3H/HeJ mice died earlier correlating with extensive cell necrosis and hemorrhage in the organs.

Supported by: FAPESP and CNPq

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5.06 Development of a microbial cell display system for the expression of heterologous lipoproteins on the surface of *E.coli*

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Introduction: The display of proteins or peptides on the surface of microbial cells has a wide range of biotechnological applications, including live vaccine development, bioadsorbents for the removal of harmful chemicals and heavy metals, biocatalysts and biosensors by anchoring enzymes, receptors or other signal-sensitive components, antigen delivery and screening of peptide or protein libraries. Several cell-surface display systems have been described in the literature. Most of them are based on anchoring motifs present on the outer membrane proteins, lipoproteins, secreted proteins or subunits of surface appendages of bacteria. **Objectives:** In the present study, we aimed to construct a cell display system for the expression of heterologous lipoproteins on the surface of *E. coli* using the signal sequence (Lpp) and lipoprotein signal peptidase recognition site (LSP) of the major *E. coli* lipoprotein. **Methods:** Four oligonucleotides were synthesized, that when annealed and ligated encode the *E.coli* Lpp signal sequence, LSP recognition site and outer membrane targeting signal. The construct was designed with overhangs to allow direction cloning into NdeI and XhoI digested pET-37b(+) vector. Five restriction sites were included downstream of the outer membrane sorting signal to provide a multiple cloning site. This new vector was named pLIPO. To test the capacity of this system to express heterologous lipoproteins, four genes encoding probable outer membrane lipoproteins from *Leptospira interogans* sorovar Copenhageni were cloned into the pLIPO. The recombinant lipoproteins were expressed and characterized by globomycin assay and their surface expression was detected by FACS analysis. **Results and Discussion:** Our results demonstrated that mature portions of four leptospiral lipoproteins cloned into the pLIPO vector were expressed, lipidated and exposed on the surface of *E.coli*. This new cell display system for the expression of heterologous lipoproteins on the surface of *E.coli* can potentially be applied for functional characterization of lipoproteins, which are important structural and functional components of bacteria.

Supported by: CNPq and FAPESP

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5.07 Studies on the mechanism of the neutralization of VapC by VapB *of* the toxin-antitoxin system VapBC of *Leptospira interrogans*

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Introduction: Toxin-antitoxin (TA) *loci* are ubiquitous in prokaryotes. The analysis of the *L. interrogans* genome allowed the investigation of the TA system VapBC. TA modules are bicistronic operons that encode a stable toxin and an unstable antitoxin. Their general function remains controversial but the most probable hypothesis is the cessation of growth under conditions of nutritional or environmental stress. The *vapBC* operons constitute the largest family of bacterial TA modules, grouped due to the homology of a PIN domain of the toxin, which is thought to act as ribonuclease. This activity was observed in VapC from *H. influenzae* and was inhibited by VapB. We have cloned the *vapBC locus* of *L. interrogans* and expressed the cognate proteins in order to study their properties. **Objectives:** The aim of this study was to determine whether VapB neutralization of the inhibition of cellular growth caused by VapC activity is in fact related to the binding of the two molecules. **Methods:** *vapC*, *vapB* and the *locus vapBC* of *L. interrogans* were amplified, cloned in pAE vector and expressed in *E. coli* BL21(DE3). Proteins were purified by Ni^{+2}-Sepharose chromatography, and refolded by pressurization when necessary. The structural integrity was analyzed by circular dichroism spectroscopy (CD). Interaction was tested by pull-down assay and affinity blotting. **Results and Discussion:** VapB was obtained in soluble form while VapC was expressed insoluble in *E. coli*. Solubilization and refolding of VapC were achieved by pressurization in the presence of L-arginine. About 5 to 15 mg/L of culture of VapB, VapC and VapBC were recovered. CD showed a mixture of α-helix, β-strand and coil in VapB secondary structure and a predominance of α-helices in VapC, suggesting that they are likely folded and functionally active. The statement that the toxic effect of VapC is neutralized by VapB was confirmed by observing that the growth of *E. coli* expressing VapC is restored by the co-expression of VapB. The hypothesis that this neutralization would be caused by the interaction between these proteins was demonstrated by a pull-down assay, where the supernatant of lysed *E. coli* cells transformed with pAE-*vapBC* submitted to Ni^{+2}-chromatography resulted in co-elution of VapB and VapC, although only VapB was fused with His-tag, indicating that the binding between these proteins may have occurred in the bacteria and could be the reason for abolishing the toxic activity of VapC. The binding between purified rVapB and rVapC was observed *in vitro* by affinity blotting, confirming that the proteins were obtained in active form, keeping their natural binding affinity.

Supported by: FAPESP, PAP/SES and Fundação Butantan

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5.08 Type IV pilus in atypical enteropathogenic *Escherichia coli*: characterization and investigation into the possible role of PilS and PilV in the interaction with epithelial cells *in vitro*

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Introduction: Enteropathogenic *Escherichia coli* is the main etiologic agent of acute diarrhea in developing countries. Pathotype strains that have been of concern cause a characteristic histopathological lesion known as attaching and effacing, which is encoded by genes located on a pathogenicity island called locus of enterocyte effacement. EPEC is divided into typical and atypical, and this ranking is based on the presence of the EAF plasmid, *bfp* gene and expression of *bundle-forming pilus* only in typical EPEC. This fimbriae pattern is responsible for localized adherence of bacteria to epithelial cells and promotes and stabilizes bacterial attachment within microcolonies. Several virulence factors are implicated in the pathogenesis of *E. coli*, among which detach the fimbrial adhesion involved in adhesion of bacteria to the enterocyte. The type IV pilus has an important role in adherence to epithelial cells *in vitro*, and while the atypical EPEC does not express the BFP adhesin, the characterization of other adhesins contribute to the understanding and study of virulence factors in this subcategory. **Objectives:** The aim of this study was to evaluate the role of PilS and PilV proteins in the interaction of the atypical EPEC isolates with epithelial cells *in vitro*. **Methods:** By PCR, we investigated the *pilS* and *pilV* genes in 72 strains of atypical EPEC. After amplification, only three isolates (BA 558, BA 956 and BA1244) were positive for both genes, which were selected for cloning and expression of PilS and PilV. After obtaining the recombinant protein Pil, this was used to immunize rabbits to obtain polyclonal antibodies. **Results and Discussion:** The three isolates were submitted to adhesion assays for phenotypic characterization using epithelial cells HEp-2, Caco-2, T84 and HT29 with incubation period of 6 h in the presence and absence of mannose. The strain showed the phenotype BA 558 AL in 4 cell lines used, the BA 956 had isolated IND phenotype in Caco-2 and T84, and HT29 cell phenotype NA, and HEp-2, BA 1244 showed the isolate phenotype in ALL cells Caco-2 and T84, and HT29 cell phenotype IND. Later, we will perform immunostaining assays and Western blotting using the polyclonal serum in bacterial isolates, as well as assays of inhibition of adhesion and biofilm formation, to define the role of type IV pilus in atypical EPEC.

Supported by: FAPESP

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5.09 The influence of kefir on biofilm production

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Introduction: Kefir is a beverage derived from milk fermentation by a symbiotic association of lactic acid bacteria and yeast maintained in a polysaccharide matrix in granular form, called kefiran. For more than 2000 years, ancient populations have empirically used kefir as an energy supplement, modulator of immune response and remedy for infectious diseases. However, kefir's properties may vary according to its fermentation cycle, origin and grain maintenance. Our earlier studies have shown that kefir studied in our laboratory produces a component resistant to lyophilization, of molecular mass under 1500 Da capable of inhibiting the proliferation of yeast and Gram-negative and Gram-positive pathogenic bacteria. **Objectives:** The objective of our study was to determine the effect of kefir on biofilm production by O111:H21, a diarrheagenic strain of *Escherichia coli*. **Methods:** Our bacterial isolate was incubated in 96-well plates with tryptic soy broth for 24 h with and without previously lyophilized and non-lyophilized kefir. After incubation, the plate was washed with PBS, fixed with 75% ethanol and the remaining bacteria contained in the biofilm were stained with crystal violet solution. After staining, the crystal violet solution was solubilized with 95% ethanol. The solution was transferred to a microtiter plate and the results obtained by optic density reading at 595 nm on a Multiskan EX plate reader. **Results and Discussion:** The results showed that only the non-lyophilized kefir was capable of inhibiting biofilm formation by the bacteria. In contrast to the component(s) responsible for inhibiting bacterial proliferation, which is (are) resistant to lyophilization, the component(s) responsible for inhibiting biofilm formation is (are) volatile or does (do) not maintain its (their) original structure(s) after lyophilization. Our next step will be the characterization of this (these) component.

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5.10 BRASIL methodology for selection of *Leptospira interrogans* adhesins

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Introduction: *L. interrogans* causes one of the most common zoonotic diseases in the world, leptospirosis. The development of vaccines has been pursued as a strategy for its prevention. At present, developed vaccines are not effective or ideal. Protein antigens, such as adhesins, conserved among pathogenic serovars, may contribute to overcoming the limitations of the currently available vaccines. **Objectives:** The aim of this study was to develop a shotgun phage display system for direct selection and identification of *L. interrogans* adhesins using the BRASIL (Biopanning and Rapid Analysis of Selective Interactive Ligands) method. **Methods:** Four libraries by insertion of randomly fragmented genomic DNA from *L. interrogans* serovar Copenhageni into the phagemid vector pG8SAET (BBT1 and BBT2) and pG3DSS (BBT5 and BBT6) were constructed. The libraries contain inserts with average size of 1500 bp (BBT1 and BBT5) and 345 bp (BBT2 and BBT6). The phage library BBT5 was used for biopanning against LLC-PK1 cells using the BRASIL method. Random clones from the second panning cycle without amplification were sequenced. **Results and Discussion:** It was possible to find only one clone in phase with the PIII protein, which code for a possible lipoprotein. Its characterization and validation as a true adhesin is underway.

Supported by: FAPESP, CNPq and Fundação Butantan

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5.11 Recombinant antibodies against EspB protein and Stx1/Stx2 toxins as tools for immunodiagnosis of enteropathogenic and Shiga toxin-producing *Escherichia coli*

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Introduction: Enteropathogenic (EPEC) and Shiga toxin-producing *Escherichia coli* (STEC) are recognized agents of infantile diarrhea and hemorrhagic colitis/hemolytic uremic syndrome, respectively. In Brazil, the diagnosis of diarrheal disease caused by EPEC and STEC is performed using traditional bacteriological methods, which depend on the isolation of the bacteria and their subsequent biochemical identification, followed by serogrouping and determination of specific virulence factors by phenotypic and/or genotypic methods. These methods are time-consuming and very expensive for our economic reality. Therefore, the development of inexpensive and rapid diagnostic methods for these important enteric pathogens, such as immunodetection, is a need for our reality. **Objectives:** This work aimed at the production of recombinant antibodies against the secreted protein EspB and Stx1 and Stx2 toxins to be used in the immunodiagnosis of EPEC and STEC, respectively. **Methods:** The hybridomas secreting antibodies against the EspB protein and Stx1, Stx2 toxin, used in this study were obtained by the Laboratory of Bacteriology (Instituto Butantan) in previous studies, and their RNA was extracted (RNeasy® Plus mini Kit QIAGEN) and transformed into cDNA (First Srand cDNA Synthesis Kit FERMENTAS). The cDNAs were used as template for amplification of mouse antibody heavy and light chains by PCR using random primers. These sequences were cloned in pGEMT-Easy vector (Promega) and sequenced to confirm the correct amplification and cloning. Both fragments, corresponding to the heavy and light antibodies chains, were later joined by a peptide linker by PCR, obtaining the scFv fragments. This recombinant antibody fragments were cloned into pAE and pSUMO vectors and transformed in *E. coli*. **Results and Discussion:** The in silico modeling of these recombinant fragments showed the correct conformation of the antibodies towards their respective antigens. The scFvs obtained were successfully cloned in two different expression vectors (pAE and pSUMO) and maintained in *E. coli* hosts. Obtaining these recombinant antibodies is a promising tool for the rapid diagnosis of these pathogenic strains, as these tend to be more sensitive and specific than monoclonal antibodies and, in addition, their production is faster and less expensive.

Supported by: FAPESP

5.12 Characterization of *Leptospira interrogans* Lp35 protein-binding adhesive matrix molecules

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Introduction: Leptospirosis is an infectious disease caused by pathogenic leptospires that are transmitted to humans through direct contact with infected animals, or through indirect contact with water or soil contaminated with the urine from infected animals. Leptospires enter the body by penetrating mucous membranes or broken skin and disseminate via the bloodstream to colonize the renal tubules of hosts. The colonization and survival of leptospires in the host require several types of surface-exposed proteins, such as lipoproteins, porins, adhesins and others. The protein chosen for this work is an outer membrane lipoprotein of *Leptospira interrogans* serovar Copenhageni that displays extracellular matrix-binding properties. **Objectives:** The aim of this work was to study the capacity of Lp35 protein to mediate attachment to extracellular matrix molecules (ECM). **Methods:** The recombinant 6xHis-tagged protein expressed in *Escherichia coli* was purified from the insoluble fraction by nickel affinity chromatography, and characterized by circular dichroism spectroscopy (CD). The capacity of recombinant purified protein to bind to ECM components was evaluated by ELISA and Western blotting methods. **Results and Discussion:** The structural integrity of Lp35 recombinant purified protein was assessed by CD spectroscopy, which revealed a predominant β-sheet secondary structure, and showed successful refolding. Furthermore, recombinant Lp35 was able to bind strongly to laminin, collagen type I, collagen type IV, cellular fibronectin, plasma fibronectin and matrigel. Lp35 protein is probably a leptospire antigen involved in adherence to host tissues.

Supported by: CNPq and FAPESP

5.13 Do atypical enteropathogenic *Escherichia coli* (aEPEC) secrete a factor capable of inhibiting the adherence to epithelial cells?

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Introduction: Our group has demonstrated that two aEPEC (O55:H7) isolates prevent phagocytosis through a secreted factor. The bacterial culture supernatants from these samples were able to prevent adhesion of bacteria, *Saccharomyces cerevisiae* and latex to macrophages. The macrophages are professional phagocytes with very distinct characteristics when compared to epithelial cells, to which bacterial adhesion is essential for the establishment of infection. EPEC colonizes the intestinal mucosa inducing a lesion called A/E lesion (attaching and effacing) that is believed to be related to diarrhea. **Objectives:** The aim of this study was to determine if the anti-phagocytic factor would also prevent adhesion of EPEC to epithelial cells. **Methods:** LB 7 and BA 320 culture supernatants from aEPEC and C600, grown in TSB or M9 media, were fractionated by solid phase extraction (SPE) eluted with 0, 25, 50, 75, and 100% acetonitrile (ACN). HEp-2 and Caco-2 epithelial cells pre-incubated with the different fractions were infected with the EPEC prototype E234869 for 3 h, washed and further incubated for 3 h. They were washed again, fixed and stained for light microscopy analysis. **Results and Discussion:** The fraction eluted with 25% ACN (containing the antiphagocytic factor) significantly inhibited adhesion of the bacteria to the cells. When added to previously infected cells, this fraction caused the release of the adhered bacteria, without interfering in cell viability, which was not observed when the cells were incubated with fractions obtained from C600, TSB or M9 Although the anti-phagocytic effect is beneficial to the bacteria, prevention of adhesion should not be, but the later effect only occurs when the supernatant is fractionated and concentrated. So prevention of adhesion may be due to the higher concentration of a factor causing it or to the absence of a necessary one, eliminated by fractionation. In addition, there is no evidence, other than both being in the same SPE fraction, that the anti-phagocytic factor and the anti-adhesion factor are the same. In any case, the existence of a soluble secreted factor capable of preventing bacterial adhesion to the epithelium raises the possibility of controlling colonization, although more is necessary to fully characterize it.

Supported by: FAPESP and CAPES

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5.14 Influence of different growth conditions on biofilm formation by atypical enteropathogenic *Escherichia coli*

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Introduction: Atypical enteropathogenic *Escherichia coli* (aEPEC) strains are emerging enteropathogens that have been detected worldwide, mainly causing infant diarrhea. The adhesion in epithelial cells forming microcolonies is one of the first steps towards biofilm development. Biofilms are formed by bacterial layers adhered in different surfaces, wrapped by an extracellular matrix, and they are associated with bacterial persistence and to antimicrobial resistance. **Objectives:** The aim of this study was to determine how different growth conditions effect biofilm formation by atypical enteropathogenic *Escherichia coli*, as well as to investigate the presence of *csgA* gene and expression of cellulose and *curli* fimbriae. **Methods:** Twenty-three aEPEC strains were tested through crystal violet colorimetric assay to determine biofilm formation on an abiotic surface, using the following culture media: LB broth, LB broth without NaCl, *E. coli* broth, minimum M9 and DMEM, at 26°C and 37°C of incubation. We observed the expression of *curli* fimbriae and cellulose after incubation in Congo red agar and in Calcofluor agar, respectively. The aEPEC strains were probed by PCR for the presence of the *csgA* gene (major subunit of *curli*). **Results and Discussion:** The aEPEC strains were able to form biofilm sy host body temperature and at environmental temperature, displaying its survival outside the host and enhancing its pathogenicity. Quantification of biofilm formation through the crystal violet assay showed that 38.5% of the strains grown at 26°C in LB broth without NaCl and in *E. coli* broth showed high adherence, whereas only 7.7% of the strains grown in DMEM showed high biofilm formation. In *E. coli* broth at 37°C, 77% of the strains displayed high biofilm formation, whereas in DMEM only 7.7% were capable of forming biofilm at the highest level. The LB and minimum M9 broths did not induce high biofilm formation. Cellulose expression was found in 13 strains. The presence of the *csgA* gene was seen only in 7 strains and the *curli* fimbriae expression was detected in 8 strains. There was no correlation between the high biofilm formation and the cellulose and/or curli expression, suggesting the possible influence of the expression of other adhesins or exopolysaccharides, which will be investigated. The best incubation conditions to determine *curli* fimbriae and cellulose expression were 37ºC for 48 h and 37ºC for 24 h, respectively. The growth conditions such as culture medium and temperature must be used carefully to effectively determine the ability of biofilm formation by aEPEC strains.

Supported by: CNPq and CAPES

5.15 The virulence factors intimin and BfpA trigger TNF-α production

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is among the most prevalent agents of acute diarrhea, affecting especially children of developing countries. In children, illness becomes very serious due to the consequent strong dehydration, which can lead to death. Intimin and bundle-forming pilus (BFP) are virulence factors of EPEC, playing an important role in the pathogenesis of the infection. Intimin, the EPEC outer membrane adhesin, mediates intimate adhesion by binding to the cell membrane via its own translocated receptor (Tir). The type IV pilus BFP is responsible for the formation of EPEC microcolonies and is involved with the initial attachment to host cells. BFP is composed of several subunits represented mainly by BfpA. Both intimin and BFP trigger immune responses after natural infection. The contribution of intimin and BFP to the macrophage activation during innate immune response is unknown. Macrophages are present in various tissues and respond rapidly to environmental signals that are generated following infection. These stimuli are recognized by receptors important to design the innate and adaptive immune response. LPS stimulates toll-like receptor 4 (TLR-4), resulting in the activation of intracellular signaling pathways and production of pro-inflammatory cytokines. **Objectives:** The aim of this study was to evaluate the ability of recombinant intimin and BfpA proteins to induce TNF-α using two models: bone marrow derived macrophages (BMDMϕ) from C3H/HeJ (tlr4 $^{-/-}$) mice and macrophage cell line J774-A1, which differ in sensitivity to endotoxin. **Methods:** BMDMϕ from C_3H/HeJ mice were differentiated for 7 days in complete RPMI medium plus L929 cell supernatant and antibiotic. On the 7th day, the adherent cells were stimulated with either intimin or BfpA (1, 5 and 10 μg/mL). J774 cell were cultured in RPMI medium plus 10% FBS and antibiotic. The cells were stimulated with intimin, BfpA (1, 5 and 10 μg/mL) or LPS (10 μg/mL) plus polymyxin B or proteinase K or BSA (10 μg/mL) as control of specificity. After 20 h of incubation, the supernatants were collected for TNF-α analysis using ELISA. **Results and Discussion:** The level of TNF-α produced by activation of J774 cell with intimin and BfpA in the presence of polymyxin B was similar to that found in BMDMϕ from C3H/HeJ mice (tlr4 $^{-/-}$). The treatment with intimin and BfpA with proteinase K inhibited the synthesis of TNF-α by J774 cells in contrast to that obtained with LPS, whose activity was blocked by polymyxin B. Both proteins induced significant production of TNF-α independent of TLR4 activation. Our data demonstrated that both intimin and BFP are able to activate macrophages during the innate immune response.

Supported by: CNPq and FAPESP

5.16 The influence of kefir on toxin release by pathogenic bacteria

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Introduction: The rising increase of bacterial resistance to antibiotics creates a constant search for molecules with antimicrobial activity. **Objectives:** Due to kefir's ability to inhibit bacterial proliferation, the objective of this study was to determine the influence of kefir supernatant on the release of hemolysin-α, caseinase, urease and arginase by a *Pseudomonas aeruginosa* sample isolated from the urine of a patient with a clinical diagnosis of urinary infection. In addition, a search was conducted for the biologically active component after the material has been fractioned by molecular mass. **Methods:** Kefir beverage derived from the fermentation of whole milk was centrifuged twice at 8000 rpm for 15 min, and its supernatant was lyophilized and resuspended in MilliQ water to obtain the original volume. Fractions of kefir containing different molecular mass were obtained by filtration through 10 to 3 kDa membranes (Centriprep-Millipore™) and by dialysis with a 1 kDa membrane. The bacterial sample was incubated for 18 h in tryptic soy broth at 37° C in the presence and absence of the kefir supernatant and its fractions. After this period, 10 μL of our bacterial sample was added to blood agar plates, which were incubated for 3 h at 37° C to evaluate hemolysin-α production by the formation of a hemolysin halo around the culture. Other 10-μL aliquots were applied to LB agar plates containing 0.6% casein. After 18 h incubation at 37°C, the release of caseinase was shown by the presence of a transparent halo around the culture after adding 3% acetic acid to the plate. The production of urease and arginase was detected by a biochemical identification system, API 20NE (Biomerieux™). **Results and Discussion:** The results showed that the kefir supernatant and its fraction with a molecular mass below 1 kDa were capable of inhibiting the release of all toxins tested. In summary, the results indicate that kefir has potential as an antimicrobial agent.

5.17 Detection of parainfluenzavirus 1, 2 and 3, in children from São Paulo, city, from 1995 to 2006

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Introduction: Human parainfluenza viruses (HPIVs) are an important cause of lower respiratory tract infections in infants, young children and immunocompromised patients, and have a worldwide distribution. These viruses belong to the order *Mononegavirales* and family *Paramyxoviridae.* There are 5 viruses distributed into two distinct genera: *Respirovirus* (HPIVI-1 and HPIV-3) and *Rubulavirus* (HPIV-2, HPIVI-4A and HIVI-4B). In the pediatric population, the characteristic illness associated with HPIV-1 and -2 is laryngotracheobronchitis (croup), but it can also be responsible for upper respiratory tract infection and pharyngitis, whereas HPIV-3 is also associated with pneumonia and bronchiolitis. The diagnosis cannot be based only on clinical signs and symptoms, because other respiratory pathogens can cause similar syndromes. Rapid laboratory diagnosis can impact positively patient care and treatment. **Objectives:** The aims of this study was to optimize a multiplex RT-PCR for the detection of HPIV-1, -2 and -3 in a single reaction and to use this method to detect the viruses circulating in children hospitalized in São Paulo city, Brazil. **Methods:** Nasopharyngeal aspirates from 2152 infants and children under five years old, hospitalized at the University of São Paulo Hospital (HU-USP) with acute respiratory illness were collected from 1995 to 2006. HPIV 1, 2 and 3 detection was performed by multiplex RT-PCR using specific primers to HN gene, labeled with FAM. **Results and Discussion:** A total of 6% (n=135) of samples were positive for one of the HPIV, and the HPIV-3 was the most prevalent virus during all years studied, corresponding to 80% (108/135) of positive cases, followed by HPIV-1 with 15% (20/135) and HPIV-2 with 7% (10/135). The positivity among the years studied ranged from 1% (1/195) in 1996 to 18% (24/154) in 1999. The average age of children infected with parainfluenza virus was 6 months. The multiplex RT-PCR proved to be a rapid, sensitive and simple method for the diagnosis of HPIV-1, -2 and -3.

Supported by: FAPESP

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

5.18 Detection of *Rickettsia bellii* in the tick *Ornithodoros brasiliensis* aragão (Acari: Argasidae)

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Introduction: Argasid ticks could be naturally infected with microorganisms and they are potential vectors of spirochaetes (*Borrelia* spp.) and arbovirus, causing diseases in humans and animals. However, they are not traditionally considered important as vectors of rickettsiosis. There are few contributions about argasid ticks and their relationship with rickettsial agents in the literature. The species *Ornithodoros moubata* (Etyopic region), *Ornithodoros kelleyi* (Nearctic region, Cuba and Costa Rica), and *Ornithodoros capensis* (Etyopic, Nearctic, Neotropical, Oriental and Palearctic regions) have been found naturally infected by *Rickettsia akari*, *R. australis* or *R. hoogstraalii*. **Objectives:** In Brazil, the endemic species *Ornithodoros brasiliensis* was investigated by the presence of pathogens because it is very aggressive to humans and domestic animals, causing fever and severe lesion at the tick bite site. **Methods:** Specimens collected on the ground of rural areas in southern Brazil were processed by PCR targeting the rickettsial *gltA* gene. **Results and Discussion:** A total of 25% of the ticks were shown to contain rickettsial DNA 99% similar to *R. bellii*. Although this *Rickettsia* species does not belong to the spotted fever group and its pathogenicity is unknown, this is the first record of a Brazilian argasid naturally infected by rickettsiae.

Supported by: FAPESP and CNPq

5.19 Preliminary identification of secreted proteins by *Leptospira interrogans* serovar Pomona strain Fromm

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Introduction: Leptospirosis is a zoonosis of worldwide distribution caused by pathogenic spirochetes of the genus *Leptospira*. The mechanisms by which leptospires invade the host and cause the disease are not fully understood, but experimental results have shown that the pathogenesis may be related to the ability of these bacteria to bind to extracellular matrix proteins, to escape the host's immune responses and to produce toxins. Up to now, there are no published data of secreted proteins by leptospires identified using proteomic approaches, despite the existence of homolog genes in the *L. interrogans* genome that encode some transport systems known in other bacteria. **Objectives:** This project aimed to identify secreted proteins by pathogenic *Leptospira interrogans* serovar Pomona strain Fromm by proteomic analyses. **Methods:** *L. interrogans* serovar Pomona strain Fromm, whose virulence was maintained by passages in hamsters, were cultured in EMJH medium at 29°C or 37°C. The supernatants were centrifuged, dialyzed and subjected to lyophilization. Protein samples were resolved first by IEF at pH 3 to 10, immobilized pH gradient 13-cm strips. Strips were then processed for the second-dimension separation on 12.5% SDS-polyacrylamide gels. Proteins from gel spots were subjected to reduction, cysteine-alkylation, and in-gel tryptic digestion, and analyzed by LC/MS/MS spectrometry. Liquid chromatography-based separation followed by automated tandem mass spectrometry was also used to identify secreted proteins. *In silico* analyses were performed using the PSORTbV.3.0 program and SignalP server. **Results and Discussion:** Our analysis resulted in the identification of 200 proteins with high confidence. Only 5 of 63 secreted proteins predicted by *in silico* analysis were found. Other classes identified included proteins that possess signal peptide but whose cellular localization prediction is unknown or may have multiple localization sites, and proteins that lack signal peptide and are thus thought to be secreted via nonconventional mechanisms or resulting from cytoplasmic contamination by cell lysis. Many of these are hypothetical proteins with no putative conserved domains detected. To our knowledge, this is the first study to identify secreted proteins by leptospires using a proteomic approach. The identification of these proteins will contribute to the elucidation of the pathogenic mechanisms and development of novel strategies for the treatment and prevention of leptospirosis.

Supported by: LNBio/LNLS, CNPq, CAPES and FAPESP

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

5.20 Influence of Hfq on the interaction of enteropathogenic *Escherichia coli* to epithelial cells *in vitro*

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Introduction: The term atypical EPEC is used to define the enteropathogenic *Escherichia coli* that does not carry the EAF plasmid (EPEC adherence factor). The atypical EPEC may belong to the traditional EPEC serogroups, but some strains belong to different serogroups. Divergent from typical EPEC, which show a localized pattern of adherence in epithelial cells in vitro, AEPEC strains can adhere to epithelial cells in different patterns, including localized-like, aggregative and diffuse adherence. Samples of typical and atypical EPEC have in their genome the pathogenicity island called LEE (locus of enterocyte effacement) region, which contains genes related to the formation of a histopathological lesion called attaching and effacing (A/E). LEE region gene transcription is regulated by quorum sensing, which is a chemical signaling process realized through the secretion of small metabolites called auto-inducers (AI). The involvement of small RNAs regulating quorum sensing processes is well established in *Vibrio cholerae,* where RNAs interact with Hfq chaperone and form a complex called Qrr (quorum sensing regulatory RNA). This complex acts in LuxR Qrr, increasing or decreasing its production, directly linked to bacterial density in biofilm formation. **Objectives:** So far, no studies show the involvement of Hfq in EPEC, and thus, Hfq mutants were analyzed for their ability to adhere to HEp-2 cells in vitro. **Methods:** Mutants of typical and atypical EPEC strains were obtained, using the lambda Red system & allelic recombination. **Results and Discussion:** Mutants showed alterations in their adherence capacity, indicating the involvement of Hfq in this process. Based on the capacity of the Hfq protein interactions with RNAs, an analysis of these mutants under different growing conditions will be performed. It is estimated that there are around 100 units, and these molecules have an essential role in post-transcriptional regulation.

Supported by: CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

5.21 Analysis of plasmid profile in samples of atypical enteropathogenic *Escherichia coli*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is one of the main agents of diarrhea in children in developing countries. This pathotype can be classified into two groups: typical EPEC (t-EPEC), and atypical EPEC (a-EPEC). Samples of both EPEC groups have in common the ability to cause injury to the intestinal epithelium known as "attaching and effacing" (A/E). The main feature that distinguishes a-EPEC is the fact that the samples of this group do not harbor the pEAF plasmid ("EPEC adherence factor"). pEAF contains the genes encoding fimbriae of the type IV BFP ("bundle forming pillus") and gives t-EPEC the phenotype of localized adherence (LA) to epithelial cells. a-EPEC is generally characterized as showing localized adherence-like (LAL), but may also show diffuse adherence (DA), aggregative adherence (AA) or maybe non-adherent (NA) or indeterminate (IND). Samples of a-EPEC are also known to compose an extremely heterogeneous group of pathogens that are able to express virulence factors described in other pathotypes of diarrheagenic *Escherichia coli* (DEC). **Objectives:** The aim of this study was to perform a plasmid profiling of 78 samples of a-EPEC. **Methods:** Plasmids were obtained using commercial kits with some modifications. **Results and Discussion:** The plasmid profiles obtained allowed us to verify that among the 78 samples analyzed, 12 did not harbor plasmids, 33 plasmids had between 50- and 90-kb plasmids and 38 had between 90- and 124-kb. The survey of incompatibility groups revealed that groups IncFIB and IncF were the most frequent among the samples of a-EPEC. RFLP analysis of plasmid DNA from strains of serotype O55: H7 suggested that there are common nucleotide sequences between plasmids. These data also allowed us to infer the existence of common plasmid DNA fragments from samples of EHEC O157: H7 and samples of a-EPEC O55: H7. The biological function of a-plasmid of EPEC and its relationship to plasmid PO157 require additional studies.

Supported by: CNPq

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5.22 Performance of Quantiswab™ in microbial monitoring of environmental surfaces

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Introduction: Surface monitoring is an important control to be performed in aseptic processing. Surface sampling may be accomplished by the use of contact plates or by the swabbing method. The swabbing method may be used for sampling of irregular surfaces, especially for equipment. Swabbing is used to supplement contact plates for regular surfaces. The area to be swabbed is defined using a sterile template of appropriate size. In general, it is in the range of 24 to 30 cm^2. The microbial estimates are reported per contact plate or per swab. Quantiswab™ includes a screw cap tube with a Nylon™ flocked swab attached to the cap and a tube filled with a specified volume of appropriate diluents (SRK). The purpose of this product is to improve microorganism recovery capacity and, moreover, not to leave a residue on the surface after contact. **Objectives:** The aim of this study was to improve the surface microbial monitoring of clean rooms and other controlled environments using Quantiswab™ by evaluating the performance on microbial release and recovery. **Methods:** Standard microbial strains were utilized at concentrations from 10 to 100 CFU/mL (*Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 14458, *Bacillus subtilis* ATCC 6633) and wild strains isolated from environmental microbial monitoring of production clean rooms (*Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp.). Tests evaluated the preservation of microbial viability in the SRK diluents, swab ability of microbial release into SRK, microbial recovery by direct plating from swab and on Quantiswab™ sensitivity to detect low levels of contamination. All tests were performed in triplicate, employing pour plate and spread plate methods and incubated at 30 °C-35 °C, for 5 days. **Results and Discussion:** We found 67 to 166% microbial recovery when evaluating the ability to preserve microbial viability in SRK. Quantiswab™ demonstrated a satisfactory capacity of microbial release into SRK, revealed by characteristic microbial growth of all microorganisms inoculated; 50 to 150 % microbial recovery obtained from direct plating and satisfactory sensitivity to detect low levels of contaminants on the surface sampled. The results allow the immediate establishment of Quantiswab™ for microbial monitoring of clean rooms, having been assured of their performance with regard to preservation, release and recovery of microorganisms and detection of low levels of contaminants.

Supported by: Fundação Butantan

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5.23 Application of BacT/ALERT 3D system in production control of adsorbed diphtheria and tetanus vaccine (dT)

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Introduction: BacT/ALERT 3D system (B/A) is an automatic equipment used for early detection of contamination, and the bottles containing culture medium provide nutritional conditions suitable for the growth of microorganisms. This system uses a colorimetric sensor that measures reflected light to monitor the presence and production of carbon dioxide dissolved in culture medium, produced from microbial metabolism. Adsorbed diphtheria and tetanus vaccine (dT) includes thimerosal as preservative in the formulation. Bacterial and fungal sterility test (ST) of dT is made by membrane filtration (MF) with visual analysis over 14 days. **Objectives:** The aim of this study was to implement B/A utilization in the control process during dT production, to demonstrate the importance of preservative (thimerosal) neutralization, and to evaluate B/A sensitivity to detect low levels of contaminants in dT. **Methods:** We used three batches of dT previously approved on ST. B/A SN and B/A SA media were used for the growth of anaerobic and aerobic microorganisms, respectively. Each bottle (B/A SN or B/A SA) was inoculated with 1.0 mL of sample, 1.0 mL of diluent neutralizing pharmacopoeic (DNP) and 1.0 mL of ATCC microorganism dilution (10-100 UFC/mL) of the following ATCC strains: *Aspergillus brasiliensis*, *Bacillus subtilis* and *Candida albicans* (B/A SA); *Clostridium sporogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (B/A SN). **C**ulture medium sensitivity was tested with negative control (sterile DNP) and positive controls (ATCC microorganism dilutions without product). **Results and Discussion:** The three batches tested did not reveal the presence of contamination, like in ST. B/A SN and B/A SA media tested with the product and inoculated with microorganism dilutions detected ATCC strains with the following average time recovery: *A. brasiliensis* 297.6 h, *B. subtilis* 15.1 h, *C. albicans* 73.5 h, *P. aeruginosa* 32.0 h, *C. sporogenes* 74.8 h, and *S. aureus* 17.9 h. Negative control showed no microbial growth and positive controls revealed the following recovery times: *A. brasiliensis* 37.4 h, *B. subtilis* 11.0 h, *C. albicans* 36.9, *P. aeruginosa* 26.4 h, *C. sporogenes* 26.4 h and *S. aureus* 15.8 h. Five microorganisms had a low average detection time of 75.0 h. *A. brasiliensis* was detected in only one of three bottles. Preservative incorporated in product formulation (thimerosal) does not prevent B/A SN and B/A SA detection of low levels of contaminants, but times for microorganism recoveries were greater because thimerosal has a bacteriostatic and fungistatic effect. The results showed that the B/A system can be used in the dT production process, reducing significantly product retention time.

Supported by: Instituto Butantan

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5.24 Validation of sterility test in isolator of diphtheria and tetanus toxoids formulated at Butantan Institute by means of bacteriostatic and fungistatic effect

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Introduction: Bacterial and fungal sterility test (ST) of diphtheria and tetanus toxoids formulated at Butantan Institute is done by membrane filtration (0.45-µm pore size) and diluent neutralizing pharmacopoeic (DNP) is used to rinse the membrane to neutralize the preservative (thimerosal). Subsequent incubation is carried out in fluid thioglycollate medium (FTM) and soybean-casein digest medium (SCM). The current standards require that all operational procedures used in quality control must be validated according to Current Good Laboratory Practice (cGLP) and this procedure ensures analysis and product quality. Diphtheria toxoid and tetanus toxoid are components of adsorbed diphtheria, tetanus and pertussis vaccine (DTP) and adsorbed diphtheria and tetanus vaccine (dT), both produced at Butantan Institute. **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied in ST to diphteria and tetanus toxoids in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of diphteria toxoid and three batches of tetanus toxoid, all previously evaluated for thimerosal concentration by a spectrophotometric method. These bulks were tested according to standard methodology and membranes were rinsed with DNP. After product filtration, an inoculum of viable ATCC microorganisms (10-100 cfu/mL) was added to the final portion of DNP used to rinse the membrane. Fluid thioglycollate medium was inoculated with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus* and soybean-casein digest medium, with *Aspergillus brasiliensis, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for not more than 5 days at 20 °C - 25 °C and 30 °C - 35 °C (SCM and FTM, respectively). **Results and Discussion:** Characteristic growth of all microorganisms was obtained after the incubation period. The methodology applied in the isolator to test bacterial and fungal sterility of diphtheria and tetanus toxoids is effective and thimerosal present in the product formulation as preservative was completely inactivated rising the membrane with DNP. We conclude that the method applied detects low levels of microbial contamination, providing the public with a save product (sterile) according to national and international standards. The sterility test may be carried out without further modifications.

Supported by: Fundação Butantan

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5.25 A variant of AAF/I fimbria of enteroagregative *Escherichia coli* is responsible for aggregative adherence in atypical enteropathogenic *Escherichia coli*

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Introduction: Atypical enteropathogenic *Escherichia coli* (aEPEC) are among the main agents of diarrhea in both developing and developed countries. In a previous study conducted by our group, an aEPEC (serotype O142:H34) harboring an unexpected combination of virulence genes was identified: the *eae* gene, which encodes the adhesin intimin of EPEC, and the *aatA* gene, which encodes a protein that is part of a protein secretion system in enteroaggregative *E. coli* (EAEC), and which is widely employed as a diagnostic probe fragment for EAEC. In addition, this strain expressed the aggregative adherence (AA) pattern in HEp-2 cells. **Objectives:** The main objective of this work was to characterize the adhesin that mediates the AA in this aEPEC strain. **Methods:** The aEPEC strain Ec46/88 (O142:H34) was selected for this work. Initially, the expression of some virulence genes of EPEC (*tir*, *eae*, *espA*, *espB* and *espD*) was determined by immunoblotting. The ability to cause the attaching-effacing (A/E) lesion, characteristic of EPEC, was evaluated by the falloidin actin staining (FAS) test on HEp-2 cells, HeLa, HT29 and T84. The presence of virulence genes of EAEC was investigated by PCR. The plasmid profile was determined by the alkaline lysis method, followed by Southern blot hybridizations with *aagA, aggR* and *aggC* probes. **Results and Discussion:** The FAS test demonstrated that the Ec46/88 strain was capable of causing the A/E lesion only in HT29 cells. The expression of some genes of EPEC by immunoblotting confirmed the characterization of this sample as aEPEC. Only the *shf*, *aggB*, *aggD* and *aggC* genes of EAEC were amplified by PCR. The *aggB*, *aggD* and *aggC* genes encode proteins involved in the biogenesis of the AAF/I fimbriae of EAEC, described in the prototype EAEC 17-2, were it is encoded by the *aggDCBA* operon. This finding indicated the presence of a variant of AAF/I, since the *aggA* and *aggR* genes were not detected with the primers used. The *aggC*, *aggB* and *aggD* amplified fragments were sequenced showing ~ 99% homology with the respective genes of EAEC strain 17-2. The analysis of the plasmid profile indicated the presence of a single plasmid of ~ 60 MDa. Since the operon *aggABCD* is located in a similar plasmid in EAEC 17-2, this finding suggested that the fimbrial operon in aEPEC Ec46/88 was in the plasmid. The results of Southern blot hybridizations demonstrated the presence of *aggA* and *aggC* in the plasmid. Moreover, unlike in EAEC 17-2, in aEPEC Ec46/88 the fimbriae that mediates AA is not regulated by AggR. Our data point out the possibility that this aEPEC strain has acquired the virulence plasmid of EAEC, thus expressing the AA pattern of adherence.

Supported by: FAPESP

6. Biotechnology

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6.01 Purification of polysaccharide from *Haemophilus influenzae* type b by hollow fiber membranes combined with enzymatic hydrolysis

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Introduction: *Haemophilus influenzae* b is a pathogenic bacterium responsible for pneumonia and meningitis in children under the age of two. The capsular polysaccharide is the main virulent agent, used as the antigen, in combination with a protein in the vaccine formulation. The classical polysaccharide purification process includes: five ethanol precipitations, phenol extraction and several continuous centrifugation/ultracentrifugation to remove the insoluble materials. The industrial installations require areas with protection against fire hazards, toxic and corrosive fumes and the capital cost investment in continuous centrifuge/ultracentrifuge and energy are very high. **Objectives:** The aim of this work was to replace the use of centrifugation during the ethanol precipitations steps introducing enzymatic hydrolysis before and after use of hollow fiber tangential microfiltration, making this process simple and reproducible. **Methods:** The supernatant from *H. influenzae* type b culture broth was diafiltered and concentrated using a tangential ultrafiltration membrane of 100 kDa cut-off. The concentrated fraction was treated with enzymes and was sequentially precipitated with ethanol to 30% final concentration and submitted to hollow fiber 0.2 μm tangential microfiltration. The precipitated fraction at 30% ethanol was discarded and the microfiltered 30% ethanol fraction was concentrated with a tangential ultrafiltration with a 50 kDa cut-off, to reduce the volume, and precipitated with ethanol to 80% of final concentration. Ethanol was removed by tangential microfiltration with hollow fiber 0.2 μm, and the microfiltered solution of 80% ethanol was discarded. The precipitated of 80% ethanol was solubilized with water and recirculated in the hollow fiber system with the permeate line closed. Extensive diafiltrations with water were introduced to recover the maximum PSb in microfiltrate fraction (2TMF0.2). The 2TMF0.2 was concentrated in 50-kDa tangential ultrafiltration membrane to reduce the working volume and submitted to enzymatic hydrolysis, and finally concentrated using a 100-kDa tangential ultrafiltration membrane to remove the residual contaminants, obtaining the purified PSb in the final fraction. The purified PSb reached the required purity, that is a minimum of 100 times, weight by weight, more PSb than protein or nucleic acids. **Results and Discussion:** The relative purity was $RP_{protein}$=141 mg PSb/mg prt±0.16 and $RP_{Nucleic\ acid}$=1155 mg PSb/mg AN±0.39 based on protein and nucleic acids, respectively. The purification of PS using hollow fiber membrane met WHO requirements for vaccine and the purification process showed technical feasibility for scaling up.

Supported by: FAPESP and Fundação Butantan

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

6.02 Characterization of immune response in mice to a novel leptospiral predicted outer membrane protein

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Introduction: Leptospirosis is an important zoonotic disease, widely spread around the world and caused by spirochetes of the genus *Leptospira*. The sources of leptospires are essentially wild and domesticated mammals harboring the spirochetes in the urinary system. With the recent sequencing of the genome of *L. interrogans*, several genes that encode proteins were identified allowing the testing of possible vaccine candidates and for the elucidation of the mechanisms involved in the pathogenesis of this disease. **Objectives:** This study aimed to evaluate the immune response in an animal model of a predicted lipoprotein, LIC13417, present in the *Leptospira*. **Methods:** The gene was cloned and expressed in *E. coli* using the expression vector pAE. The recombinant protein tagged with N-terminal hexahistidine was purified by metal chelating affinity chromatography and the secoundary structure analyzed by circular dichroism (CD) spectroscopy. Four-week-old BALB/c mice were immunized subcutaneously twice, at a 2-week interval and bled from the retro-orbital plexus, and the antibody response was evaluated by ELISA and Western blotting. The animals were sacrificed, and the splenocytes isolated for evaluation of lymphocyte proliferation and cytokine profiles in response to prime boosted antigen. The capacity of these proteins to mediate attachment to ECM and serum components was evaluated by binding assays. **Results and Discussion:** Our results with immunization assays showed that the recombinant protein rLIC13417 elicited a Th2 response as revealed by the increase in antibody titers during subsequent boosters. Altogether, a moderate Th1 response was stimulated as demonstrated by lymphoproliferation and production of IFN-gamma. The recombinant protein showed a strong attachment to plasminogen while no adhesion to extracellular matrix components was detected. We obtained a recombinant protein that will be used in immunization assays in hamsters followed by evaluation of immunoprotection activity.

Supported by: FAPESP, CNPq and Fundação Butantan

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

6.03 *Schistosoma mansoni*: Molecular characterization of Alkaline Phosphatase and expression patterns across life cycle stages

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Introduction: Schistosomiasis is a tropical neglected disease affecting more than 200 million people in the world, and the search for a prophylactic vaccine is the main goal established within the efforts made to eradicate the disease and morbidity caused by the blood fluke *Schistosoma mansoni*. As a preliminary screening of potential vaccine candidates, we cloned and characterized *Schistosoma mansoni* alkaline phosphatase (SmAP), previously identified in the tegument of adult worms. **Objectives:** The aim of this study was to characterize the molecular traits, to produce the recombinant protein and to evaluate the expression patterns of the SmAP along the parasite's life cycle; and to investigate the surface enzymatic activity in different parasite stages and its putative inhibition by antisera. **Methods:** Quantitative PCR (qPCR) was performed in a 7300 Real Time PCR System (Applied Biosystems). Expression of the recombinant protein was done in *E. coli* BL21 Star (DE3) pLysS, followed by purification in a 5-mL HisTrap column (GE Healthcare). For antibody production, rats were immunized with 100 μg of the purified recombinant protein following a three-dose scheme and these antisera were used for Western blotting, immunolocalization and activity inhibition experiments. Deglycosylation was done with PNGase F (New England Biolabs) and GPI-anchor detection with phosphatidylinositol phospholipase C (Sigma). Activity assays were done using the synthetic substrate p-nitrophenyl phosphate (pNPP, Sigma). **Results and Discussion:** SmAP encodes a complete sequence composed of 536 amino acids containing an N-terminal signal peptide, five N-glycosylation sites and a GPI anchor signal, similar to that described for mammalian orthologs. Real-time RT-PCR and Western blot experiments suggestted a rapid translation as soon as cercariae were transformed into schistosomula. Immunolocalization analysis shows that the protein is widely distributed in the worm tissues, with increased concentration in the vitelline glands of female parasites. It is N-glycosylated and GPI-anchored. There is an isoform only present in eggs, which might be O-glycosylated. Furthermore, the surface localization of this enzyme was quantitatively supported by its enzymatic activity in live *ex vivo* or cultured parasites throughout the life cycle stages. The fact that cercariae accumulate large amounts of SmAP mRNA, which rapidly translates into protein upon schistosomula transformation, indicates it may have an important role in host invasion.

Supported by: FAPESP

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

6.04 Induction of the immune response in mice to leptospiral surface proteins expressed in *Escherichia coli* in fusion to DnaK

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Introduction: Leptospirosis is an important global disease of human and veterinary concern. Caused by pathogenic *Leptospira*, the illness was recently classified as an emerging infectious disease. Humans are accidental hosts that can be infected by exposure to urine of chronically infected animals. Currently available veterinary vaccines do not induce long-term protection against infection and do not provide cross-protective immunity. Outer membrane proteins (OMPs) are attractive alternatives because of their antigenic conservation across leptospiral species and serovars. Several studies have suggested the use of DnaK as an antigen in the formulation of vaccines, due to an exceptional degree of immunogenicity (humoral and cellular) and the ability to stimulate T cells to produce IL10. **Objectives:** We focused on four surface proteins, LIC10368, LIC10494, LIC12690 and LIC12730, previously shown to be involved in host-pathogen interactions. Our goal was to evaluate the immunogenicity of the proteins genetically fused with DnaK in an animal model. **Methods:** The recombinant proteins were expressed in fusion with a 6xHis-tag and DnaK at the N-terminus. After purification by nickel-charged Sepharose beads, the recombinant proteins were analyzed by circular dichroism (CD) spectroscopy, which revealed well folded proteins. Four-week-old BALB/c mice were immunized subcutaneously twice with a 2-week interval, bled from the retro-orbital plexus to evaluate antibody response and sacrificed to isolate splenocytes for lymphocyte proliferation and cytokine profiles in response to prime boosted antigen. **Results and Discussion:** Our results demonstrate that the protein Dnak elicited a high immunogenic response as revealed by the enhanced production of IgG1 and IgG2a, lymphocyte proliferation and significant amounts of IFN-gamma and IL-10, and no detection of IL-4 production in splenocytes. All these results indicate a Th1 response. The co-administration or the use of the protein fused to DnaK was shown to have potential to promote specific immunogenicity against the second antigen and to stimulate the production of IL-10. Our studies show that DnaK, genetically fused with the proteins, induced an immune response in mice and constitutes a good strategy to improve the expression of proteins that are normally difficult to express. In addition, the use of this strategy to deliver proteins may reveal novel antigens against leptospirosis.

Supported by: FAPESP, CNPq and Fundação Butantan

6.05 Molecular characterization of *S. mansoni* venom allergen-like protein 6

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Introduction: Schistosomiasis, caused by trematodes of the genus *Schistosoma,* is one of the world's prevalent tropical parasitic diseases, affecting over 200 million people. The *Schistosoma mansoni* venom-allergen-like proteins (SmVALs) are members of a diverse protein superfamily containing a highly conserved SCP/TAPS (Sperm-coating protein/ Tpx-1/Ag5/PR-1/Sc7) domain. SCP/TAPS proteins may be important in key biological processes, including host-pathogen interactions and defense mechanisms. **Objectives:** In this study, we cloned the SmVAL6 cDNA and produced the recombinant protein in *E. coli*. We also characterized its expression profile and distribution in the tegument fractions. **Methods:** The cDNA for SmVAL6 was obtained by RT-PCR and cloned into expression vector pAE. The recombinant protein was expressed in *E.coli,* purified by affinity chromatography and used to generate polyclonal antibodies in mouse. The protein expression profile of SmVAL6 over the life cycle stages was investigated by Western blotting. **Results and Discussion:** The cDNA for SmVAL6 contains an open reading frame of 1,209 bp encoding a protein of 402 aa. The recombinant protein was expressed as inclusion bodies and purified under denaturing conditions. Western blotting revealed that SmVAL6 is upregulated in cercariae and adult male worms. Furthermore, the protein is enriched in the tegument fraction, showing several additional isoforms as compared to stripped worms. Based on the data of tegument fractionation, our hypothesis is that the protein should be anchored to the tegument plasma membrane by palmitoylation on exon junctions 33/34 or 32/34, and experiments are underway to confirm this hypothesis.

Supported by: FAPESP, Fundação Butantan and CNPq

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6.06 Effects of a new set of culture conditions on the production of *Haemophilus influenzae* type b capsular polysaccharide

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Introduction: *H. influenzae* type b is a worldwide prevalent Gram-negative bacterium and etiological agent of bacterial meningitis and pneumoniae infections mainly in children under 5 years of age. Its capsular polysaccharide, a polymer of polyribosyl ribitol phosphate (PRP), effectively prevents infection when used as a vaccine. Optimization of cultivation conditions is expected to improve also the subsequent steps of purification and conjugation with a protein. **Objectives:** The aim of this study was to evaluate the effects of: 1. constant dilution rate; 2. constant volumetric aeration rate; 3. presence of baffles; and 4. a new impeller configuration on the final concentration and molecular weight of PRP of a fed batch cultivation of *H. influenzae* in comparison to similar runs without these features. **Methods:** The fed batch cultivation of *H. influenzae* strain GB3291 was conducted in a New Brunswick Bioflo 2000 bioreactor with the following conditions: dilution rate at 0.0146 h^{-1} and volumetric air flow rate (air flow/volume of medium) at 0.75 min^{-1} kept constant through mass balance calculations, dissolved oxygen tension at 30% of air saturation controlled by agitation frequency, pH at 7.5 controlled by 5 M NaOH addition, temperature at 37°C, initial culture volume at 6.4 L. The impeller was composed of a shaft with 3 Rushton turbines: one at the minimal height and the others at 3.5 and 7.0 inches above. Four baffles were present. Total cultivation time was 19.5 h, during which hourly samples were collected for optical density (540 nm), dry cell mass, viability, glucose, acetic acid and PRP concentrations and PRP molecular mass determinations. Glucose and yeast extract enriched culture medium was fed with a variable flow rate by peristaltic pump. **Results and Discussion:** The final PRP concentration was 1,546 mg/L, similar to previous runs. Before the end of cultivation, the PRP concentration did not reach a plateau as in previous experiments, suggesting that a longer cultivation time would result in increased concentrations. The initial cell growth was slower but resulted in similar final values of optical density, dry cell mass and viability. The presence of baffles and the new configuration of the Rushton turbines decreased the maximum agitation frequency from 600 to 450, which decreased shear rate and may have a positive effect on the molecular mass of the produced PRP. Less antifoam was required and the total volume added was approximately 1.8 mL. The overall effect of these conditions on the molecular mass of the PRP produced is to be determined.

Supported by: Fundação Butantan

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6.07 Choice of a method for determination and evaluation of mixing time at different combinations of agitation, aeration and volume of liquid in bioreactor

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Introduction: Mixing time (T_m) is a parameter directly related to mass transfer in agitated vessels, a critical aspect in submerged cultivations. It is defined as the time required to achieve a given degree of homogeneity starting from a completely segregated state, and can therefore be measured by the injection of a detectable tracer into the reactor at a given set of conditions followed by the monitoring of its concentration until a given level, usually between 90 and 95 % of the interval. Common tracers include solutions of acids, bases, electrolytes, dyes, warm liquids and radioactive isotopes. T_m is a useful parameter for the selection of agitation, aeration, volume of media and geometrical parameters combination that optimize mixing. **Objectives:** The aim of this study was to adjust the method for mixing time determination and to evaluate how different combinations of agitation frequency, air flow rate and liquid volume affect T_m. **Methods:** The bioreactor used, NBS Bioflo 5000, had the following configuration: vessel of 80 L nominal volume, impeller with 3 Rushton turbines, one at the minimal height and the others at 11 and 22 cm above, 4 baffles and calibrated pH and oxygen probes. Boiling water (for temperature variation) and the solutions of 10 M NaOH and 5 M H_2SO_4 (for pH variation) were tested as tracers in 28°C purified water. Two levels of water (30 and 60 L), 2 levels of volumetric air flow rate (0.30 and 0.75 min^{-1}) and 3 levels of agitation frequency (100, 300 and 500 rpm) were combined in a full factorial experiment and T_m was tested in quadruplicate. A fixed volume of tracer liquid was added to the reactor at stable pH and temperature conditions and the time for 95% of the pH or temperature step was determined through analyses of the graphs generated from NBS software Biocommand Plus logged values of temperature and pH. The data was analyzed in Microsoft® Excel for Mac 2011. **Results and Discussion:** The data generated from the different combinations of agitation, aeration and volume of liquid is under evaluation. Acid and base solutions were found to be better tracers than hot water because of decreased tracer volumes, faster probe response time, easy and fast adjustment to desired pH values and the possibility of measurement with both increasing and decreasing step variations. This simple method makes it easier to compare variations of mixing efficiency at different bioreactor conditions and is therefore recommended for the optimization of bioprocesses.

Supported by: Fundação Butantan

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6.08 Consistency of the pilot scale production process for the pneumococccal whole cell vaccine

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is a Gram-positive encapsulated bacterium that colonizes the human respiratory tract. It causes pneumonia, meningitis and sepsis, and the risk groups are children, the elderly and immunocompromised people. Nowadays, pneumococcal vaccines are made with polysaccharides from different strains. However, the process of conjugation with a protein, to improve the immune response, is expensive for production in poor countries. Instituto Butantan, Children's Hospital Boston, and PATH joined to develop a production process in order to obtain a low cost whole cell vaccine, based on a genetically modified non-encapsulated pneumococcus strain. This new vaccine would induce a serotype-independent immune response and protect all at-risk groups. **Objectives:** This work aimed to evaluate the production process of different lots of the whole cell vaccine produced at Instituto Butantan from 2009 to 2010. **Methods:** Thirteen lots were produced in a 60-liter fermentor of the pilot plant under GMP conditions. Pneumococcus was grown in a culture medium with 2% enzymatically hydrolyzed soybean meal (EHS) in batch fermentation with subsequent product recovery and purification via tangential flow microfiltration in hollow fibers 0.1 μm. Cells were harvested at the end of exponential phase, concentrated to 1/6 of the initial volume and submitted to 6 washing steps with Ringer lactate plus 0.2% glucose. After eliminating the culture medium, the cell suspension was inactivated with β-propiolactone (1:4000 v/v). All lots were compared in relation to: growth curve, maximum specific growth rate, metabolic production of organic acids, glucose consumption and yield of tangential microfiltration. **Results and Discussion:** Analyses of whole cell vaccine production lots showed consistency, comparable growth curves and production of acetic and lactic acids. UFC at the beginning of concentration was around 10^9 per mL for all lots. The average of maximum specific growth rate during exponential phase was 1.0 h^{-1}. The product recovery was 80 to 120% after concentration/diafiltration. Our results showed the consistency of the production process of pneumococcal whole cell vaccine, which enables large scale production for human immunization.

Supported by: PATH, Fundação Butantan and Instituto Butantan

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6.09 Analysis of antiviral activity of a recombinant protein obtained from the caterpillar *Lonomia obliqua*

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Introduction: Viral infection control, mainly those related to by influenza viruses, is of great interest in public health. Several studies have shown the presence of active compounds in the hemolymph of arthropods, some of which are of interest for the development of new pharmacological drugs. Recently, we demonstrated the existence of a potent antiviral in the hemolymph of *Lonomia obliqua* caterpillar. This purified protein reduced virus production ($TCID_{50}$ ml^{-1}) more than 157-fold (from $3.3\pm1.25x10^7$ to $2.1\pm1.5x10^5$) in measles virus, 61-fold in polio virus ($2.8\pm1.08x10^9$ to $4.58\pm1.42x10^7$) and 64-fold in H1N1 influenza virus. **Objectives:** This study aimed to build recombinant bacmids containing sequences encoding this antiviral protein in baculovirus/SF-9 cell system and and test the antiviral activity of recombinant protein. **Methods:** To synthesize cDNA, RNA of *L. obliqua* was extracted with Trizol reagent and used in polymerase chain reactions using reverse transcriptase polymerase (RT-PCR) with specific primers for the antiviral protein, based on the sequence of the cDNA libraries of *L. obliqua* tegument and spicules, using all possible translation frames of each cDNA. Restriction sites were inserted in the cDNA for connection to the donor plasmid pFastBac1™ (Invitrogen). The recombinant plasmid was selected in *Escherichia coli* DH5α and subsequently used in the transformation of DH10Bac *E. coli*, to obtain the recombinant bacmids. This bacmid, containing the sequence of a protein with antiviral activity was used for expression of this protein in baculovirus/SF-9 cell system. **Results and Discussion:** In order to investigate the antiviral effects on picornavirus (EMC encephalomyocarditis), whole hemolymph and recombinant protein (1% v/v) were added to the L929 cells cultivated on 96-well plates, 1 h before infection with 100 TCID50 of virus. Samples of the cell cultures were collected daily and analyzed to determine the percentage of cells with cytopathic effect (*CPE*). The recombinant protein was able to block the replication of 100 TCID50 of picornavirus (EMC), showing that the recombinant antiviral protein remained fully active.

Supported by: FAPESP, CAPES and CNPq

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6.10 Production of the recombinant protein *Sm*-TSP-2 on a pilot scale - A Sabin technology transfer

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Introduction: Schistosomiasis is an important parasitic disease in the world, and considered among the neglected tropical diseases. In Brazil it is estimated that 25 million people are living in risk areas and 10% already have symptoms of the disease. Treatment by chemotherapy is available but effective administration is difficult and it does not prevent reinfections. A vaccine is desired and pursued by researchers in different countries. A fragment of a protein from *Schistosoma mansoni*, tetraspanin-2 (*Sm*-TSP-2), was presented in 2006 as a promising vaccine antigen. The gene coding this fragment of *Sm*-TSP2 was cloned into yeast Pichia Pink® for production of the recombinant protein. A process for *Sm*-TSP-2 production by fermentation of the recombinant yeast at a 10-L scale, followed by cell separation and chromatography for protein purification was developed by the R&D team of Sabin Vaccine Institute. **Objectives:** The aim of this study was the production of *Sm*-TSP-2 on a pilot scale, under GMP conditions, for stability tests, formulation, filling and pre-clinical trials. **Methods:** All the parameters for fermentation were designed by the Sabin R&D team. *Sm*-TSP-2 expression was induced by methanol and the protein was secreted into the supernantant. Cell separation was performed by microfiltration using hollow fiber cartridges. The protein was purified using three steps of chromatography: cation exchange, hydrophobic and gel filtration. **Results and Discussion:** The full process was scaled up at the Laboratório Piloto de Produtos Biológicos Recombinantes of the Instituto Butantan under GMP conditions in 60-L scale fermentor. *Sm*-TSP-2 induction was about 260 mg per liter of culture, from which 2.5 g was purified as final product, while about 8 g were separated in purification tests. The next steps include tests by Quality Control, formulation and filling, stability tests and pre-clinical trials before completing the documentation to submit to regulatory agencies for future clinical trials. The experience of *Sm*-TSP-2 production at the pilot facility of the Instituto Butantan helps to continue the discussion about different aspects of process development for new vaccines and biopharmaceutics, which may be of interest to the Institute to produce formats of technology transfers, specific needs for different products, including legal questions, human resources and financial agreements, to accomplish Butantan´s goal of improving public health.

Supported by: Sabin Vaccine Institute and Fundação Butantan

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6.11 Determination of capsule polysaccharide molecular weight profiles during fermentation of *Haemophilus influenzae* type b

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Introduction: *Haemophilus influenzae* type b (Hib) is a bacterium responsible for invasive diseases as pneumonia, bacteremia and meningitis in infants, elderly and immunodeficient individuals. Prevention against Hib infections can be achieved with vaccination using the capsular polysaccharide as antigen. The polysaccharide, a chain of ribosylribitol linked by phosphodiester bonds, is obtained by fermentation of the bacterium and purification of the polymer from the supernatant. In this laboratory, the purification process has been established based on tangential micro and ultrafiltrations, ethanol precipitation and enzymatic hydrolysis. A low yield in the ultrafiltration steps suggests that the molecular weight of the polysaccharide is low. **Objectives:** This study focused on the characterization of the molecular weight profiles of the polysaccharide over the fermentation. **Methods:** Fermentations were conducted in MP medium, at 37°C and pH 7.5, in 7- and 13-liter bioreactors - nominal capacity. Sodium hydroxide (5 M), Na_2CO_3 (20%) and NH_4OH (14%) solutions were used for pH control. Batches were fed either with concentrated media or glucose solution. Samples were collected for dry cell mass, polysaccharide concentration (by Bial`s method), organic acids and carbohydrates concentration (by HPLC) and molecular weight determination (by HPSEC). **Results and Discussion:** The characterization of molecular weight would demonstrate whether the polymer is degraded over time, by enzymatic or alkaline hydrolysis. These determinations will be considered in the development of a process for higher molecular weight polysaccharide production and improvement of polysaccharide recovery in the purification process. Relative molecular weight values varied from nearly 1,000 kDa at 5 h of fermentation to values as low as 350 kDa at later samples, decreasing constantly throughout the culture. Respective samples for the different base solutions showed no significant variation, suggesting that local alkaline hydrolysis in the culture broth has little or no effect whatsoever. One specific fermentation was kept in a starvation state once glucose was totally depleted, presenting no variation in polysaccharide concentration over nearly 2 h. However, molecular weight shows a decrease of about 100 kDa. This is evidence of the presence of a hydrolyzing agent in the broth. Pulse addition of glucose seems to cease the hydrolysis, suggesting that the agent is an enzyme that may have its expression regulated by the availability of substrate.

Supported by: Fundação Butantan

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6.12 A whole-cell pertussis vaccine with reduced LPS content protects mice against infection with *Bordetella parapertussis*

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Introduction: Despite the high effectiveness of the current whole cell and acellular pertussis vaccines in preventing disease caused by *Bordetella pertussis*, some studies indicate that these vaccines have little or no efficacy against infection caused by *Bordetella parapertussis,* and it is not yet completely understood how *B. parapertussis* may respond to selective pressure exerted by global pertussis vaccination. Several epidemiological studies have shown that *B. parapertussis* is commonly found in patients from a population vaccinated against *B. pertussis*. The Butantan Institute has recently developed a simple and low-cost procedure, producing a non-toxic and safer whole cell pertussis vaccine (Plow), with low LPS content. **Objectives:** This work evaluated the impact of the vaccination with Plow in the colonization of mice's upper respiratory tract with *B. parapertussis*. **Methods:** Groups of 7 to 14 female Balb/c mice were subcutaneously immunized, in two independent experiments, with two doses of Plow (1/2 human dose of 200 µl in PBS mixed with aluminum hydroxide 1.2 mg/ml), with a 15-day interval. The control group received 200 µl of aluminum hydroxide, 1.2 mg/ml in PBS. Two weeks after the last injection, the animals were intranasally challenged by live *B. parapertussis* 12822 (10^7 CFU/20 µl in PBS). The colonization of the upper respiratory tract was evaluated by the number of CFU in lung-tracheal wash in the immunized animals and controls. Total IgG was measured by ELISA against Plow, in individual sera before and after the challenge. **Results and Discussion:** Immunization with Plow vaccine induced high levels of IgG homologous antibodies. Although the challenge with *B. parapertussis* did not increase the anti-pertussis antibody levels in the immunized animals and controls, a decrease in *B. parapertusssis* colonization rates was detected, on the average of greater than 80%, in the animals immunized with Plow, strongly suggesting a protective cross effect. Our data raise the possibility of that this new whole cell pertussis vaccine with low content of LPS can induce an optimal clearance of *B. parapertussis*, possibly maintaining a cross protective efficacy against *B. pertussis* and *B. parapertussis* infections.

Supported by: FAPESP, CNPq and Fundação Butantan

6.13 Expression and characterization of two predicted membrane proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is a zoonosis of global importance that has been considered a major emerging infectious disease. Studies have been conducted to characterize antigens for the development of a vaccine. **Objectives:** Our goal was the expression and characterization of surface proteins of *L. interrogans* serovar Copenhageni, LIC11834 and LIC12253. **Methods:** The following were carried out: bioinformatics analysis of the sequences encoded by LIC11834 and LIC12253; design of primers; genomic DNA extraction and RNA extraction and amplification by PCR to study the conservation of selected genes in *Leptospira* serovars; cloning of PCR products in pGEM-T vector; subcloning in pAE expression vector; sequencing analysis; expression of recombinant proteins in *E. coli* strain BL21 SI; analysis of solubility of the recombinant proteins; purification of the recombinant proteins; protein analysis by circular dichroism spectroscopy; production of polyclonal antibodies by immunization of mice and immunogenicity tests by ELISA; and evaluation of the capacity of these proteins to mediate attachment to ECM by binding assays. **Results and Discussion:** The gene LIC11834 was present in almost all leptospiral species tested, but transcription was restricted to *L. interrogans*. The gene LIC12253 is found in all species tested, while the transcription was restricted to the pathogenic species. The proteins were expressed with a 6xHis tag at the N-terminus, making them suitable for metal-affinity chromatography purification. The recombinant proteins rLIC11834 and rLIC12253 were expressed in the soluble and insoluble form, respectively. Polyclonal antibodies raised in mice for both proteins yielded high serum titers, indicating their highly immunogenic activities. The proteins bound strongly to immobilized laminin in a dose-dependent and saturable manner. The data suggest that these proteins may be involved in leptospiral pathogenesis, although other tests must be conducted. Moreover, immunoprotection in animal model will allow us to evaluate whether these proteins are suitable for vaccine development.

Supported by: FAPESP, CNPq and Fundação Butantan

6.14 Isolation and biochemical characterization of the active antimicrobial factor present in kefir

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Introduction: Kefir is a beverage derived from milk fermentation by a symbiotic association of lactic acid bacteria and yeast maintained in a polysaccharide matrix in granular form, called kefiran. Earlier studies have shown that kefir is capable of inhibiting the proliferation of pathogenic bacteria resistant to up to nine different types of antibiotics. **Objectives:** The aim of this study was to identify the component responsible for inhibition by high pressure liquid chromatography (HPLC). **Methods:** kefir drink derived from fermented whole milk was centrifuged twice at 8,000 rpm for 15 min and the supernatant was lyophilized and resuspended in distilled water to obtain again the original volume. The kefir supernatant was separated according to molecular mass by centrifugation in Centripeps with a 10-kDa membrane and dialysis across a 1-kDa membrane. The fractions obtained were lyophilized and processed at a later stage. Initially, a two-phase partition was performed, in which the greater part of the material was retained in the aqueous phase and a small fraction in the organic phase. To identify the fraction responsible for inhibiting bacterial proliferation, the fractions were resuspended in distilled water, diluted twofold in tryptic soy broth and incubated for 18 hours at 37°C in the presence of O6:H1, a uropathogenic *Escherichia coli.* The test was done in 96-well culture plates and bacterial growth was determined by measurement of optical density at 595 nm in a Mutiskan EX plate reader. **Results and Discussion:** Results showed that the aqueous fraction was definitely responsible for the antimicrobial activity of kefir. In the aspect of refining the fractions, a kefir solution was fractioned by HPLC with elution timing. We observed a mass scale predominance of hydrophilic molecules resulting from the chromatographic separation. At the present time, the active fraction is being separated by chromatography to identify the biologically active molecule(s).

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6.15 Immunological evaluation of three *Leptospira interrogans* membrane lipoproteins produced in *Escherichia coli*

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Introdution: Leptospirosis is a widespread zoonosis caused by the spirochete of genus *Leptospira*, which includes 8 pathogenic species. Veterinary vaccines protect for a short-time and only for serovars included in the preparation. There is a critical need for the development of a vaccine, because so far, only Cuba has licensed a human vaccine, similar to the veterinary ones. Thus, the identification of conserved outer membrane proteins among different pathogenic strains is the main target for vaccine research. Among these, the lipoproteins are very important because several of them are involved in bacterial pathogenesis and may have immunogenic or immune protection properties. **Objectives:** The goal of this work was to clone, express and evaluate leptospiral lipoproteins for immunological response in an animal model. **Methods:** DNA inserts corresponding to the genes LIC10973, LIC10731 and LIC10645 were obtained by PCR from genomic DNA of *L. interrogans* sorovar Copenhageni. The inserts were cloned in pGEM-T vector and subcloned in pAE expression vector. Expression of the proteins was induced in *E. coli* BL21 DE3 Star pLyS transformed with confirmed DNA constructions. After protein solubility analysis, the recombinant proteins were purified by nickel affinity chromatography. Secondary structure of purified proteins was assessed by circular dichroism analysis. Immunogenicity of the proteins was evaluated by immunization of mice followed by ELISA titration. **Results and Discussion:** All three recombinant proteins were expressed in their insoluble form. Protein purification was performed by urea denaturation followed by refolding. The purified proteins had a final concentration of approximately 0.1 μg/μL. Circular dichroism analysis of rLIC10973, known as OmpL1 protein, revealed a predominance of beta-strands in its secondary structure. After immunization of mice, high antibody levels were detected by ELISA. The results obtained allow us to conclude that these proteins are highly immunogenic in mice. Similar assays will be performed in hamsters followed by immunoprotection assays in order to see if these proteins are good targets for vaccine production or for development of diagnostic methods.

Supported by: FAPESP, CNPq and Fundação Butantan

6.16 E6 and E7 oncoprotein-based antigens for the development of a therapeutic treatment of cervical cancer

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Introduction: Cervical cancer is the second most common cancer among women worldwide and HPV infection is a major risk factor for its etiopathogenesis. HPV16 is the most commonly identified papilloma type in cervical cancer and the E6 and E7 oncoproteins, involved in proliferation control and cellular transformation, have been of particular interest for the development of therapeutic vaccines. **Objectives:** In view of this, our objective was to develop a vaccine with immunogenic epitopes of E6 and E7 to enhance a cellular immune response against established HPV16 infection. **Methods:** We constructed vectors for expression of epitopes from E6 and E7 proteins of HPV16. The synthetic DNA sequence coding for E6E7 epitopes containing C-terminal 6X-histidine tag to facilitate its purification was subcloned into the pAE vector. After transformation into BL21(DE3)pLys, cultures were grown at 37ºC and induced using IPTG. Cells were harvested and lysed by sonication. The protein was purified by Ni2+-chelating Sepharose resin in denatured condition. Groups of C57BL/6 mice were vaccinated 2 times with 15 μg E6E7 or PBS and challenged with TC1 tumor which carries E6 and E7 transforming oncoproteins. **Results and Discussion:** The E6E7 protein appeared in inclusion bodies, and was dissolved in a solution containing 8 M urea. The purified protein was dialyzed against PBS and 2 M urea and showed an apparent molecular weight of 17.5 kDa, as estimated by SDS-PAGE. The peptide integrity was checked by mass spectrometry. In an immunization experiment, 100% of mice immunized with E6E7 did not develop tumor, while all mice injected with PBS developed tumor. These results are promising and, although preliminary, indicate the potential of E6E7 protein as an immunogen, for a therapeutic vaccine against cervical cancer induced by HPV16.

Supported by: FAPESP and CAPES

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6.17 Influence of the carbon source on bacterial growth and capsular polysaccharide production by *Streptococcus pneumoniae* serotype 14

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Introduction: *Streptococcus pneumoniae* is a major cause of meningitis, pneumonia, and bacteremia, especially among young children and older adults. The capsular polysaccharides of pneumococci have been shown to be essential for their virulence and their production depends on the regulation of their metabolic pathways. Sugars are the most preferred source of carbon and energy. Genome analyses suggest that *S. pneumoniae* possesses pathways for catabolism of pentitols, as well as for fructose, galactose, glucose, sucrose and other carbohydrates. **Objectives:** The aim of this study was to evaluate the influence of glucose and sucrose separately and simultaneously on cell growth and polysaccharide serotype 14 (PS14) production by *S. pneumoniae*. **Methods:** Fermentations were carried out in shaker flasks and in bioreactors. The following carbon sources and concentrations were tested in the bioreactor: glucose 10 g/L and 20 g/L, initial glucose 10g/L with pulse of additional 10 g/L after 4h of cultivation, and sucrose 10g/L. In shaker flasks, glucose and sucrose, 5 g/L each, were used simultaneously. The culture medium was chemically defined (Van der Rjin & Kessler, 1980). The biomass was measured by optical density at 600 nm and the production of PS14 was measured by capture ELISA according to a method established in our laboratory. Sugar consumption and production of organic acids was determined by HPLC. **Results and Discussion:** Comparing the bacterial growth at different glucose concentrations and using sucrose as a carbon source, the highest specific growth rate (0.75 h^{-1}) was obtained with initial glucose concentration of 10 g/L with later pulse, whereas in other conditions with glucose, the growth rates were around 0.66 h^{-1} and with sucrose, 0.63 h^{-1}. For the production of PS14, there was a slightly higher production using sucrose as the sole carbon source. According to Salminen et al. (2004), for dextran production by *Ln. mesenteroides*, sucrose is cleaved by a cell wall-associated enzyme and the glucose moiety is used for dextran synthesis and fructose moiety is fermented in the usual manner. The same phenomenon could explain a higher PS14 production using sucrose, but it is necessary to carry out more fermentations to confirm these results. HPLC analysis of the samples from the fermentation using glucose and sucrose simultaneously showed that both carbon sources were consumed concurrently. A cost analysis showed that glucose and sucrose have equivalent prices, allowing us to use any of them as a carbon source in culture with chemically defined medium for *S. pneumoniae* serotype 14.

Supported by: CAPES and FAPESP

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6.18 Production of recombinant egfp-tagged scFv anti-glycoprotein g from rabies virus for diagnostic purposes

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Introduction: Rabies is acute, progressive and incurable viral encephalitis. The causative agents are neurotropic RNA viruses in the family Rhabdoviridae, genus Lyssavirus. Globally, there are about 55,000 cases of rabies each year (WHO, 2010). Most of the deaths occur in the absence of post-exposure prophylaxis. Since rabies is nearly always fatal when left untreated, an accurate diagnosis is of paramount importance to decrease mortality. The envelope glycoprotein G of rabies virus induces neutralizing antibodies, which are important in protection against rabies, and therefore, the recombinant single chain fragment variable antibodies (scFv) against this glycoprotein fused with the enhanced green fluorescent protein (EGFP) could be a promising diagnostic tool. **Objectives:** The aim of this study was to clone scFv from anti-rabies virus monoclonal antibody glycoprotein in fusion with EGFP to generate a biomarker molecule to be used in diagnosis. **Methods:** The EGFP gene was fused to the N-terminal of scFv against glycoprotein G, using overlapping primers and several rounds of PCR. The amplicon containing the construction (EGFP+scFv) was submitted to gel purification, digested with BamHI and XhoI endonucleases and inserted into pET 20 b+ expression vector, previously digested with the same enzymes. DH5α competent cells were transformed and screened in LB-agar plates containing ampicillin (100 µg/mL). The ampicillin-resistant colonies were PCR screened and a positive clone had its plasmid isolated and submitted to DNA sequencing. **Results and Discussion:** The construct pET20EGFPscFv was then transformed into different strains of *E. coli* (BL21 DE3, BL21 DE3 star, C43, SI) and tested under different growth temperature conditions (25°, 30° and 37°C). C43 *E. coli* at 30°C showed the best results in the expression tests, since the IPTG-induced bacteria showed a band with the expected molecular weight in the SDS-polyacrylamide *gel electrophoresis* (*SDS-PAGE*). Further purification procedures are being conducted and the recombinant EGFPscFv will be evaluated in the future by fluorescence microscopy analysis, using cells infected with rabies virus.

Supported by: FAPESP, CNPq and INCTTOX

6.19 Antifungal activity of crude extract and fractions of an endophytic fungus from *Rhizophora mangle*

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Introduction: The frequency and diversity of severe fungal infections are increasing, around the world. Microorganisms are a source of important bioactive secondary metabolites of economic impact, and in this aspect, the Brazilian mangrove is an unexplored biome. In a previous study, 83 fungi were isolated from mangrove plants of Bertioga, São Paulo State, Brazil, and maintained under laboratory conditions. Among them, the endophytic fungus of *Rhizophora mangle* coded as R-2BI-8 produced a crude extract with promising antifungal activity. **Objectives:** The aim of this study was to purify the crude extract produced by the fungus R-2BI-8 and perform physico-chemical characterization of the antifungal molecules isolated. **Methods:** The fungus R-2-BI-8 was cultivated in potato dextrose broth at 28ºC, 150 rpm for 14 days. The culture supernatant was extracted with hexane (HEX), dichloromethane (DCM) and ethyl acetate (EA). Fractions and subfractions of the crude extracts R-2BI-8-HEX and R-2BI-8-DCM were obtained by high performance liquid chromatography (HPLC). The antifungal activity of the subfractions or fractions were evaluated *in vitro* by the microdilution assay (minimal inhibitory concentration - MIC) at concentrations lower than 500 µg/mL against *C. albicans* ATCC 36802/IOC 3704, *T. rubrum* IOC 4527, *C. neoformans* IOC 4528 and *A. fumigatus* IOC 4526. The MIC was defined as the lowest concentration that inhibits at least 90% of the pathogens' growth. **Results and Discussion:** The crude extract R-2BI-8-EA did not inhibit the growth of any pathogens at up to the highest concentration tested (1000 µg/mL) while the R-2BI-8-HEX and R-2BI-8-DCM extracts were the most effective, showing promising antifungal activity with MIC values ranging from 31.25 to 250 µg/mL. R-2BI-8-HEX and R-2BI-8-DCM crude extracts resulted in 9 and 8 fractions, respectively. The fraction R-2BI-8-HEX F9 showed MIC values of 1.5 and 125 µg/mL against *C. neoformans* and *T. rubrum*, respectively. The subfractions R-2BI-8-DCM F3-4, F3-5, F3-6, F5-1, F5-2, F5-3 and F5-4 inhibited the growth of at least one pathogen at concentrations less than 125 µg/mL. The most effective was the subfraction R-2BI-DCM F5-3, whose MIC value was less than 2.8 µg/mL against all pathogens. Purifications are under development to obtain the most effective fractions and to physico-chemically characterize the isolated molecules by nuclear magnetic resonance (NMR ^{1}H and ^{13}C) and mass spectrometry. The taxonomic identification of the fungus R-2BI-8 is in progress by 26S rDNA sequencing.

Supported by: FAPESP

6.20 Novel approach to study the metabolism of *Streptococcus pneumoniae*

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Introduction: *Streptococcus pneumoniae* is responsible for millions of deaths every year and its capsular polysaccharide (PS) is the major virulence factor, currently used as a vaccine antigen. Despite its importance for PS production, very little attention has been devoted to pneumococcal metabolism. Three important substrates are directly involved in *S. pneumoniae* metabolism: glucose, choline and glutamine. Glucose is the source of energy and is oxidized to pyruvate via glycolysis. Due to the absence of respiratory metabolism, the main end-product of glycolysis is lactate, which inhibits cell growth. Choline is present in teichoic and lipoteichoic acids as phosphocholine residues, required for cell division and the infection process. The role of glutamine is not completely understood; however there are 7 different ABC transporters predicted for glutamine, which suggests that glutamine plays other roles besides being a component of proteins. **Objectives:** This study aimed to evaluate the effect of glucose, choline and glutamine on growth, PS production and carbon metabolism of *S. pneumoniae* serotype 14 (the most common pediatric serotype in Brazil) using a novel approach to this microorganism: continuous cultivation in a bioreactor. **Methods:** Three continuous reactor cultivations were carried out at 0.5 h^{-1} dilution rate with 1-L of chemically defined media, increasing the concentration of each substrate in the feed medium: (i) glucose (2.5-30 g/L), (ii) choline (10-500 mg/L) and (iii) glutamine (0.02-1 g/L). Samples were taken to measure optical density, viable count, glucose, organic acids, and PS in the supernatant (PS_S) and cell-bound (PS_C) and to check for contamination. **Results and Discussion:** Glucose was the substrate with major impact on PS production, and a positive effect on PS_S production was observed in excess glucose. Under this condition, there was also the uncoupling of growth and acid production. In excess choline, glucose consumption increased twofold, probably due to an enhancement of the synthesis of teichoic and lipoteichoic acids, but contrary to that described in the literature, the viable cell concentration did not increase. Excess glutamine did not have impact on PS and cell growth and its role remains unknown. It was also determined that the yield factors of PS and biomass per glucose consumed as well as the yield factors of PS per biomass produced increased in limitation of glucose/choline/glutamine. This novel approach to study pneumococcal metabolism allowed us to identify that substrate limitation is the most efficient condition to improve the conversion of substrate into biomass and PS and to avoid the uncoupling of growth and by-product formation.

Supported by: FAPESP and CAPES

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6.21 Growth of *Neisseria lactamica* and production of outer membrane vesicles (OMV) with and without addition of amino acids in bioreactor

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Introduction: *Neisseria lactamica* is a commensal microorganism whereas *Neisseria meningitidis* can be a pathogen occasionally invading the nasal mucosa to cause septicemia and meningitis. *N. lactamica* colonizes the nasopharynx, particularly in young children, and its presence has been implicated in naturally acquired immunity against *N. meningitidis*. These bacteria cells naturally release outer membrane vesicles (OMV) during growth. An alternative approach to a vaccine for serogroup B meningococcal disease has been the development of an OMV vaccine based on the commensal *N. lactamica.* **Objectives:** The focus of this study was to analyze growth and OMV yield produced by *N. lactamica* in a bioreactor, in modified Catlin medium (MC), with or without amino acids. **Methods:** *N. lactamica* was cultured in MC as a minimal defined medium, with or without amino acids (5 constitutive amino acids of MC: L-glutamic acid, glycine, L-arginine, L-serine, L-cysteine) containing 1 g/L of yeast extract in batch cultivations for 12 h. Process conditions comprised: 7-L bioreactor, 36ºC, 0.2 bar, overlay air flow rate of 1 L/min, agitation varying from 250 rpm to 850 rpm and oxygen controlled (DO) at 30% of air saturation condition. Samples were collected every hour and biomass was determined by dry weight (g/L). OMV were purified from culture medium by centrifugation followed by ultracentrifugation. OMV yield was determined by Lowry's method. **Results and Discussion:** The maximum cell concentration (X_{max}) was similar in MC without amino acids, 1.06 g/L, and in MC with amino acids, 1.38 g/L. The maximum OMV yield (P_{max}) was almost twice in MC with amino acids (85.16 mg/L) when compared to MC without amino acids (45.70 mg/L). OMV productivity ($Prod_p$) was 4.26 mg/L.h in MC without amino acids and 7.64 mg/L.h, in MC with amino acids. The conversion factor OMV/biomass ($Y_{p/x}$) was 52.60 mg/g in MC without amino acids and 68.28 mg/g in MC with amino acids. The results suggest that the constitutive amino acids in MC are important for growth and especially important for OMV yield.

Supported by: CNPq

6.22 Effect of pH and ammonium chloride on coagulation factor VIII and protein C purification by immobilized metal ion affinity chromatography

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Introduction: Fractionation of human plasma provides a wide range of biological products that can be used in therapeutic treatments. Deficiency in coagulation factor VIII (FVIII) causes the inherited bleeding disorder called hemophilia A, while patients deficient in protein C (PC) are at risk of deep vein thrombosis. The recommended treatment for these diseases consists in replacement using the corresponding protein concentrate. Most of the licensed plasma-derived concentrates are produced by the Cohn method, which consists in sequential precipitation of the proteins and requires expensive equipment and facilities, such as refrigerated centrifuges and cold rooms. Alternatively, direct chromatography of plasma has been found to be particularly advantageous for fine and rapid capture of plasma proteins. In this context, we proposed the purification of FVIII and PC from human plasma using ion-exchange followed by immobilized metal ion affinity chromatography (IMAC). Ion-exchange is an inexpensive separation technique while IMAC has relatively high specificity and great potential for difficult protein separations. In previous studies with IMAC-Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} and Fe^{3+}, it was observed that FVIII and PC are well separated by the first two columns when imidazole was the eluent. **Objectives:** The aim of this study was to examine the possibility of using NH_4Cl and variation of pH as alternative methods to desorb FVIII and PC from the IMAC-Cu^{2+} and Zn^{2+} columns. **Methods:** Human plasma was directly applied to an anion-exchange ANX-Sepharose Fast Flow (FF) column. The eluate was applied to IMAC-Cu^{2+} or -Zn^{2+}. Analytical methods: Bradford, for protein content; chromogenic method, for FVIII and PC activities. **Results and Discussion:** Using IMAC-Zn^{2+}, FVIII and PC could be separated by elution with pH change and NH_4Cl. When the proteins were loaded at pH 7.0, PC was mainly eluted with pH 6.0 and FVIII mainly with pH 5.0. In the experiments with NH_4Cl at pH 6.0, PC did not bind to the metal and the FVIII was desorbed with 1 M NH_4Cl. On the other hand, in IMAC-Cu^{2+} it was not possible to elute these proteins by decreasing pH or increase in the NH_4Cl concentration up to 1 M. FVIII has 75 histidine residues in the primary sequence while PC only 15. This explains why FVIII is much more strongly attached to the metal ions than PC. Comparing the binding with Cu^{2+} and Zn^{2+}, these two proteins are more weakly attached to the IMAC-Zn^{2+} column, allowing the elution under milder experimental conditions. The results indicate that the IMAC-Zn^{2+} column is more suitable for the separation of FVIII and PC.

Supported by: PAP/SES, CNPq and Fundação Butantan

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6.23 Development virus pseudoparticles and recombinant Semliki Forest virus particles carrying the hepatitis C virus nonstructural protein 3

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Introduction: Brazil has approximately 3.5 million people infected with hepatitis C virus (HCV) and approximately 70% of cases become chronic. HCV pseudoparticles (HCVpp) are used for different purposes, ranging from studies of the mechanisms of viral infection to immunization. These particles are formed by the core of murine leukemia virus and E1 and E2 glycoproteins of HCV. In addition, these pseudoparticles carry *mRNA*. Another kind of virus particles based on Semliki Forest virus (SFV) is used for the production of heterologous proteins by mammalian cells. Our purpose was to construct HCVpp and SFV particles carrying an *mRNA* coding for an HCV nonstructural protein to immunize animals and stimulate the immune system against the HCV nonstructural protein 3 (NS3). **Objectives:** The aim of this study was the construction of vectors containing the serine protease of the NS3 of HCV genotype 1a (NS3p1a-Ab) and production of HCVpp and SFV containing the *mRNA* coding for NS3p1a (HCVppNS3p1a-Ab and SFVNS3p1a-Ab). **Methods:** To construct the HCVpp vector containing the NS3p1a-Ab, we removed a GFP gene from the vector pTG13077 (kindly supplied by Cossette) and inserted the sequence of NS3p1a-Ab synthesized by GeneArt® based on the reference sequence (GenBank: AF009606) forming the pTGNS3p1a-Ab vector. To construct the SFV vector containing NS3p1a-Ab, we digested the cloning vector pSFV2genC (kindly supplied by Renaud Wagner) and inserted the sequence of NS3p1a-Ab forming the pSFVNS3p1a-Ab vector. For the generation of HCVppNS3p1a-Ab, the three vectors pCMVGagPol, pCMVE1E2-1a (kindly supplied by Cossette) and pTGNS3p1a-Ab were co-transfected in HEK 293T cells, using liposomes, polymers, precipitated calcium or electroporation. For the generation of SFVNS3p1a-Ab particles, the two vectors pSFVHelper (kindly supplied by Renaud Wagner) and pSFVNS3p1a-Ab were transcribed *in vitro* and co-transfected in BKH-21 cells, using liposomes or electroporation. **Results and Discussion:** The vector pTG13077 was digested to remove the GFP gene and also the vector NS3p1a-Ab (GeneArt®) to isolate the NS3p1a-Ab fragment. The ligation of the fragment NS3p1a-Ab to the pTG vector was done by T4 DNA ligase. These same methods were used for the ligation of the NS3p1a-Ab fragment to the pSFV vector. Both constructs (pTGNS3p1a-Ab and pSFVNS3p1a-Ab) were confirmed by banding pattern. We then performed a series of transfections of HEK293T cells or BHK-21 cells. After 24 h, the HCVppNS3p1a-Ab or SFVNS3p1a-Ab were collected and stored at -80°C. These particles will be tested for the presence of NS3p1a-Ab *mRNA* by infection of human hepatocytes (Huh7.0).

Supported by: FAPESP and CNPq

6.24 Tank clean holding time in hepatitis B vaccine production plant

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Introduction: Endotoxins released by gram-negative bacteria are frequent contaminants of aqueous and physiological solutions and their levels must be within the limits outlined by regulatory agencies. Injectable products require that water, solutions and receptacles used in the final steps of production be tested for pyrogenic activity. Stainless steel tanks are widely applied in the pharmaceutical industries. The tanks used in the hepatitis B vaccine production plant are treated with sodium hydroxide (NaOH) and rinsed with purified water. This method has been used for more than ten years and has proved to be effective for endotoxin removal. **Objectives:** The aim of this work was to establish the clean holding time for our standard tank cleaning method. **Methods:** Standard chemical treatment consists in spraying the inner surfaces of the clean tanks with a NaOH solution (1 N), which is washed out with purified water after 1 h. Rinse water samples are collected in pyrogen-free tubes and sent to the Quality Control Service of Instituto Butantan, for testing with the Limulus amebocyte lysate (LAL) gel clot method. The acceptable limit for endotoxin detection is 0.125 EU/mL (EU = endotoxin units). To determine the clean holding time extra rinses were repeated over one week and samples were collected and tested as above. **Results and Discussion:** The treated tanks proved suitable for use after at least five days. This is particularly relevant for the vaccine production routine, considering that these tanks are frequently required in days that the Quality Control Service is restricted. We adopted the limit of five days for clean holding time.

Supported by: Fundação Butantan

6.25 Expression and Purification of Recombinant BPV-1 L1 structural protein

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Introduction: Papillomaviruses are associated with different carcinogenic processes in humans and other animals. In cattle, bovine papillomaviruses (BPV) are associated with malignant neoplasias of the upper gastrointestinal tract and urinary bladder. These viruses have a genome of approximately 7900 bp of double-stranded DNA, with at least nine potential reading frames, divided between the early (E) and late (L) regions. The E region encodes the replication and transcription of regulatory proteins, E1 and E2, and the transforming proteins E5, E6 and E7, which are associated with the uncontrolled proliferation and loss of differentiation of the infected cells. The L region corresponds to the structural proteins L1 and L2 that assembles into the viral capsid during the maturation process. **Objectives:** In order to improve the development of a prophylactic vaccine for BPV-1 targeting L1 protein, our aim was to clone the BPV-1 L1 gene and achieve its expression in *E. coli* BL21 strain. **Methods:** BPV-1 L1 was amplified by PCR using upstream primer including a SalI restriction site and the downstream primer including a EcoRI site. The L1 codon sequence of BPV-1 was cloned into pGEM-T (Promega), excised with SalI and EcoRI enzymes, and subcloned in pGEX plasmid (GE) for protein expression. BPV-1 L1 sequences were verified in alignment, using ClustalX 1.83, revealing that sequence identity with the BPV-1 L1 gene. **Results and Discussion:** The detection of L1 expression and its preliminary purification in the AKTA system (GE) was achieved. These results indicate that this bacterial system is appropriate for the expression L1 gene, which could be used for further vaccine development.

Supported by: CAPES, CNPq and FAPESP

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6.26 Influence of different culture medium compositions on capsular polysaccharide production by *Streptococcus pneumoniae* serotype 1

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Introduction: The bacterium *S. pneumoniae* is the main infectious agent of pneumonia and causes millions of deaths worldwide. The capsular polysaccharide (PS), as the major virulence factor, is the antigen of current vaccines. The serotype 1 is prevalent among children in Brazil and is frequently associated with severe invasive diseases. *S. pneumoniae* shows high nutritional requirements, since its metabolic apparatus does not synthesize all precursors necessary for cell replication, hence the importance of elaborating an adequate culture medium to achieve an efficient production process for PS-based vaccines. **Objectives:** The aim of this study was to evaluate the effect of different nitrogen sources and different concentrations of culture medium key components on the production of PS of *S. pneumoniae* serotype 1(PS1). **Methods:** Two series of experiments in a bioreactor (5 L) were performed. The first one evaluated three sources of nitrogen: Casaminoacids, Soytone and Phytone. For the second series, using the nitrogen source previously selected, different concentrations of yeast extract (2-10 g/L), nitrogen source (5-15 g/L), glutamine (0.03-0.7 g/L) and asparagine (0.135-0.435 g/L) were tested to determine optimal conditions. Cell growth was measured by optical density (DO) at 600 nm; organic acids production and glucose consumption were analyzed by HPLC; and PS1 concentration was measured by the *m*-hydroxydiphenyl method. **Results and Discussion:** The first series of experiments showed similar biomass production (4.4 g/L) for all nitrogen sources tested, although the organic acids and PS1 production was higher with Phytone (19.8 g/L lactate, 18.8 g/L acetate and 298 mg/L PS1) than with the other ones. Therefore, Phytone was selected as a nitrogen source for the second series of experiments. The results showed that the maximum specific growth rate (μ_{max} = 1.4 h^{-1}) and PS1 production increased with the concentration of Phytone. The yeast extract had low impact on PS1 production, but positively influenced the μ_{max} (1.4 h^{-1} versus 1.2 h^{-1} in central point), although the DO_{max} was lower (7.3) than that of the central point (10.5). The yield factors, Yx/s, Ylac/s and Yps/s, were lower in the experiment with the lowest concentration of the nutrients tested. The culture conditions with 10 g/L yeast extract, 15 g/L Phytone, 0.435 g/L asparagine and 0.7 g/L glutamine led to the highest production of lactate (15.5 g/L), acetate (17.3 g/L) and PS1 (275 mg/L). This production of PS1 was about nine times higher than that obtained by Kim *et al.* (1996) for the same serotype, 15% higher than that obtained by De-Sheng *et al.* (2009) after optimizing conditions for serotype 3.

Supported by: CAPES and FAPESP

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6.27 Study of antiapoptotic effect in cells treated with a protein obtained from the hemolymph of *Lonomia obliqua*

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Introduction: Mitochondria have an essential role in the cell death process called apoptosis. This mitochondrial pathway of apoptosis may suffer impairment, which causes various diseases such as cancer, diabetes, ischemia and neurodegenerative diseases, e.g., Parkinson's and Alzheimer's. During signal transduction of apoptosis in a cell, there is a change in membrane permeability of mitochondria, which may be caused by loss of mitochondrial electrochemical potential ($\Delta\Psi m$). The formation of pores in the mitochondrial membrane leads to release of cytochrome c and apoptogenic proteins into the cytoplasm, which activate proteolytic proteins known as caspases and DNases and the death process becomes irreversible. The molecules that interfere with the process of apoptosis may be used in the biotechnology industry, especially in the development of products employed in cell culture. Thus, the discovery of new antiapoptotic proteins, as well as the discovery of their mechanisms of action is essential for further progress in this field. **Objectives:** This study aimed to demonstrate the antiapoptotic effect found in a protein isolated from the hemolymph of the caterpillar *Lonomia obliqua*. **Methods:** The protein fraction with antiapoptotic activity was identified and isolated by ion exchange chromatography and gel filtration. *Spodoptera frugiperda* Sf-9 cells were treated or not with 1% (v/v) crude hemolymph or purified fraction, and after 1 h of exposure, apoptosis was induced with baculovirus or different concentrations of H_2O_2 and terbutyl (t-BHP). Samples of cultures were collected daily to determine the concentration and viability. The determination of apoptosis was performed by staining with propidium iodide/acridine orange and Hoechst 33324, and the assessment and quantification of the mitochondrial membrane potential ($\Delta\Psi m$) was performed by FACS (FACSort Becton Dickinson-Ar laser) after labeling with DIOC6 (3) and by fluorescence microscopy after staining with JC-1 and Hoechst 33324. **Results and Discussion:** The results showed that all cultures treated with the purified fraction and crude hemolymph showed an increase in cell number as well as a prolongation of cell viability and were able to maintain a high mitochondrial membrane potential, reducing cell death by apoptosis induced by chemical and viral agents. The protein from the hemolymph of *Lonomia obliqua* was able to affect the process of apoptosis, probably maintaining the high mitochondrial membrane potential, not allowing the formation of pores in the membranes of mitochondria and thus preventing the release of cytochrome c and apoptogenic proteins into the cytoplasm.

Supported by: FAPESP and CAPES

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6.28 Expression of recombinant BPV-1 E6 protein in bacterial system: first report

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Introduction: Papillomaviruses (PVs) are double stranded circular DNA viruses, with a genome size between 5300 and 8000 nucleotides, divided into three regions: LCR region, required for DNA replication and transcription; E region, associated with viral early transcripts encoding non-structural proteins; and L region, associated with late transcription capsid structural proteins. The correlation between PVs and infection process is associated with the expression of viral oncoproteins (non-structural proteins). Bovine papillomaviruses (BPVs) are the agents of relevant chronic diseases, epithelial neoplasias, and tumors in skin and mucosa (esophagus and bladder cancer and cutaneous papillomatosis). Among the eleven BPVs described, BPV-1 causes fibropapillomas in its natural host (cattle). However, BPV-1 is also associated with the equine sarcoid. The BPV-1 E6 gene encodes a 137 amino acids protein that acts as a transcriptional activator, potentially oncogenic. The development of vaccines to bovine papillomavirus is a very relevant procedure (prophylactic or therapeutic). For this purpose, improvement of our knowledge of viral proteins is a specific challenge. **Objectives:** The aim of this study was to produce the recombinant protein E6/BPV-1 in a bacterial system. **Methods:** After amplification of the E6 gene of BPV-1, subcloning into pET28a expression vector, plasmid sequencing, *E. coli* BL21 strain transformation and induction of recombinant protein expression, a portion of the cell extract was subjected to electrophoresis on SDS-page and Western blotting. **Results and Discussion:** As far as we are concerned, the results showed that the E6/BPV-1 was for the first time expressed in a bacterial system. The purified protein will be sequenced and submitted to crystallography. The detailed study of the E6 protein improves, in addition to unpublished data, the ability to produce biotechnology material such as antibodies for diagnostic devices to evaluate immune response to vaccines and also to be used in vaccine production.

Supported by: CAPES, CNPq and FAPESP

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6.29 Variants of anti-digoxin monoclonal antibody obtained by phage display

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Introduction: Digoxin is a pharmaceutical used in the control of cardiac dysfunction. Its therapeutic window is narrow, with therapeutic dosage very close to the toxic dosage. To counteract the toxic effect, a polyclonal anti-digoxin Fab fragment (anti- Dig Fab) is commercially available. The work presented herein was based on a monoclonal anti-digoxin antibody, which would account for a product with a specific potency and more precise dosage for the detoxification of patients under digoxin treatment. The phage display technology allows the selection of high affinity and specificity antibody sequences to a determined antigen and its production in unlimited amounts. This technology makes use of filamentous phages able to incorporate fragments of exogenous DNA and expose the synthesized protein on its surface as antibody fragments, which can be selected by the appropriate antigen. **Objectives:** The aim of this study was to obtain variants by phage display and characterize anti-digoxin Fab fragments by their affinity. **Methods:** An anti-digoxin mAb was generated at the Heart Institute (Sao Paulo, Brazil). This work started with total RNA extraction for cDNA synthesis. Specific primers were used for the light chain and Fd amplifications, then cloned sequentially in a phagemid vector (pComb3XTT) for the combinatorial Fab library construction. Clones displayed on the surface of phages were selected by the binding to the antigen (digoxin-BSA conjugate). Randomly selected clones were evaluated for the presence of light and heavy chains and the positive clones were sequenced. The clones displaying variation in the variable regions were induced to produce soluble Fabs. **Results and Discussion:** The constructed library was analyzed for anti-digoxin expression. Out of 10 clones randomly chosen, 6 were positive. Sequencing showed 2 identical clones, and one showed a pseudogene in the light chain. Four clones displaying variations in framework 1 were induced to express soluble Fabs, which were positive for anti-digoxin binding in ELISA assays. The clones will be further analyzed for production capacity, and kinetic analyses will be performed by Biacore technology, to evaluate affinity association and dissociation constants.

Supported by: CNPq

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6.30 Generation of a Chinese Hamster Ovary cell line producing recombinant human glucocerebrosidase

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Introduction: Impaired activity of the lysosomal enzyme glucocerebrosidase (GCR) results in the inherited metabolic disorder known as Gaucher disease. Current treatment consists of enzyme replacement therapy by administration of exogenous GCR. Although effective, it is exceptionally expensive and patients worldwide have a limited access to this medicine. In Brazil, the public healthcare system provides the drug free of charge for all Gaucher's patients, which costs as much as $ 84 million per year. **Objectives:** However, the production of GCR by a non-profit institution in Brazil would significantly reduce the therapy costs and the near expiration of the GCR patent prompted us to generate a Chinese Hamster Ovary (CHO) cell line for future production of this enzyme. **Methods:** Here, recombinant GCR was expressed in CHO-DXB11 (dihydrofolate reductase-deficient) cells after stable transfection and gene amplification with methotrexate, using traditional methods to screen high producer clones. **Results and Discussion:** As expected, glycosylated GCR was detected by immunoblotting assay both as cell-associated (~ 64 kDa) and secreted (63-69 kDa) form. Analysis of subclones allowed the selection of stable CHO cells producing a secreted functional enzyme, with a calculated productivity of 5.14 pg/cell/day for the highest producer. To the best of our knowledge, this is the first report describing a robust protocol for the generation of a cell line producing recombinant human GCR intended for therapeutic use. Although being laborious, traditional methods of screening high-producing recombinant cells may represent a valuable alternative to generate otherwise expensive biopharmaceuticals in countries with limited resources.

Supported by: FAPESP, CNPq and Fundação Butantan

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6.31 Lsa66, the novel OmpA-like protein that mediates binding to laminin, plasma fibronectin and plasminogen

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Introduction: *Leptospira interrogans*, the etiological agent of leptospirosis, is a highly invasive bacterium that colonizes target organs after penetrating the host. However, the mechanisms by which *L. interrogans* invades and colonizes are poorly understood. The search for novel leptospiral antigens that could be relevant in host-pathogen interactions could help our understanding of the molecular mechanisms of leptospiral pathogenesis and could also facilitate the identification of novel vaccine candidates. **Objectives:** The aim of this study was to study three genes selected from the genome sequences of *Leptospira interrogans* serovar Copenhageni and to evaluate the binding ability of the recombinant proteins to extracellular matrix (ECM) components and plasminogen. **Methods:** The gene sequences of LIC10258, LIC12880 and LIC12238 were amplified by PCR methodology from genomic DNA of *L. interrogans* serovar Copenhageni. The DNA inserts were cloned into the *E. coli* expression vector pAE and inserted in BL21 SI *E. coli* strain for protein expression. The recombinant proteins were purified using affinity chromatography. The capacity of the recombinant proteins to mediate attachment to ECM components and plasminogen was evaluated by ELISA-based assay. **Results and Discussion:** Recombinant proteins were expressed and purified as a major band, as assessed by PAGE-SDS. Evaluation of the binding capacity of the recombinant proteins with laminin, collagen type I, collagen type IV, cellular fibronectin and plasma fibronectin showed that rLIC10258 possessed the OmpA-like domain that binds to laminin and plasma fibronectin ($P < 0.05$). Binding was dose-dependent and saturable, and therefore it was called Lsa66 (Leptospiral surface adhesin of 66 kDa). No specific interaction to the ECM components was detected with rLIC12880 and rLIC12238. BSA and fetuin were employed as negative controls. All recombinant proteins were able to bind plasminogen and to generate plasmin, showing specific proteolytic activity. No PLG binding and no plasmin activity was detected when the negative control BSA was used. The results suggest that LIC10258 is a novel protein with dual activity that may promote attachment to the host via ECM and may help the leptospires to overcome tissue barriers by plasmin generation. To our knowledge, this is the first leptospiral OmpA-like protein with ECM- and PLG-binding properties reported to date.

Supported by: FAPESP, CNPq and Fundação Butantan

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6.32 Expression of recombinant human colony stimulating factor (rhG-CSF) in *Brevibacillus choshinensis* and *Escherichia coli*

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Introduction: The recombinant granulocyte colony-stimulating factor (rhG-CSF) is a clinically important protein for the treatment of neutropenia caused by chemotherapy, radiotherapy or medication. Filgrastim (generic name of non-glycosylated rhG-CSF), whose patent expired in 2006, has become a target for the pharmaceutical industry. Currently, Brazil is completely dependent on importation of this medicine. Aiming to produce this recombinant protein, we evaluated two different expression systems: one based on the bacteria *Escherichia coli* and the other on *Brevibacillus choshinensis,* using the vectors pAR-Kan I and pNCMO2, respectively. The former system is based on a novel *E. coli* expression vector created by our group. It has a *par* sequence that ensures that all daughter cells have the plasmid. The latter is a *Brevibacillus choshinensis* expression system, which has an excellent ability to secrete various types of proteins, while providing several advantages such as low production of extracellular proteases. **Objectives:** We aimed to express the *rhg-csf* gene using both expression systems, since both models may be useful to optimize rhG-CSF industrial production in the future. **Methods:** The *rhg-csf* sequence gene was synthesized based on the sequence described in GenBank (Accession E07167). The gene was cloned in the expression vectors pNCMO2 and pAR-KanI, and the consctructs were used to transform *B. choshinensis* and *E. coli*, respectively. The transformed bacteria were selected on culture plates with ampicillin (pNCMO2) or kanamycin (pAR-KanI). Selected colonies were grown in 10 ml of appropriate liquid media. The supernatant was separated from the cells by centrifugation and the cells were resuspended in the same volume of PBS. Samples of supernatant and cell fraction were analyzed for the presence of the recombinant protein by SDS-PAGE and Western blotting. **Results and Discussion:** As confirmed by sequecing, the *hg-csf* gene was successfully cloned in both plasmids used in this work. Moreover, the expression of rhG-CSF was also obtained in both expression systems, as confirmed by Western blotting. In *E. coli* (BL21 SI and Star PlysS), the protein was expressed as an inclusion body, leading to the need of a refolding step to obtain a soluble protein. On the other hand, rhG-CSF was secreted by *B. choshinensis*, and thus, it was in a soluble form. The results suggest that both systems are able to express this biopharmaceutical, but more tests are needed to prove the viability of industrial production.

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6.33 Cloning and expression of a single-chain fragment variable against heat-labile toxin of enterotoxigenic *Escherichia coli*

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Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is responsible for at least 400 million acute diarrhea episodes and 700,000 childhood deaths per year. ETEC is also a prevalent cause of traveler's diarrhea, which affects tourists that visit endemic areas. Heat-labile (LT) and heat-stable (ST) toxins are the main ETEC virulence factors and infection diagnosis is based on their detection by molecular biology or immunoserological methods. Immunoserological assays have some advantages when using specific antibodies 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 cloning immunoglobulin heavy and light variable domains (HV and LV) as a single-chain fusion interspaced by a flexible linker, allowing the correct interaction between the domains and preserving the antigen-binding site. **Objectives:** In this study, our aim was the construction of a scFv based on hybridoma cells that produce an anti-LT monoclonal antibody followed by its production in bacteria. **Methods:** After total RNA extraction from hybridoma cells, reverse transcription, PCR-amplification and sequencing, heavy and light chains were fused to a linker corresponding to $(Gly_4Ser)_3$, obtaining the scFv-LT coding region. This insert was subcloned into pET28a vector, with subsequent transformation of BL21-C43 competent cells. After induction of T7 promoter-associated transcription by IPTG, scFvLT recombinant antibody was submitted to metal affinity chromatography and step-wise elution, and after refolding, it was tested against LT toxin recognition by immunoassays. **Results and Discussion:** A single-chain variable fragment with 723 bp was obtained and expressed as a protein with apparent molecular weight of 30 kDa. By capture ELISA, dot-blot and immunofluorescence, we determined minor concentrations of refolded scFvLT, which was able to detect LT toxin released from ETEC strain H10407 and also purified LT. However, scFvLT was not able to recognize LT toxin subunits by immunoblotting or neutralize its activity in Y1 adrenal cells. Our results showed that scFvLT recombinant antibody obtained by bacterial expression was able to recognize ETEC heat-labile toxin by immunoassay methods, indicating its use as a tool in ETEC diagnosis. Although the refolding method used in this study was efficient to produce a recombinant antibody with biological activity, its production as soluble molecule is recommended since the refolding processes are time-consuming and expensive.

Supported by: FAPESP

6.34 Evaluation of treatment of fermentation waste from a recombinant hepatitis B vaccine plant

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Introduction: Hepatitis B vaccine produced at Instituto Butantan is composed of the major virus surface recombinant antigen. A methylotrophic yeast (*Hansenula polymorpha*) has been transformed with the integrative vector constructed with the corresponding gene (S). CTNBio, the National Technical Commission of Biosafety, determines the guidelines for storage, handling, transporting and discarding of products or residues that may contain recombinant organisms. A genetically modified organism (GMO) must be totally confined in the producer plant, and therefore, the fermentation derived residues should be decontaminated before their release to the environment. **Objectives:** The present study sought to evaluate the treatment used for fermentation processes residues generated by the hepatitis B vaccine production plant, with the aim of conforming to biosafety guidelines of CNTBio. **Methods:** The decontamination procedures of all the fermentation residues are performed using moist heat (autoclave or in place). Samples of treated residues were collected under aseptic conditions, and 0.1 mL was added to TSA solid medium. Plates were incubated at 30 °C and examined for growth after 24 and 48 h. **Results and Discussion:** None of the incubated plates showed colonies after 48 h, attesting to the efficiency of the decontamination methods. The evaluation of treated residues has been performed since 2003, for all the produced batches. The sensitivity of the TSA medium for *H. polymorpha* growth has also been tested. Thus, the decontamination method used prior to effluent release was shown to be consistently suitable for the GMO elimination.

Supported by: Fundação Butantan

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6.35 Evaluation of rabies virus glycoprotein expression in mammalian cells using a recombinant Semliki Forest virus carrying the respective *mRNA*

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Introduction: The present study aimed to use the Semliki Forest virus - SFV, as expression system in order to assess gene expression in mammalian cells. The rabies virus glycoprotein (RVGP), recognized as an antigen capable of conferring immune response against rabies, was chosen as a target gene in this approach. **Objectives:** The aim of this study were the following: to establish a protocol for *in vitro* transcription and transfection of RNA to produce recombinant SFV (SFV-RVGP); to analyze the RVGP expression in cells infected by SFV-RVGP using ELISA; and to determine the best conditions for cell culture and viral infection for the expression of the heterologous protein. **Methods:** Two different plasmids were used: an expression plasmid containing SFV genes coding for nonstructural proteins and the RVGP gene, and a helper plasmid containing SFV genes coding for structural proteins. *In vitro* transcription was performed and RNAs were co-transfected in BHK-21 cells, for generation of SFV-RVGP. They were then activated and used to infect BHK-21 cells, and induce the heterologous protein (RVGP). Expression evaluation was done by ELISA. **Results and Discussion:** Using the SFV-RVGP method of expression, we evaluated the process of *in vitro* transcription, the time of SFV-RVGP generation, and the RVGP production after infection. The experiments were performed in duplicate in 6-well plates in a CO_2 incubator at 37 °C. The cell inoculum was of 7x1E5 cells/well with a working volume of 2 mL. The amount of protein produced was of 3.7 µg/1E7 cells. An important fact is that the production of protein 48 h after infection was higher when compared to that obtained after 24 h. New experiments will be done under these conditions.

Supported by: FAPESP

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6.36 Rabies virus glycoprotein expression using viral pseudoparticles

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Introduction: Rabies virus glycoprotein (RVGP) has been recognized as an antigen able to induce neutralizing antibodies, conferring protective immunity against rabies. Gene expression in cells has been a powerful tool in biotechnology, and several biological products have been generated through the construction of gene vectors that upon cell transfection can be expressed and give rise to active proteins. Some viral particles (viral-like particles-VLP or pseudoparticles-pp) have been produced in cell cultures to express proteins of different viruses. In this work, we used HCVpp-RVGP to study RVGP expression in human cells. **Objectives:** The aim of this study was the establishment of an expression system using pseudoparticles (pp) with E1 and E2 hepatitis C virus (HCV) glycoproteins, GAG and POL proteins of MLV (murine leukemia virus) and the mRNA of the rabies virus glycoprotein. **Methods:** The pTG-RVGP vector was constructed by digestion of RVGP DNA fragment extracted from pMtiGPV and by ligation with pTG13077. Three vectors (pTG-RVGP, pGagPol, and pE1E2) were co-transfected in HEK 293T cells for production of pseudoparticles HCVpp-RVGP. Different transfection reagents were compared, Cellfectine (Invitrogen); Superfect (Qiagen); calcium phosphate-DNA co-precipitation and ExGen500 (Fermentas). Different storage temperatures were evaluated. The HCVpp-RVGP produced was used to infect hepatocarcinoma Huh7.0 cells and the RVGP expressed was measured by ELISA. **Results and Discussion:** The pTG-RVGP vector obtained had the RVGP gene under the control cytomegalovirus promoter and the MLV encapsidation signal. We produced pseudoparticles containing RVGP mRNA in HEK 293T cells after co-transfection procedure. Pseudoparticles samples stored at temperatures of -20^{0}C and -80^{0}C were used to infect hepatocarcinoma cells (Huh7.0). Expression levels of RVGP produced by infected cells reached 0.4 – 1.4µg/ 10^7 cells. The preliminary results with RVGP expression by HCVpp-RVGP showed the system to be efficient, but it must be further developed and optimized so that HCVpp-RVGP can be used to infect model animals to determine immunological responses.

Supported by: FAPESP

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6.37 Development of an immunochromatographic test for diagnosis of Shiga toxin-producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC)

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Introduction: Diarrhea is a major public health problem and even the cause of high mortality, especially in children. One of the main causes of this disease is *Escherichia coli.* Among the six *Escherichia coli* categories associated with intestinal diseases, Shiga toxin-producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC) produce toxins harmful to humans. In Brazil, there are no rapid, practical and inexpensive tests to identify ETEC or STEC infections. Thus, a method for detection of these pathogens is extremely important to prevent new outbreaks and allow a correct treatment. The immunochromatographic (IC) test could be an interesting diagnostic method since there is no requirement of sophisticated equipments or specially trained personnel. **Objectives:** The aim of this study was the development of a rapid and sensitive IC assay to identify STEC and ETEC based on the detection of Shiga toxin 2 (Stx2) and heat-labile (LT) toxins, respectively. **Methods:** Murine monoclonal (Mab) and rabbit polyclonal antibodies against each toxin were previously obtained and characterized in our laboratory. Different concentrations of each Mab were evaluated for conjugation to colloidal gold particles. Afterwards, the ideal polyclonal antibody concentration was studied to establish the positive reaction and the revelation time. When all these parameters were established, Mab-colloidal gold probe specific to each toxin were applied to glass fiber, and anti-LT or Stx2 polyclonal antibodies applied to nitrocellulose. The IC test strips were assembled using a sequence of three types of papers: cellulose fiber, glass fiber and nitrocellulose HiFlow (Millipore®). After assembly, the strips were tested by immersion of the cellulose pad in their respective toxins. The toxin presented in the sample migrates through the strip and reacts with Mab-colloidal gold probe. This complex migrates and is captured by the polyclonal antibodies generating a red line. The reaction was considered positive when the sample test and control test showed the characteristic color. **Results and Discussion:** The IC test showed a positive reaction after 15 min and the detection limit was estimated to be 9 ng/mL for LT and 18 ng/mL for Stx2. The results demonstrate that the method developed represents a promising tool for rapid diagnosis and, in the future, could be used in clinical laboratories for the detection of pathogens involved.

Supported by: FAPESP

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6.38 Antimicrobial activity of silver nanoparticles produced by mangrove fungi

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Introduction: The mangrove is an ecosystem with high competition for nutrients, and due to the extreme conditions, microorganisms are adapted to produce secondary metabolites important for their survival. Additionally, the lack of oxygen supplies enables many oxidation-reduction reactions to occur. **Objectives:** Therefore, our study focused on a biological synthesis of silver nanoparticles (Ag NP) by some fungi isolated from São Paulo State's (Brazil) mangrove and evaluation of Ag NP antimicrobial activity on pathogenic microorganisms important in hospital infections. **Methods:** Fourteen fungi were cultivated in potato dextrose broth at 28°C and 150 rpm for 72 h. The biomass was filtered and incubated under the same conditions with water (0.1 g/mL). The synthesis of Ag NP was performed by adding 1 mM $AgNO_3$ to the fungal filtrate. The suspensions were kept in the dark at 25°C, and aliquots of 2 mL were removed for absorbance measurements (200-800 nm) every hour for 96 h. The formation of nanoparticles was confirmed by a plasmon resonance band at 440 nm. The silver nanoparticles were characterized by TEM, size and zeta potential, and protein portion adhered to Ag NP was analyzed by SDS-PAGE electrophoresis. Antimicrobial activity was evaluated against some Gram-negative and Gram-positive bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, and on *Candida albicans* (ATCC 36802), *Candida albicans* (IOC 4556), *Candida albicans* (IOC 4525), *C. krusei* (IOC 4559), *C. glabrata* (IOC 4565), *C. guillermondi* (IOC 4557), *C. parapisilosis* (IOC 4565) and *C. tropicalis* (IOC 4565) using microdilution assays. **Results and Discussion:** Five fungi were able to produce Ag NP with promising antimicrobial activity, with minimal inhibitory concentrations ranging from 0.5 to 125 μM. Ag NP were obtained with satisfactory yields, spherical morphology and size in the range of 10-30 nm. TEM and SDS-PAGE revealed the presence of protein, and hopefully, further investigations will elucidate the importance of proteins in Ag NP formation.

Supported by: FAPESP

6.39 Protection assays of a whole cell pneumococcal vaccine

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Introduction: Currently available pneumococcal vaccines, based on the capsular polysaccharide, offer low protection for the high-risk groups, are too expensive and may not cover all the dominant serotypes. Instituto Butantan is developing a killed whole cell pneumococcal vaccine (SPWCV) derived from the non-encapsulated mutant Rx1E PdT Δ*lytA* of *Streptococcus pneumoniae*, originally a serotype 2 strain, autolysin negative, carrying a kanamicin resistance and a pneumolysin defective gene. **Objectives:** In this work we evaluated two different ways of immunization and challenge of mice to compare the feasibility of the methods to be included in the quality assurance assays of the vaccine. **Methods:** In the first set of experiments, Balb/c female mice were immunized by the intranasal route (100 μg/dose of 10 μl) in three doses with one-week interval, using cholera toxin subunit B (CTB) (10 μg, 5.0 μg or 2.5 μg per dose) as adjuvant. The animals were challenged (*i.n.*) three weeks after the last immunization, with a virulent S. *pneumoniae* strain 603/6B and sacrificed after 5 days for determination of nasopharyngeal colonization. In another set of experiments, Balb/c female mice were subcutaneously immunized with SPWCV (1 μg or 10 μg/animal, in 200 μl of Ringer lactate and aluminum hydroxide - 1.2 mg/ml), in two doses with 15-day interval. Two weeks after the last dose, the mice were 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. One day before the challenge the animals of both groups were bled for IgG evaluation. **Results and Discussion:** The lowest nasopharyngeal colonization were obtained in the group immunized with the vaccine formulated with the lowest dose of CTB (2.5 μg per dose). The control group of nonimmunized mice showed an average of 59 colonies/animal, those injected with the vaccine alone 6.5 colonies/animal, and those immunized with vaccine+CTB 2.5μg 8.8 colonies/animal; the vaccine elicited very low IgG titers. When injected by the subcutaneous route, the higher dose of SPWCV (10 μg) elicited 85.7% and 60% survival after challenge, in two independent experiments, while the lower dose protected on average 14%. In these groups, significant levels of IgG were detected, with an apparent dose-response against the entire vaccine. Despite that our results demonstrated protective activity of the vaccine by both routes, the large standard deviations and the low reproducibility of the assays using intranasal route led us to choose subcutaneous immunization with intraperitoneal challenge as the more promising.

Supported by: Fundação Butantan, FAPESP, CNPq and PATH

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6.40 Cloning, expression and purification of three predict outer membrane lipoproteins of *Leptospira interrogans*

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Introduction: Leptospirosis is an emerging infectious disease caused by pathogenic species of the genus *Leptospira*. Human infection is accidental and results from direct or indirect exposure to urine of infected animals. *L. interrogans* serovar Copenhageni is the predominant serovar causing disease in humans. Its genome has been sequenced and several predicted outer membrane proteins have been identified. Proteins involved in host-bacteria interaction, such as membrane proteins, should provide new insights into leptospiral pathogenesis. **Objectives:** This study aimed to clone and to evaluate the expression of three genes encoding hypothetical proteins (LIC11360, LIC11009 and LIC13011) using *E. coli* vectors. **Methods:** The genes were amplified without the signal peptide sequence by PCR of *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genomic DNA, as a template, and complementary sequence primers. The DNA inserts subcloned in vector pGEM-T were subsequently cloned into the pAE expression vector at *BamHI* or *XhoI* and *HindIII* restriction cloning sites. The recombinant proteins were expressed with 6x His-tag at the N-terminus, thus facilitating their purification by metal-affinity chromatography and purified after urea denaturation by nickel-charged Sepharose beads. **Results and Discussion:** The choice of the coding sequences was mostly based on their cellular localization and the fact that three selected are predicted to be outer membrane proteins. The recombinant proteins rLIC11360, rLIC11009 were expressed in *E. coli* BL21 SI, and rLIC13011 in *E. coli* BL21 DE3 with the expected sizes of 22.5 kDa, 25.6kDa and 27.5 kDa, respectively. The proteins were expressed in the insoluble form, as inclusion bodies, and purified after refolding by dilution. The immunogenicity of these recombinant proteins will be further evaluated in mice.

Supported by: FAPESP, CNPq and Fundação Butantan

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

6.41 Rabies virus glycoprotein expression by transient gene transfection of *Drosophila melanogaster* S2 cells

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Introduction: *Drosophila melanogaster* S2 cells (S2) stably transfected have been used in the production of many heterologous proteins and have been studied for production of rabies virus glycoprotein (RVGP), as it is recognized as an antigen capable of conferring a immune response against rabies. This approach involves the selection of a stable cell population, a procedure that requires considerable periods of time (months). In the last decade, many systems focused on the expression of heterologous proteins in transient gene approaches were analyzed, because they allow obtaining considerable amounts of recombinant protein in a short period of time. For transient transfection, a variety of methods and reagents are known, such as electroporation, use of liposomes or cationic polymers or formation of DNA calcium phosphate precipitates, and are available for important comparisons depending on cell type and final product. **Objectives:** The aim of this study was to develop a protocol for transient gene transfection, evaluating vehicles based on transfection lipids, polymers and calcium phosphate, in S2 insect cells for the expression of rabies virus glycoprotein recombinant. **Methods:** In order to determine the most efficient transfection agent, experiments were performed transfecting the cells with different concentrations of pMtiRVGP vector (expression under the control of the inducible promoter metallothionein (pMt)) and two different transfection agents: ExGen500 (Fermentas) and Cellfectin (Invitrogen). The transfections were done in duplicate in 6-well plates. After 24 h of transfection, CuSO4 (700 μM) was added to induce gene expression, and 48 h later, a sample of 1E6 cells was removed for ELISA. **Results and Discussion:** We analyzed the influence of DNA concentration, amount of transfection reagent and cell density on the expression of the rabies virus glycoprotein. Productivities ranging from 50-90 ng/1E7 cells were obtained in different experiments. The comparison between the transfection agents showed no significant differences. There was a decrease in protein production when using amounts of DNA over 15 μg.

Supported by: CNPq and FAPESP

7. Cellular Biology and Genetics

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

7.01 Distinct bone marrow progenitor replenishment under an *in vivo* inflammatory stimulus in mice phenotypically selected for acute inflammation

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Introduction: Neutrophils are an important participant in the acute phase of inflammatory response. In homeostatic conditions, the bone marrow (BM) continuously produces granulocytes and stores mature neutrophils. In injuries, such as acute inflammation or infections, the normal leukocyte production is altered. Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response exhibit a significant difference in the resistance and susceptibility to infections and chemically induced tumors. **Objectives:** The aim of this study was to investigate whether the differential inflammatory capacity between AIRmax and AIRmin mice may be correlated with the commitment of the BM progenitor cells under an in vivo potent inflammatory stimulus. **Methods:** The inflammatory exudate and BM cellularity were evaluated by cell count in basal mice and 1.5, 3, 6, 12 and 24 h after s.c injection of nonimmunogenic and non-biodegradable polyacrylamide beads (Biogel). Mature cells were depleted by magnetic beads and the dynamics of the progenitors further analyzed by flow cytometry: hematopoietic stem cell (HSC), common myeloid progenitor (CMP) granulocyte-macrophage progenitor (GMP) and megakaryocyte - erytrocyte progenitor (MEP). **Results and Discussion:** Cell number in inflammatory exudate increased after 3 h in AIRmax mice and was 20-fold higher than in AIRmin mice after 24 h. In AIRmax mice, a significant increase in whole BM cellularity was observed at 1.5 and 3 h (32.3±3.79 and 33.3±2.84 cells x 10^6/ml, respectively), and a significant decrease occurred at 24 h (13.7±0.66 cells x 10^6/ml) compared to basal AIRmax (22.4±1.3 cells x 10^6/ml). In contrast, no significant change at any time point after inflammation was observed in BM from AIRmin mice, which suggests that only AIRmax undergoes emergency granulopoiesis elicited by Biogel. FACS analysis revealed 2-fold CMP (Lin^- $Ckit^{high}Sca1^{high}Fc\gamma RIII/II^{med}CD34^{high}$ cells) and 4-fold GMP (lin^- $ckit^{high}Sca1^{high}Fc\gamma RIII/II^{high}CD34^{high}$) expansion (13.8±1.4% and 16.2±1.2%, respectively) in AIRmax mice at 1.5 h after inflammation over basal mice (7.0±0.5% of CMP and 3.8±0.5% of GMP). On the other hand, AIRmin mice showed the same GMP percentage, 2-fold decrease in CMP and 5-fold increase in MEP (lin-ckithighSca1highFc³ III/II+CD34-) at 1.5 h compared to basal AIRmin. The Biogel treatment had no effect on the percentage of HSCs ($lin^-ckit^{high}Sca1^{high}$) in both lines. Taken together, the results suggest that there is a correlation between lineage commitment and the ability to develop an acute inflammatory response.

Supported by: CNPq

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7.02 Pro-survival mechanism of recombinant Losac in serum-dreprived HUVECs: cell signaling and microarray analysis

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Introduction: Losac is a procoagulant protein from *Lonomia obliqua* caterpillar. It shows high similarity with hemolins, cell adhesion molecules from lepidopters, and with members of the L1-family from humans, crucial for embryonic development and metamorphosis (insects). Previous studies demonstrated that Losac is able to inhibit endothelial cell death in HUVECs. **Objectives:** Using a model of serum deprivation, we investigated the molecular mechanism by which recombinant Losac (rLosac) produced in bacteria, exerts its protective effect in endothelial cells (HUVECs). **Methods:** Cell survival was analyzed by the MTT assay; cell cycle (PI), proliferation (KI-67) and mitochondrial membrane potential (rhodamine-123) were analyzed by flow cytometry. Cell signaling pathways were examined by immunoblot analysis. Gene expression analysis was performed using Whole Human Genome Expression Microarray (4x44K-Agilent). **Results and Discussion:** Cell survival was better observed after 48 h of treatment (Losac: 0-30 nM). Immunoblot analysis showed that rLosac induces p44/42 MAPK phosphorylation in the first minutes after treatment. The amount of apoptotic DNA was reduced and the number of cells in the S phase was increased (48 and 92 h). Microarray analysis showed down-regulation of transcription factors related to cell death and apoptosis. All these results indicate that rLosac triggers cell viability through the activation of survival pathways in the early hours of cell contact with Losac and subsequent gene expression, DNA synthesis and proliferation. A potential biotechnological application of rLosac is minimizing cell death of animal cell culture (production purposes) and consequently increasing productivity.

Supported by: FAPESP, CAPES and CAT/CEPID

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7.03 A new Kunitz-type inhibitor able to induce cell death of human breast cancer: Amblyomin-x

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Introduction: Breast cancer is a type of malignant cancer that can grow in surrounding tissue or spread to distant areas of the body in a process known as metastasis. We produced in our laboratory a recombinant protein originating from the salivary gland of the tick *Amblyomma cajennense* called amblyomin-X, capable of inducing cell death in various tumor cell lines. **Objectives:** The objective of this study was to evaluate the effect of amblyomin-X (Ambly-X), a Kunitz-type inhibitor with ~ 14 kDa obtained in recombinant form (Toxicon 823:34, 2008), in cultured MCF-7 human breast cells through cytotoxicity assays and microinjection technique. **Methods:** MCF-7 cells were grown and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cells were treated with 0.01, 0.03, 0.1, 0.3 and 0.5 μM Ambly-X. Growth medium was used as a negative control. The mitochondrial potential, indicative of cell viability, was measured by MTT assay. Another test performed for evaluation of death, was the Ambly-X microinjection of 0.5 mM in MCF-7 cells with an InjectMan NI 2 micromanipulator (Eppendorf, Hamburg, Germany), connected to FemtoJet (Eppendorf, Hamburg, Germany). At 2 and 4 h after injection, cells were stained with Hoechst (1g/ml) for evaluation of morphological changes in nuclei. **Results and Discussion:** The MTT assay at concentrations of 0.3 and 0.5 μM induced reduction in the number of cells in culture to 74.62% and 36.85%, respectively, when compared to control (ANOVA followed by Dunnett's test, $p<0.01$). The same concentrations induced reduction in cell culture and morphological changes such as rounding and detachment, observed by light microscopy. On the other hand, when 0.5 mM Amblyomin-X was injected directly into cells, we observed 13% of changes as pyknosis and nuclear fragmentation after 2 h of treatment and 31.3% after 4 h, suggesting time-dependence for these effects. Amblyomin-X was able to reduce the population of tumor cells in vitro. The microinjection of the molecule suggests that it needs to be internalized to produce its cytotoxic effects, which are time-dependent, and that cell death can be triggered by the mechanism of apoptosis.

Supported by: FAPESP, CAPES and CNPq

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7.04 DNA replication control in trypanosomes

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Introduction: During S phase of the cell cycle, DNA must be replicated precisely once. The start points of DNA replication (called origins of replication) are established by the formation of the pre-replication complex (PRC). In metazoans, PRC is composed of ORC1-6, Cdc6, Cdt1 and Mcm2-7, which are assembled at origins of replication in G1 phase, where they are activated only in S phase, allowing DNA replication. After DNA replication initiation, PRC needs to be disassembled, preventing re-firing of the same origin of replication. In trypanosomes, Orc1/Cdc6 and Mcm2-7 form the PRC. **Objectives:** Our laboratory has already shown that Orc1/Cdc6 does not seem to be involved in DNA replication control, since it is in the nucleus, bound to the DNA, during the entire cell cycle. Thus, we investigated the possible role of the Mcm2-7 complex in DNA replication control in trypanosomes. **Methods:** Antibodies against *Trypanosoma cruzi* Mcm 3/4 and 7 subunits were raised. We performed immunofluorescence assay using antibody against *Plasmodium falciparum* Mcm4, which is able to recognize Trypanosome brucei Mcm4. **Results and Discussion:** The TbMcm4 is located at the nucleus of some cells with one nucleus, one kinetoplast, and one flagellum (1N1K1F - G1 phase); and one nucleus, two kinetoplasts and two flagella (1N2K2F - S and G2 phase). However, some of these cells did not show TbMcm4 in the nucleus. From this data, it is possible to suppose that cells in beginning of G1 phase do not have Mcm4 in the nucleus. As cells progress to the end of G1 phase, TbMcm4 is recruited to the PRC and can be found in the nucleus until the beginning of S phase. During S phase, TbMcm4 is displaced from the nucleus, and therefore, cells in G2 phase do not show Mcm4 in the nucleus. We are now labeling early/late S cells by Edu incorporation in order to test this hypothesis. These recent data from our laboratory clearly show that DNA replication control in trypanosomes may rely on the Mcm2-7 complex.

Supported by: FAPESP

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7.05 Characterization of neuron-like stem cells derived from the tick *Amblyomma cajennense* (Acari: Ixodidae)

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Introduction: The species *Amblyomma cajennense* is a hard tick distributed in the neotropical region. This species is the most relevant in terms of medical importance, because it is a common parasite of domestic animals and is responsible by the largest number of records biting humans. In vitro tick cell culture is an important tool to isolate and to study the causal agents of transmitted diseases. Previously, the isolation of cell populations of different tick species has been reported. However, tick stem cells have not yet been isolated. **Objectives:** The aim of this study was the derivation and characterization of primary culture of stem cells from *A. cajennense*. **Methods:** The cell culture was obtained from 18- to 23-day-old eggs. The egg masses were washed using ethanol and antibiotic/antimycotic agents. They were then crushed in L-15 modified medium with addition of nonessential amino acids, vitamins and minerals and adjusted to pH 6.4 and pH 7.2. The expression of markers of undifferentiated cells, maturation and differentiation was determined by flow cytometry. Cell viability was evaluated by MTT assay. **Results and Discussion:** When these cells were derived from older egg masses (>23 days), they showed a predominantly nerve-like phenotype. Only after isolation of young cell mass from eggs, did the cells grow as a monolayer and form cell aggregates, which grew in suspension and were mechanically transferred and maintained growing in suspension, even though some of the cells adhered. These cells demonstrated fast proliferation, high viability and were able to undergo approximately 15 passages without presentation of signs of senescence. They demonstrated spontaneous differentiation into adipocytes, melanocytes, and neuronal-like cells. Our data indicate that we succeeded in isolating neuronal-like stem cells, which mimic neurosphere formation in vitro, similar to primary culture of cells from the central nervous system. It seems that these cells show self-renewing capacity, due to multiple passages, and high differentiation potential. Our findings will contribute significantly to the study of pathogenic mechanisms of ticks, which can be involved in cell proliferation and differentiation. To the best of our knowledge this is the first report about isolation of stem cells from a South American tick.

Supported by: FAPESP and CNPq

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7.06 Crotamine-like genes in Brazilian poisonous snakes

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Introduction: Crotamine is a small basic myotoxin of the rattlesnake *Crotalus durissus terrificus* venom, with a β-defensin scaffold, it is a cationic peptide structured by three disulfide bridges. The crotasin gene also expresses a β-defensin-like peptide that is abundantly expressed in several rattlesnake tissues, but hardly in the venom gland. Crotamine (Crt-p1) and crotasin (Cts-p2) genes have three exons and two introns. They have shown high similarity in the untranslated regions (UTR), introns and signal peptide sequence but not in exons 2 and 3. Defensins are present in all vertebrates and their genes were described in mammals, turtles, platypus, and lizards but are scarcely known in snakes. Due to their small size, defensin genes provide excellent targets for PCR amplification. **Objectives:** The aim of this study was to investigate crotamine-like genes in snakes of Bothrops and Lachesis genera. **Methods:** These sequences were amplified by PCR using the primers H010 and 3UTRas, which were designed based on the signal peptide (SP) and 3'UTR sequences from crotamine gene. The annealing temperature ranged from 55 to 58°C depending on the species template used. **Results and Discussion:** Eight crotamine-like sequences were analyzed, and as Crt-p1 and Cts-p2, they are organized into three exons and two introns, one long and another short. The size of the long intron varied from 475 to 1650 nt and the short intron from 148 to 153 nt. The SP sequences showed 58 nt with 97% of similarity when compared to Crt-p1 and 93% to Cts-p2. Exon 2 showed approximately 118 nt coding a prosequence of three amino acids (aa) and about 37 aa of mature peptide. It showed similarities of ~58% when compared to Crt-p1 and 65% in relation to Cts-p2. Exon 3 encodes the last four aa and showed similarities of ~67% when compared to crootamine and 70% to crotasin. The mature peptides have the six cysteines conserved in the β-defensin family, basic amino acids in the carboxy-terminal region, a C-terminal lysine, and positive net charge. The comparison between synonymous and non-synonymous substitutions suggested accelerated evolution acting on the mature protein, mainly on exon 2.

Supported by: FAPESP and INCTTOX

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7.07 Markers related to the differentiation potential of tooth germ cells of human fetus

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Introduction: Technological and scientific advancement has brought considerable benefits to mankind through the introduction of new therapies, and therewith, much research has been conducted on the use of stem cells, yielding encouraging results with regard to the benefits of treatment and in some cases even cure. Stem cells are cells capable of generating different cell lines and can be collected from different adult or embryonic tissues. Among the adult tissues that have a large number of viable stem cells, easily obtained in a method considered less invasive, are the stem cells obtained from the pulp of deciduous teeth. Stem cells derived from the pulp of deciduous teeth have been widely studied because of their ability to differentiate into a variety of cell lines in the presence of various chemical mediators. The formation of teeth in humans begins around the third month of intrauterine development. **Objectives:** The aim of the present study was to analyze the expression of several proteins related to cell differentiation potential of cell populations that make up the mandibular tooth germ of human fetuses. **Methods:** We evaluated 20 human fetuses whose inclusion criterium was the absence of genetic or morphological changes according to the pathological report, we collected 20 mandibles of both genders and different gestational periods. The mandibles were embedded in paraffin, sectioned and stained with hematoxylin and eosin to determine the different stages of development of the tooth germ. The material was subjected to immunohistochemistry for the following markers: OCT-4, NANOG, STAT-3, SOX-2 and PDGF. **Results and Discussion:** The aim of this study was to use the antibodies studied to determine the differential staining of the various anatomical structures, showing different degrees of expression and thus the action of these marker proteins during the stages of tooth development.

Supported by: FAPESP

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7.08 Boron neutron capture therapy induces apoptosis by caspase-3 increase in human melanoma cells

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Introduction: Metastatic melanoma is a highly lethal skin cancer, with incidence increasing more than any other cancer type. In the majority of cases, the existing treatments do not control its metastatic potential, making it necessary to search for new therapy alternatives. Boron neutron capture therapy (BNCT) is a radiotherapy procedure where a compound containing 10B is administered to cancer patients and it is preferentially accumulated in tumor tissues. Boronophenylalanine (BPA) is the agent responsible for delivering boron to the tumor tissue, a process based on the biosynthesis of melanin. The selective boron accumulation in tumor cells is due to high proliferative response and high melanin synthesis. When the tumor is irradiated with thermal neutrons the 10B is converted to 11B, which decays by emission of an alpha particle. Thus, with the administration of BPA, BNCT is used as local radiotherapy for melanoma treatment. **Objectives:** This work was aimed at the evaluation of antiproliferative and antioxidant effects by BNCT in SKMEL-28 human melanoma cells. **Methods:** SKMEL-28 were grown for 24 h, and then treated with different concentrations of BPA and, after 90 min, irradiated with thermal neutron flux up to a dose of 8.4 Gy. The cellular viability was determined by MTT assay for calculation of the 50% inhibitory concentration (IC50%). The oxidative stress over the unsaturated lipids in cell membranes was evaluated. The electric potential of the cell mitochondrial membrane was analyzed by rhodamine 123. The cell populations were analyzed by their DNA content, the check point marker cyclin D1 and phosphorylated caspase 3 by flow cytometry in order to determine cell cycle progression and apoptosis. **Results and Discussion:** The IC50% value found in SKMEL-28 cells was 17.7 mM. BNCT induced oxidant potential by producing up to 20 times more degradation in tumor cells. In addition, BNCT induced a substantial increase of cell death in SKMEL-28 cells by means of DNA fragmentation and decreasing of cells in the G2/M phase. Expression of the marker cyclin D1 decreased, indicating reduction of the cell proliferative capacity. There was a significant decrease in rhodamine 123 capture, evidencing the occurrence of severe damages in mitochondria and resulting in cell death. Furthermore, there was an increase in caspase 3 phosphorylation, which would indicate cell death by apoptosis. In this context, BNCT could be an attractive tool to improve response over the standard radiotherapy for cancer treatment, by raising selectivity for tumor cells.

Supported by: FAPESP

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7.09 Dll4 in collateral artery formation

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Introduction: Despite progressive insights into the pathologies underlying coronary and peripheral arterial diseases, these conditions continue to be the major cause of morbidity and mortality in developed countries. Collateral arteries are direct arteriole-to-arteriole anastomoses, interconnecting adjacent major arterial trees. It is now recognized that these unique vessels can serve as "endogenous bypass vessels", providing substantial protection when a main artery occlusion occurs. However, little is known about collateral vessel formation. **Objectives:** The objective of this study was to understand the molecular mechanisms controlling development of collateral arteries. In particular, we investigated the role of dll4 in collateral growth, as Delta-Notch pathway is critical to maintain arterial endothelial gene expression. **Methods:** Initial assessment of collateral density in wild type (WT) and dll4 heterozygous (dll4+/-) pial vasculature showed that dll4+/- brains had higher collateral numbers at all post-natal ages. Microphotographs of whole mount Connexin40-GFP (CX40-GFP) brains in WT and dll4+/- mice at post-natal day 0(P0) to post-natal day 21 (P21) were quantitated for the number of collaterals in WT and dll4+/- pial circulation. Developmental parameters were obtained by dissection of embryos belonging to both phenotypes from E 11.5 to E 16.5, dll4 expression was additionally modulated pharmacologically by DAPT treatment and Notch signaling by the analysis of loss and gain of function inducible mice. **Results and Discussion:** Dll4+/- mice show delay in initiation of arterialization (E11.5-E13.5), increased and disorganized arterial branching (E13.5 - E15.5), increased arborization of arterial network (E15.5 to birth), persistence or maintenance of arterial arborizations/collaterals to adulthood, and pharmacological Notch inhibition by DAPT; endothelial-specific inducible Notch-1 deletion generates a phenotype similar to dll4+/-, dll4 GOF appears to produce opposite phenotype (i.e., less arterial branching, reduced number of collaterals), and partial inhibition of dll4 does not improve post-ischemic recovery after MCAO.

Supported by: Fundação Butantan and INSERM

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7.10 Cell cycle and cell death markers of bovine yolk sac during embryonic development

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Introduction: The development of the embryo is complex and influenced by several factors, where it is totally dependent on an adequate balance between proliferation, differentiation and cell death. The yolk sac is attached to an embryo, present in all vertebrate species, and plays important functions during embryonic development. This process produces many cells that are driven to death, contributing to the formation of organs and tissues. **Objectives:** The purpose of this study was to determine the mediators involved in the induction of apoptosis during involution of the yolk sac of bovine embryos. **Methods:** Twenty-one yolk sacs of embryos from cattle slaughterhouses in the state of Goiás and Sao Paulo were studied according to gestational age obtained by calculating the crow rump (CR) in the following groups: Group I (22 - 27d), Group II (28 - 32d), Group III (33 - 37d), Group IV (38 - 42d) and Group V (43 - 47d). The cell cycle phase was determined by flow cytometry and cell death by expression of annexin V/PI, caspase 3, r-TNF, cytochrome C, electrical potential of the mitochondria, synthesis of HSP 47 and receptors involved in angiogenesis VEGF-R1. **Results and Discussion:** The results of samples showed that there is an increased presence of haploid cells with fragmented DNA in Group II. An ability to arrest in G2/M in groups III to V showed that the greater the gestational age is the lower the capacity for cell proliferation is. The expression of markers involved in programmed cell death and synthesis positively correlated with increase in the activity of phosphorylated caspase 3 in Group I (40%), Groups II and III (10%); Group IV (20%) and Group V (10%). Decrease in mitochondrial electrical potential (rhodamine 123) and released cytochrome C were also accompanied by the expression of TNF receptor. The proportion of cells dead by apoptosis (Annexin V) and necrosis (PI+) indicated that the measure that has an increase in gestational age and there is an inversion of the proportion of necrosis in relation to late apoptosis. The capacity for synthesis as assessed by expression of HSP 47 protein showed a significant increase in the lowest gestational age (60%). The R1 receptor expression of VEGF was increased in Groups I and II (80%), while others showed a decrease in the remaining periods. We conclude that the involution of the yolk sac is correlated with induction of apoptosis and increased expression of VEGF in the first pregnancy and possibly signs of hematopoiesis, which will occur in the fetal liver.

Supported by: CNPq

7.11 Subtyping, sequencing and phylogenetic analysis of influenza in samples isolated from wild, migratory and resident birds, in São Paulo state

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Introduction: Avian influenza virus belongs to the family Orthomyxoviridae. In the last years, several low pathogenic avian influenza subtypes have caused outbreaks and epidemics in humans and poultry. Wild and migrating birds may be participating in the maintenance and interspecies transmission of the sixteen subtypes of the hemagglutinin and nine neuraminidases in nature. **Objectives:** The aim of our study was to subtype the positive samples for influenza A isolated from migrating and wild birds by molecular techniques and hemagglutination inhibition test (HI). **Methods:** Samples from the species *Elaenia mesoleuca* (6712, 6715), *Sporophila lineola* (6744) *Sporophila caerulescen* (6745), *Vireo olivaceus* (6751), *Columbina talpacoti* (6780, 6781, 6784) and *Paroaria dominicana* (6782, 6783) were collected in reserves and experimental field stations located in São Paulo State - Brazil, during the years 1997 and 1998. The samples were identified by HI test (according to WHO) using the 20 antibody patterns of anti-influenza A type and one for the influenza type B, and RT-PCR and sequence analysis of hemagglutinin and neuraminidase genes**. Results and Discussion:** The HI test demonstrated that nine samples showed a close antigenic relationship with A/HongKong/1/68(H3N2), A/ Equine/Miami /63(H3N8) and A/Duck/ Ukraine/ 63 (H3N8) antiserum. The sequencing analyses of hemagglutinin and neuraminidase genes of these nine isolates revealed a high homology with H3N2. Phylogenetic analysis and genetic variability compared with GenBank sequence representing several countries, showed that our current samples submitted close homology to that circulated in Victoria (1990), Siena (1991) and Beijim (1989). Amino acid variability analysis compared our samples with representative GenBank samples; all mutations are synonymous. Our laboratory is currently characterizing six other gene segments in these isolates for ongoing active surveillance, for early identification of recombination events which could lead to the emergence a novel H3N2 influenza virus with potential to infect human.

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7.12 Myogenic potential of multi-colony derived immature dental pulp stem cells

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Introduction: Stem cell therapies can be applied to repair and replace damaged skeletal muscle using different cell sources such as, muscle-derived satellite cells, mesoangioblasts or pericytes, embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs). Our studies and those of other authors have revealed that some stem cells derived from dental pulp (DPSCs) share similar properties with both MSCs and ESCs. Due to their nature, these cells are able to differentiate into different mesoderm lineages. Recently, it has been proposed by J. Mao group and that single cell colony derived DPSCs show more robust myogenic differentiation, than those isolated from multi-colony populations. **Objectives:** We focused our study on in vitro myogenic potential of multi-colony populations of pluripotent immature cells (IDPSCs). **Methods:** Human IDPSCs from deciduous and adult teeth were characterized and maintained under conditions previously described. Differentiation towards muscle cells was performed following protocol developed by us. Morphological studies were performed using hematoxylin/eosin. For immunofluorescence analysis, differentiated cells were fixed in 4% paraformaldehyde and characterized using anti-human antibodies: anti-ABCG2, anti-syndecan-3 and 4, anti-myosin heavy chain, anti-alpha actinin, anti-titin, anti-desmin, anti-MyoD1 and others. The expression of muscle-specific proteins and genes was studied by confocal microscopy and by RT-PCR, respectively. **Results and Discussion:** IDPSCs growing under basal culture medium showed MSCs morphology and expressed muscle cell progenitor markers, such as syndecan 3 and 4. After induction of differentiation, these cells start to show cell alignment and fusion. Next, small myotube-like formation and finally robust myofiber-like structures as well as myosacs were observed. Following the process of myogenic differentiation of IDPSCs, down-regulation in expression of syndecans was detected. On the other hand, there was up-regulation of expression of immature muscle markers, such as MyoD1 and myogenin. We observed that these muscle-like cells can be maintained in culture for a long period, and whole process of differentiation took at least 20 days. At the end of differentiation process the expression of mature muscle cell markers was evidenced in myofiber-like structures derived from IDPSCs. In contrast to previously reported, multi-colony derived pluripotent IDPSCs were able to undergo almost uniform and robust myogenic differentiation. These cells expressed a wide range of muscle-specific proteins, which suggests their capacity to produce skeletal, smooth and cardiac cells under the same conditions. These and our previous findings support the therapeutic potential of multi-colony derived IDPSCs in muscle regeneration.

Supported by: FAPESP and CAPES

7.13 Equine adipose tissue-derived stem cells. Their potential for the treatment of endometrosis

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Introduction: Endometriosis is a progressive and irreversible disease which is defined as active or inactive periglandular and stromal endometrial fibrosis, including glandular alterations. Modifications induced by the disease alter the surface of endometrium, which consequently leads to infertility. Conventional treatments do not reduce the fibrotic process or even help to restore fertility. Stem cell (SC) therapy in horses is a promising tool for tissue reconstruction, which also provides additional benefits, such as anti-inflammatory and immunosuppressive activity. There is no information about the use of stem cells derived from equine adipose tissue, for the treatment of endometriosis. **Objectives:** The aim of the present work was to test the capacity of equine adipose tissue-derived stem cells for the treatment of endometriosis. **Methods:** In order to reduce the inflammatory process and to remodel periglandular fibrotic tissue affected by the disease, equine heterologous adipose tissue-derived stem cells, previously obtained and characterized by our group, were used. Six estrus synchronized mares (four for treatment and two for control) were used. The diagnosis had been established by histomorphological and immunohistochemical analyses, performed using formalin-fixed, paraffin-embedded uterine biopsies. The sections were stained with hematoxylin/eosin and alcian blue. Equine stem cells were stained using a vital dye (Vybrant CFDA-SE Cell Tracer Kit®). These cells were infused into the uterus of the four treatment mares. Each one of these animals received $2.5x10^7$ cells. The two control mares were infused with a placebo. **Results and Discussion:** After 7, 21 and 61 days, new uterine biopsies were obtained and remodeling of uterine tissues by stem cells was analyzed. Histological changes were observed in the uterine biopsies of the 4 treatment mares. Analysis performed 7 days after treatment confirmed the presence of injected cells in the uterine tissues, by green fluorescent signal provided by vital dye, while control animals did not show fluorescence. No signs of immunologic reaction in response to cell application were observed, since the presence of T-cells infiltrating the site of cell graft was not evident. The presence of equine stem cells within uterine tissues was confirmed. Further investigations are needed in order to prove the remodeling of uterine tissue through stem cell differentiation.

Supported by: CAPES and FAPESP

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7.14 Ultrastructural mapping of HPV16 capsid proteins in transfected Human epithelial cells

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Introduction: HPVs (human papillomavirus) are currently associated with genital warts and several types of cancer. The most common is the cervical cancer, one of the leading causes of cancer death in women worldwide, caused mainly by HPV16 which is responsible for nearly 50% of all cases. Presently, available HPV vaccines are based on the L1 major capsid protein, which self-assembles into virus-like particles (VLP). In spite of being extremely effective, commercial vaccines are too expensive for public distribution in developing countries. As vaccines based on the minor capsid protein L2 proved to provide protection against different HPV types but were little immunogenic, we developed the production of L1 and L2 by the same cell, so that these two proteins can self-assemble in a more stable VLP, similar to the native HPV capsid. Here, we describe the production of HPV16 L1 and L2 proteins by human cell cultures and demonstrate the expression of the proteins by immunodetection methods. **Objectives:** The aim of this study was to map the HPV16 L1 and L2 protein distribution in transfected and co-transfected human cell cultures to evaluate this method for HPV vaccine production. **Methods:** Cell cultures of HEK 293T were transfected with DNA expression vectors encoding for L1 and L2 proteins of HPV16. The intracellular protein expression was detected by transmission electron microscopy using anti-HPV16 L1 and anti-HPV16 L2 specific primary antibodies and secondary antibodies conjugated with 5-nm and 10-nm colloidal gold particles for immunolabeling. The cell cultures were also analyzed by immunofluorescence with laser scanning confocal microscopy and Western blotting. **Results and Discussion:** We successfully established an efficient system of recombinant L1 and L2 gene expression. The L1 and L2 proteins were detected within the cell nucleus and cytoplasm, likewise co-transfection assays showed double labeling for L1 and L2, confirming the protocol efficiency by cellular and molecular procedures. Vaccines formulated with HPV16 L1L2 VLPs will be tested in animals to evaluate their capability to induce immune responses with antibodies specific for HPV16 and also other types. These findings have implications for the development of a national human papillomavirus prophylactic vaccine that could embody public health programs especially in developing countries where the prevalence of HPV-related cancer is a significant cause of morbimortality.

Supported by: FAPESP, Instituto Butantan, Fundação Butantan, CAPES and PAP/SES

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7.15 Pre-rRNA *trans*-splicing in trypanosomatids: new targets for old mechanisms

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Introduction: Trypanosomatids regulate gene expression mainly at the post-transcriptional level. Large polycistronic units are converted to monocistronic mature messages by spliced-leader (SL) addition trans-splicing and polyadenylation. Preliminary results suggested that SL addition trans-splicing is not an exclusive pre-mRNA processing mechanism, but it can also use Typanosoma cruzi and *Leishmania (Leishmania) amazonensis* 5'ETS pre-rRNA sequences as acceptor substrates. **Objectives:** The objective of this study was the characterization of pre-rRNA trans-splicing acceptor sites in trypanosomatids. **Methods:** A semi-nested RT-PCR strategy was used to determine pre-rRNA acceptor sites in four different trypanosomatid species: *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania (Leishmania) amazonensis* and *Crithidia fasciculata*. RNase protection and primer extension approaches certified the RT-PCR strategy in *Leishmania (L.) amazonensis*. qRT-PCR was used to determine SL-5'ETS copy number in *Leishmania (L.) amazonensis* promastigotes treated with sinefungin or 5-fluoruracyl. 3'RACE was used to determine poly(A) sites. **Results and Discussion:** We first *identified* and characterized these acceptor sites in *T. brucei, T. cruzi, L. (L.) amazonensis* and *C. fasciculata* using a semi-nested RT-PCR strategy. Sequence analysis of RT-PCR products showed that generation of SL-5'ETS hybrid molecules follows the same rules as for pre-mRNA trans-splicing. RNAse protection and primer extension experiments showed that 5'ETS acceptor sites indeed correspond to native processed products in *L. (L.) amazonensis*. Real-time RT-PCR experiments (qRT-PCR) using *L. (L.) amazonensis* total RNA extracted from cells treated with trans-splicing inhibitor sinefungin showed that SL-5'ETS molecules products diminish in concentration while non-spliced 5'ETS molecules remain constant. Treatment of the cells with the exosome inhibitor 5-fluoruracyl resulted in accumulation of 5'ETS molecules in a dose-dependent manner, suggesting they can be discarded, at least in part, by exosome degradation route. RT-PCR experiments addressing acceptor splice sites in *L. (L.) amazonensis* internal transcribed spacer I (ITSI) showed three other different pre-rRNA trans-splice sites. Finally, we detected polyadenylation products upstream from 5'ETS addition sites. This polyadenylation event was expected since trans-splicing and polyadenylation are accomplished mechanisms in trypanosomatids. The detection of trans-spliced pre-rRNAs indicates broad RNA joining properties regardless of the polymerase used for transcription and may be indicative of an ancient gene expression strategy used by organisms with partially specialized RNA polymerases.

Supported by: FAPESP and CNPq

7.16 Numerical and structural chromosome abnormalities in primary cell lines from BPV-affected bovines

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Introduction: Bovine papillomavirus (BPV) is an epithelial oncogenic DNA virus, species and tissue specific. Studies have reported the virus genome presence in other non-epithelial tissues, and recently, much evidence of expression of these DNA sequences has led to discussions about their infection potential. One of them is the high level of chromosomal abnormalities found in lymphocytes from chronically infected animals and the presence of virus DNA in fibroblast cells in vivo and in vitro cultures. **Objectives:** The aim of this study was to evaluate the frequency of chromosome abnormalities in cell cultures obtained from normal skin, cutaneous papillomas, esophagus papillomas and fragments of bladder with enzootic hematuria. **Methods:** Cytogenetic studies were performed in samples from affected bovines and in a normal skin sample of an asymptomatic animal (control). DNA virus detection and BPV-1 antibody labeling were developed. These cells were labeled with anti-BPV-1 antibody. To establish a primary cell line culture, fragments of the tumors were incubated in Dulbecco Modified Eagle medium, supplemented with 10% fetal bovine serum and kept at 37°C. In both procedures, colchicine was added for 1 h; the material was then centrifuged and treated with hypotonic solution (0.075M KCl) and fixed (methanol:acetic acid, 3:1). The slides were stained in 2% Giemsa in phosphate buffer and analyzed with a light microscope. The detection of BPV genome sequences in warts and through culture passages was investigated by PCR, using generic and specific primers. For immune-fluorescence, we used as primary antibody mouse monoclonal [BPV-1-1H8] and anti-mouse IgG antibody linked to FITC as secondary antibody. **Results and Discussion:** There was a higher frequency of cells with chromosome abnormalities in the affected animals compared to control, both numerical ($p>0.05$) and structural ($p>0.01$).The most frequent structural aberrations were: chromatid breaks (Ctb) and the rearrangement caused by the addition or deletion of segments (add/del), followed by acentric fragments and telomeric and centromeric fusions. The frequencies of aneuploid and polyploid cells were higher, but only in the bladder cells was this statistically significant. Immunofluorescence was positive for BPV-1 in cutaneous, esophageal papilloma cells and in hematuric bladder, more evident in the nuclear and perinuclear region. This localization of L1 could be very similar to that observed in cells infected with L1 proteins and cells that produce L1 for VLP particles. These findings could point out that virus genome sequences found in the cultures seem to be active, causing damage to host chromatin and impairing cell cycle control.

Supported by: CNPq, FAPESP, Fundação Butantan and CAPES

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7.17 Immature dental pulp stem cells can differentiate in spheres with retinal characteristics *in vitro*

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Introduction: Retina is the light-sensitive eye tissue which converts captured energy into image by highly specialized neuronal cells. If these cells are damaged, vision capacity is permanently lost, since they are unable to regenerate. Currently, treatments aim only to decrease retinal damages instead of promoting an effective vision recovery. Considering the difficulties of obtaining stem cells (SC) from the 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:** We aim to evaluate the potential of IDPSC to develop structures similar to retinal spheres, which can represent a new source for treatment of retinal degenerative diseases. **Methods:** Undifferentiated IDPSC, previously established and characterized by us, were analyzed by immunocytofluorescence to evaluate CD73 expression, an early photoreceptor marker. We also analyzed the expression of specialized retinal neuron antibodies: anti-rhodopsin, anti-calbindin, anti-PKC and anti-Phd. In addition, these cells were submitted to progenitor neural differentiation using protocols developed by us. The capacity of IDPSC to differentiate towards structures similar to retinal spheres was evaluated by immunofluorescence using anti-nestin, anti-β-III-tubulin and anti-Pax-6 antibodies. Co-culture of differentiated IDPSC with retinal pigmented epithelium cells is being developed. **Results and Discussion:** Undifferentiated IDPSC reacted positively to CD73 and were negative to specialized retinal neurons antibodies. We also observed the positive reaction of anti-nestin and anti-β–III-tubulin in retinal spheres, indicating that these structures have previous commitment with neural lineage. Anti-Pax-6, a marker of early stages of eyes and other sensory organ development, was also positive in retinal spheres. Nevertheless, some factors that can facilitate the induction of mature retinal characteristics are still required. We demonstrated that IDPSC have the potential of developing structures similar to retinal spheres, with neural and retinal properties in vitro, which can be maintained viable for a long time in 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.

Supported by: CNPq and FAPESP

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7.18 Cytochemical and morphologic features of bone marrow hematopoietic cells in *Oxyrhopus guibei* (Ophidia: Dipsadidae)

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Introduction: Hematopoietic stem cells are characterized by the ability to self-renew and differentiate into all mature blood lineages. Studies related to hematopoietic cells in reptiles are very scarce. Bone marrow of vertebrae and ribs is the main hematopoietic focus in the newborn and adult snakes, while in the early embryo it starts in the yolk sac blood islands and in a region of dorsal aorta. Currently, there are many markers of different cell lineages used to identify and characterize hematopoietic cells, but most of these markers are specific to humans and rodents and are not always useful for other groups such as snakes. Therefore, the cytochemical reactions are widely used to evaluate the morphological characteristics of the maturation of these cells. **Objectives:** The aim of this study was to identify and characterize the cytochemical features on bone marrow hematopoietic cells of adult and newborn snakes *Oxyrhopus guibei*. **Methods:** Fragments of vertebrae of newborn snakes (n= 8) were collected to obtain bone marrow. The material was fixed in Bouin, formol calcium or 4% paraformaldehyde + 2% glutaraldehyde and embedded in resin or paraffin. Tissue sections and imprint of bone marrow and blood smears without anticoagulant were stained with May-Grunwald-Giemsa (Rosenfeld) or HE (hematoxylin and eosin) or methylene blue and examined with a light microscope. The cytochemical reactions performed were: periodic acid-Schiff (PAS), toluidine blue (TB), Sudan black B (SBB), benzidine peroxidase (BP) and acid phosphatase (AP). **Results and Discussion:** Presence of an active hematopoietic focus was observed in bone marrow of vertebrae and ribs. Precursors of all lineages of blood cells were identified. Acidophilic granulocytes are the most abundant cells in bone marrow. Cytochemical reactions were useful for the differentiation of immature cells. TB was positive in the basophilic granules of immature and mature basophils. Cytoplasmic PAS positive cells were identified as thrombocytes; basophilic leukocytes showed PAS-positive granules. Heterophils and azurophils showed strong positive reaction for lipids staining with SBB. Similar results were obtained for BP. AP was found on azurophils in various stages of maturation. Therefore, we conclude that the rib or vertebral bone marrow is a principal hematopoietic site in the newborn and adult *O. guibei* snakes and that cytochemical characteristics are useful to identify and analyze different stages of maturation of various blood cells. Additional cytochemical and ultrastructural studies will be carried out to identify other lineages of blood cells in snakes.

Supported by: CNPq and FAPESP

7.19 Identification of glyceraldehyde 3-phosphate dehydrogenase as a DNA-binding protein in *Trypanosoma*

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Introduction: Recent studies have established that the glycolytic protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional protein with defined functions in numerous subcellular processes. New roles for GAPDH include transcriptional control, nuclear membrane fusion, recognition of fraudulently incorporated nucleotides in DNA, and its mandatory participation in the maintenance of telomere structure. **Objectives:** The aim of this study was to evaluate GAPDH binding to DNA in *Trypanosoma*. **Methods:** Fractionated extract was used in order to separate soluble proteins from DNA-binding proteins and perform the electrophoretic mobility shift assay. **Results and Discussion:** We demonstrated that GAPDH is found in the DNA bound fraction from *Trypanosoma brucei*. In *Trypanosoma cruzi*, GAPDH is only found in DNA bound fraction when cells are treated with leptomycin B, which blocks nucleus – cytoplasm transport. Preliminary data using *T. cruzi* recombinant GAPDH protein in electrophoretic mobility shift assay showed that it binds double strand telomeric DNA, but not single strand telomeric DNA. These data suggest that the role of GAPDH is more than energy production even in *T. cruzi* and *T. brucei*, which are early divergent eukaryotes.

Supported by: FAPESP

8. Animal Biology

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8.01 The influence of *Wolbachia* on *Culex quinquefasciatus*: a study case in São Paulo, Brazil

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Introduction: *Wolbachia* are α-proteobacteria that were first reported in *Culex pipiens* mosquitoes early in the twentieth century. Heretofore, these endosymbionts have been detected in dozens of invertebrate species. However, the effects of *Wolbachia* on their host's reproduction have been increasingly investigated, given the extreme complexity of this interaction, new study cases are welcome to enhance its understanding. The present work addressed the influence of *Wolbachia* on *Cx. quinquefasciatus*, the cosmopolitan member of that *Culex* complex. **Objectives:** The aim of this study was to evaluate and compare physiological and reproductive differences between *Cx. quinquefasciatus* infected and uninfected by *Wolbachia*. **Methods:** Samples of a *Cx. quinquefasciatus* colony ($wPip^+$) originating from individuals naturally infected by *Wolbachia pipientis* B strain, were cured with tetracycline, yielding a *Wolbachia*-free colony ($wPip^-$). Both the presence of bacteria and the efficiency of bacterial removal were checked by PCR of the *wsp* gene and ovary ultrastructure. Some fitness aspects, shape and the wing length, of mosquitoes from both colonies were measured. Moreover, *Wolbachia* present in mosquitoes from some states of Brazil and from our colony are phylogenetically classified by two genes (*wsp* and *coxa*). **Results and Discussion:** Reproductive aspects were also comparatively evaluated between colonies**.** No bias in the sex ratio was observed in any colony. Concerning the oviposition time during the first gonotrophic cycle, $wPip^+$ females laid eggs earlier than did $wPip^-$ females ($p< 0.01$). Reproductive fitness was higher among $wPip^-$ than $wPip^+$ females regarding the following parameters. Fertility: rafts/fed females ($p< 0.001$); fecundity: eggs/raft ($p< 0.001$), and viability: larvae/eggs ($p< 0.001$). Conversely, longevity of $wPip^-$ females was shorter ($p < 0.001$). Summarizing, although the infected mosquitoes have the advantage of a greater longevity, they have lower reproductive fitness. The comparison of wing shape shows no differences, but the length of the wing from individuals from $wPip^+$ is greater ($p< 0.001$). *Wolbachia* of *Cx. quinquefasciatus* in Brazil are genetically identical for the two genes tested. Our results are partly distinct from some other reports on *Aedes* and *Culex* mosquitoes.

Supported by: CNPq

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8.02 Patterns of selection and use of the nest site in Neotropical snakes

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Introduction: In oviparous reptiles, nest site location has received increasing interest from evolutionary ecologists because it may affect fitness in several ways. For example, hatchling phenotypes linked to survival are strongly influenced by thermal and moisture conditions of nests. These variables are directly dependent on nest characteristics as level of sun exposure, nest depth, type of soil and type of nest. Thus, knowing the types of microhabitat utilized by oviparous females to lay their eggs is central for understanding snake nesting ecology, besides providing important knowledge for conservation of breeding sites. Nevertheless, nest sites of Neotropical snakes are poorly known and there are only sparse reports of natural nests in Brazil. Mostly, this reflects the difficult task of finding snake nests in nature only by visual search. **Objectives:** Here, we focused on answering three questions: (1) What are the types of microhabitats used by gravid snakes to oviposit? (2) Are the nests used individually or communally (i.e., shared with two or more females)? (3) Is there a pattern of selection and use of the nest site? **Methods:** We performed an extensive literature survey of egg-laying in nature. In addition, we included six original observations of nest sites. We gathered 127 observations for 20 genera and 29 species of Neotropical snakes. **Results and Discussion:** In all instances, females seem to be unable to construct a nest and thus rely on pre-existing sites for egg-laying. However, such sites were widely diverse; anthill, under rocks and logs, within preformed subterranean chambers or litter fall. Anthills (86%) were significantly more used. Such micro-habitats show high and invariable temperature and moisture and thus provide an adequate environment for egg development. Nest use (communal or individual) was 50-50%. However, sharing nests appears to be more recurrent in some taxa than others. For example, *Sibynomorphus* spp. (n = 5) nests were communal in all instances whereas all *Oxyrhopus* nests (n = 6) were used individually. Nevertheless, for some groups (e.g., Philodryadini and Xenodontini) both patterns of use were observed. Both communal and individual nests have advantages and disadvantages. The adoption of one type of nest versus the other suggests that fitness benefits resulting from the trade-offs between them vary among species.

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8.03 Femoral glands in the lizard *Micrablepharus maximiliani* (Squamata, Gymnophthalmidae)

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Introduction: The blue-tailed gymnophtalmid lizards *Micrablepharus maximiliani* are diurnal and have fossorial habits, living in open fields or regions covered with foliage. Like most lizards, it makes use of the chemical communication mediated by pheromones secreted by femoral glands to obtain and interpret information from the environment in which they live. In this species, one of the most basal in relation to other genera of the group, in which the loss of limbs is very prominent, the appendicular reduction is present in the forelimbs, but restricted only to the absence of the inner finger. **Objectives:** The aim of this study was to better understand the morphology of the femoral glands of *M. maximiliani* and their role in chemical communication, comparing to the most derived lizards of the family Gymnophthalmidae. **Methods:** The glands were fixed in Karnovsky or Bouin solution, and embedded in historesin or paraffin. The sections were stained with toluidine-fuchsin or HE for general study of glandular morphology. Other sections were stained with picro-sirius and Mallory trichrome for the identification of collagen and muscle fibers. The following histochemical stainings were performed: periodic acid-Schiff (PAS) and alcian blue, pH 2.5, for neutral and acidic carbohydrate identification, respectively, and bromophenol blue, for protein identification. **Results and Discussion:** The glands are located in a row below the dermis, forming a glandular cord in the femoral region only in males of the species. Each gland is divided into lobules by a thin layer of connective tissue and composed of peripheral germinative cells and secretory cells in the internal region. At the end of their maturation, the secretory cells are totally filled with granules, die and desquamate from the secretory epithelium, becoming part of the solid secretion plug, which obstructs the duct. This process is typically holocrine. The secretion of rigid nature, but brittle, is deposited into the environment through 10 secreting pores.The secretory cells and secretion plug are strongly stained by PAS and bromophenol blue, which indicates that the secretion is mainly composed of glycoproteins. The glandular structure was slightly positive with alcian blue staining. Until now, conspicuous interspecific differences concerning the morphology and size of secretion granules are quite noted among Gymnophthalmidae lizards. This feature should reflect differences in the structure and composition of the secretion and in the different ways in how the pheromone is released into the environment.

Supported by: FAPESP and CNPq

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8.04 Evaluation of ovarian and uterine development by ultrasonography in Brazilian boids

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Introduction: Information about the reproductive biology of Neotropical Boidae is limited to examples preserved in collections. The boid snakes are viviparous and have a seasonal reproductive cycle. The evaluation of reproductive status with the naked eye and palpation are imprecise, while the sonography is a very valuable method because it permits the visualization of the follicular development process until birth of the offspring. **Objectives:** The objective of this paper was to evaluate follicular development during the vitellogenic process, ovulation and fertilization and the embryonic development **Methods:** The snake reproductive status was evaluated with three ultrasound machines: (1) portable ultrasound Fukuda 4500, (2) with color Doppler ultrasonography in the IVI (Veterinary Institute of Image) and (3) with ultrasound My Lab 30 Gold Vet and My Lab Five Vet (Cimex-Esaote). The vitellogenesis evaluation was done every three months and the embryological development was observed monthly. **Results and Discussion:** Follicular development was observed in the sonographic examinations and two phases were visualized: primary and secondary vitellogenesis. The development of the embryonic process starting from fertilization up to yolk absorption and bone formation was observed in *Boa constrictor* and *Corallus hortulanus*. In *Eunectes murinus* and *Epicrates cenchria* only the vitellogenesis process was observed. Ultrasonography is an important tool to determine developmental phases, reproduction seasonality, vitellogenesis period, fertilization and pregnancy of the species studied.

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8.05 Where are my eggs? Sonographic and Radiographic evaluations of the vitellogenesis in *Python regius* and *Python molurus*

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Introduction: Pythoninae is a subfamily restricted to the old world, occurring in Africa, Asia, Solomon Islands, Australia and New Guinea. They are strong animals which kill their prey by constriction. They have a quite complex reproduction, with oviparity, incubation of eggs and the males performing combat dance before mating. The literature is controversial about the phenomenon of follicular reabsorption, whether it occurs in the ovary or in the oviduct. **Objectives:** The objective of this study was to report the visualization of secondary vitellogenesis and the formation of eggs that have been absorbed before the posture. **Methods:** Vitellogenesis was assessed by radiography and ultrasonography in two specimens of Python: a *Python regius* and an albino *Python molurus*. **Results and Discussion:** Radiography and ultrasonography were performed in *Python regius* after a combat dance and mating. Five follicles were observed in the secondary vitellogenesis after copulation. A year later, there was no posture and follicle reabsorption was observed in the ovary. In *Python molurus* about 23 follicles measuring from 3.5 to 4.9 cm were observed in the ovary, which measured from 0.6 to 0.7 cm 4 months later. This reabsorption and lack of posture may be related to the lack of necessary conditions, such as adequate moisture and temperature, which may cause the animal to reabsorb the follicles.

Supported by: Fundação Butantan

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8.06 Lymphoma in tree frog (*Phylomedusa distincta*, Anura, Amphibia): a case report

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Introduction: *Phyllomedusa distincta* is a tree frog from the family Hylidae, which lives in close vegetation near to water bodies in the Brazilian rain forest, from the south of São Paulo to Rio Grande do Sul. It has an olive-greenish coloration, big yellow eyes, with male individuals reaching 55 mm in length. Its population is in decline due to natural habitat loss to agriculture activities and deforestation. Neoplasias, spontaneous or not, although rarely reported, are a common illness in amphibians, usually species or population specific. About 50% of amphibian neoplasias have a tegumentary origin. The malignant lymphomas are common among animals, with great mortality rate. They originate as white, fleshy and solid masses which destroy the lymphoid organ located in the parenchyma of the viscera. The malignant cells show variable aspects, such as normal small lymphocytes and large blastic cells. The pathogenic factors responsible for the release or proliferation of neoplastic cells are unknown and the lymphomas may progress to leukemia. **Objectives:** The objective of this paper is to report a lymphoma case in a tree frog, *Phylomedusa distincta*. **Methods:** After the animal's death, a necropsy was performed and the collected material was sent for histopathological analysis to IVI (Instituto Veterinário de Imagem) and to the Pathology Comparative Laboratory FMVZ- USP. **Results and Discussion:** The tree frog (*Phylomedusa distincta*), maintained in captivity in the Museu Biológico of Instituto Butantan for 4 years, had a bleeding rostral wound. Topical treatment was performed and the animal died on the following day. At necropsy, the liver showed a neoplasic process, congested vessels and a white mass. The histopathologic examination showed compression of hepatocyte plaques with cell loss, multifocal degeneration and aggregated melanophages. In other organs, cellular pleomorphism, evident nucleolus and rare atypical mitotic activities were found. The alterations determined the diagnosis of widespread lymphoma. Malignant lymphomas are a common cause of death in amphibians, but the origins of these neoplasias are unknown, and thus, there is a need for further studies.

Supported by: Fundação Butantan

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8.07 Use of acupuncture in the therapy of traumatic disease in iguana (*Iguana iguana*): case report

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Introduction: Acupuncture is an ancient therapeutic practice of Traditional Chinese Medicine which consists in the stimulation of certain points on the body. The treatment has immunomodulatory, antiinflammatory, analgesic and regenerative effects. It stimulates wound healing and interacts with the autonomic nervous system through a visceral cutaneous reflex, which influences the internal organs through stimulation of a somatic point. Points already known in humans and other animals are transposed to reptiles, and the same therapeutic effects were observed as in other animals. **Objectives:** This paper demonstrates the use of acupuncture as a complementary therapy in a case of an iguana (*Iguana iguana*) that suffered trauma, osteomyelitis and bone loss in the left forelimb. **Methods:** A 10-year-old female Iguana (No. MIB 4291) was treated with acupuncture. The animal had a history of osteomyelitis due to traumatic subluxation in the left thoracic limb and had been treated with antiinflammatory and antibiotic drugs. After one year of this treatment, acupuncture was performed weekly, for three months, using an electrical apparatus (EA), at the points R3, F3, E36, VG34, B23, B14, P5, IG10 and IG4. The parameters "activity on the premises" and "proportion of time with closed eyes" was observed. **Results and Discussion:** In the last radiography before the acupuncture treatment (March 2011), the forelimb showed carpal osteopenia, organized bone discontinuity of the proximal epiphyses of the metacarpals and distal epiphysis of the radius with loss of articular relationships, mainly radio-carpal and carpal-metacarpal. The animal showed decreased activity and increased of time with closed eyes, without lameness or evident pain on palpation of the affected limb. With the treatment, subjective values varied according to the room temperature, and a decrease in the proportion of time with closed eyes and increased activity of the animal were observed. Acupuncture is an efficient and feasible complementary therapy as well as an alternative technique in reptiles because it is a well-tolerated treatment with very minor side effects.

Supported by: Fundação Butantan

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8.08 On the revision of the genus *Ariadna* Audouin, 1826 in Brazil (Araneae: Segestriidae)

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Introduction: The genera *Ariadna* Audouin, 1826, *Segestria* Latreille, 1804 and *Gippsicola* Hogg, 1900 comprise the spider family Segestriidae, today with 120 species described taxonomically. They are haplogynae six-eyed spiders, with the third pair of legs forth. Nowadays, they belong to the Dysderoidea clade, composed of four families: (Segestriidae (Dysderidae (Oonopidae + Orsolobidae))). *Ariadna* contains 98 species, which can be found in all continents (except Antarctica), with the greatest diversity in tropical and subtropical region. In Brazil, there are eight described or recorded species, where only five remain taxonomically valid: *Ariadna obscura* (Blackwall, 1858), *A. crassipalpa* (Blackwall, 1863), *A. mollis* (Holmberg, 1876), *A. boliviana* Simon, 1907, and *A. gracilis* Vellard, 1924. Three species are considered *nomina dubia*: *Ariadna dubia* Mello-Leitão, 1917, *A. conspersa* Mello-Leitão, 1941 and *A. spinifera* Mello-Leitão, 1947. **Objectives:** The aim of this study was to revise the Brazilian species of *Ariadna*, redescribe and illustrate their genitalia, and present a new diagnosis for them, eventually describing new species. **Methods:** Type and non-type material of 10 national and international collections were examined. Two types of the species described for Brazil were examined. The types of *A. obscura, A. gracilis* and *A. dubia* are lost, but we analyzed the types of *A. campinensis* and *A. taperae*, both synonyms of *A. obscura*. The description is the standard in the recent revisions in Segestriidae. To study the female genitalia, the anterior portion of the abdomen was dissected, and then cleaned and digested in lactic acid 85%. The drawings were made with a camera lucida on a Leica MZ 12.5 stereomicroscope and the multifocal photos were taken with a Leica MZ 16A stereomicroscope with a Leica DFC 500 digital camera attached. **Results and Discussion:** We detected that *A. gracilis*, *A. conspersa*, *A. campinensis* and *A. taperae* are synonyms of *A. obscura*. *A. spinifera* consists of a male of *A. mollis* and will be synonymized. The allotype of *A. crassipalpa* was analyzed and, by the original description of the male, this species appears to be a senior synonym of *A. mollis*, but this is still on study. The same occurs with *A. dubia*, whose description is not clear enough, but because of its occurrence being in the range of *A. crassipalpa*, it could be considered a junior synonym of this species. The current list is composed of five of the previously described species: *A. boliviana*, *A. obscura*, *A. mollis A. crassipalpa* and *A. dubia*. *Ariadna calilegua* Grismado, 2008 is recorded for the first time for Brazil, and two new species are described, one for the state of Minas Gerais and other for the state of Rio Grande do Sul.

Supported by: CAPES and CNPq

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8.09 Ovarian cycle of *Bothrops atrox*

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Introduction: The reproduction of venomous snakes in captivity has become increasingly necessary. Several species are difficult to obtain or are even threatened with extinction, making studies of their poisons very difficult. Knowing the cycle and determining the best reproductive management of these species in captivity increases breeding success. *Bothrops atrox* is a species found in Guyana, Suriname, French Guiana, Venezuela, Colombia, Ecuador, Peru, Bolivia and northern Brazil, where it is responsible for most snake bite accidents that occur in these locations. **Objectives:** The aim of this study was to monitor the ovarian cycle of females of *B. atrox* born in captivity to determine the best reproductive husbandry of this species. **Methods:** During a period of 17 months (March/2010 to July/2011) six females of *B. atrox,* born in captivity in 2006, were monitored monthly by ultrasound equipment, using a linear transducer of 6 to 13MHz, to observe the development of their ovarian follicles. The females were contained in plastic tubes and immersed in warm water for the examination. The follicles were divided into four stages according to their size: stage I (follicles <0.6), stage II (follicles 0.6 to 1.1cm), stage III (follicles 1.2 to 2.0cm) and stage IV (follicles ≥ 2.1cm). **Results and Discussion:** The follicles in stages I and II (primary vitellogenic follicles) were present in all months of the year, but in a larger percentage in the months of December to March (summer), follicles in stage III (secondary vitellogenic follicles) were seen mainly from April to December (autumn, winter and spring) and stage IV follicles (secondary vitellogenic follicles) were seen in the months of June to October (winter and spring). As the males of this species shows a "combat dance" that stimulates copulation, from May to August/2010, groups of two males and one female were released into a heated room with a 24-h monitoring system. In June 2010, two females were mated and in September of the same year fertilized eggs could already be seen in the oviducts. In the females not mated there was a progressive decrease in the number of secondary vitellogenic follicles as of October, and in February/2011, only follicles in stage I were seen. In the absence of mating, females reabsorb secondary vitellogenic ovarian follicles but sometimes atretic ovarian follicles can be eliminated. In December/2010, the offspring of both females were born, a clutch with 6 and another with 9 healthy babies. It was observed that the month of June had a higher success of mating rate, coinciding with the greatest amount of ovarian follicles in stage III encountered in the females that copulated.

Supported by: INCTTOX / FAPESP and INCTTOX / CNPq

8.10 First record of *Quadraseta brasiliensis* (Brennan & Gettinger, 1989) (Trombiculidae: Trombiculinae: Schoengastiini) in *Euryoryzomys russatus* (Wagner, 1848)

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Introduction: Many species of Trombiculidae (chiggers) were described in the past century. The last revision of the Neotropical and Nearctic chiggers was done in the 1980s. Eighty-seven chigger genera were then reported for the Western Hemisphere, including *Quadraseta*, described in 1970 and known to occur exclusively in the Neotropical region. Fourteen species are placed in *Quadraseta*, all ectoparasitic on mammals and birds: *Q. antillarum, Q. azula, Q. macarenae, Q. mackenziei, Q. pazca, Q. trapezoids, Q. tachirensis, Q. mirandae, Q. rotstieri, Q. falconensis, Q. akadonica, Q. brasiliensis, Q. brennani, Q. brasiliensis.* In collections of wild rodents in the Atlantic Forest of the state of Paraná, southern Brazil, some specimens of trombiculid were found on *Euryoryzomys russatus* (Thomas). **Objectives:** The aim of this work was to describe a new record of *Q. brasiliensis* of Brazil in a new host. **Methods:** Some of those specimens were slide-mounted for morphological studies under light and confocal microcopy; they were identified as *Q. brasiliensis*. Some specimens were also prepared for molecular studies to compare their DNA sequence with that of other trombiculid species. **Results and Discussion:** *Idiosoma*. A pair of humeral setae; dorsal idiosomal setae arranged 8-8-8-8-6-4-2. The SIF is 4B-3-N-2/3111.0000. *Scutum.* Sparsely punctuate; subrectangular, with sinuous anterior and posterior margins; 5 setae; sensilla subcapitate with few setules. Eyes 2/2. *Gnathosoma*. Cheliceral blade with tricuspid cap; papal tarsus with 4B (branched setae); palpal claw trifurcate. Palpal setal formula B/B/BBB/4B. *Legs* 7-7-7; coxae unisetose; 3 genualae I, genuala II and III; tibiala III; subterminala and parasubterminala I. This is the first record of this species from Adrianópolis, Paraná, Brazil; this is also the first record of *E. russatus* as its host.

8.11 Calcium metabolism during ontogenesis in *Bothropoides jararaca*

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Introduction: Embryos of snakes oviparous receive a substantial amount of calcium from the eggshell (54%) and viviparous species from yolk or placenta (85%). Viviparity has evolved independently in multiple lineages of squamates. Viviparous species should produce calcium-rich yolk and embryos should not be dependent on eggshell calcium. In one group consisting of the chelonians and birds, where the amount of calcium in the yolk and albumen of eggs is insufficient, embryos need to resorb calcium from the eggshell. In another group comprising the squamate reptiles, calcium required for embryogenesis apparently comes from reserves present in the yolk at oviposition. **Objectives:** The aim of this study was to apply neutron activation analyses to determine the quantity of calcium in plasma, egg yolk and embryo samples of *Bothropoides jararaca* snake that could be used in the nutrient transport through the placenta during embryonic development. **Methods:** Gravid females (N = 13) of *B. jararaca* were dissected and samples of plasma, yolk of the follicle vitellogenin, ovulated eggs and embryos were removed. Neutron activation analyses were performed on samples of lyophilized plasma, yolk and embryos. Quality control of the analytical results was evaluated using the certified reference material, NIST 1566b Oyster Tissue, provided by the National Institute of Standards and Technology (NIST), USA. **Results and Discussion:** The average size egg weight was 11.0 g. Of this weight, the yolk constitutes 64.0% and embryos 26.0%. The total quantity of calcium in plasma varied with reproductive time: 0.9 mg/ml, vitellogenic stage; 2.0 mg/ml for eggs without embryos and 0.7 mg/ml for embryos at different stages of development. The analyses of *B. jararaca* eggs obtained during vitellogenesis showed an average of 1600 mg Ca/100 g egg. In relation to the yolk: 37.0 mg/egg of calcium for vitellogenic follicles; 31.0 mg/egg for eggs in the oviduct and 14.0mg/egg with embryos. Yolk dry mass was relatively stable until stages 34 or 36, but yolk of stage 40 embryos contained less dry mass than earlier stages. Patterns of ontogenetic variation in total calcium in the stage 40 embryos were lower in the stage 34/36. Compared with other reptiles (*V. berus*: 713 mg Ca/100g egg; *T. sauritus*: 1400 mg Ca/100g egg; *L. vivipara*: 916 mg Ca/100g egg), *B. jararaca* showed a higher concentration of yolk calcium. Unlike bird embryos, *B. jararaca* does not use this source of calcium; it simply deposits the yolk for the embryo at the time of formation of the skeleton.

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8.12 Influence of diet on post-embryonic development of *Loxosceles gaucho* Gertsch, 1967 (Araneae, Sicariidae)

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Introduction: Spiders of the genus *Loxosceles* Heineken & Lowe, 1832 are considered of interest in health, as they are responsible for serious accidents to humans and pets. Studies on different aspects of the species of *Loxosceles* are important to understand which factors may influence the population growth in urban areas, aimed at control solutions in areas with high incidence of these spiders. The *Loxosceles gaucho* Gertsch, 1967, is distributed in southern and southeastern Brazil, and venom of this species is used for the preparation of anti-arachnid serum. **Objectives:** The aim of this study was to evaluate the post-embryonic development of *L. gaucho* on the mono and polyspecific feeding. **Methods:** Two hundred spiderlings from different egg sacs were individualized in plastic containers (120 ml) and divided into four groups with 50 individuals. The diets were offered weekly, where three groups were fed a monospecific diet: group I - *Gryllus* sp. (cricket), II-*Phoetalia pallida* (cockroach) III-*Tenebrio molitor* (beetle larvae), and group IV with polyspecific diet (varied: three alternative prey). The sequential molts of each individual were stored in an Eppendorf (2 ml) with 70% alcohol. The width and length of the cephalothorax of molts were measured with a stereoscopic microscope with ocular micrometer for comparison of growth between groups of spiders. **Results and Discussion:** It was found that growth was higher in group II spiders, followed by groups IV, I and III. Spiders reached maturity between 6th and 7th instar. The mortality was higher in group III, especially in the 3rd instar. The data provided evidence that diet composed of cockroach had a positive influence on the development of spiders, promoting greater and faster growth compared to other diets.

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8.13 Aspects of the fecundity of oviparous snakes of the families Colubridae and Dipsadidae

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Introduction: The fecundity of female snakes can be analyzed using information of the litter size, offspring size, relative clutch mass (RCM), frequency of reproduction and litter relation with female body size. **Objectives:** The aim of this study was to determine the aspects of the fecundity of some colubrid and dipsadid specimens. **Methods:** Oviparous females clearly ready to lay eggs were kept in captivity in the Laboratório de Herpetologia. The eggs were numbered, weighed, measured and incubated in the laboratory. Recorded were the date of oviposition and incubation period. The newborns were sexed, measured and weighed. **Results and Discussion:** We analyzed 29 egg-layings of 11 different species of snakes (3 colubrid and 8 dipsadid). Most of the ovipositions ocurred in the spring (83%), while some species made their ovipositions in the summer (14%) and only one (3%) in the fall. The percentage of hatching was 59%, and the period recorded as of oviposition until the eggs hatched varied between different species, but generally ranged from 68 to 124 days. For *Oxyrhopus guibei* (N = 9 eggs), a relation was found between female SVL and the number of eggs ($F_{0.05(2),8} = 10.4950$, $p < 0.05$) and between female SVL and total litter mass ($F_{0.05(2),8} = 11.6873$, $p < 0.05$). For *Philodryas patagoniensis,* these relations were not observed. Generally, the RCM observed for arboreal species (N = 6) was lower than for terrestrial species. The highest values of RCM were observed in *Erythrolamprus aesculapii* and *Liophis typhlus*. The largest and heaviest newborns were for the species *Spilotes pullatus*, the largest body size in samples. The analysis of a greater number of egg-layings would allow more precise conclusions of the relationship between the RCM and the habits of snakes.

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8.14 Morphological characterization of the parotoid macrogland in the toads *Rhaebo guttatus* and *Rhinella marina*

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Introduction: The parotoid macroglands are accumulations of venom glands mainly present in toads, symmetrically located in the post-orbital region. They act in the chemical defense system against predators, producing several different toxic compounds. The parotoids are described as large accumulations of secretory alveoli embedded in the dermis, arranged side by side, forming a honeycomb-like structure. We recently demonstrated that these macroglands need an external mechanical pressure for the venom to be released in the form of jets. When biting the parotoids the predator is therefore responsible for its own intoxication, which characterizes the "passive defense" of toads. In contrast, it was found that the species *Rhaebo guttatus* has an atypical type of defense: when disturbed this toad shows a stereotypical sequence of behaviors consisting of stretching out the front legs, inflating the lungs and aiming one of the parotoids towards the aggressor. This sequence culminates with the voluntary launching of venom from the parotoids in the form of jets. **Objectives:** The aim of this study was to examine and compare the morphology of the parotoids in *Rhinella marina*, featuring typical toad passive defense and in *Rhaebo guttatus*, capable of voluntarily ejecting venom. **Methods:** The macroglands of both species were embedded in paraffin. The sections were stained with HE, Mallory's trichrome and picrosirius and submitted to PAS, alcian-blue, pH 2.5, bromophenol blue and von Kossa histochemical reactions. **Results and Discussion:** The general morphology and the histochemistry of the parotoids were very similar in the two species. The dermis that underlies the alveoli is composed mainly of collagen type II, which is responsible for the honeycomb-like arrangement of the macrogland. Each alveolus contains a syncytial secretory unit surrounded by a myoepithelium and a duct, which is surrounded by differentiated glands and obstructed by an epithelial plug. The alveoli content is highly reactive to alcian blue, indicating the presence of a great amount of glycosaminoglycans. The secretion coming from the differentiated glands contains acid and neutral mucosubstances and proteins produced by different cell types. The main difference between the parotoids from the two species is the absence of a calcified dermal layer in *R. guttatus* (by its abrupt interruption on the edges of the macrogland) and the thick myoepithelial layer surrounding each one of the secretory units. The voluntary launching of venom in this species is possibly a consequence of these morphological features together with its peculiar sequence of defensive behaviors.

Supported by: CAPES, CNPq and INCTTOX

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8.15 Wing morphological evaluation of *Aedes aegypti* from São Paulo city

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Introduction: *Aedes aegypti* (Diptera: Culicidae) is the most important vector of dengue virus in Brazil. Low larval density, high availability of food and low temperatures induce development of adult mosquitoes with larger body sizes and with higher vectorial capacity. Analysis of the morphological variation in *Ae. aegypti* may be helpful in disease control strategies, since the phenotypic development may influence the transmission of dengue. **Objectives:** The aim of the present study was to evaluate, for three months, the morphological variation in size and shape of *Ae. aegypti* wings, in the Butantan neighborhood of São Paulo city. **Methods:** A total of 117 female and 139 male larvae were collected from March to May 2011, and were allowed to develop under appropriate laboratory conditions. Wing size and shape were assessed by geometric morphometrics. Left wings were mounted on a slide-coverslip and digitally photographed. By using TpsDig V.1.40 software, positional coordinates of each of the 18 anatomic points were taken. From these data, for males or females, centroid sizes were computed using TpsUtil 1.29, TpsRelw 1.39 and InStat3 software and canonical variations were determined using MorphoJ software. **Results and Discussion:** In the present work, higher temperatures were measured in the months of March and April, in comparison to May. ANOVA indicated that centroid sizes of both male and female mosquitoes collected in March or May were significantly different. At lower temperatures, the centroid sizes were shown to increase, suggesting a possible correlation between temperature and size, as similarly observed by other researchers. According to this data, specimens collected in May would have higher vectorial capacity and this would be a critical period to enhance control strategies in dengue virus spreading. Moreover, variations in wing shape were observed in specimens collected in the same month and in different months, possibly due to a genetic drift. Altogether, our results indicate that such genetic restriction of populations and environmental variations may be sufficient to produce metric differentiation in geographically restricted populations in a short time course. Further studies are necessary to evaluate all hypotheses proposed here.

Supported by: FAPESP

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8.16 Comparative morphology of the integument of *Rhinella major*, from Amazonia, and *Rhinella granulosa*, from Caatinga, Brazil (Anura: Bufonidae)

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Introduction: Amphibian skin morphology shows characteristics that allow the group to disperse over terrestrial environments. Amphibian cutaneous glands produce secretions with an important role in respiration and protection against predators and desiccation, besides acting as a protection against microorganisms. Together with morphology, a variety of behavioral and physiological traits are responsible for reducing water evaporation, granting success for the dispersion of anurans. **Objectives:** The aim of this study was to compare the structure of the skin of *Rhinella granulosa* (Caatinga) and *Rhinella major* (Amazonia). Results were analyzed in the light of the species' behavior and environment. **Methods:** Skin samples of dorsal, ventral, inguinal and mental regions were embedded in paraffin wax, and 5-μm sections were stained with hematoxylin and eosin (HE). For morphometric evaluations, five male individuals from each population were used. The following parameters were measured: total thickness, thickness of epidermis, of the calcified layer and of the *stratum spongiosum* and *stratum compactum*, number of vessels and number of granular (or poison) glands and mucous glands. Comparisons were performed using one-way ANOVA and Mann-Whitney U test, followed by a Tukey HSD and Dunnett's T3 tests. Differences were considered significant when $p<0.05$. **Results and Discussion:** Comparing the two species, we observed significant differences in the morphological structure of the skin. *Rhinella granulosa* presents more blood vessels in the inguinal region ($x=7.41 \pm 1.01$; Tukey $p=0.000$) than *R. major* ($x=3.16 \pm 0.10$). *Rhinella major* shows a thicker epidermis than *R. granulosa*. The calcified layer was thicker in *R. granulosa* than in *R. major*. Thickness of the skin of the mental region was significantly higher in *R. granulosa* ($x=154.79 \pm 11.94$) than in *R. major* ($x=104.79 \pm 9.12$, Tukey $p=0.047$). The inguinal region, which displays discontinuous calcified layer and high concentration of *verruca hidrophilica*, is considered responsible for the absorption of water by the animal. The largest number of vessels in this region, observed in the population of the semi-arid environment, can be directly related to the water balance, since water absorbed by the skin can be quickly transported by vessels. *Rhinella granulosa* shows a thicker calcified layer than *R. major* on the dorsal and ventral regions, and a thinner layer in the inguinal region, which may be indicative of adaptation to a semi-arid climate, thus reducing water evaporation through the dorsum and facilitating water absorption by the inguinal region.

Supported by: CNPq, CNPq/INCTTOX

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8.17 The Amazonian species of *Acanthoscurria* (Araneae, Theraphosidae, Theraphosinae)

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Introduction: Twelve species of the genus *Acanthoscurria* Ausserer, 1871 were described for the Amazonian region: the type species *A. geniculata* (Koch 1841) from Rio Branco, Roraima; *A. theraphosoides* Doleschall in Ausserer, 1871, from Maranhão; *A. ferina* Simon, 1892 from Tefé, Amazonas; *A. brocklehursti* F.O.P. Cambridge, 1896 from Pará; *A. tarda,* Pocock, 1903 from Tefé, Amazonas; *A. fracta* Chamberlin, 1917 from Pará; *A. juruenicola* Mello-Leitão, 1923 from Juruena River, Mato Grosso; *A. xinguensis* Timotheo da Costa, 1960 from Alto-Xingu, Mato Grosso; *A. transamazonica,* Piza, 1972 from Marabá, Pará (all from Brazil); *Acanthoscurria minor* Ausserer, 1871 from Guyana; *A. insubtilis* Simon, 1892 from San Mateo, Bolivia; and *A. simoensi* Vol, 2000 from French Guyana. **Objectives:** The aim of this study was to revise the Amazonian species of the genus *Acanthoscurria* including the type-material and to redescribe the species, illustrate their sexual organs and present new diagnoses for these species. **Methods:** The specimens of *Acanthoscurria* studied herein are deposited in the following institutions: Instituto Butantan, São Paulo (IBSP); Museu Paraense Emilio Goeldi (MPEG); Laboratório de Pesquisas Zoológicas, Faculdades Integradas do Tapajós (FIT); Instituto Nacional de Pesquisas da Amazônia (INPA); Natural History Museum, London (MNHN); Museum für Naturkunde Berlin (MNB) and Naturwissenschaftliches Museum Wien (NWMW). Collections were also made in Belterra and Santarém, Pará, Brazil on May and October, 2010, using pitfall traps filled with 70% alcohol and random searches during day and night. All measurements are in millimeters. Female seminal receptacles were dissected and bleached in lactic acid for the observation of internal structures. The length of leg segments was measured between joints in dorsal view. The drawings were made on a Leica MZ 12, with a camera lucida. The latitude and longitude data were based on Google Earth program and the map was made in the Diva GIS 5.2 mapping program. **Results and Discussion:** We include *A. insubtilis* and *A. simoensi* as a new record for the Brazilian Amazonian region and describe the first female of these species. Five synonymies are established and seven species are considered valid: *Acanthoscurria geniculata*, *A. tarda*, *A. juruenicola, A. theraphosoides, A. simoensi, A. insubtilis,* and a new species from Santarém, Pará, Brazil is described, based on both males and females. The holotype of *Acanthoscurria minor* has not been located and is considered *species inquirendae*. The distribution range of these species is extended.

Supported by: CNPq/INCTTOX

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8.18 Report: Consumption of small rodents in *Chironius bicarinatus* (Serpentes: Colubridae) Wied, 1820 held captive in the exhibition of the Museu Biológico – Instituto Butantan

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Introduction: The genus *Chironius* includes species that forage actively. They have an arboreal habit, naturally inhabiting forests, where they feed on especially arboreal frogs, and occasionally small lizards and birds. *Chironius bicarinatus* is distributed primarily in the southern highlands of Brazil and in Rio Grande do Sul, is dispersed to the central depression, both east and west, reaching the northwest of Uruguay, where it is very rare. In exhibitions, it often draws visitors' attention because of its gaudy coloration and intense. **Objectives:** The aim of this study was to describe the consumption of small rodents by *C. bicarinatus* kept in captivity at the Biological Museum of the Instituto Butantan. **Methods:** The male specimen (CL + SVL = 93cm +54 cm), 150 g, kept in a terrarium (1.20 m x 0.72 m x 0.85 m), T ± 23 °C and RH> 70%. Tadpoles were offered weekly, juveniles with complete metamorphosis (*Lithobates catesbeianus)* (10% to 15% of their weight in food). Neonates were offered, three (3 g) of the species *Mus musculus*, to supplement the diet. **Results and Discussion:** After offering the neonates in the dry area of the terrarium, the individual was attracted by the movement of prey, darting from 20 to 30 times per minute. In the beginning of the recognition of prey, the specimen approached the neonate, pushing the prey with its head. Soon after, the boa struck, snapping up and swallowing the prey. Although frogs are considered as main food item of some colubrids, they may eventually find small mammals in their search for food. It is appropriate to report the event as it occurred in a confined environment, where reptiles, especially snakes, are more susceptible to adverse conditions imposed by captivity. Studies on the management and adaptation of snakes in captivity are important for ex-situ conservation.

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8.19 Occurrence and diversity of snakes recruited in Instituto Butantan, São Paulo, Brazil: an overview on the twentieth century

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Introduction: Instituto Butantan (IBUT) is a reference center for research with venomous animals, public health and environmental issues. Production of antivenom started in 1901, where donations of animals were the basis for obtaining venom. People have brought in snakes to help snakebite prevention. **Objectives:** The aim of this study was to record and compare the occurrence and diversity of snake fauna at the beginning (1916 - 1932) and at the end (1990 - 2000) of the XX century in the state of São Paulo. **Methods:** Register books from Lab. Herpetologia were surveyed. **Results and Discussion:** A total of 19,792 snakes were received from 7,140 donations (69.0% *Bothrops*, 23.2% *Crotalus*, 0.4% *Micrurus* and 7.2% non-poisonous snakes) in the beginning of the XX century (2.77 specimens/donation). In the nineties, recruitment was 58,400 specimens from 42,846 donations (28.0% *Bothrops*, 26.2% *Crotalus*, 3.2% *Micrurus* and 42.6% non-poisonous snakes) (1.36 specimens/donation). Considering donations as captures, data show an increase of 6 times in the collection effort between the two periods and an increase of 2.95 in snake recruitment. In this period, the state of São Paulo had a demographic growth of 17.5 times. The ratio snake/donation diminished about 50% comparing the two periods. Decline in captures were most pronounced in *Bothrops* (40.6%), whereas among *Crotalus* a slight increase was observed (12.9%), but *Micrurus* and non-poisonous ones showed significant capture ratio increase of 800% and 600%, respectively. Habitat destruction of the Atlantic Forest associated with camouflage ability and fast mobility could explain the decrease of *Bothrops* captures. It shows ontogenetic change in feeding habits, since young snakes demand ectothermic prey (also very dependent on vegetated areas). *Crotalus* however, is well adapted to open/agricultural areas and does not show ontogenetic change in diet (rodents), which may have contributed to the stability with slight increase in population received between periods. Regarding *Micrurus,* their dependence on the forest, particularly the litter, shelter and source of food, could convey the expectation of a decreasing capture ratio. However, their slow mobility and bright colors would favor encounters and captures. Reduced sample size of *Micrurus* in both periods (n=1,936) compared to other groups should be taken into consideration for discussion. Significant increase in non-poisonous snake collection could be related to their generalist habits and availability of prey and niche both in urban and rural areas despite deforestation. We are continuing this study to gather information on number and possibly species that might have become extinct in the last hundred years.

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8.20 Malformations in *Bothropoides jararaca* (Serpentes, Viperidae)

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Introduction: Like other vertebrates, snakes show a number of malformations in newborns. The developing embryo is the result of expression of the genome under the influence of environmental factors such as temperature, humidity and environmental contaminants, possible teratogenic agents for ectothermic species. Organogenesis, the most complex stage in embryonic development, is most sensitive to exogenous agents, and therefore, it is a very important period for the occurrence of malformations. **Objectives:** The aim of this study was to determine the frequency of malformations that occur in jararaca, correlating with the sex of the newborns and the gestation period in captivity. **Methods:** Pregnant specimens of *Bothropoides jararaca* delivered to the Instituto Butantan were kept in captivity until the birth of their offspring. These newborns were weighed and measured and evaluated for the presence of malformations. At first, this evaluation was done by external morphology, but after the death of these animals, necropsies were performed and internal organs were evaluated. **Results and Discussion:** Between the years 2007 and 2010, 113 jararacas gave birth to1881 snakes, of which 66 (3.5%) had a congenital anomaly. Snakes that were born dead but had no obvious abnormalities (87) were not considered. Males and females had the same rate of malformations (47% and 44% respectively; six individuals could not be sexed). The anomalies of the spine (kyphosis, lordosis and scoliosis) were the most common (48.5%) followed by fissure in the ventral midline with organ extruded or not (23.7%). Tip of the tail wound was an abnormality found nine times (13.6%), the same number found for shortened lower jaw (prognathism). The fusion of the ventral scales was seen in 10.6% of newborns and eye problems, such as microphthalmia or bufophthalmos, 6%. Two snakes had bicephaly (3%). Few animals showed abnormal internal organs. The most common was the increased size of some organs such as heart (5 animals), liver (2), gallbladder (1) and kidney (1). Even in captivity, the majority of snakes with malformations die in days. In the wild, these animals are easy prey.

Supported by: INCTTOX/FAPESP and INCTTOX/CNPq

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8.21 Report: Ichthyophagy in *Chironius bicarinatus* (Serpentes: Colubridae) Wied, 1820 in the exhibition of the Museu Biológico – Instituto Butantan

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Introduction: The genus *Chironius* consists of non-poisonous species, having daytime foraging, actively being predominantly batrachophagous while using other types of prey in their diet, such as small lizards and birds. They have a wide distribution in the Neotropics, from Nicaragua to northeastern Brazil and Argentina, inhabiting tropical forests, rainforests and eventually altitude areas of open savanna, occurring from sea level to 2,800 m altitude. *Chironius bicarinatus* snakes are medium to large, reaching more than 120 cm snout-vent length, colored green on the dorsal anterior and posterior vertebral brown with light streaks. **Objectives:** The aim of this study was to report ichthyophagy in *C. bicarinatus* in a captive environment in the Biological Museum. **Methods:** The male specimen (CL + SVL = 93cm +54 cm) weighing 150 g kept in a terrarium (1.20 m x 0.72 m x 0.85 m) with artificial tank (capacity of 3 liters) with T ± 23 ° C, and RH> 70%. Their diet was mainly composed of young tadpoles and the species with complete metamorphosis *Lithobates catesbeianus* being offered weekly from 10% to 15% of their weight in food. To supplement the diet of the snake, *Brycon* sp and fry of *Ctenopharyngodon idella* were offered. **Results and Discussion:** By introducing the fish in the tank, the snake darted from 10 to 25 times per minute, following the movement of potential prey. The snake engulfed prey capturing it with the mouth open with semi-zigzag movements. In captivity, we can eventually replace the main food item for another type of prey in the absence natural prey or for dietary supplementation of copies kept. Data on the feeding of captive snakes are of great value for improving management and conservation studies.

9. History, Education and Science Dissemination

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9.01 Evaluation of the temporary exhibition Vaccination and Human Rights

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Introduction: The "Primavera dos Museus" is an event coordinated by Instituto Brasileiro de Museus. Each year a theme is proposed as a guideline for the development of events in all participating museums and institutions, thus allowing discussion and increasing public awareness about current events. In 2010, the theme was "Museums and Human Rights" and at the Instituto Butantan an exhibition about Vaccination and Human Rights was created by the Museu Histórico. The work presented here is an exploratory audience study, developed from December 11, 2010 to January 13, 2011, in this temporary exhibition. **Objectives:** The aim of this study was to record and evaluate the different ways in which the visitors interacted with the exhibition, their behavior and their preferences. **Methods:** Data were collected using two different methods, namely the record of the visitors' route and interviews with those visitors. The sample included 44 adult visitors (older than 15 years) not associated with organized groups. The tracking of visitors was recorded by marking their routes in the floor plan of the museum, while behavior, such as the choice of the way through the exhibition, the pauses of more than 30 seconds, text reading and conversations between visitors, was noted. After recording the route, the researchers interviewed the visitors. The questionnaire was divided into 3 parts: (1) questions about previous experiences and knowledge related to the Museu Histórico and the Instituto Butantan; (2) questions about the visiting experience (motivation, preferences, readings, opinions); and (3) questions about the sociodemographic profile of the visitor. **Results and Discussion:** The results of the 44 interviews and 37 observations on tracking indicated that most of the visitors came accompanied, were first-time visitors, female and took 6 to 10 minutes to visit the exhibition. The respondents considered the theme "interesting." The historical part of the exhibition was the most appreciated and some of them named the theme of the "Revolta da Vacina"(Vaccine Rebellion) as the best one. Most of the suggestions were related to the inclusion in the exhibition of themes and exhibits more suitable for children, such as folders and interactive displays. The results of this exploratory research revealed some patterns and data that can be confirmed and more thoroughly analyzed with the development of new audience studies at the Museu Histórico and in other public areas of Butantan. The more we know about the visitors' experience, the better we are able to receive them and also achieve the objectives of yjr dissemination of knowledge obtained by researchers from Instituto Butantan and other institutions through the use of the museum's collections.

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9.02 Museums and memories: Museum Week at Butantan Institute

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Introduction: From May 18th to 22nd, the Centro de Desenvolvimento Cultural of the Instituto Butantan promoted a special event to celebrate the 9th Semana Nacional dos Museus. Three tents were set up at the park as a place to record the recollections of Instituto Butantan's employees and visitors, related to the history of the Instituto Butantan. The public and employees came to the tents and recorded their testimony, immortalizing their memories for the 110th anniversary of the Institute, bringing also photographs taken at the park. Recollections of the years of work of the employees, of friendships, investigations developed and unforgettable visits were recorded during the testimonies. **Objectives:** The aim of this study was to record the recollections of employees and visitors related to Instituto Butantan, to encourage the recording and documentation of the institutional history, and to promote activities integrating employees and researchers from different areas of Instituto Butantan. **Methods:** During semi-structured in-depth interviews that were filmed and recorded, employees and visitors were encouraged to report their memories of Instituto Butantan. The photos exhibited around the tents inspired employees to bring their own pictures and to talk about them. These pictures were digitized and their contents and authors were recorded, before returning them to the owners. The testimonies and photographs are available at Butantan website (www.butantan.gov.br). **Results and Discussion:** In five days, 137 testimonies of 2 to 23 minutes were collected, of which 78 were from employees and 59 were from visitors. Most of the respondents were female (54% of the employees and 66% of the visitors). Members of the Scientific Division were the most frequent participants, totaling 34 participants, followed by the Cultural Division, with 17 and Production with 10. All visitors interviewed live in São Paulo State, with 47 residing in the Capital, 10 in the Metropolitan Region and 3 in the countryside. It was possible to observe the respect and admiration of Instituto Butantan both by the external and internal public. The response by both visitors and employees exceeded our expectations. This experience contributes to the development of a larger project of institutional history.

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9.03 "Congressos de Ciências Sociais e Humanas em Saúde da Abrasco": memories and history as central theme

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Introduction: The History of Health in Brazil using the memories of physicians and other health professionals is traditional. The production is based on historical memories must always take into account the social position of the observers and expanding their informants and sources. The relations between history and health has gained ground over the past 25 years, especially with the foundation of Casa de Oswaldo Cruz in FIOCRUZ, Rio de Janeiro State. At the same time, the movement for democratization and the "Movimento pela Reforma Sanitária" occurred in Brazil. During this period, much has been searched, analyzed and produced; the aspects have been varied and include the memories and history of medical practice and care, the history of education, care and research, the history of disease and the architecture of hospitals and places of care. **Objectives:** The aim of this study was to identify and analyze the memory issue in the scientific production in Congressos de Ciências Sociais e Humanas em Saúde da ABRASCO (1995-2007) and its importance in public health. **Methods:** We performed a documentary analysis of the papers presented at conferences with the theme of memories, in different categories of study (research or experience report), mode of presentation, methodology, institutional affiliation of the author and source region. **Results and Discussion:** A great number of works were academic and used qualitative methodology. The number of papers presented at the Congress has increased. Most of the work was produced in the southeast of Brazil, but there did appear to be a trend towards increased participation from other regions. The memories of health in Brazil is little studied and preserved. It is necessary to make it public and to salvage different approaches.

Supported by: CNPq

9.04 Pharmacists in Sao Paulo state (1892-1934)

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Introduction: The history of pharmacy and pharmacists in Brazil has attracted the interest of pharmacists, historians and sociologists. More systematic studies have been made in recent years, following the line of study of the health professions and science history which began and greater focus on medicine and doctors. This study is limited to Sao Paulo from 1892 to 1934, during which the state had a rapid growth and rapid urbanization, resulting from the expansion of trade and services. The first course of health care in Sao Paulo was the Escola Livre de Pharmácia, founded in 1898 in the capital. **Objectives:** The aim of this study was to evaluate the profiles of qualified pharmacists registered in "Livros de Fiscalização do Exercício Profissional" of the "Serviço Sanitário do Estado de São Paulo," which was responsible for registering pharmacists, physicians, dentists, nurses, etc. **Methods:** Documents and data were analyze in spreadsheets with the following: name of the pharmacist, place and date of birth, institution of education, sex, date and place of birth, and date of the registration in the Serviço Sanitário do Estado de São Paulo. Information related to the pharmacists was collected in the records from 1892 to 1934. The books were consulted at the Museu da Saúde Pública Emilio Ribas of Instituto Butantan. **Results and Discussion:** Noted were the importance of the Escola Livre de Pharmácia de São Paulo for education in Pharmacy in the first decades of the twentieth century, the emergence of a large number of pharmacy schools in this period and the mobility of students and professionals throughout the country. The records show that the majority of the professionals who worked in São Paulo were natives of Sao Paulo State, with a few from other countries, and that in this period there was significant participation of women in the area. The information collected allows professionals to analyze the number, place of birth, sex ratio, training site, main educational institutions, and age of entry into the labor market of São Paulo, significantly expanding the understanding of the processes of transformation in the performance of health care in São Paulo.

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9.05 Environmental education with venomous animals in the Museu Biológico do Instituto Butantan: Workshop on "Saiba Mais".

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Introduction: Vital Brazil is one of the pioneers in the organization of environmental education with venomous animals and also worked on campaigns with primary focus on the themes "anti-snakebite serum is specific, unique and effective," "accident prevention - use of heavy boots" and "Preserve predators." This education program produced, such as a 50% reduction in mortality in rural areas, and also created educational information on snakes and accident prevention. Following Vital Brazil's ideas, in 2001 the Biological Museum was one of the few museums to have a permanent live collection of invertebrates such as insects and arachnids and vertebrates such as amphibians and reptiles. The Biological Museum has *biodioramas*, displays and posters where visitors can see and understand more about "Poisonous Animals." Besides the permanent and temporary exhibitions and activities with the character of environmental education, the Biological Museum has a workshop entitled "Saiba Mais" with the primary mission to demystify the fear of these animals. **Objectives:** The aim of this study was to characterize the public who frequents the workshop on vacation, take visitors on the subject of venomous animals and evaluate the workshop. **Methods:** The evaluation was performed in the period of January 5 to 30 of 2011, twice a day (in the morning and afternoon) with a maximum of 20 participants per activity. Workshop exhibition was set up in which educators address issues related to the biology of snakes, spiders, scorpions, caterpillars and frogs. Issues such as identification of venomous snakes in Brazil, accident prevention, first aid and conservation, with the aid of objects as replicas, stuffed and live animals. **Results and Discussion:** "Saiba Mais" on vacation had 536 visitors. Of these, 145 were interviewed, showing 63% female and 37% male, 25% with graduate degrees, 35% with higher education, 4% with technical education, 19% high school graduate, 15% with elementary education and 2% with early childhood education, and 83% of respondents were from Sao Paulo. With regard to the workshop "Saiba Mais," the expectation of 93 respondents indicated the application of the workshop as very satisfactory and 51 satisfactory; 97% of visitors felt that the workshop clarified their doubts on the subject. What drew most attention was what visitors found most relevant: 22% the teacher's explanation, 21% touching the living animal, 16% accident prevention, 15% first aid, 14% the replicates used for the explanation, and 12% the biological material. Contact with stuffed animals, biological materials and live animals, promote the learning by the public, giving them through direct experience, ties between nature and humans.

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9.06 Health, development, technological innovation and regional cooperation: Instituto Butantan - Technology transfer for production of a vaccine for influenza

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Introduction: The purpose of the project "Health, Development, Technological Innovation and Regional Cooperation" is to contribute to the formulation of public policies in the areas of health, science and technology. It also tries to induce the development of sectors of the industrial economic complex of health that are strategic for Brazil, to increase the regulatory power of the State, to fortify the public institutions so that they produce technological innovations in the area of health, and to establish new standards of regional cooperation. The project has four studies that show the experiences of São Paulo. In the case of the Instituto Butantan, the study shows the technology transfer for production of the vaccine for influenza. **Objectives:** The aim of this study was to evaluate and characterize the process of technology transfer in Instituto Butantan. We searched the institutional bases and factors of context that contributed to its development. **Methods:** A case study was conducted where the questions "How" and "Why?" were to be answered in non-controlled events, searching for uniqueness of data gotten by means of interviews with key people involved in the transfer process. We carried out an archive analysis of the process and analysis of context factors that can show the various steps of the process, the institutional impediments, the legal restrictions and the economic parameters involved. In the first phase of the research, four interviews were conducted. Moreover, we did a bibliographical survey of the subject. **Results and Discussion:** From the interviews already done and the bibliographical review of the subject, it was possible to identify some of the intervening factors of the process, such as decision-making on the part of the Health Department and the construction of formal mechanisms in the public administration of the institute, given the absence these mechanisms, the dependence on the entrepreneurship of their leaders, the absence of policies of human resources compatible and sustainable, capable of supporting the transfer. The conditions of this transfer and the cost of this process are still being studied.

9.07 Building of meanings by the visually impaired person in a science museum

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Introduction: Educational practices have always been of the utmost concern to museum and science center administrators. Dealing with the general public, it is a must for these administrators to have in place programs to adequately convey the institution's assets, and enhance the comprehension of the material on exhibition, to all the visitors, including those with physical disabilities. To this endeavor, the Museum of Microbiology, in partnership with other related institutions, has developed a specific program for the visually impaired person, called Micro-touch Program. It comprises three-dimensional models of the Museum building and microorganisms, audio information and brochures in Braille. However, some questions remained unanswered: what kind of knowledge may the blind and the visually impaired gain from this material? How do they decode and decipher the materials they are handling? **Objectives:** The aim of this study was to identify and study the various and different meanings that the visually impaired person learns from educational proposals such as The Micro-touch Program. **Methods:** Three semi-structured interviews took place with visually impaired persons, carried out by one of the partners of the program, before defining proper materials, models and instruments for the project. Seven more interviews were conducted afterwards, which were audio, photo and videotape duly recorded. Of these seven, three were transcribed and analyzed by the Allen (2002) method of categories of learning-talk. **Results and Discussion:** Three hundred and fourteen conversations were obtained, distributed among the 15 subcategories. The most frequent was Strategic Use (11.8%), when the interviewers related their impressions on the program materials and also had the opportunity to suggest their amelioration. Following this category was the subcategory Perceptual Feature (10.8%), where visitors described specific properties of the materials submitted to them, such as dimensions and shapes. Two other subcategories were also relevant: Affective Pleasure (10.2%) and Perceptive Identification (8.6%). The handling of the three-dimensional objects by the visitors, plus the audio material, enhanced their ability to understand the concept behind the artifacts. The assistance of highly qualified professionals to develop and make these instruments and the excellent quality of the materials used had markedly improved the way the visually impaired persons form mental images in a museum. From the results of this research it is possible to create communication strategies to maximize the interaction between visitor and the Museum expographic discourse promoting learning development.

Supported by: FAPESP

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9.08 Expographic resources and learning discussions at the travelling exhibition "The Great Epidemics"

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Introduction: Large epidemics have occurred in the past due to deplorable sanitary conditions in large cities and to deficient knowledge of their etiologies at that time. Today, rapid disease dissemination has been facilitated by advances in transport and by mass movements of populations for many different reasons. Itinerate expositions are important tools for promoting communication between science museums and the public, and help familiarize people with the culture of science. Within this context the Museum of Microbiology prepared an itinerant exposition focusing on some of the largest epidemics (the Plaque, Small Pox, Meningitis, Influenza, and AIDS) with a video, sound-effects, special lighting, statistical presentations, and three-dimensional displays.
Objectives: The aim of this study was to evaluate the significance given to the exposition by its visitors and the learning possibilities offered by its open discussions.
Methods: Semi-structured interviews (before and after visits), video tracking of visitors, photographic records of visitation situations, and field annotations were employed. Recordings were transcribed and analyzed following Allen (2002), whose methodology establishes interpretative categories called "learning conversations."
Results and Discussion: The eight groups considered in the present analysis comprised 27 visitors and 674 conversations distributed into 15 conversational subcategories. The subcategory most observed was "Identification Perception" (21.8%); this was followed by "Simple Conceptual Inference" (19.3%), represented by conversations in which visitors performed cognitive interpretations of objects, displays, videos or texts seen in the exposition. Additional categories, including "Connection with Life" (8.6%) and the "Complex Conceptual Inference" (8.5%), were also developed. This latter category is considered difficult to observe because it presupposes the construction of hypotheses and generalizations or conceptual elaborations. This category appeared with significant relative frequency in the present case study, suggesting that the exposition was efficient in stimulating the elaboration of hypotheses and generalizations. It was noted that the learning conversations analyzed during the temporary exposure provoked different cognitive manifestations in the visitors, with different degrees of complexity. Additionally, affective and connective conversations within the same social groups appeared to strengthen the values and concepts shared among their members. The use of different expographic resources was identified as being essential to the development of different conversational elaborations.

Supported by: FAPESP

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9.09 Profile and frequency of visitors to the museums of Instituto Butantan: analysis and new perspectives

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Introduction: In museums, visitor profile research is an essential tool for institutional management. The identification of the audience characteristics, their needs and expectations contribute to the definition of the direction of science dissemination in museums. In this sense, the Centro de Desenvolvimento Cultural of the Instituto Butantan initiated an analysis of the frequency and the profile of visitors from 3 of 4 museums, based on data collected by the Divisão de Administração, through the tickets revenues, and data collected at the entrance of the museums. **Objectives:** The aim of this study was to determine the audience profile and the variation of the frequency over different periods of time, seeking to understand the problems that resulted in this variation. **Methods:** Data of sale/distribution of tickets of museums were obtained from 2006 to 2011. Moreover, data relating to the type of audience (preorganized groups or spontaneous visitors, schooling level, origin and disabled people) were collected at the entrance of museums during the first semester of 2011. All data were tabulated and graphs and tables of the distribution of the frequencies are presented. **Results and Discussion:** The frequency of the 3 museums of Instituto Butantan suffers large variation throughout the year. This variation coincides in part with the school holiday periods, but this does not explain fully the monthly variability. The data also point to a tendency towards a decrease in the frequency of the visitors, around 30% between the years of 2006 and the first half of 2011. This brings up the possibility of a non public loyalty, suggesting the need for a systematic renewal of exhibitions in museums. It is still possible to determine the characteristics of the scheduled groups, in which over 50% came from São Paulo City, the vast majority were of basic schooling and 12% had some kind of disability. It is considered that the continuation in the collection of these data will enable us to increase the quality of education and communication activities in the museums of Instituto Butantan.

9.10 The archive's diagnosis as aid to an acquisition policy – the Museu de Saúde Pública Emílio Ribas archive's construction

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Introduction: The Museum of Public Health Emílio Ribas (Musper) of the Butantan Institute was created in 1965 by the Secretaria de Estado da Saúde de São Paulo (SES) with the objective of having a reference to the memory of Dr. Emílio Marcondes Ribas. Throughout the years, it had its activities extended, including the preservation and diffusion of the history of São Paulo public health. Since 2010, it has been tied with the Butantan Institute when it started to integrate the activities. **Objectives:** This work is an integrated part of the first stage of the diagnosis that is being developed at the Museum. The objective was to do a survey of the institutional history and the process of formation of its archive, identifying how much this trajectory influenced its formation, constitution and identification. This way, we tried to determine what were the acquisition policies adopted, contextualizing the archive and its importance for the memories of public health in São Paulo, to establish criterion for making available the construction of a new policy, suiting the museum's new project. **Methods:** The first step of the diagnosis was based on surveying the documents that represent the documents entrance, also interviews with former directors and professionals who worked in the archives' constitution and identification. **Results and Discussion:** As a result, we determined that the main nucleus of the archive was formed from the works of the Comissão dos 100 anos de Saúde Pública Paulista, in 1984, and after this period the entrance flow was reduced. We found that the archive of the museum was formed in the diverse agencies of the SES without any archiving criteria established by the State, since the record management process is unfinished. Today, the Museum Emílio Ribas keeps contact with other institutions of education and research in health that saw this possibility or found in their archives unique documents about the history of São Paulo public health.

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9.11 Institutional challenge of preserving scientific documents: the creation and activities of the Núcleo de Documentação do Instituto Butantan

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Introduction: The Núcleo de Documentação of the Butantan Institute was created in January 2010 with the mission to disseminate and to conserve its archives, beyond developing research about organization of scientific archives, supporting research in the areas of history of science and museology in the institution. **Objectives:** Thus, one of its responsibilities was to create policies for the identification and valuation of the institutional scientific patrimony. **Methods:** The archive today under the responsibility of the Núcleo de Documentação started to be established at the end of the 1970s, when a commission was organized with the purpose of assembling the institutional archive with historical value. For this, the commission visited and stimulated the various sectors of the Butantan to direct its documentation for archiving. This work is part of the first activities of the Núcleo de Documentação, about the contact with the archive and the challenges found in relation to preserving, archiving and making the documents accessible. All of this passes through a reflection about what should be preserved as institutional memories. **Results and Discussion:** From a previous study of this documentation and the recording of the donations received, it was possible to understand that one is mainly about registrations proceeding by means of activities of the institution. This way, we noticed that the commission's activities were an important initiative to the conservation of institutional documents. Nevertheless, it was not effective in recognizing which documents should be preserved to exemplify the works developed in the research laboratories. We conclude that the difficulty of archiving and preserving this documentation is related to the problem of not recognizing from the research that they produce what are institutional documents, raising the idea that the scientific documentation, produced by the researcher is private.

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9.12 Educational activities and dissemination of scientific concepts undertaken by the Microbiology Museum in 2011

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Introduction: Seeking to up-date and diversify the scope of public information available about microbiology, the Museum mounted two temporary expositions directed towards the general public in 2011: "Friends are Friends, Microbes are Microbes" and "Today, You are the Scientist." Additionally, the museum re-inaugurated the "Scientist's Park," an open area that holds a number of busts honoring famous scientists who contributed to microbiology and immunology. **Objectives:** The aim of this work was to inform the public about illnesses that can be acquired through contact with familiar animals, and to expose visitors to the world of science. **Methods:** The temporary exhibition "Friends are Friends, Microbes are Microbes" presented multimedia games, informative posters, and a 3D movie. The "Today, You are the Scientist" exposition was designed to use our sense of touch as a learning tool. The exposition also had a life-sized replica of a scientist for photos and a video emphasizing the importance of vaccinations. The "Scientist's Park" with 10 busts of important investigators that contributed to the field of microbiology was remodeled to provide space for educational activities promoted by the Museum. **Results and Discussion:** The temporary exposition "Friends are Friends, Microbes are Microbes" presented information to the public in a ludic form about illnesses caused by microbes found in animals and how to prevent them. Approximately 38,885 people (including 9,729 school children) visited the temporary expositions until June 2011. The "Scientist's Park" was visited by 17,014 people (including 6,206 students) between May 2011 (when it re-opened) and the end of June. The interest in science shown by the visitors was very clear, reinforcing the educational role of the Microbiology Museum and its promotion of active citizenship.

Supported by: FAPESP/CAT-Cepid and Fundação Butantan

10. Others

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10.01 Computerization and accessibility of the Zoological Collections of Instituto Butantan

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Introduction: The Zoological Collections of Instituto Butantan are distributed into four significant collections: Herpetological (reptiles and amphibians); Entomological (insects); Arachnological (spiders, scorpions, myriapods and opilionids) and Acari (mites and ticks). During the 1910s, the herpetological and entomological collections were organized by the eminent scientist Prof. Dr. Vital Brazil. In 1924, Prof. Jean Vellard began the arachnid collection. As early as 1933, Dr. Flávio da Fonseca began the Acari collection. The collections are known to be of great value to science in Brazil and worldwide, because of their specificity in animals of medical and veterinary importance, and also for containing type species. Because of the importance of scientific and historical collections, the Butantan Institution in 2011 received support from Fundação Butantan and FAPESP to develop the INFRA Project (09/54921-4), entitled "Computerization and Accessibility of the Zoological Collections of Butantan Institute." **Objectives:** This project aimed to enter and organize the data of the collections, which are gathered from index cards and collection books and entered into a computer database, making them available online in both English and Portuguese. The project also contemplates the transfer of collections to the new building, remounting of damaged slides, replacing 70% alcohol with absolute alcohol, standardization and implementation of barcode labels, scanning of all type species images, and development of the Collections Standard Operating Procedure, which will control data organization. **Methods:** The work implies the following: entering all information contained in the records into an Excel program, as the basis to a computer program, which can be applied on the internet; taxonomic species update through the literature and related articles; and conference on the distribution and geographic coordinates using the IBGE database. **Results and Discussion:** The entomological collection includes 3,000 data, of which 100% were already computerized. Approximately 70% of the 11,000 lots (about 150,000 specimens) of the Acari collection were computerized so far. The Arachnids and Myriapods with more than 500,000 specimens, Opiliones with 10,549 specimens, and the herpetological collection with 85,000 specimens are in the initial process of computerization. The computerized process of all four collections should be completed by early 2012, coinciding with the completion of the building that will store the collections. The other objectives of this project should be implemented over the next six years as announced by FAPESP.

Supported by: FAPESP

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10.02 Optimization of SILAC in Y1 cells: the effect of dialyzed FBS

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Introduction: The eukaryotic cell cycle is divided into four phases, G1, S, G2 and M, which are regulated by cyclin-dependent kinases. Fibroblastic growth factor 2 (FGF2) is associated with proliferation and carcinogenesis, but it shows anti-proliferative and tumor suppressive functions depending on the cellular context. In Y1 murine adrenocortical carcinoma cells, FGF2 promotes G0/G1 transition but delays S-phase and permanently blocks cells in G2/M. To better understand the molecular mechanism induced by FGF2 in the cell cycle, we decided to perform mass spectrometry-based quantitative proteomics using SILAC. The latter is a method to label cells with stable amino acid isotope in cell culture allowing the quantification of proteins by mass spectrometry. In order to avoid contamination with the natural amino acid isotopes, the use of dialyzed fetal bovine serum (dFBS) is required. Dialysis of FBS, however, also eliminates small molecules that are important for cell growth and is the main reason that some cell lines are unable to grow in SILAC medium conditions. **Objectives:** The aim of this study was to optimize the SILAC technique in order to quantify proteins through cell cycle of Y1 cells treated with FGF2. In order to do that, we first analyzed the growth impact and the effect of FGF2 on cell cycle using Y1 cell maintained in dFBS. **Methods:** FBS were extensively dialyzed against several changes of 0.15 M NaCl through a 10 kDa cut-off membrane. To analyze cell proliferation and viability, $1.2x10^4$ cells/cm^2 were cultivated in DMEM medium supplemented with 10% dFBS for 9 days at 37ºC and the cell growth was evaluated daily by automatic counter, trypan blue staining, light microscopy and flow cytometry (FACS). To evaluate the progression of cell cycle after FGF2 treatment, the Y1 cells were synchronized in G0/G1 in the absence of dFBS for 48 h. Afterwards, the cultures were maintained in DMEM medium with 10% dFBS with (or without) 10 ng/ml of FGF2 for 0, 15, 20 and 48 h. The cells of at each time point were fixed and stained with 50 µg/mL propidium iodide. The histograms of DNA contents were evaluated by FACS. **Results and Discussion:** Growth curves of Y1 cells cultivated in DMEM with dFBS or FBS showed no significant statistical differences. Preliminary analyses indicated that the number of dead cells were slightly lower in cells cultivated with dFBS. By FACS and light microscopy, we observed that FGF2 treatment induces G0/G1 transition and G2/M arrest in cells cultivated in FBS or dFBS. Taken together; these results indicate that Y1 cells can efficiently grow in the presence of dFBS and show the same cell cycle blockage induced by FGF2. Therefore, it will be possible to evaluate quantitatively the proteome of Y1 cells by SILAC.

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10.03 Evaluation of the sensitivity of *Biomphalaria glabrata* (Say, 1818) adults and embryos to potassium dichromate

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Introduction: To evaluate the accuracy of methods for laboratory acute toxicity assays, reference substances are used, allowing the establishment of an acceptable range of results, which in turn allows the identification of changing conditions of test organisms as well as estimating the accuracy and reliability of the results. This tool is required to be applied in toxicity assays for the development of molluscicide screening and ecotoxicological assays performed at the Malacology Laboratory of Butantan Institute. **Objectives:** In this work, assays with the freshwater snail *Biomphalaria glabrata*, intermediate host of schistosomiasis, were performed to evaluate the sensitivity of animals to the reference substance, potassium dichromate ($K_2Cr_2O_7$). **Methods:** Adults and embryos at blastula, gastrula, trochophore and veliger stages were exposed for 24 and 48 h to potassium dichromate to determine the LC_{50} values (concentration that affected 50% of the exposed organisms). Mortality and malformation were the endpoint. **Results and Discussion:** For adults the LC_{50} values were 61.24 mg/L with 24 h of exposure and 59.46 mg/L with 48 h. Embryos at the blastula, gastrula and trochophore stages exposed for 24 h showed similar response to $K_2Cr_2O_7$ – 8.39 mg/L and 8.84 mg/L LC_{50} values, while for veliger, concentration was less than 6.25 mg/L. Blastula and gastrula stages exposed for 48 h were less sensitive than trochophore and veliger (13.24 mg/L, 12.42 mg/L and 8.85 mg/L and less than 3.43 mg/L, respectively). Embryos were more sensitive than adults and among the developmental stages, veliger was most susceptible to the substance. In addition, we observed the induction of teratogenicity by potassium dichromate in all embryonic stages. These preliminary data are part of the elaboration and interpretation of the control chart of *B. glabrata* that indicates if the sensitivity of organisms reared in the laboratory is under control.

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10.04 Effects of therapeutic ultrasound on pain and functional recovery after nerve crush injury in rats

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Introduction: Peripheral nerve injury is common in our daily life. It may result in motor, sensory, and autonomic impairment, and often in neuropathic pain characterized by spontaneous pain and/or burning, accompanied by allodynia and hyperalgesia. In order to try to accelerate peripheral nerve regeneration several physical agents, such as therapeutic ultrasound, have been shown to be promising. **Objectives:** The aim of this study was to compare three different parameters of therapeutic ultrasound on pain and functional recovery in rats submitted to nerve crush injury. **Methods:** Male Wistar rats were submitted to sciatic nerve injury, performed by clamping the nerve, according to the technique described by Monte-Raso et al. (2008). The animals were divided into four groups with five animals each, namely: Group 1: dose of 0.2 W/cm^2, Group 2: dose of 0.5 W/cm^2, Group 3: dose of 1.0 W/cm^2, Group 4: no treatment. Groups 1, 2 and 3 were treated on the first postoperative day, for 21 consecutive days. To evaluate the functional activity, we used the sciatic functional index (SFI) method proposed by De Medinaceli et al. (1982, 1984). For assessment of mechanical hyperalgesia, we used the paw pressure test in rats, conducted according to the method described by Randall & Sellito (1957). The assessment of thermal hyperalgesia was performed by the plantar test (Hargreaves et al., 1988). Allodynia was assessed by quantitative assay, in response to tactile stimulation applied to the paws of the rats, using the method described by Chaplan et al. (1994). Statistical analysis was performed using the two-way ANOVA method and a Bonferroni post-test with a significance level of $p < 0.05$. **Results and Discussion:** Statistical differences SFI were observed between Group 4 and Groups 2 (day 21, $p < 0.05$) and 3 (day 14, $p < 0.05$ and day 21, $p < 0.01$) and between Groups 2 and 3 (day 21, $p < 0.01$). In the assessment of mechanical hyperalgesia, there was partial reversal of the decrease in the nociceptive threshold between Group 2 and control group ($p < 0.05$), and total reversal in group 3, compared with all other groups ($p < 0.001$). The same results were obtained for thermal hyperalgesia. We observed that on day 21, partial reversal of allodynia occurred in Groups 1 and 2 ($p < 0.01$). Group 3 displayed total reversal of allodynia ($p < 0.001$) Therapeutic ultrasound at a dose of 1.0 W/cm^2 showed benefits in the control of nociception and functional recovery in this experimental model.

Supported by: CAPES

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10.05 Start-up wastewater treatment plant in Influenza Laboratory

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Introduction: The raw sewage discharges generated by domestic and untreated industrial waste, when launched in spring contribute to its degradation, affecting their quality. To preserve water resources and prevent contamination of the fraction of consumable water, the treatment of wastewater and sewage effluent is essential. Currently, the Influenza Laboratory of Butantan Institute implements a Wastewater Treatment Plant employing the activated sludge process. Using aerobic bacteria to oxidize the organic matter in the effluent in order to favor the reduction of BOD (biochemical oxygen demand) and meet the parameters required by legislation for effluent discharge receiving waters. **Objectives:** The aim of this study was to establish the Start-Up Wastewater Treatment Plant and adapt to the characteristics of the effluent legislation. **Methods:** To start-up structural improvements, electrical and automation modifications were made to facilitate and expedite the process performance. Tests to characterize the effluent were also carried out two times a month (every 15 days). To perform initial tests for reduction of organic matter in the effluent, and as there was no time to generate its own sewage sludge, a biological sludge received from SABESP Wastewater Treatment Plant was inserted in the reactors. It also made it possible to characterize the sludge. The process control analysis was carried out daily for pH and temperature along with the Imhoff Cone test. **Results and Discussion:** In three months of start up, the results showed that the factory effluent enters into treatment plant with lower pH values in the morning and higher pH values in the afternoon relative to that required by environmental rules. The findings may be related to the type of activity that occurs in the Influenza Laboratory during these periods. In the afternoon when the production process is finished, cleaning of the areas begins using detergents that end up giving this basic feature to the effluent. The results of the reduction of BOD (organic matter) were not satisfactory, since it was observed that there was no continuous generation of wastewater reactors to feed and maintain the activated sludge.

Supported by: Fundação Butantan

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10.06 Influenza viruses in Dogs from Brazil

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Introduction: Concerns over interspecies transmission of influenza viruses, as well as the emergence of new pandemics, led to the first study on influenza viruses in domestic animals in 1970. Birds and mammals, including humans, are their natural hosts; however, other animals may also play a role in the virus epidemiology. Infection with an influenza A type virus originated in horses has been reported in dogs in the USA. **Objectives:** The objective of this work was to investigate the incidence of influenza viruses in adult dogs raised in rural (9, 19.56%) and urban (37, 80.43%) areas in the state of São Paulo, Brazil. **Methods:** Dog serum samples were collected and were examined for antibodies to influenza viruses by the hemagglutination inhibition (HI) test using the corresponding antigens from the circulating viruses in Brazil. **Results and Discussion:** Nearly 90% (88.88%) of rural dogs showed protective titers of ≥40 HIU in response to human influenza A (H1N1). All rural dogs responded to human influenza A(H3N2), and equine influenza A (H7N7) and A(H3N8). About 84% (83.78%) and 91.89% of urban dogs responded to human influenza A (H1N1) and A (H3N2), respectively. About 92% and 100% of urban dogs were positive for antibodies against equine influenza A (H7N7) and A (H3N8), respectively. According to our results, rural and urban dogs are more susceptible to infection by human subtype A (H3N2) and equine subtypes A (H3N8) and A (H7N7) than by human subtype A (H1N1). In conclusion, our results indicate that both human and equine influenza viruses can be transmitted to dogs, which, like other mammals, may play a role in interspecies transmission and the spread of the viruses. The present study provides evidence, for the first time, that influenza viruses circulate among dogs in Brazil.

Supported by: CNPq

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10.07 Use of silicone filling with taxidermy techniques of representatives of the herpetofauna

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Introduction: Taxidermy is an old technique, initiated by the Egyptians around 2500 BC. Despite being an old record nowadays, it is a modern tool to support environmental education. Its multi-disciplinary nature involving several areas of knowledge such as ecology, biology, chemistry, anatomy, behavior, geography, ecology and fine arts, among others, in order to return the original form of copies in their natural behavior allowing contact with the work piece and more detailed observations. **Objectives:** The aim of this study was to examine the application of the silicone filling techniques in representatives of the herpetofauna. **Methods:** Using herpetofauna animals which have already been fixed or suffered natural death at the Institute, the leather was removed, treated with sodium tetraborate, filled with silicone and shaped. **Results and Discussion**: With the silicone filling process, it is possible to get stuffed flexible parts with greater durability, giving an appearance more like that of the living animal. These stuffed animals are used for the public's benefit in environmental education activities. Stuffed animals help students with learning, showing them through direct experience the links between nature and humans. When using all the senses, the individual has a chance to experience emotions and sensations, and, at the same time, get an impression of the animal in its most complex form. This combination is important because it can be a basis of new values that include environmental awareness and quality of life. Environmental education with the use of stuffed animals is more dynamic and attractive. Classes or activities that have this theme can count on this tool to help hold the attention of children and adults. Before, learning was only through pictures in books or zoos, and now, these tools are available to enrich the learning of all.

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10.08 Environmental enrichment and its influence in the behavior BALB/c mice

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Introduction: The objective of research on environmental enrichment is to identify the influence of the environment on the behavior of animals. In this context, the enrichment of the environment can be defined as a tool used to promote well-being and for decreasing behaviors considered abnormal for the species. The knowledge obtained in zoology, psychology and mostly in ethnology is fundamental for research and should be the foundations of choice concerning the kind and methods of enrichment. The development of alternative handling systems should take into account the behavioral needs and their motivations, in order to preserve the well-being of captive animals. **Objectives:** The aim of this study was to evaluate the influence of environmental enrichment in the behavior of *BALB/c* mice. **Methods:** The experiment lasted 310 days, from the first mating to weaning of the last litter. Sixty mouse couples from the *BALB/c* strain were used (divided in two groups), with controlled health status, raised and kept under standardized environment. Cardboard tubes, approximately 10 cm long and with a diameter of 4 cm, were previously packed and autoclaved and used as cage enrichment. Characteristics relating to fertility, longevity and survival were analyzed. **Results and Discussion:** According to the results obtained in the experiment, the use of cardboard tubes as material for nest building worked satisfactorily, stimulating maternal care. In the enriched cages, pre-weaning mortality was lower (P=0.01) than in non-enriched cages (0.80 *vs.* 1.87 deaths, respectively). The cardboard tubes worked effectively as shelters, serving as burrows that allowed the rodent to hide when they felt threatened or just safe in their nests. They also helped the movement of the animals, stimulating exploratory behavior and playful manifestations. Therefore, the cage enrichment allowed the manifestations of behaviors closer to what is expected as natural. Effects on the other characteristics analyzed were not detected. The results suggest that the implementation of an environmental enrichment program for the isogenic mouse, in an intensive production system will improve the production levels because it will reduce the pre-weaning mortality rate, besides contributing to well-being and manifestation of behaviors characteristic of the species.

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10.09 Cloning and characterization of genes coding for C3 and factor B complement system components from *Loxosceles laeta* spider venom glands

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Introduction: The human complement system is composed of more than 30 proteins, many of which have conserved domains that allow us to trace phylogenetic evolution based on linear amino acid sequences and their tertiary structures. The studies of vertebrate and invertebrate genomes revealed that many domains of mammalian complement components are found in both deuterostomes and protostomes. The origin of the complement system probably occurred with the appearance of C3 and factor B (Bf), the only components found in some protostomes and in cnidarians, suggesting that the alternative pathway represents the most ancient complement pathway. Recently, the transcriptome of *Loxosceles laeta* spider venom gland was found to contain C3-like and factor B-like genes of invertebrates of the families Limulidae and Strongylocentrotidae, respectively. These findings suggest that the central components of the complement system can be expressed in venom gland from brown spiders, perhaps playing a role in defense mechanisms. **Objectives:** This project aimed to clone and characterize the C3- and factor B-like from the *L. laeta* venom gland. **Methods:** Eighty *L. laeta* female spiders were subjected to food restriction to stimulate the production of mRNA in the venom glands. After 5 days, the spider venom glands were collected and frozen at -80ºC until use. cDNA fragments coding for components C3 and Bf were amplified from mRNA obtained from the venom gland of *Loxosceles* spiders and cloned in pGEM 11zf^{+} plasmid at EcoRI/NotI sites. Plasmid DNA was isolated and sequenced on an ABI 3100 sequencer. **Results and Discussion:** Using bioinformatics tools, it was possible to characterize two domains in the deduced amino acid sequences in both components, in other words, the A2M_receptor and C345R domains for C3-like (Lox-C3) and the vWFA (von Willebrand of type A) and serine proteases domains for factor B-like (Lox-Bf). Similarity analyses indicated that Lox-C3 shares a major identity (~40%) with horseshoe crab C3-like sequences, while Lox-Bf shows identity ranging between 27% and 30% with invertebrate Bf-like sequences. Alignments made with these and other sequences present in the database showed strong conservation of such domains in other organisms. The results, although preliminary, allow us to infer that the components C3 and factor B from *Loxosceles* spiders have the same composition of domains in organisms of the families Limulidae and Strongylocentrotidae, respectively.

Supported by: FAPESP, CNPq and INCTTOX

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10.10 São Paulo state: an ethnoarachnologic approach

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Introduction: Considering the lack of studies that address the interaction between man and arachnids in the state of Sao Paulo, Brazil, we carried out a pioneering study of the ethnoarachnologic imprint in this location. **Objectives:** The objective was to initiate and encourage the development of works that address this issue, so that we can understand how different localities of São Paulo state interact with their spiders and scorpions. **Methods:** Data were collected through open interviews and semi-structured interviews with 50 men and 50 women, with ages ranging from 5 to 90 years. The data were analyzed with emphasis on the emotional, cognitive and behavioral aspects. **Results and Discussion:** Regarding the cognitive aspect, we recorded ethnotaxonomy, natural history and beliefs related to these animals, as well as the myth that, if a person was bitten by a snake and has not died, he will be persecuted and bitten by a spider, which will finish the "snake's work." For the behavioral aspect, the use of arachnids as a therapeutic resource was discussed, for example the "garrafada de escorpiões," which consists of pickled scorpions in brandy, for the treatment of sexual impotence, frequently used by people of Lorena city, São Paulo. As for the emotional aspects, it has been observed that people attribute anthropomorphic qualities to these animals (ugly, beautiful, brave, intelligent), such negative qualities are broadcast by the media, the main entity responsible for the feeling of fear among the majority of the respondents. People include the arachnids in the ethnozoology field as "insect" and live together with case accidents of arachnids, which can be treated by home made "remedies." such as rubbing garlic or dropping sugar on top of the bite. We conclude that traditional knowledge is not only a privilege of the community called "traditional," because although the vast majority of respondents are part of the dominant social culture associated with the great influence of the media, the effective presence of folk medicine, legends, myths and beliefs, ethnobiology can start within each individual through a unique insight, vision, feeling and own experience.

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10.11 Study of molluscicidal activity and toxicity of Piperaceae amide - a possible natural molluscicide

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Introduction: Schistosomiasis is a parasitic and endemic disease that occurs in 54 countries, mainly in the South America, the Caribbean, Africa and East of the Mediterranean. In Brazil, it affects over 8 million people and about 30 million live in hazardous areas due to the presence of *Biomphalaria* infected snails. One of the most efficient methods to control this disease is the application of molluscicides that eliminate or reduce the intermediate host population. Environmental preservation, high costs and recurrent resistance of snails to synthetic molluscicides have stimulated the study of molluscicides from plant origin. The family Piperaceae has species with a diversity of chemical compounds, some of which with bioactive properties, such as amides. **Objectives:** In the present study, the molluscicide, ovicidal and schistosomicidal activities of an amide from *Piper* genus (Piperaceae) were evaluated. **Methods:** The activities were evaluated on the adult and embryo stages of the *Biomphalaria glabrata* snail, miracidium and cercaria, the free-living larval stages of *Schistosoma mansoni.* Acute toxicity assays were performed on the bacterium *Vibrio fischeri*, microcrustaceans *Daphinia simillis* and fish *Danio rerio* for the evaluation of toxicity in different trophic levels. **Results and Discussion:** The amide showed molluscicidal activity at concentrations lower than that recommended by WHO for molluscicide screening and evalution (LC_{90} 7.18 ppm and 0.99 ppm for adult and embryo snail, respectively). However, at the same concentrations, the amide was not active on miracidium and cercaria. Acute toxicity was evaluated and the amide was toxic to all organisms tested. The molecular structure of the amide is being studied and modified in order to increase the molluscicidal and schistosomicidal activities and decrease the toxicity.

Supported by: FAPESP and CAPES

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10.12 Description of larva of *Amblyomma romitii* Tonelli-Rondelli, 1939 (Acari: Ixodidae) by scanning electron microscopy

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Introduction: The species *Amblyomma romitii* was originally described in six females, one male and seven nymphs collected on a capybara, in British Guiana. The species was also collected in 1957 on a human from the state of Pará. **Objectives:** The aim of this study was to describe the morphology of the larva of *A. romitii* by means of scanning electron microscopy. **Methods:** Larvae of *A. romitii* were reared from a female collected on *Hydrochaerus hydrochaeris* from Rurópolis (04º05'45''S, 54º54'33''W), state of Pará. The female was maintained at 27ºC and high humidity (almost 100%), scotophase. A sample of 10 unfed larvae was prepared for optical microscopy in order to obtain measurements. Five specimens were also processed for scanning electron microscopy. For determination of the frequency of integumentary pores, each idiosomal side was independently analyzed. Thus, a total of 30 idiosomal sides were analyzed. Several characters are presented including the chaetotaxy and porotaxy of idiosoma, chaetotaxy of palpi and Haller's organ, number of campaniform sensillum on dorsum and festoons and also measurements. **Results and Discussion:** The larval chaetotaxy of *A. romitii* is similar to that of other species of *Amblyomma*. However, the porotaxy of this species showed specific characteristics and distribution. Dorsally, the larva of the *A. romitii* has 3 pairs of dorsal wax glands located at the lateral marginal idiosoma, 11 pairs of lyrifissures and 60 pairs of small glands. Ventrally, it has 5 pairs of wax glands, 13 pairs of lyrifissures and 34 pairs of small glands. The larvae of the genus *Amblyomma* have an arrangement of large wax glands, with 4 pairs observed on the ventral surface, 3 located behind each coxa, 1 pair in the 5th festoon, and 1 pair located dorsally on the posterior lateral margin of the body. Nevertheless, in *A. romitii,* there is one additional ventral pair of large wax glands on the 4th festoon. This was also reported for 3 Asian species and for 3 Brazilian species, *A. aureolatum*, *A. longirostre* and *A. ovale*. In these species the gland is located next to the lateral margin of idiosoma. Furthermore, *A. romitii* has two additional pairs on the dorsal surface, one in segment IV and other in segment V. The numerical and topographical pattern for lyrifissures on surface dorsal and ventral has been found to be similar among *Amblyomma* species. However, in *A. romitii,* the lyrifissure on ventral segment XIV was observed in series 4, while in the other species it has been located in series 2.

Supported by: FAPESP, CAPES and CNPq

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10.13 Effects of bioactive compounds from rosemary (*Rosmarinus officinalis* L.) on biochemical markers and antioxidant enzymes in liver and kidneys in diabetic animals

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Introduction: Rosemary is much appreciated for its aroma and flavor and is rich in the following phenolic compounds: carnosic acid, carnosol, rosmarinic acid, caffeic acid, and hydroxycinnamic acid ester. These compounds can play an important role in reducing tissue peroxidation that is associated with diabetes. **Objectives:** This study aimed to evaluate the effect of an aqueous extract (AE) of rosemary on streptozotocin-induced diabetes in rats. **Methods:** Twenty-four male Wistar rats were distributed into three experimental groups: control (water), diabetic (water) and AE diabetic (50 mg /kg aqueous extract). Water and the aqueous extract were administered by orogastric intubation for 60 days. The animals were then sacrificed. The blood was collected for analysis of biochemical markers. The liver and kidney tissues were collected for the determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. Statistical analysis was performed by one-way analysis of variance (ANOVA) and Tukey's test. **Results and Discussion:** Rats in the diabetic group showed polyuria, polyphagia, polydipsia and weight loss. The following changes in markers in diabetic animals were noted: altered antioxidant enzyme activity; and a significant increase ($p<0.05$) in blood glucose, glycosylated hemoglobin (Hb-G), triglycerides, total cholesterol and creatinine levels. The administration of the aqueous extract at a concentration of 50 mg/kg resulted in an 8.1%; 16.8%; 24.6%; 25.3% and 49.8% reduction in blood glucose, Hb-G, creatinine, total cholesterol and triglycerides levels, respectively; and a 17.43% increase in HDL-cholesterol levels, compared to those in the diabetic group. The aqueous extract also reduced the activity of SOD and GPx, compared to that in diabetic animals; however, CAT activity behaved differently. The results indicate that the effects of these enzymes on diabetes are still quite controversial and that adjuvant therapy with aqueous extracts of rosemary can be used in the prevention of oxidative stress in experimental diabetes.

Supported by: CNPq

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10.14 Effect of toxins from *Phoneutria nigriventer* spider venom on release of neurotransmitters in brain tissue *in vitro*

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Introduction: *Phoneutria nigriventer* spider venom has many peptides with distinct pharmacological actions that can stimulate or block ion channels, altering the release of neurotransmitters in the central and peripheral nervous system. Many symptoms are described in poisoning caused by the spider, such as intense pain, muscle spasms and tremors. **Objectives:** In this study, we aimed to perform a compound screening in search of active substances on neurotransmitter release. **Methods:** We employed labeled neurotransmitter release in vitro by superfusion of striatal tissue. The striata of rats, dissected and cut into wedges with a Mcllwain tissue chopper were washed in Krebs-Ringer-bicarbonate (KRB) buffer at 37°C. We then added ^{14}C- or ^{3}H-radiolabeled neurotransmitters for uptake and loading. The toxins were diluted in KRB and perfused using a Suprafusion 2,500 (Brandel – USA) for automatic superfusion. The equivalent of 10% of the total fraction obtained after HPLC fractionation of 100 mg of crude venom was tested in each experiment. Results are expressed as fractional release. **Results and Discussion:** The fractions investigated contained peptides with a size range of 2,300 to 6,600 Da (previously mapped by mass spectrometry). Three of the twenty fractions produced a decrease in KCl-stimulated glutamate and acetylcholine release. Fractions 2, 6 and 15 contained potentially active peptides and will be further purified to identify the active compound.

Supported by: FAPESP

11. PIBIC/PIBITI

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11.01 Recombinant expression and characterization of the metallopeptidase neprilysin (EC3.4.24.11, NEP)

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Introduction: Neprilysin (NEP), also known as endopeptidase, is a membrane protein that activates or inactivates oligopeptides such as natriuretic hormones (ANP, BNP and CNP), the endothelins, angiotensin I and bradykinin. The major function assigned to NEP is related to tumor progression and metastasis promotion, processes in which there is an evident increase in this enzyme's expression. **Objectives:** The aim of this study was to express the enzyme NEP in a prokaryotic expression vector and to purify it to apparent homogeneity. **Methods:** The cDNA cloned into pGEX vector, which allows expression of fusion proteins with glutathione S-transferase (GST), was transformed using *E. coli* BL21DE3. Protein expression was induced with 1 mM IPTG at 37°C for 24 h. Cells were collected by centrifugation at 3500 rpm, at 4°C for 15 min, and suspended in lysis buffer (50 mM Tris HCl, pH 7.5, 2 mM $MgCl_2$, 0.25 mg/mL of lysozyme and benzonase nuclease 25 U/uL). The cells were lysed by sonication, and the pellet containing the inclusion bodies was subjected to solubilization and a dialysis protocol. The purification was finally accomplished by affinity chromatography on a glutathione-Sepharose column. The eluate containing GST-NEP was digested with thrombin, followed by membrane filtration using Centricon 50. The expression of NEP was analyzed by SDS-PAGE and Western blot, and SDS-PAGE analysis demonstrated NEP bands. They were cut out, macerated, destained (40% methanol and 10% acetic acid) and washed 3 times with 1 mL of H_2O and once with 1 mL of PBS. The pGKJE8 molecular chaperone was employed as the same methods of expression in vector pGEX4T2 with different conditions of expression of inducers, such as 10 ng/mL tetracycline, 4 ng/mL L-arabinose and IPTG, less aggressive techniques to obtain the soluble protein. **Results and Discussion:** Through comparative methods, we obtained approximately 8 μg/mL of NEP without catalytic activity, which was used for immunization of the genetically selected H_{III} mouse line to obtain antibodies for immunochemical characterizations. It is widely known that the co-expression of chaperones can assist protein folding and prevent the aggregation of newly formed protein until it folds into its native form, and in some cases, this may lead to increased production of native proteins, such as NEP. These studies will improve our performance in protein production, avoiding aggressive protocols for the solubilization of inclusion bodies, and make NEP with catalytic activity through the use of chaperones. Thus, different forms of expression are in progress.

Supported by: CNPq/PIBIC and FAPESP

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11.02 Glutamatergic inhibitory effect on melatonin synthesis and secretion involves interactions between pinealocytes and astrocytes via a soluble factor

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Introduction: The glutamatergic modulation of melatonin synthesis is well established in the literature as well as the importance of astrocytes in mediating glutamatergic signaling in the central nervous system. In our laboratory, we demonstrated that the inhibitory glutamate effect on melatonin synthesis is dependent on paracrine interactions between pinealocytes (the secretory cells that synthesize melatonin) and astrocytes (the main glial cell in the pineal gland). **Objectives:** The objective of this work was to investigate the glutamate receptors involved in this glutamate inhibitory effect and the nature of the interactions between astrocytes and pinealocytes. **Methods:** Young male Wistar rats were sacrificed by decapitation and their pineal glands were isolated and dissociated using the Papain Dissociation System kit. The pinealocytes in association with astrocytes (co-culture) were kept in culture (DMEM medium + 10% BSF) and were then submitted to the pharmacological treatments for 5 h. The cells were stimulated with norepinephrine (1 μM) combined with glutamate (600 μM) or with the defined agonists to AMPA (AMPA – 50 μM) or NMDA (NMDA – 100 μM) ionotropic receptors, to type I metabotropic receptors (DHPG – 50 μM), or to type II metabotropic receptors (L-CCG – 10 and 100 μM). Moreover, the cells in co-culture were physically isolated using inserts and were stimulated with norepinephrine (1 μM) and glutamate (600 μM). The cells were also stimulated with norepinephrine (1 μM) combined with glutamate (600 μM) and BB1101 (10 μM) which is the TNF-α inhibitor. Melatonin was quantified by HPLC with electrochemical detection. **Results and Discussion:** The inhibitory effect caused by glutamate on melatonin synthesis and secretion was also observed when NMDA agonist was used. The other agonists (AMPA, DHPG, L-CCG) did not modify melatonin synthesis. When the cells were separately cultured using inserts, the glutamate inhibitory effect also occurred. The inhibitory effect caused by glutamate on melatonin secretion was not observed when the TNF- α inhibitor was used. The results obtained support the idea that glutamate modulation of melatonin synthesis involves paracrine interactions between pinealocytes and astrocytes through the stimulation of NMDA receptor and the diffusion of a soluble factor, probably TNF-α, which inhibits melatonin synthesis.

Supported by: FAPESP and CNPq

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11.03 Isolation of pro- and anticoagulant proteins from *Bothrops jararaca* snake plasma

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Introduction: Hemostasis is the ability of the organism to control the flow of blood following vascular injury. It comprises the process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue. Some coagulation factors are vitamin K-dependent proteins (VKDPs), such as protein C, factor X and prothrombin, and they can regulate blood coagulation. **Objectives:** The aim of this work was the purification and characterization of these VKDPs from *B. jararaca* plasma. **Methods:** The process of purification was achieved by a combination of ion exchange (Hi Trap DEAE FF) and affinity (HiTrap Heparin HP) chromatographies. Protein concentrations were determined spectrophotometrically, and fractions eluted from Hi Trap DEAE FF, which reportedly contained VKDPs, were pooled and concentrated by centrifugation and then applied to a HiTrap Heparin HP column. **Results and Discussion:** The presence of prothrombin on the purified material was detected according to Rob (1997) and the presence of factor X and protein C, according to Lindhout (1978). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), in presence and absence of beta-mercaptoethanol, did not show the isolation of VKDPs yet, due to the presence of contaminants in the analyzed material. The next steps of this work are to improve the process of VKDP purification.

Supported by: FAPESP and CNPq

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11.04 Research on the gene, mRNA and protein corresponding to factor XII in chicken species

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Introduction: In mammalian species, the Hageman factor or factor XII (FXII) is considered the centerpiece of several mechanisms that activate the body's defense systems such as blood coagulation, fibrinolysis, blood pressure regulation and complement. Because patients with deficiency of Hageman factor have no bleeding symptoms, this factor has been considered dispensable for hemostasis. On the other hand, many studies have recently reported its involvement in the pathogenesis of thrombotic processes in the intravascular space. Therefore, its pharmacological inhibition has been suggested as a new therapeutic strategy for the treatment of thrombotic episodes, which are potentially fatal. Whether the FXII plays a physiological role is crucial, because if it is important for hemostasis, its pharmacological inhibition will surely lead to harmful side effects. FXII gene and activity have not yet been detected in the avian genome and plasma, respectively. Functional FXII activity appears to be absent also in the plasma of *Bothrops jararaca*, contrary to the case, curiously, of the plasma of *Crotalus durissus* and *Boa constrictor constrictor*. In previous work, we detected the possible presence of similar FXII activity bound to the surface of thrombocytes (platelets) of the chicken *White leghorn*. **Objectives:** The aim of this study was the detection of partial sequence of the active site of FXII in *W. leghorn* chickens and *B. jararaca* and *B. c. constrictor* snakes. **Methods:** The practical activities involved the extraction of DNA and agarose gel analysis, and then the amplification of sequences of interest by PCR, cloning of amplified products and sequencing. We used the primers PB1d and PB1r, designed according to common sequences found in the alignment of exon 10 of the FXII gene from *Mus musculus* and *Homo sapiens*. **Results and Discussion:** The result of PCR showed a band of 160 bp in the DNA of *W. leghorn* and *B. jararaca*, which after sequencing and analysis by the program *Clustal W* revealed 100% sequence identity with part of the coding region of the active site of FXII in both *W. leghorn, B. jararaca* and *B. c. constrictor*. The results suggest the unexpected presence of the FXII gene in these vertebrates. Further studies are being carried out to confirm our findings.

Supported by: CNPq

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11.05 Niche stem cells from fetuses x niche adult stem cells of the brain

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Introduction: Stem cells (SC) can be isolated from the embryo, fetus and adult organism. SC are found in the physiological microenvironments composed of specialized cells that control SC proliferation called niches. Adult niches are fixed microenvironments while embryo–fetus niches are considered transient, where they are essential for ontogenesis. Embryonic-fetal niches consist of hierarchical pool types of SC [pluripotent stem cells (PSC), multipotent stem cells (MSC) and progenitors]. Different types of SC were isolated by us from fetal brain, as well as similar embryonic stem cell (ESC) and progenitor cells. To our knowledge, the presence of PCS from the central nervous system (CNS) has not yet been reported. Additionally, adult multipotent neural stem cells are located in the sub-ventricular and sub-granular zone of the dentate gyrus of the hippocampus. Therefore, there is a need to identify the CNS niches, which will contribute to our knowledge about basic processes of SC development and neurogenesis. **Objectives:** We aimed to identify the PSC niches from brains of embryos, fetuses and adults. **Methods:** Mouse embryo of 129 strains with 10 days of development and adult brain samples were used. The samples were included in paraplast and hematoxylin-eosin stained. Immunohistochemistry was performed using Oct3/4, NANOG and SSEA1 antibodies. **Results and Discussion:** The embryo with 10 days of development is macroscopically a single cell mass (embryoblast). Therefore, it was reported that the embryos with 10 days of development showed features of the most advanced developmental stage. In this period, the embryo reacted positively to pluripotent markers: Oct3/4, nanog and SSE1. Interestingly, we observed Oct3/4 immunopositive cells in the cerebellum. However, in the adult niche (subventricular zone), Oct3 /4 positive cells were not identified, as well as in other brain regions. The results suggest that it is the presence of PSC after morulae stage and is possible to isolate these cells in vitro. Future studies are needed for a more detailed investigation of the niches of SC in adult brain, as it is believed that the adult CNS is incapable of undergoing cell renewal and structural remodeling.

Supported by: CNPq/PIBIC

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11.06 Mouse toxicity screening of the Cyanobacteria Culture Collection of the Institute of Botany of São Paulo

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Introduction: Cyanobacteria are prokaryotic photosynthetic organisms that can be found in various habitats, such as terrestrial and aquatic, and can also grow under extreme conditions (very high or low temperature, low humidity, etc). Depending on the environmental conditions, cyanobacteria can form blooms in water reservoirs for human consumption. This causes great concern since, besides altering the organoleptic conditions of the water (color, smell, taste, etc), toxins can be released in the water and may cause severe and lethal intoxications. These toxins are classified according to the symptoms: hepatotoxins (microcystins and nodularins), neurotoxins (saxitoxins and anatoxins), cytotoxins (cylindrospermopsin) and dermatotoxins (LPS). **Objectives:** The aim of this study was to investigate the toxins produced by the cyanobacteria from the Culture Collection of the Institute of Botany of São Paulo, using acute toxicity tests in mice (i.p.), observing the symptoms after administration, and performing postmortem examinations, looking for structural alterations in the vital organs. **Methods:** Extract preparation: the cultured cyanobacteria cells were filtered with a AP-20 filter and freeze-dried. The resulting material was then extracted with 0.1 M acetic acid (4x) or MeOH/H_2O (75:25, v/v) (5x) by ultrasonication (4 x 10 s, 50 W) and centrifugation. The supernatant was concentrated under reduced pressure, and the extracts were maintained at -20°C until they were used. The toxicity tests (i.p.) were performed in male Swiss-Webster mice (19-21 g). The mouse symptoms were observed for up to 7 days after administration. After death by acute toxicity or by euthanasia, necropsy was performed and tissue samples were taken from the liver, kidneys and lungs, fixed and used for histopathological analysis. **Results and Discussion:** In the present work, 25 extracts (of 14 strains) were tested. The extracts of four strains caused death in mice within a few to ninety minutes after administration. The extracts of three strains did not cause macroscopic alterations in the mouse organs after seven days of observation, although they had induced intoxication symptoms right after the sample injection. The other extracts induced macroscopic alterations mainly in the liver (stains, swelling and adhesion to the intestines) and in the lungs (hemorrhage). The symptoms and signs displayed by the animals after the administration of extracts indicate that these cyanobacterial strains produce hepatotoxins and neurotoxins. Histopathological and chemical analyses are ongoing.

Supported by: CNPq/PIBIC and CNPq

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11.07 Characterization of toxin isolated from *micrurus lemniscatus* venom with activity on muscarinic receptors in the rat hippocampus

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Introduction: The muscarinic acethylcholine receptors (mAChRs) play important roles in regulating the activity of many essential functions of the central and peripheral nervous systems, such as heart rate and force, contraction of smooth muscles, glandular secretion and cognitive functions such as learning and memory (J. Med. Chem., 43: 4333, 2000). Several small proteins isolated from the venom of the African green mamba (*Dendroaspis angusticeps*) have been found to bind highly and selectively to mAChR subtypes (for review, see Biochimie, 82: 793, 2000). Some of them were shown to have the ability to activate mAChR, while others act as muscarinic antagonists (Toxicon, 41: 207, 2003). **Objectives:** The aim of this study was to search for the components associated with mAChR in the venom of the Brazilian snake *Micrurus lemniscatus*, and to isolate and characterize a purified component with affinity for mAChR obtained from the venom of *M. lemniscatus*. **Methods:** Previously, we isolated the toxin Mlx-8 from the venom of the *M. lemniscatus* snake by HPLC in a C8 reversed-phase column. The molecular weight was determined (13,531 Da) by mass spectrometry. In saturation experiment, hippocampus membranes, obtained from male Wistar rats (3 months old), were incubated with [3H]QNB (0.05-8.0 nM) in the absence and presence of atropine (1 μM) (30°C/1 h). In competition experiments, hippocampus membranes were incubated with [3H]QNB, in the absence and presence of increasing concentrations of the purified component Mlx-8, obtained from the venom of *M. lemniscatus*, and atropine (control) (30°C/1 h). The intracellular [3H]-inositol phosphate content was also measured in the hippocampus of rats as previously described (Mol. Cell. Endocrinol. 160:17, 2000). **Results and Discussion:** Scatchard analysis of specific binding yielded a dissociation constant (KD)=0.88 ± 0.13 nM and binding capacity (Bmax)=1459.40 ± 235.26 fmol/mg protein (n=5). Mlx-8 and atropine indicated a statistical preference for a one-site rather than two-site fit. Thus, both Mlx-8 and atropine defined one muscarinic binding site with high affinity (respectively, pKi = 7.38 ± 0.15, n=4, and pKi = 8.96 ± 0.08, n=4) to [3H] QNB in the hippocampus. Purified component Mlx-8 (10-7 M) reduced the total [3H]-inositol phosphate accumulation induced by 10-5 M carbachol in hippocampus. The results suggest that the purified component Mlx-8 obtained from *M. lemniscatus* snake venom may not only have affinity for mAChRs but also have the ability to inhibit mAChRs in rat hippocampus.

Supported by: FAPESP and CNPq

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11.08 Artificial feeding of *Amblyomma cajennense* (Acari: Ixodidae) with blood of suspected Baggio-Yoshinari syndrome (BYS) patients

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Introduction: Considering that the vectors amplify the pathogen, the artificial feeding of *Amblyomma cajennense* through capillary tubes with blood of suspected patients of Baggio-Yoshinari syndrome (BYS), is a promising method for the isolation and identification in cells of the etiologic agent of the disease in Brazil. Blood samples from suspected patients with clinical and laboratory evidence of BYS were collected in EDTA at the Laboratory of Rheumatology, FMUSP (Reference Laboratory) and donated to the study. **Objectives:** The aim of this study was to artificially feed females of *A. cajennense* partially engorged with blood of suspected patients of Baggio-Yoshinari Syndrome (BYS) and blood with inoculated with *Borrelia burgdorferi*. **Methods:** After the 5th day of feeding on rabbits, females of *A. cajennense* mated and partially engorged were removed by twisting and washed with water. They were then dried on filter paper, weighed on a precision scale to four decimal places and set in trays with double-sided tape. The tubes with blood samples of patients were removed from the refrigerator and kept at room temperature just before the start of feeding. The filled capillaries were placed on the mouthparts of the ticks and they were kept in an incubator (27 ± 1 ºC and humidity above 80%) for up to 12 h during the feeding period. Every 3 h, the capillary tubes were replaced and each hour they were cleared with a needle. At the end of the feeding period, the females were weighed again to assess the weight gain after feeding. Some of the females were fixed in absolute alcohol at different days (ranging from 1 to 3) to be used for PCR. The others were kept alive in an incubator until the end of oviposition to use the eggs in the primary culture of embryonic cells. The third part was fixed in fixative solution of 4% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2, to perform transmission electron microscopy. In addition to the artificial feeding of patients' blood, feeding with blood of rabbit inoculated with *B. burgdorferi* was also performed to help in the standardization of PCR and also for viewing in cell cultures of *Borrelia*. **Results and Discussion:** Samples of females fed with blood of patients have so far been negative in PCR as well as primary cultures of embryonic cells. On the other hand, the PCR of females fed with blood inoculated with *B. burgdorferi* (*Bb* 1:10 dilution/mL) were positive; however, ovipositions have not yet been performed.

Supported by: CNPq and FAPESP

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11.09 Cruentatoxins: antimicrobial peptides from the venom of *Nephilengys cruentata*

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Introduction: Venomous animals incapacitate their prey using complex venoms that can contain hundreds of unique protein toxins. Many of these toxins may have pharmaceutical potential due to their remarkable potency and selectivity against target receptors. The research for potential new drugs has led to an explosion in the number of novel toxins being discovered and characterized. From an evolutionary perspective, spiders are one of the most successful venomous animals and they maintain the largest pool of toxic peptides found to date. However, there are only a few reports of antimicrobial peptides from spider venoms, peptide toxins which display a high selective antimicrobial activity. **Objectives:** Therefore, the aims of this study were to purify and characterize those peptide toxins from *Nephilengys cruentata* venom that display antimicrobial activity. **Methods:** The venom was obtained from spiders stimulated by a low-voltage electrical stimulator and then centrifuged, and the soluble part was dried by vacuum centrifugation and reconstituted with 1 mL of acidified water (TFA - 0.05% trifluoracetic acid). The soluble part was subjected to pre-purification in two disposable Sep Pak C18 columns connected in series. Purification of the material was achieved by reversed-phase chromatography (RP-HPLC), using a Jupiter semi-preparative C18 column. Elution was performed with different linear gradients of ACN/0.05% TFA over 60 min at a flow rate of 1.5 mL. The column effluent was monitored by absorbance at 225 nm and the activities were determined by liquid growth inhibition assay against *Micrococcus luteus* A270, *Escherichia coli* SBS 363 and yeast *Candida albicans* MDM8. Purification of the material was achieved by reversed-phase chromatography (RP-HPLC), using a Jupiter semi-preparative C18 column. When necessary, further purification was done submitting the fractions to RP-HPLC, using a Jupiter analytic C18 column. All fractions with activity had their homogeneity determined by mass spectrometric analysis. **Results and Discussion:** According to our results, four fractions inhibited the growth of the yeast *C. albicans* MDM8, the Gram positive *M. luteus* A270 and the Gram negative *E. coli* SBS363. These broad spectrum fractions will be purified in an analytical column to homogeneity. We also aim to determine their sequence, minimal inhibitory concentrations and spectrum of activity against clinically relevant bacterial and fungal strains.

Supported by: CNPq/PIBIC and FAPESP

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11.10 Strategies for standardizing the expression of iNOS and MAPKinases in *Bordetella pertussis*-activated macrophages

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Introduction: Host cells recognize specific pathogen-associated molecules that act as sensors and transduce signals that alert and activate the immune response. However, many pathogens have learned how to subvert the host defense mechanism. Nitric oxide (NO) and arginase are molecules involved in the control of several infections and inflammatory processes. iNOS is induced by cytokines (e.g., IFN-γ) and/or microbial products such as LPS. The mechanisms involved in NO regulation in *B. pertussis*-activated macrophages are unknown and may involve pathways controlled by MAPkinases. **Objectives:** The aim of this study was to standardize a method for detecting the expression of iNOS and MAPkinases p38 and ERK1/2 in *B. pertussis*- or LPS-activated macrophages combined or not with IFN-γ. **Methods:** Bone marrow-derived macrophages (BMDMϕ) from C57/BL6 mice were differentiated for 7 days in RPMI medium supplemented with 10% FBS, 20% L929 fibroblast supernatant, 50 U/mL penicillin, 50 μg/mL streptomycin and nonessential amino acids. BMDMϕ were activated with soluble protein from *B. pertussis* (30 μg/mL) or LPS from *E. coli* for 1 or 20 h, with or without IFN-γ as a control. The level of nitrite in the culture supernatants was determined by the Griess reaction. Western blotting of macrophage whole-cell lysate for iNOS and MAPkinases expression was performed using a polyclonal rabbit antiserum against iNOS or MAPkinases p38 and ERK1/2. **Results and Discussion:** We could not detect iNOS and MAPkinases expression in *B. pertussis*-activated macrophages. The same results were observed when the cells, used as a positive control, were treated with LPS + IFN-γ, despite the high production of nitrite. We conclude that the specific antibodies did not recognize the enzymes due to their non-functionality. Other protocols are being used to define the MAPkinase contribution in iNOS regulation during the activation of macrophages with *B. pertussis*.

Supported by: CNPq/PIBIC and FAPESP

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11.11 Maternal exposure of Wistar rats to *Tityus bahiensis* scorpion venom during lactation

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Introduction: Accidents with scorpions have shown an alarming increase in tropical and subtropical countries including Brazil. Previous studies have demonstrated that the venom of the scorpion *Tityus bahiensis* causes alterations in physical and behavioral development in offspring of mothers that received the venom during pregnancy. **Objectives:** This study aimed to evaluate the effects of the venom in reflexological and behavioral development of offspring of mothers envenomed during the lactation period. **Methods:** Pregnant females were separated into three control groups (n=10) injected with saline on the 2nd (2C), 10th (10C) or 16th (16C) postnatal day and into three experimental groups (n=10) injected with venom (2.5 mg/kg, s.c.) on the 2nd (2E), 10th (10E) or 16th (16E) postnatal day. The reflexological development of the pups (n= 22 to 32 per group) were analyzed on postnatal days (PN) based on the following parameters: palmar grasp (PN 4, 6, 8); righting reflex (PN 4, 6, 8, 10); negative geotaxis (PN 6, 8, 10, 12) and general and locomotor activity (PN 10, 14, 18, 22). In adulthood (n=4 to 8 animals per group), the parameters measured were: forced swim, activity box, enriched environment, social interaction and discriminative avoidance in elevated plus maze. On the pre-determined days, animals of both sexes were assessed, with n ranging from 4 to 8 animals. **Results and Discussion:** Animals of both sexes from the group 2E had a reduction in latency time of palmar grasp reflex on PN4 and PN6 and an increase in PN8. In tests of righting reflex, an increase in latency time was observed on PN4, PN6, PN8 and PN10, and in negative geotaxis, the increase was on PN6, PN8, PN10 and PN12. In the activity box, males showed a decrease in locomotion and general activity on PN10. The females had an increase in general activity on PN22. In the group 10E, only females showed a reduction in general activity on PN14. In animals from the group 16E, no alterations were observed. The alterations observed in this study reinforce the data previously obtained in our laboratory, highlighting the harmful effects of the venom in the development of offspring of mothers treated during lactation, mainly during the initial period.

Supported by: CNPq/PIBIC and Fundação Butantan

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11.12 *Bothrops jararaca* snakebites in São Paulo State, Brazil: the influence of biological variables

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Introduction: Snakebites are major public health problems in Latin America, especially Brazil where there are about 20,000 accidents with snakes annually, mostly with *Bothrops* genus (about 90% of all accidents). *B. jararaca* is responsible for almost 93% of all *Bothrops* accidents. **Objectives:** The present work aimed to determine the epidemiological profile of accidents caused by *B. jararaca* and to evaluate the interference of biological variables responsible for snakebites in São Paulo State. **Methods:** *B. jararaca* specimens that have caused accidents from 1995 to 2010 are preserved at "Coleção Vital Brazil" in Instituto Butantan. All these snakes were dissected and examined. Data related to seasonal activity, mating season, sexual maturity and diet were collected and analyzed. **Results and Discussion:** Our data showed that 71% of the accidents were caused by juveniles, whereas 29% were caused by adults. Accident seasonal patterns are different between *B. jararaca* adults and juveniles. Preliminary analysis showed that juveniles and adult females caused more accidents than did juveniles and adult males. The juveniles (male and female) caused more accidents during the spring and summer. Adult females caused more accidents than did adult males. Analyzing the female reproductive status of the snakes that caused the accidents, reproductive females were found during the summer and pregnant females were found as of the end of the spring. The juveniles were born in the fall. Adult males caused more accidents during the fall, whereas adult females caused more accidents during the summer. These periods coincide with this species reproductive pattern, mainly for females which must feed heavily during this period, so they can have enough energy for vitellogenesis. During the fall, males are looking for females (mating period). Snake stomachs were dissected to check whether or not they had stomach contents. These data revealed that *B. jararaca* male juveniles fed mainly during the spring, whereas female juveniles fed mainly during the winter. *B. jararaca* male juveniles had 128 identifiable items in their stomachs (110 endothermic and 18 ectothermic prey, 86% and 14%, respectively), whereas female juveniles had 110 identifiable items in their stomachs (98 endothermic and 12 ectothermic prey, 89% and 11%, respectively). Most of the accidents occurred during the day, between 6 am and 6 pm. However, many accidents occurred during the night period, between 7 pm and midnight. Therefore, when the snake is in foraging activity (e.g., searching for food; searching for partners) it can be more active, thereby increasing the probability of causing snakebites.

Supported by: CNPq/PIBIC

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11.13 Anti-Sat antibody: An essential tool for the study of the Sat toxin expression in atypical enteropathogenic *Escherichia coli* (aEPEC)

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Introduction: Sat is a 107-kDa toxin encoded by the *sat* gene, and belonging to the serine protease autotransporter family of *Enterobacteriaceae* (SPATEs). This toxin, first described in uropathogenic *Escherichia coli* (UPEC) and more recently in diarrheagenic *E. coli* with a diffuse adhesion pattern (DAEC), has been related to the pathogenicity of both pathotypes. Atypical enteropathogenic *E. coli* (aEPEC) is considered an emergent pathogen, still little studied. Our group recently identified the *sat* gene in aEPEC samples CB5304 (0125:H6), CB3338 (0125:H6) and CV323/77 (0125:H21). The cytotoxic effect induced by these samples suggests the action of the Sat toxin, but neutralization of the effect with anti-Sat antibodies is essential for the continuity of this work. **Objectives:** The aim this study was to produce an anti-Sat monospecific antibody. **Methods:** A New Zealand rabbit was immunized with the culture supernatant from sample DAEC FB 114 (*sat*+) concentrated with an Amicon Ultra filter with a 100-kDa cut off, in the presence aluminum hydroxide (V/V). The rabbit received three immunizations at intervals of 15 days. Total bleeding was performed by cardiac puncture 15 days after the last immunization. Immunoblotting against the supernatant from sample FBC114 was performed in the presence or not of PMSF, after adsorption of the rabbit's serum to the enteroaggregative *E. coli* prototype sample (EAEC) 042. **Results and Discussion:** A 107-kDa band was identified, the molecular weight of Sat toxin, when using the supernatant from sample FB 114 in the presence of PMSF. On the other hand, no band was detected after the supernatant was kept without inhibitor for 24 h at ambient temperature. The results demonstrate the specificity of the antibody obtained in this work, since Sat toxin is a serine protease. This antibody's capacity to neutralize the cytotoxic effect of *sat*+ aEPEC samples is essential for the study of the expression of this important toxin in aEPEC.

Supported by: CNPq/PIBIC

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11.14 *Neisseria lactamica*: Study of growth in different concentrations of yeast extract and amino acids

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Introduction: An alternative approach to producing a vaccine for *Neisseria meningitidis* serogroup B is the development of an outer membrane vesicle vaccine based on the commensal bacteria *N. lactamica,* closely related to *N. meningitidis*. Outer membrane vesicles, OMV, are constantly being released from the surface of the bacteria during growth. It is currently postulated that colonization by *N. lactamica*, which possibly shares antigens with *N. meningitidis*, can contribute to the natural development of immunity against *N. meningitidis*. Studies of the growth of *N. lactamica* and studies about purification and productivity of OMV from *N. lactamica* may provide a key to large-scale culture and OMV production. **Objectives:** The aim of this work was to study the growth kinetics of *N. lactamica* in a shaker, in Catlin medium (MC) with different concentrations of yeast extract and amino acids, and to analyze and compare the yield of OMV, obtaining a medium that can be used in bioreactor studies. **Methods:** *N. lactamica* was cultivated in a shaker, at 250 rpm and 36°C, for 8 h in the first group of assays and 12 h in the second group of assays. Assays were performed in triplicate. The culture medium tested in the first group was Catlin defined medium (MC) with the addition of ultrafiltrate of yeast extract (YE) at concentrations of 0.2, 1.0 and 2.0 g/L. In the second group, MC was tested with and without amino acids, and the addition of 1.0 and 2.0 g/L YE in each condition. Biomass was measured by reading optical density at a wavelength of 540 nm (OD_{540}) and the yield of OMV was determined by Lowry's method. **Results and Discussion:** In the first group of assays, growth determined in OD_{540} was approximately 1.7, 2.6 and 2.8 at 8 h of cultivation and final OMV yield about 15.8; 36.1 and 33.7 mg/L in MC with 0.2, 1.0 and 2.0 g/L YE, respectively. At the second group growth at OD_{540} was approximately 1.9, 2.5, 2.9, 2.7 at the twelfth hour of cultivation and final OMV yield was 22.8; 30.0, 40.5, 30.6 mg/L in MC without amino acid plus 1.0 g/L of YE, or plus 2.0 g/L of YE, MC with their constituent amino acids plus 1.0 g/L of YE, or plus 2.0 g/L of YE, respectively. Based on the results, the best growth of *N. lactamica* and the best OMV yield was obtained in MC with amino acids plus 1.0 g/L YE when compared to the other media. The current results suggest that there is a relationship between the amount of amino acids in MC and yeast extract ultrafiltrate in the culture medium to achieve a better OMV yield.

Supported by: CNPq/PIBIC

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11.15 Quantitative determination of porcine lung surfactant lipid by DE-TLC

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Introduction: Among the analytical methods for the separation of drug substances, TLC is so far the most used for the separation of biological lipids due to its practical, easy and low cost features. The natural lung surfactant is a mixture of 90% lipids and 10% proteins. Total lipids are 80% phospholipids (PL), and among them, phosphatidylcholine (PC) is the major component. Surfactant deficiency at birth caused by premature delivery is responsible for the development of neonatal respiratory distress syndrome (NRDS). The surfactant replacement therapy is the adopted clinical care. It is essential to guarantee quality control of the drug by adopting the best methodology to assay it. **Objectives:** The aim of this study was to demonstrate that thin layer chromatography (TLC) is still a good and low-cost method to separate lipid classes in surfactant, combined with digital image analysis using a public domain computer program. **Methods:** Surfactant preparations are generally obtained from natural sources by organic solvent extraction. A surfactant sample was then separated and developed. Its PL profile characterization was assessed by conventional TLC, on silica gel 60 using chloroform/methanol/water as the solvent system. PLs were visualized on the plate with ammonium molybdate spray and used for subsequent determination of the phospholipids content. The combination of digital photography with regular TLC (DE-TLC) and the program TLC Analyzer produced multispectral scans, densitograms, and calibration curves used for quantitative determination. **Results and Discussion:** Our results showed good correlation values compared to those obtained by phosphorus levels. The mass error value calculated from surfactant PC spot after the optical density analysis was around 15% (64 μg) from the expected mass value (57-66 μg). The experience with this TLC modality will permit future investments in more professional and sophisticated equipments (densimeters, dosimeters, scanner and documentation system).

Supported by: FAPESP, Fundação Butantan, Sadia, CNPq/PIBIC

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11.16 Purification of plasma proteins using monolithic columns

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Introduction: Deficiency in coagulation factors can cause a number of diseases that are treated with concentrates of the deficient protein. Plasma fractionation provides a wide range of biological products that can be used in therapeutic treatments. Chromatography is a cost-efficient method to obtain such proteins. Monolithic based supports present some advantages over the commonly used bead based columns. Because of its low resistance to flow, it is particularly advantageous to use for the purification of viscous samples such as plasma. Also higher working flow rate is very much considered when biomolecules are handled as the stability can be a critical parameter. Finally, as with the conventional resin processes, purification methods can be scaled up from laboratory to production scale. **Objectives:** To compare the results previously obtained with the anion exchange bead based columns with the monolithics columns for the purification of coagulation factor VIII. **Methods:** Plasma was directly applied to the anion-exchange columns and fractions were collected with the increasing of salt concentration. Analytical methods: Bradford, for protein content; chromogenic, for FVIII activity, SDS-PAGE, for the protein profile of the collected fractions. **Results and Discussion:** The monolithic support used was a 0.34 mL disc. It was possible to work with flow rates of up to 4 mL/min, with very low back pressures, even using such a small device. The maximum flow rate allowed by the manufacturer with the 1 mL pre-packed bead based columns is 1 mL/min. Other important parameters such as loading capacity and protein and activity recoveries still have to be evaluated. Preliminary results indicate that the purification profile is similar to that observed with the bead based column.

Supported by: CNPq/PIBITI and Fundação Butantan

11.17 Establishing the plasma fractionation plant at Instituto Butantan

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Introduction: It is under construction at Instituto Butantan a plasma fractionation plant. Five large groups of installations are planned for the next year: tanks, process lines, water system, automation system and electric system with the corresponding validations and qualifications. Meanwhile, pilot tests are being carried out aiming the production of biologicals and the improvement of biotechnological processes. **Objectives:** Prepare the technical documentation for bidding of automation services, electrification, process lines and for equipments and instruments purchasing. Perform the tests and processes carried out in pilot scale. **Methods:** Analysis and evaluation of the technical specifications related to the installation project of the plant. **Results and Discussion:** As part of the first activities for the installation of the automation system, technical and engineering information about the plant´s system were gathered and the documentation for the bidding of the equipments was prepared. Training in fermentation, microfiltration and chromatography was received during the processes that were carried out at the hemoderivative as well as recombinant protein pilot plants of the Instituto Butantan.

Supported by: CNPq/PIBITI

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11.18 Revision of the species of *Acanthoscurria* Ausserer from the southeastern Brazil (Araneae, Mygalomorphae, Theraphosidae)

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Introduction: The genus *Acanthoscurria* Ausserer, 1871 is represented by nine species in southeastern Brazil, where the majority were described by Mello-Leitão. In 1923, he described six new species: *Acanthoscurria cunhae*, *A. chiracantha, A. violacea, A. paulensis, A. gomesiana* and *A. melanotheria*. In 1924,Vellard described *A. pugnax,* and Piza described two more species, one in 1939, *A. aurita,* and the other in 1972, *A. guaxupe*. **Objectives:** The aim of this study was review the species of the genus *Acanthoscurria* from southeastern Brazil. **Methods:** The material examined is deposited in the following institutions: Instituto Butantan, São Paulo (IBSP), Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), Museu Nacional do Rio de Janeiro, Rio de Janeiro (MNRJ), and Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte (UFMG). All measurements were in millimeters. Female seminal receptacles were dissected and cleared in lactic acid for observation of internal structures. The length of leg segments was measured between joints in dorsal view. Length and width of carapace, eye tubercle, labium and sternum represented maximum values. Total body length excluded pedicel and spinnerets. The drawings were made on a Leica MZ 12, with a camera lucida. **Results and Discussion:** We redescribed and illustrated the appearance of the sexual organs of the male and female of *A. gomesiana* and established the junior synonymies of *A. violacea, A. aurita* and *A. pugnax. A. cunhae* and *A. melanotheria* were considered species inquirenda, because the types are lost and the descriptions are too poor to identify the species. Of the nine described species from southeastern Brazil only two are considered valid: *A. gomesiana* and *A. paulensis*. The male of *Acanthoscurria gomesiana* can be distinguished from *A. paulensis* by the appearance of the keels of the embolus which join in the central portion; the female shows a singular aspect of the receptacles, where the basal membrane involves the two very small lobes totally. The general appearance of this specimen is brown, and the color of the carapace and dorsal side of the abdomen, including the femora is dark brown. Legs and chelicerae are lighter brown. The dorsal side of the abdomen is covered with orange or yellow short hairs. *Acanthoscurria gomesiana* is a very common species in the city of São Paulo and surroundings. These spiders are aggressive and when disturbed bite and easily throw urticating setae, and they can display a defensive behavior by raising the anterior legs, palps and opening the chelicerae, exposing red ventral setae and releasing poison droplets in some cases, besides giving false attacks, mainly among males. The distribution range of *A. gomesiana* is enhanced.

Supported by: CNPq/INCTTOX

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11.19 Secondary metabolites produced by *Colletotrichum gloeosporioides*

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Introduction: Current data show an increase in the number of opportunistic fungal infections in immunocompromised patients, whose mortality rate of around 40%, already exceeds that for bacterial infections. It is also important to note that treatment for fungal infections is limited due to the small number of available drugs. Endophytic fungi represent an important source for new compounds. In this approach, Brazilian biodiversity is very rich and little explored, showing huge opportunities for the discovery of bioactive secondary metabolites. **Objectives:** In this study, an endophytic fungus isolated from coffee trees was employed to search for new antifungal agents. **Methods:** The endophytic fungus coded as BG9-IId3 and isolated from coffee trees was cultivated for 7 days for crude extract production in potato dextrose broth at 28°C, 150 rpm and at least 3,200 mL weekly. Extract was obtained by extraction with hexane, and the secondary metabolites were purified by high performance liquid chromatography (HPLC) in two steps. The crude extract and the isolated fractions were evaluated for antifungal activity by the minimal inhibitory concentration (MIC) assay against *Candida albicans* ATCC 36802/IOC 3704, *Cryptococcus neoformans* IOC 4528 and *Trichophyton rubrum* IOC 4527. Physico-chemical characterization of the substances obtained is being done by nuclear magnetic resonance (^{1}H and ^{13}C), infrared and by high resolution mass spectrometry (HR-MS). The fungus BG9-IId3 was taxonomically identified using the 26S rDNA gene sequencing technique. **Results and Discussion:** The crude extract was purified resulting in three isolated compounds (F2a, F2b and F2c). Compound F2c did not inhibit the growth of any pathogens even at the highest concentration tested (250 µg/mL). Compounds F2a and F2b showed MICs of 125-250 µg/mL for *C. albicans*, *C. neoformans* and *T. rubrum*. The fungus BG9-IId3 was taxonomically identified as *Colletotrichum gloesosporioides*.

Supported by: PIBIC/CNPq, FAPESP and EMBRAPA

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11.20 Expression of antiviral protein of *Lonomia obliqua* by recombinant DNA technology in *Escherichia coli*

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Introduction: Viral infections are of great interest in public health, mainly those caused by influenza. Studies with hemolymph of arthropods have shown the presence of active principles of interest for the development of new pharmacological drugs such as antivirals. Recently, we have demonstrated the existence of a potent antiviral in the hemolymph of the caterpillar *Lonomia obliqua*. This purified protein reduced virus production ($TCID_{50}$ ml^{-1}) by more than 157-fold (from $3.3\pm1.25x10^7$ to $2.1\pm1.5x10^5$) in measles virus, 61-fold in polio virus ($2.8\pm1.08x10^9$ to $4.58\pm1.42x10^7$) and 64-fold in H1N1 influenza virus. **Objectives:** The aim of this study was the cloning and expression of the gene coding for a protein with antiviral activity present in the hemolymph the *L. obliqua* caterpillar in a bacterial system. **Methods:** To synthesize cDNA, RNA of *L. obliqua* was extracted with Trizol reagent and used in polymerase chain reactions using reverse transcriptase polymerase (RT-PCR) with specific primers for the antiviral protein, based on the sequence of the cDNA libraries of *L. obliqua* tegument and spicules, using all possible translation frames of each cDNA of 3 proteins (P1, P2 and P5). Standardization was performed with temperature gradient PCR to find the best condition for amplification of each sequence. Restriction sites were inserted in the cDNA for connection to the plasmid pET28a (Novagen). The first reaction was performed with the restriction enzyme BamHI for all sequences, followed by digestion with the enzymes HindIII (P1 and P2) or EcoRI (P5). The vector pET28a was digested with the same enzymes for each protein. After binding the insert in the vector, the recombinant plasmid was selected in *Escherichia coli* BL21. **Results and Discussion:** The fragments corresponding to the sequences P1, P2 and P5 were amplified by PCR with the sequences for appropriate restriction enzymes. The initial digestions were ineffective in generating the fragments for cloning, which requires a standardization step. Now attempts are being made to clone inserts in expression vector. After cloning and confirmation of the sequences, expression will be induced and activity tests will be performed with the purified protein.

Supported by: FAPESP and CAPES

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11.21 Evaluation of the painful sensation in accidents caused by spiders, scorpions and lepidopteran larvae (caterpillars): cases at Hospital Vital Brazil, Butantan Institute for the Health Secretariat of São Paulo

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Introduction: In Brazil, in 2009, 45,721 accidents caused by scorpions were reported, 18,687 by spiders and 3,387 by Lepidoptera larvae. The key characteristic of these three injuries is the local pain, usually acute and intense. Sometimes there are other flogistic abnormalities. **Objectives:** The objective of this study was to compare the epidemiological, clinical and therapeutic accidents caused by spiders, scorpions and caterpillar seen at the Hospital Vital Brazil (HVB). **Methods:** This was a prospective observational study, where information was collected on the animal that caused the accident, the variables related to patients and also the circumstances of the accident, the clinical picture (mainly the pain), the treatment applied and the final outcome. **Results and Discussion:** The period of data collection was from July 2009 to October 2011. By June 2011, 162 protocols were completed, of which 75 met the inclusion criteria and reviewed in this summary. The epidemiological variables of the patients showed that 39 (52%) were males and 62 (83%) accidents occurred in urban areas. The accidents were caused by 35 spiders (46%), 17 scorpions (23%) and 23 caterpillars (31%). Regarding the assessment of pain intensity, a numerical scale ranging from 0 to 10 was used, resulting in a median of 5 for spiders, 7 for scorpions and 7 for caterpillars. Our study aimed to include all patients admitted to the HVB after being injured by spiders, larvae of Lepidoptera or scorpions, regardless of whether or not the animal was brought. This project will propose some routines to the health professionals of HBV and other health facilities that encounter envenomations: 1 - Establish an assessment of pain as the 5th vital sign; 2 - Therapeutic approach of the painful phenomenon based on the guidelines already established in the literature and evaluation of its effectiveness; 3 - Add items 1 and 2 in the routine care of all accidents caused by venomous animals; 4 - Allow the implementation of future projects of comparative evaluation of different therapeutic approaches, from the standpoint of efficiency and economic concerns.

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11.22 New aspects of peptidase inhibitors in Arachnida

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Introduction: Insects represent the spending of billions of dollars annually due to agricultural crop consumption, transmission of human, animal and plant diseases and with chemical and biological control strategies. The development of new strategies to control herbivorous and vector insects is essential. Owing to the co-evolution process between plants and insects, the use of plant protein inhibitors does not show a high efficiency in crop protection. Nevertheless, the use of animals as an inhibitor source has been hardly explored. Previous results have shown that spiders possess peptidase inhibitors in their digestive tract and hemolymph. **Objectives:** This project aimed to identify Arachnida inhibitors affecting insect digestive enzymes involved in the digestion of proteins and peptides. **Methods:** Midguts were dissected from *Periplaneta americana* (adult), *Musca domestica* (larva), *Spodoptera frugiperda* (larva), *Aedes aegypti* (larva and adult), and *Anopheles aquasalis* (larva and adult) and homogenized. All these homogenates were used as sources of trypsin (benzoyl-Arg-p-nitroanilide; BApNa), carboxypeptidase A (carbobenzoxy-Gly-Phe; Z-GlyPhe) and aminopeptidase (leucine p-nitroanilide; LpNa). *Nephilengys cruentata* (Aranae) digestive juice and digestive tract homogenate samples and *Tityus serrulatus* midgut and midgut gland homogenate will be used as inhibitor sources. Enzymatic assays were carried out at 30°C for different time intervals in appropriate buffers according to each enzyme tested. The release of p-nitroaniline was followed at 410 nm in a Spectramax190. The release of Phe was quantified by the method of L-amino acid oxidase (LAOR). Inhibition was tested by the mixture of enzyme and inhibitor source, with pre-incubation for 30 min at 30°C. Controls were also carried out. Absolute and specific activities and percentage of inhibition were measured. **Results and Discussion:** All insects tested showed activities of carboxypeptidase A (*S. frugiperda*>*M. domestica*>*P. americana*> *Ae. aegypti* larva>*An. aquasalis* adult>*An. aquasalis* larva), aminopeptidase (*An. aquasalis* larva>*Ae. aegypti* adult>*Ae. aegypti* larva> *S. frugiperda*); and trypsin (*S. frugiperda*>*Ae. aegypti* adult>*An. aquasalis* adult>*Ae. aegypti* larva> *An. aquasalis* larva). *N. cruentata* digestive juice and hepatopancreas showed trypsin inhibition as already described. *T. serrulatus* also inhibited insect trypsin (by at least 70%). Inhibition assays of trypsin using spider or scorpion as inhibitor source are easily analyzed due to the absence of trypsins in the digestive process of Arachnida species. However, the same is not true for carboxypeptidase or aminopeptidase. Besides that, pre-incubation at 30°C leads to the loss of carboxypeptidase activity. Standard conditions for metallopeptidase inhibitors are being established.

Supported by: CNPq, FAPESP and CNPq/PIBIC

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11.23 Myeloid-derived suppressor cells after urethane-induced lung tumor in AIRmax and AIRmin mice

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Introduction: Studies suggest that myeloid-derived suppressor cells (MDSC) play a critical role during the progression of cancer. These cells are derived from bone marrow and can be characterized by flow cytometry using a combination of antibodies directed to specific surface molecules, CD11b/GR1/F480. Tumors alter the normal hematopoiesis as well as causing the MDSC accumulation at the tumor site and in lymphoid organs. Besides, MDSC has the ability to differentiate in tumor-associated macrophages within the tumor micro-environment. This micro-environment secretes cytokines, chemokines, and other soluble molecules which can induce MDSC recruitment and increase their maturation into suppressive cells. **Objectives:** Considering that mice genetically selected for the maximal (AIRmax) or minimal (AIRmin) acute inflammatory reaction differ in maturation and myeloid cell production in bone marrow and also exhibit a dichotomy in susceptibility to the development of chemically induced tumor processes, in this work we compared the MDSC population and cytokine gene expression profile in these lines of mice after induction of lung tumor by urethane. **Methods:** Mice were treated by two ip injections of urethane at a 48-h interval for tumorigenesis induction and observed for 120 days after treatment. The tumor lesions were determined by macroscopic observation, the cellular infiltrate in lung was evaluated by specific antibodies, GR1/CD11b/F480, and interleukin gene expression of IL-1β, IL-6, IL-10 and IFN-γ was assessed by quantitative real-time PCR. **Results and Discussion:** At 40 days after treatment, we observed an important infiltrate of $CD11b^{+}/GR1^{+}/F480^{+}$ cells ($1.6x10^{5}$ cells per ml) in AIRmin lung parenchyma, whereas in AIRmax mice the same infiltrate reached $0.6x10^{5}$ cells per ml at the same time. Furthermore, the expression of IL-1β, IL-10, IL-6 and IFNγ cytokine genes were upregulated (8-fold) only in AIRmax mice at 10 days of treatment, returning to baseline levels after 40 days. These differences between AIRmax and AIRmin in cytokine gene expression and cell infiltrate are related to the onset of the lesions in AIRmin lung suggesting, along with others factors, the action of MDSC in the susceptibility of the AIRmin line during tumorigenesis induced by urethane.

Supported by: CNPq/PIBIC

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11.24 Study of the behavior of different atypical enteropathogenic *Escherichia coli* in the interaction with lineage macrophages

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Introduction: Mechanisms of evasion of the immune system have been reported for several microorganisms. Among them, escaping phagocytosis has been described as an important virulence factor in the establishment of disease by many pathogens. Our group has demonstrated that atypical EPEC, considered as an emergent pathogen, secretes a thermostable anti-phagocytic factor, with a molecular weight under 2 kDa, that considerably prevents phagocytosis of bacteria, *Saccharomyces cerevisiae*, and inert latex particles. On the other hand, this effect was described for two atypical EPEC (LB 7 and BA 320), both of the O55:H7 serotype. It is still unknown if this anti-phagocytic mechanism is a virulence factor common to all atypical EPECs or if it is restricted to serotype O55:H7. **Objectives:** The aim of this study was to investigate the behavior of different aEPEC serotypes in the interaction with macrophages J774.A1. **Methods:** Macrophage cultures were infected with the following samples: LB 12 (O119:H2), BA 487 (O55:H7), BA 2103 (O26:H11), LB 5 (O26:H11), LB 14 (O55:H7), LB 13 (O111ab:H9), BA 580 (O119:H2), BA 3157 (O119:H2), and 1649 (O11:H38) of atypical EPEC. aEPEC sample LB 7 (O55:H7) was used as a positive control and typical EPEC samples LB 28 (055:H6) and E2348/69 (O126:H7) were used as negative controls. After 10, 30 and 60 min of infection, the cultures were washed and incubated for 1 h with gentamicin (100 μg/mL) for determination of the number of intracellular bacteria. **Results and Discussion:** Of the nine samples studied, BA 487, LB 14, BA 580 and LB 5, were less phagocytized than the negative control, showing a similar behavior as LB7 (positive control). Interestingly, samples of the O55:H7 serotype (BA 487; LB 14) were among the less phagocytized. On the other hand we cannot say that the anti-phagocytic mechanism is exclusive to this serotype, since samples of O26:H11 and O119:H2 serotypes behaved similarly compare to those of the O55:H7 serotype. We will now study the effect of the culture supernatant from these samples on the phagocytosis of bacteria that do not prevent phagocytosis by themselves. It is worth mentioning that the serotypes used here are considered classic aEPEC serotypes, and therefore, they are common in epidemiologic studies, which confers even more importance to this study.

Supported by: CNPq/PIBIC

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11.25 Standardization of culture conditions for enteropathogenic and enterohemorrhagic *Escherichia coli*: evaluation of EspB expression and its use as a diagnostic tool

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Introduction: Diarrheagenic *Escherichia coli* (DEC) are responsible for several cases of acute diarrhea, especially among children in developing countries, with a substantial public health effect. Among the six pathotypes of DEC, two can be highlighted: enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). Both of them produce EspB, a protein involved in the assembly of a type III secretion system, an essential step for EPEC/EHEC pathogenesis. Therefore, EspB is an important virulence factor and a potent target for detection of these pathogenic strains. Therefore, early diagnosis of EPEC/EHEC infections is essential in order to prevent possible outbreaks and determine the best method of treatment. In order to develop a rapid diagnostic test for DEC using dipsticks, it is important to standardize the conditions that improve EspB expression. **Objectives:** The aim of this study was to evaluate different culture media to determine the most appropriate conditions for expression of EspB. **Methods:** We tested 72 strains of atypical EPEC, 35 typical EPEC and 25 EHEC, using *E. coli* broth, M9 medium and Dulbecco's Modified Eagle medium (DMEM) under different pH conditions and time of growth, in the presence or not of ciprofloxacin and CO_2. The expression of EspB in culture supernatants was analyzed by ELISA using a polyclonal anti-EspB rabbit serum. **Results and Discussion:** Our results showed that the increase in medium acidity (around pH 5.5) leads to a decrease in EspB expression, while the increase in CO_2 was favorable. Among the evaluated conditions, the most favorable for the expression of the protein was the growth in DMEM with 1% tryptone, pH 7.2, and 37 °C, with 5% CO_2 for 24 h. This condition leads to the detection of EspB in 94.4% of the typical EPEC, 65.3% of the atypical EPEC and in 68% of the EHEC. The protein expression was validated by immunoblotting of the culture samples against the polyclonal anti-EspB rabbit serum. Our results are promising, since it was possible to determine the culture conditions that enhanced EspB expression.

Supported by: CNPq/PIBIC and FAPESP

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11.26 Bee venom contains dipeptidyl peptidase IV and inhibits plasma neutral aminopeptidase

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Introduction: One of the alternative treatments recommended for rheumatoid arthritis (RA) is acupuncture using *Apis mellifera* bee venom (BV). Recent studies demonstrated the involvement of three aminopeptidases (APs): basic (APB), neutral (APN) and dipeptidyl peptidase IV (DPPIV) in the development of RA. **Objectives:** To reveal potential mechanisms of anti-inflammatory action of BV, this investigation evaluated the direct effects of BV on these APs in plasma and soluble (SF) and solubilized membrane-bound (MF) fractions from peripheral blood mononuclear cells (PBMCs) of normal healthy rats. **Methods:** Blood was drawn from adult male Wistar rats, 150-160 g, to obtain plasma and PBMCs. PBMCs were fractionated into SF and MF by ultracentrifugation. Samples were then pre-incubated in the absence and presence of BV and, thereafter, incubated with specific naphthylamide derivative substrates. **Results and Discussion:** BV itself showed DPPIV activity at levels of 1.1 to 2.8 pmol substrate hydrolyzed/min/mg. In plasma, BV did not alter APB, but it decreased APN (50%) at a concentration of 5000 µg/mL and increased DPPIV at concentrations of 500 and 5000 µg/mL (250% and 800%, respectively). The viability of PBMCs was inversely related to the concentration of BV. The results for cell viability are 97±0.6% (n=9) saline; zero (n=3) 5µg/mL BV; 73±2% (n=3) 0.5µg/mL BV; 86±1% (n=11) 0.05µg/mL BV. The activities of APs in SF and MF of PBMCs incubated with the sublethal BV concentration (0.05 µg/mL) or saline did not differ. In conclusion, BV displays DPPIV activity and an unexpected inhibitory effect on plasma APN activity.

Supported by: FAPESP and CNPq/PIBIC

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11.27 Endophytic fungi of Caatinga as source of antifungal secondary metabolites

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Introduction: The Caatinga is a biome exclusive to Brazil in the semi-arid region. This ecosystem is almost completely unexplored regarding the biotechnological potential, and in this biome, endophytic microorganisms of several species of plants can be investigated as a source of bioactive molecules. **Objectives:** This study was performed to select endophytic fungi able to produce antifungal secondary metabolites. **Methods:** Seventy-one fungi isolated from the stem and leaves of the plant *Croton blanchetianus* were cultivated in potato dextrose broth at 28°C and 180 rpm for 15 days. The biomass was discarded and the secondary metabolites in the supernatant were extracted by solid phase extraction (SPE) using a C18 (18%) cartridge and methanol as mobile phase. All the 71 crude extracts were evaluated for antifungal activity by the minimum inhibitory concentration (MIC) assay against *Candida albicans* ATCC 36802/IOC 3704, *Cryptococcus neoformans* ATCC 90112/IOC 4528 and clinical strains *Aspergillus fumigatus* IOC 4526 and *Trichophyton rubrum* IOC 4527, at concentrations of 3.9 to 500 µg/mL. The four fungi producing the most effective extracts were taxonomically identified by sequencing 26S rDNA. **Results and Discussion:** Of the 71 crude extracts, 29 showed antifungal activity in the range of 31.25 to 500 µg/mL against the pathogens *C. albicans*, C. *neoformans, T. rubrum* and *A. fumigatus*. Among the promising extracts, the ones produced by the fungi coded as FV3, FV5, FV9 and FV10 were the most effective against *C. neoformans* with MIC of 31.25, 62.5, 62.5 and 125 µg/mL, respectively. 26S rDNA sequencing data are being analyzed for taxonomic identification of the fungi FV3, FV5, FV9 and FV10.

Supported by: CNPq/PIBIC and EMBRAPA

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11.28 Behavioral and reflex assessment in offspring of rats exposed to *Tityus serrulatus* scorpion venom during lactation

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Introduction: Accidents caused by scorpions have shown an alarming increase in various tropical and subtropical countries including Brazil. It was demonstrated that the venom of *Tityus serrulatus* scorpion causes changes in physical and behavioral development in offspring of mothers envenomed during pregnancy. **Objectives:** The present study aimed to evaluate the behavioral and reflexological effects in the postnatal period and in adulthood of offspring of dams that received the venom during lactation. **Methods:** In the experimental group dams (n=10) received venom injection (3.0 mg/kg) on the 10th day of lactation (10E). The control group (n=10) received 1 ml of saline on the same day (10C). Pups were evaluated for their physical and behavioral development. The parameters measured were: palmar grasp, postural reflex, negative geotaxis and general and locomotor activity (n ranging between 22 and 32 animals). In adulthood the parameters measured were: forced swim, activity box, enriched environment, social interaction and discriminative avoidance in elevated plus maze (n ranging between 4 and 8 animals). **Results and Discussion:** Experimental puppies had no significant alterations in palmar grasp, postural reflex and negative geotaxis. General activity increased on the 18th day of life of young females (Females: C 223.0±129.1; E 319.7±200.5). In adulthood, the animals did not show alterations in the activity box. Social interaction in females and males decreased (Females: C 97.3±32, E 64.4±5.1; Males: C 81.8±32.5, E 31.1±12.0). Forced swim showed an increase in the latency to stop swimming for both sexes (Female: C 10.5±9.3, E 85.4±31.7; Males: C 35.2±33.4, E 111.8±65.0). In the plus maze, females and males had an increase in the number of entries in non-aversive arm (Females: 4.1±2.8, E 7 7±1.7; Males: C 5.0±1.8, E 8.2±2.0). The results demonstrate that the injection of *Tityus serrulatus* scorpion venom during lactation is able to cause behavioral alterations in both developing and adult animals.

Supported by: CNPq/PIBIC

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11.29 Evaluation of the role of pneumococcal surface antigens A and C (PspA and PspC) in a pneumococcal respiratory lethal challenge in mice

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is one of the major agents of respiratory acute diseases, accounting for about 1 million deaths per year, worldwide. Among the virulence factors expressed by the bacteria, the pneumococcal surface proteins A and C (PspA and PspC) are involved in the colonization step and in bacterial evasion of the immune system. We have previously established a pneumococcal respiratory model in mice, using a virulent serotype 3 pneumococcal strain (ATCC 6303). This strain is able to invade the bloodstream after nasal inoculation in mice and kills the animal in 5 days. **Objectives:** The aim of the present work was to analyze the role of PspA and PspC in pneumococcal virulence, after a respiratory challenge in mice. **Methods:** Mutant pneumococcal strains derived from ATCC 6303, which do not express PspA, PspC or both, were constructed. The DNA sequence of the flanking regions of each gene was determined and fragments of DNA, comprising 1000 bp upstream of the genes, the entire coding sequence of the genes and 1000 bp downstream of the genes, were amplified by PCR and cloned into the pGEM-T easy vector. The *pspA* or the *pspC* genes were further removed by reverse PCR and substituted for the fragments encoding the erythromycin or the spectinomycin resistance expression cassettes. Virulence of the mutant strains was determined by intranasal inoculation of naïve mice and evaluation of survival. **Results and Discussion:** The final plasmid was used to transform competent ATCC 6303 and the mutants were obtained by homologous recombination between the flanking regions of the genes, present in the plasmids, and the flanking regions in the chromosomal DNA. Deletion of *pspA*, *pspC* or both genes in the recombinant clones was confirmed by PCR using specific primers, as well as by Western blots using anti-PspA and anti-PspC antibodies. The mutant bacteria as well as the parental strain ATCC 6303 were inoculated through the intranasal route in mice and survival was followed for 15 days. The PspC mutant strain was able to kill 100% of mice, as in the wild type strain. On the other hand, PspA and the double mutant were able to kill only 50% of mice. Our results show an attenuation of pneumococcal virulence in the absence of PspA, during the lethal respiratory challenge in mice. Conversely, deletion of PspC did not affect pneumococcal virulence in this model. Further studies to characterize the inflammatory responses and invasion of these bacteria into the bloodstream in mice are in progress.

Supported by: CNPq/PIBIC, FAPESP and Fundação Butantan

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11.30 Life cycle of *Ornithodoros mimon* (Acari: Argasidae): 4th generation under laboratory conditions

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Introduction: The life cycle of argasid ticks includes: embryonated egg, larva, two or more nymphal instars and adults. During feeding, adults can eat several times, usually before copulation and oviposition. Ecdysis in some specimens in the genus *Ornithodoros* can occur without blood-feeding. **Objectives:** The aim of this study was to obtain data from the biological cycle of the 4th generation of *O. mimon*, under laboratory conditions, to compare with previous generations, and thus to analyze the average times of pre-feeding, feeding, molting in the different biological stages of the 4th generation, as well as to monitor the period of oviposition and hatching of larvae of the 5th generation. **Methods:** Fourth generation larvae and nymphs of each of the three instars, originating from earlier generations, were fed on New Zealand rabbits from the Central Animal Facility, IBU. Feeding of larvae and each nymphal instar of *O. mimon*, was carried out in containment chambers fixed on the back of rabbits previously prepared (shaved at the site of attachment of the containment chambers). A plastic collar was placed around the neck of each animal, limiting the movement and removal of the chambers. During feeding, the pre-feeding and feeding times of the ticks were observed. Groups of fed larvae and nymphs were kept separately as stage and maintained in an incubator with controlled temperature and humidity (27 ° C ± 1 ° C and 90%, respectively). Adults, after molting, were separated in couple to feed, mate and obtain eggs. **Results and Discussion:** Larvae, nymphs (each instar) and adults were given 30 days apart pre-feeding time between feeding and others. The average time of larval feeding was similar in the four generations, but the pre-molt average of larvae to N1 decreased to 3 to 7 days, whereas in previous generations it was 5 to 8 days. The N1 and N2 of the 4th generation had the same time of pre-fixation in the host (5 min) but N3 took a longer time (> 10 min) to settle. The average time of feeding of this instar 28.39±11.90 min (11 to 50) was also higher than those observed in previous generations. However, all generations had similar nymphal molting time, ranging from 1 to 2 days. The N2 of the 4th generation changed by nine days and this ecdysis resulted in 14 females, 50 males and 92 nymphs (N3). The N3 of the 4th generation advanced to adult in 9 days, but interestingly there was a greater number of males (60) than females (32). Until the 3rd generation, the ecdysis of N3 resulted in a higher number of females. However, this difference did not affect the feeding time and these females gave rise to 300 larvae of the 5^{th} generation.

Supported by: FAPESP and CNPq/PIBIC

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

11.31 Antiproliferative effect of the peptide INKKI in orthotopic model of murine breast cancer

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Introduction: Cytochemical studies have provided evidence that protein-derived peptide hydrolysis can affect the viability and growth of cancer cells. Peptides have a high potential in the field of cancer because of their high target selectivity and vast applications. INKKI peptide is derived from bovine beta casein amino acid fragment 26-30, and is a peptide that could have a role as an anticancer compound, but the mechanisms are poorly understood. **Objectives:** The aim of this study was to determine the antitumor effects of the INKKI peptide therapy on growth and metastatic spread of murine orthotopic breast cancer. **Methods:** Human breast cells (MCF-7, MDA-231, T47D) and murine tumor (Ehrlich's) were grown in RPMI-1640 medium, supplemented with 10% inactivated fetal bovine serum, 2 mM L-glutamine, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. The cells were allowed to grow for 24 h, and treated with concentrations of INKKI and cell viability was determined by the MTT colorimetric assay. In vivo, the orthotopic model of murine breast adenocarcinoma (Ehrlich's tumor) cells was previously described (Whitehurst B, Int J Cancer. 2007). Briefly, $5x10^4$ cells were implanted into the mammary fat pad of female BALB-c mice. Every 2 to 3 days, perpendicular tumor diameters were measured by digital caliper and used to calculate tumor volume. The animals were divided into three treatment groups: INKKI, Taxol and control, and were followed for 40 days. After treatment, the DNA content in the cell cycle phases and mitochondrial electric potential was analyzed by flow cytometry. Peripheral blood was obtained by cardiac puncture using a heparinized/EDTA capillary for hematological analysis (erythrocytes, leukocytes and platelets). **Results and Discussion:** The IC50% obtained in human (MCF-7, MDA-231, T47D) and murine (Ehrlich's) cells lines were respectively 1.75, 1.53, 1.26 and 1.78 ug/mL compared to Taxol (3.75 mM). Treatment with the peptide reduced tumor volume by 42% compared to the group of animals treated with Taxol and control groups. The analysis of the cell cycle phases showed an inhibitory effect on proliferation capacity and an increase in the proportion of cells with fragmented DNA and increased mitochondrial activity. The hematologic evaluation showed that the animals treated with peptides did not develop anemia, a decrease in platelets and increase in total leukocytes. The breast cancer model mimicked the biological behavior of human cancer in terms of local growth as well as invasion or metastasis. INKKI peptide is cytotoxic in vitro and in vivo and induces significantly increased DNA fragmentation and decreased proliferative capacity.

Supported by: CNPq/PIBIC

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11.32 Glycine as a neurotransmitter in rat striatal tissue: signaling and transmitter interactions

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Introduction: Parkinson disease is a neurodegenerative disorder affecting substatia nigra dopaminergic cells. Striatal tissue is largely involved in motor control under dopaminergic afferences. Although widely studied, many striatal circuits are still poorly understood. Previous investigations have shown that glycine interferes in striatal acetylcholine release, suggesting the importance of glycine as a neurotransmitter in this brain area. **Objectives:** We aimed at investigating whether glycine acts as a neurotransmitter or a co-transmitter, together with GABA, in striatal tissue. This study approached the initial characterization of glycine release. **Methods:** Male Wistar rats were used. After decapitation, brains were removed and striatal tissue was dissected and kept in ice-cold Krebs Ringer-bicarbonate (KRB) previously gassed with carbogen. Tissue was cut in prisms using a McIlwain tissue chopper, suspended and pre-incubated at 37°C for 5 min. ^{3}H-glycine was added and incubated for 20 min. Tissue was filtered, washed twice with KRB and distributed into 18 parallel superfusion chambers (Brandel SF2500 – USA). Drugs were included in the superfusion medium according to the experimental protocol. Results were expressed as fractional release. **Results and Discussion:** Glycine was released at a steady baseline rate representing about 4.5 – 5% of the total loaded amount. Depolarization effected by 35 mM KCl induced a further 6% release that was partially calcium-dependent. Calcium channel blockers (L-type) calciceptine, (N-type) w-ConoMVIIc had no effect on stimulated release. Drugs that were ineffective in changing the stimulated release included nicotine (cholinergic), muscimol (GABAa agonist), NMDA (glutamate agonist), MK801 (NMDA antag.), glutamate itself, Go6976 (PKC inhib.), kainate (Glu agonist), and tetrodotoxin. The dopaminergic D1 agonists apomorfine and SKF39393 increased release while D2 agonist bromoergocriptine was ineffective; D2 antagonist spiperone decreased release as did W-7, a calmodulin antagonist. Dopamine seems to influence glycine release through D2-type receptors and calmodulin seems to modulate it. Glutamate, acetylcholine and GABA do not significantly modify glycine release. The characterization of glycine release including second messenger signaling and neurotransmitter interactions should help in proposing new therapeutic strategies to treat Parkinson disease.

Supported by: FAPESP and CNPq/PIBIC

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11.33 Effect of *Crotalus durissus terrificus* snake venom and crotoxin on the production of superoxide anion and hypochlorous acid by neutrophils stimulated with PMA

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Introduction: Previous studies showed that *Crotalus durissus terrificus* snake venom (CdtV) and crotoxin (CTX), its main component, modulate macrophage function inhibiting cell spreading and phagocytic activity, but increasing the oxidative burst of these cells. Recently, CdtV and CTX were shown to inhibit carrageenan-induced inflammatory response and phagocytosis by neutrophils. Despite these findings, CdtV and CTX do not alter hydrogen peroxide production by neutrophils, but other reactive oxygen species have not yet been investigated. **Objectives:** The purpose of this study was to investigate the *in vitro* and *in vivo* effects of CdtV and CTX on the production of two important reactive oxygen species by neutrophils: superoxide anion and hypochlorous acid. **Methods:** For in vitro studies, neutrophils (4.10^5/mL) were obtained 4 h after the intraperitoneal administration of carrageenan (4.5 mg/kg) in male Wistar rats (CEUAIB 705/10) and incubated with different concentrations of CdtV (0.25, 0.5 or 1.0 μg/mL) or CTX (0.02, 0.04 or 0.08 μg/mL) for 1 h. For in vivo studies, the animals were pretreated 2 h before the injection of carrageenan (4.5 mg/kg) with CdtV (0.18 mg/kg) or CTX (0.1 mg/kg). The production of superoxide anion was measured by the reduction of cytochrome c, and the production of hypochlorous acid was evaluated by the oxidation of tetramethylbenzidine (TMB), resulting in the formation of a blue product. For all assays, neutrophils were stimulated with PMA (phorbol 12-myristate 13-acetate, 25 ng) for 1 h. **Results and Discussion:** The incubation of neutrophils with different concentrations of CdtV or CTX did not alter the production of superoxide anion or hypochlorous acid by these cells, after stimulation with PMA. Similarly, the pretreatment of animals with CdtV or CTX, 2 h before the injection of carrageenan did not alter the production of the same reactive oxygen species by neutrophils, after stimulation with PMA. These results demonstrate a dual effect of CdtV and CTX on neutrophils, since they inhibit phagocytosis, but do not modify NADPH oxidase activation and the production of reactive oxygen species in response to PMA. Although the association between the process of phagocytosis and microbicidal activity, differences in the signaling pathways involved in these processes could explain the inhibitory effect of CTX particularly on phagocytosis. Furthermore, considering the difference between our results and those reported in the literature for macrophages, these data may indicate differences in the mechanisms of microbicidal activity between these cells.

Supported by: CNPq/ PIBIC, CAPES, FAPESP and INCTTOX

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11.34 Amblyomin-X heterologous expression and toxicity studies using GLP

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Introduction: Amblyomin-X is a Kunitz-type inhibitor identified and produced from a cDNA library, which was constructed from the salivary glands of the tick *Amblyomma cajennense*. It is a protein that inhibits activated factor X (FXa) of the blood clotting and it has a cytotoxic effect against tumor cells, decreasing the mass of some solid tumors such as melanoma, *in vivo*. Initially, the expression of the molecule (containing a histidine tail) was performed in bacteria *Escherichia coli*. Considering its potential as a new therapeutic tool, new proof-of-concept has to be performed *in vitro* and *in vivo*. In order to meet the requirements of regulatory agencies before the use of the compound, a new cloning and expression of Amblyomin-X is being performed using the yeast *Pichia pastoris*. **Objectives:** The aim of present study was to optimize the expression and purification of this recombinant protein (without the histidine tail) in search for better parameters, such as temperature and concentration of methanol in the culture medium, controlling its purity and activity performing standardized tests, and its use in the development of acute toxicity testing. **Methods:** The expression occurred in a shaker at 350 rpm for 24 h. Three different temperatures were used to perform the expression of the protein: 26 °C, 28 °C and 30 °C. The concentration of methanol in the culture medium was 0.25%, 0.5% and 1.0%. After the period of expression of the protein, the medium with the products of the expression was separated from the cells by centrifugation. The pellet was discarded, and the resulting supernatant was filtered and concentrated. The purification steps were performed in a Source-Q ion exchange resin and affinity chromatography in a heparin-Sepharose column (FPLC system). **Results and Discussion:** Preliminary results show that the most suitable conditions for the growth of *Pichia pastoris* cells are a temperature of 28 °C and 0.5% methanol concentration. Performing the FXa inhibition test, it was found that there is an increase in specific activity after the purification steps. After the first purification step (Source-Q), 0.5 μg protein caused an inhibition of ~14%, and after the second step (heparin-Sepharose), the inhibition was ~23%. Tests in tumor cells showed that the protein obtained (without histidine tail) retained its cytotoxic activity.

Supported by: CNPq/PIBIC and FAPESP

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11.35 Characterization of gometoxin-1 of the *Acanthoscurria gomesiana* venom

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Introduction: Currently, there are few studies dedicated to research on new molecules with antimicrobial activity obtained from spider venom. Antimicrobial peptides with broad-spectrum activity are produced by a wide variety of plants and animals. Since the number of microorganisms that are resistant to antibiotics has been increasing steadily, the need for combating these pathogens requires new pharmaceutical agents. In our studies, the venom of *A. gomesiana* fractionated by HPLC and tested against microorganisms has shown several fractions with activity. **Objectives:** The objective of this study was to characterize the molecule we designated as gometoxin-1 obtained from the venom of the spider *A. gomesiana* and to confirm its antimicrobial action. **Methods:** The purification of the crude venom of the spider *A. gomesiana* was performed by reverse-phase liquid chromatography using a semi-preparative Jupiter C18 column. 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)*. Fractions with more than one mass were repurified by reverse-phase liquid chromatography, using a Jupiter C18 analytical column. **Results and Discussion:** A new molecule was isolated and named gometoxin-1 with a molecular mass of 5462.4 Da. It showed antimicrobial activity only against *Candida albicans*. This molecule will be subjected to Edman degradation for amino acid sequencing and complete characterization.

Supported by: CNPq/PIBIC

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11.36 Interspecies variability of snake venom proteomes: exploring the glycosylation profile of Bothrops genus

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Introduction: Interspecies variability is a well known feature of snake venom proteomes. In this context, *N*-glycosylation is a major post-translational modification (PTM) and contributes to the diversification of the proteome as a whole. Although PTMs are widely studied because of their importance in many biological phenomena, the diversification of venom proteins by PTMs is less understood. **Objectives:** The aim of this study is to explore the sub-proteome of venom glycoproteins of seven *Bothrops* species. **Methods:** Crude venoms of *B. cotiara*, *B. jararacussu*, *B. moojeni*, *B. neuwiedi*, *B. jararaca*, *B. insularis*, and *B. erythromelas* were submitted to *N*-deglycosylation and *O*-deglycosylation for release of glycans, and their proteomic profiles were analyzed by SDS-PAGE. Affinity chromatography to Concanavalin A (Con A) and lectin from *Triticum vulgaris* (LTV) was used in order to isolate glycoproteins containing mannose and N-acetylneuraminic acid/N-acetyl-D-glucosamine, respectively. Proteins with affinity for lectins were digested with trypsin in solution and submitted to liquid chromatography coupled to mass spectrometry in *tandem* (LC-MS/MS) for identification. **Results and Discussion:** After removal of *N*-linked carbohydrate chains, the venom proteins showed a significant shift in the molecular mass indicating a high level of *N*-glycosylation in all Bothrops venoms. On the other hand, *O*-glycosylation does not seem to occur in most venom proteins of the seven *Bothrops* species analyzed, as no significant change in the pattern of protein migration was observed after incubation with *O*-glycosidase. Interestingly, the venom electrophoretic profiles of proteins with affinity for Con A and LTV are rather different indicating the presence of variable carbohydrate chains and variable levels of glycosylation in *Bothrops* venom toxins. On the basis of the identification of lectin-binding proteins by in-solution trypsin digestion and LC-MS/MS analysis, some generalizations can be made as to what classes of proteins undergo glycosylation in the venom gland of *Bothrops* venoms. Using as a criterion for protein identification the number of unique peptides and restricting identification to those proteins for which at least two peptides matched a protein in the database, we identified various metalloproteinases, serine proteinases, L-amino acid oxidase and C-type lectins among the proteins with affinity for lectin-Sepharose resins. Taken together, our results indicate that the varying glycosylation levels occurring in proteins within different toxin families considerably contributes to the complexity of *Bothrops* venom proteomes.

Supported by: CNPq/PIBIC and FAPESP

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11.37 Expression of laminin-5 and integrins in actinic cheilitis and squamous cell carcinomas of the lip

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Introduction: The progression of carcinogenesis entails the detachment of cells, invasion, and migration of neoplastic cells. Alterations in epithelial adhesion and basement membrane proteins may mediate the early stages of carcinogenesis. **Objectives:** The aim of this study was to determine the expression of adhesion molecules and the basement membrane protein laminin-5 in actinic cheilitis (AC) and squamous cell carcinoma of the lower lip to understand early photocarcinogenesis. **Methods:** Ln-5γ2 chain and β1, β4, and α3 integrin expression was measured by immunohistochemistry in 16 cases of AC, 16 cases of superficially invasive squamous cell carcinoma (SISCC). Ln-5γ2 chain was evaluated in 18 cases of invasive squamous cell carcinoma (ISCC) and β1, β4, and α3 integrins in 11 cases. **Results and Discussion:** Most AC cases showed reduced expression of β1, β4, and α3 integrins, and most SISCCs and ISCCs cases lacked β1, β4, and α3 integrins in the invasive front. AC cases were negative for the Ln-5γ2 chain. Five cases of SISCC (31%) and 13 cases of ISCC (72%) showed heterogeneous Ln-5γ2 chain expression in the invasive front of the tumor. Integrin α3, β1, and β4 expression is lost during the early stages of lip carcinogenesis. Expression of Ln-5γ2 in the invasive front in cases and its correlation with tumor progression suggest that it mediates the acquisition of the migrating and invading epithelial cell phenotype.

Supported by: FAPESP and CNPq/PIBIC

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11.38 Action of jararhagin on endothelial cells cultured on different extracellular matrix proteins

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Introduction: Endothelial cells are important targets of snake venom metalloproteinases (SVMPs). These toxins interfere with cell-matrix adhesion leading to apoptosis and induce capillary disruption frequently related to SVMPs-induced hemorrhage. Recently, our group showed that the strong hemorrhage induced by jararhagin, a P-III SVMP from *B. jararaca*, is related to its binding to and hydrolysis of collagen *in situ*. However, some results obtained *in vitro* are not in agreement with the biological effects of these toxins in animal models. *In vitro* studies have mostly been carried out on tissue culture plastic, a static and rigid surface that does not accurately model the *in vivo* microenvironment, indicating that different cell culture models must be employed to understand the action of SVMPs on endothelial cells. **Objectives:** Study the action of jararhagin on endothelial cells cultured on different extracellular matrix proteins. **Methods:** Human umbilical vein endothelial cells (HUVECs) were plated on fibronectin, collagen I and IV, matrigel, gelatin and non-coated tissue culture plates. Cell proliferation and viability (MTT assay) and morphology of the cytoskeleton (immunofluorescence) were compared before and after jararhagin treatment. **Results and Discussion:** The proliferation rate and spreading of HUVECs cultured on fibronectin, collagen and matrigel were than 2-fold greater compared to cells grown on gelatin or non-coated plates. The proliferation of cells cultured on matrigel-coated plates was drastically affected by 5 nM jararhagin. On collagen I and IV, fibronectin, gelatin and non-coated plates, similar results were only observed when 200 nM of toxin was used. The reduction of cell viability by jararhagin was also dependent on ECM substrate. On collagen I and IV and matrigel, 800 nM jararhagin completely abolished cell viability after 24 h, whereas on fibronectin, gelatin and non-coated plates, same concentrations of jararhagin induced only a moderate decrease in cell viability. Jararhagin was also more effective on disrupting the F-actin cytoskeleton and focal adhesions on cells plated on collagen matrices. These effects were all dependent on catalytic activity of jararhagin. Our results clearly showed that cellular response to stress induced by jararhagin was affected by biochemical matrix composition. This effect could be explained by the high affinity of this toxin to collagen that would enhance the detachment of endothelial cells. Moreover, the disruptions of cell-matrix adhesions by jararhagin could be dependent on the stability of focal adhesions conferred by different ECM components.

Supported by: CNPq/PIBIC and FAPESP

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11.39 Preliminary inventory post fire in the herpetological collection Alphonse Richard Hoge, Instituto Butantan, Brazil

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Introduction: The herpetological collection Alphonse Richard Hoge, Instituto Butantan, Brazil, has been a reference for Neotropical snake fauna studies. Records point out 85,000 specimens before the fire of May 2010, which also destroyed accessory inner collections such as skull, hemipenis and tissue samples stored since 1908. **Objectives:** The of this work was to take an inventory of the remaining specimens in order to record possible geographic distribution of species, direct specimen exchange procedures with other institutions, and select priority localities to do field work. **Methods:** We screened about 25% of the total material removed from ashes, about 1500 snakes in acceptable conditions of handling and proper identification. **Results and Discussion:** Accounts indicate there are about 5000 specimens remaining. The genus *Caudisona* (n=608) was the predominant snake rescued, followed by *Bothrops* (n=480), *Rinocerophis* (n=106), *Bothropoides* (n=62) and *Sibynomophus* (n=43). Specimens from the *Bothrops atrox* (n=335) complex were also retrieved. We intend to carry out scale counting and other morphological analyses to re-identify uncertain specimen records and to map their distribution in order to determine and confirm new possible occurrences.

Supported by: CNPq/ PIBIC

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11.40 Allergy to *Loxosceles* spider venom as occupational disease in arachnologists

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Introduction: It is sometimes suggested that spider bites can cause hypersensitivity reactions. Despite this common notion, reports on allergic reactions from spiders are rare. However, contact with spiders has been reported to cause urticarial reactions, almost exclusively from large spiders from the family *Theraphosidae*. To our knowledge, there are no reports of allergic-type reactions occurring following contact with other groups of spiders. Moreover, several workers in the Laboratory of Arthropods of the Butantan Institute have complained of allergic symptoms related to contact with spiders (or its venom) from the genus *Loxosceles*. **Objectives:** The aim of this work was to investigate the prevalence and predictors of venom allergy among workers exposed to spiders from the genus *Loxosceles* and to confirm the involvement of IgE-mediated mechanisms in this condition. **Methods:** A cross-sectional study of 16 adult laboratory workers, scientists, technicians, and trainees, all employees of the Laboratory of Arthropods was carried out. All of the subjects in the study sample had been exposed to spider venom or to the spiders themselves, most of which were of the genus *Loxosceles*. All 16 subjects completed a physician-administered questionnaire containing questions regarding their personal history of allergy, spider bites, and contact with spider venom, as well as their work history and work-related symptoms. The quantification of specific IgE and IgG antibodies to *Loxosceles* spider venom were determined by ELISA. **Results and Discussion:** Of the 16 subjects evaluated, 11 (68.8%) were female. The mean age was 41.0 ± 14.8 years. The median length of employment was 7.2 years (interquartile range 1.3-22.5). Of the 16 subjects interviewed, 13 (81.3%) had a history of atopy. Moreover, twelve subjects (75.0%) showed allergic symptoms when exposed to spiders of the genus *Loxosceles* (or its venom). These allergic symptoms were associated with the maintenance of spiders in captivity (p=0.018), but not with other specific tasks, primarily the handling of dried venom (p=0.399) and handling of liquid venom (p=0.399), personal history of atopy (p=0.136) and length of employment (p=0.558). None of the individuals had specific IgE antibodies to *Loxosceles* spider venom, but eleven (68.8%) had specific IgG antibodies. Our observations suggest that exposure to spider of genus *Loxosceles* can result in allergic sensitization in arachnologists. The prevalence rate of this condition appears to be high among these workers, and the maintenance of spiders in captivity is an important predictor of its occurrence.

Supported by: CNPq/ PIBIC

12. PAP program

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12.01 Activated thrombocytes trigger the intrinsic pathway of coagulation in chickens in the absence of factor XII

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Introduction: Recent findings suggest that the inorganic polymers polyphosphates (polyP) released from dense granules of activated platelets seem to be the endogenous activator of factor XII (FXII), leading to fibrin or thrombus formation. For over forty years, investigators in hemostasis have not considered FXII important because its deficiency is not associated with bleeding. However, data from genetically altered animal models strongly suggest that FXII plays a decisive role in pathological thrombus formation in mammalians. The mechanism by which this may occur has not been precisely described, but is believed to be related to polyP. Ablation of FXII and targeting polyP with phosphatases interfered with the procoagulant activity of activated platelets, blocked platelet-induced thrombosis and protected mice from polyP-triggered lethal pulmonary embolism. **Objectives:** FXII gene and activity are absent in the avian genome and plasma, respectively. *In vitro* stimulation with the Ca^{2+} ionophore A23187 leads to aggregation and release reaction of washed chicken thrombocytes. This animal model can be considered useful for studying the relative importance of polyP/FXII in the dynamics of fibrin generation. **Methods:** Blood samples were drawn from the brachial wing vein of chickens into syringes containing 1 mL of citrate solution (3.2%, 1/10). A modified recalcification test was performed on all whole blood (WB) or thrombocyte-rich plasma (TRP) samples by thrombelastometry (TEM), using the TEM Coagulation Analyzer Model ROTEM®, which measures the elastic shear modulus of clotting blood. The initiation of the clotting process can be studied by analyzing the clotting time (CT) parameter of the TEM profile. By preincubating WB or TRP samples with inhibitors, we determined if chicken blood clotting process involves polyP, FXII and FXI-like substances. **Results and Discussion:** Stimulation of thrombocytes with the Ca^{2+} ionophore A23187 (3×10^{-7}M) before recalcification reduced CT by 98 ± 15% (mean ± SD, $p<0.0001$, $n = 8$). The stimulation of clotting by A23187 was not prevented by corn trypsin inhibitor (100 µg/ml, $p>0.5$, $n = 7$), but it was significantly reduced by alkaline phosphatase (PSP, $p<0.0001$, $n = 8$) and by aprotinin ($p<0.0001$, $n = 8$). PSP did not alter CT in unstimulated TRP samples, and the clotting induced by Russell's viper venom was not affected by these inhibitors. Our results suggest that the intrinsic pathway of coagulation in chickens is dependent on polyP- and FXI-like substances, but not on FXII. We propose that the study of avian clotting mechanisms may be helpful in understanding the role of polyF and FXII in hemostasis and thrombosis.

Supported by: FAPESP and PAP/SES

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12.02 Liposomal phosphoethanolamine effects on gene expression of human melanoma cells

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Introduction: Melanoma (MM) is a very aggressive type of skin cancer that is hardly cured. Research on the synthetic drug liposomal phosphoethanolamine (PHS) is being developed in search of new alternatives for MM treatment. *In vitro* and *in vivo* tests have demonstrated efficient anti-carcinogenic effect of PHS in murine B16F10 MM cells. **Objectives:** This project aimed to evaluate the cytotoxicity of PHS in human MM cells and control fibroblasts (FN1), as well as its effects on gene expression concerning signaling pathways (*ILK, AKT, FAK* and *β-CAT* genes), tumor suppressors (*TP53, CDKN2A, CDKN1A* genes), and genes related to apoptosis (*CASP-3* and *BCL-2*). **Methods:** The MTT colorimetric assay was performed to determine the toxicity of the drug. The cultures of FN1 and SK-Mel-28 cells were incubated for 24 h with PHS at the concentrations 0.05 μg/μl, 0.11 μg/μl and 0.22 μg/μl. The RNA extracted from the cultures was converted into cDNA, and gene expression was assayed by conventional PCR using primers corresponding to the signaling pathways genes. **Results and Discussion:** The IC50 concerning cell viability was obtained with the PHS concentration 1.76 μg/μl for both FN1 and SK-Mel-28 cells. Genes *ILK, β-CAT, FAK* and *AKT* were active in both cell types. *ILK, AKT* and *β-CAT* were more expressed in FN1 control cells than in SK-Mel-28, which in turn had a higher *FAK* expression. The PHS treatments at the concentrations/time used did not affect the expression of the above mentioned genes. These results would suggest that PHS does not influence the signaling pathways related to these genes to induce cell death, which was demonstrated to occur by the MTT procedure. Alternatively, the exposure time and concentrations of the PHS should be adequately changed in order to be effective in the modulation of these genes. New conditions in the experimental assays, along with the inclusion of apoptotic and tumor suppressor genes for expression analysis, will be carried out to further validate the PHS effect on normal/tumor cells.

Supported by: PAP/SES and FAPESP

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12.03 Isolation and identification of natural peptides in human milk

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Introduction: Human milk is thought to be the best form of nutrition for neonates and infants. The properties of human milk facilitate the transition of life from *in utero* to *ex utero*. Milk is the first and principal food offered to neonates, showieng three different phases, which differ by the constituents: colostrum (day 0 to day 7 after birth), intermediate milk (day 7 to day 10 after birth) and mature milk (beyond 10 days after birth). There is a great number of studies that demonstrate the importance of knowledge of the possibility of generation of peptides with biological activities in milk. **Objectives:** The aims of this study were to compare the chromatograms of two different phases of milk (colostrum and mature), and to isolate the natural peptides and to identify their sequences and their precursor proteins. **Methods:** The samples of human milk were obtained from the Milk Bank of Hospital of the Servidor Público Estadual de São Paulo. The samples were treated according to the Ministério da Saúde Manual, indentified by phase and refrigerated (-20° C). The samples were lyophilized, weighed and precipitated with acetic acid (10%). After this step, the samples were first purified using a Sep-Pak (C_{18}) cartridge, and after washing the cartridge, the material eluted with acetonitrile was lyophilized, re-suspended in water (Milli-Q), filtered (0.45 μm) and analyzed by HPLC (C_{18} RP column). The peaks obtained by HPLC were collected and sequenced by Edman degradation. The obtained sequences were identified using BLAST. **Results and Discussion:** The analysis of the chromatograms showed an increase in peak intensity of the colostrum samples and similar retention times for some of them when compared to mature milk, suggesting that they may represent the same peptides in both milk phases but in different relative amounts. Seven peaks from mature milk were then selected, purified and sequenced by Edman degradation. After analysis using BLAST, 6 sequences were found to contain the ß-casein chain and only one in the α-lactoalbumin chain. This study showed the difference between human milk and bovine milk and also showed differences between the milk phases and also confirmed that mature milk contained more ß-casein than serum proteins (α-lactoalbumin). Certain bioactive substances and living cells in milk appear to influence neonatal gut maturation and growth through their transfer of developmental information to the newborn. Although most of these bio-substances have been identified in mother's milk in quantities that exceed maternal serum levels, their exact role in human newborns is uncertain, and most current information is from animal models whose development may be significantly different.

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

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12.04 Skin morphology and sexual dimorphism in *Dermatonotus muelleri* (Amphibia, Anura, Microhylidae)

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Introduction: Among amphibians, the integument is one of the main organs involved in the conquest of the terrestrial environment. Amphibian integument is characterized by the presence of mucous and granular glands related to many vital functions, such as the protection against predators and microorganisms and gas exchange for respiration. Microhylids are generally small fossorial anurans with ovoid body and small head and mouth. The skin is very mucous and the forelimbs are very short. Besides the two regular types of glands present in all amphibians, in the microhylids there is another glandular type which has been related to the secretion of adhesive substances probably used for the males to grip the female during the amplexus. **Objectives:** The aim of this study was to describe and compare the skin glandular types and their respective morphology in males and females of the species *Dermatonotus muelleri*, focusing on the mapping of the adhesive glands. **Methods:** Fragments (N = 38) of male and female specimens of *Dermatonotus muelleri* were removed, from different points of the dorsum, ventral region and limbs. The samples were embedded both in paraffin and glycol methacrylate. Sections of 5 μm (paraffin) and 4 μm (glycol methacrylate) were stained with HE, toluidine blue-fuchsin, PAS, bromophenol blue, von Kossa and alcian blue. **Results and Discussion:** Throughout the body, males and females showed a skin rich in granular (poison) glands of large dimensions, mainly in the dorsal region. These glands are positive to bromophenol blue indicating that the poisonous secretion is mainly constituted by protein. Two types of mucous glands are present, one positive to PAS and the other to alcian blue, indicating the presence of neutral and acidic mucosubstances, respectively. Besides granular and mucous glands, the skin of the males revealed the presence of adhesive glands distributed in six out of the 38 examined fragments, corresponding to the anterior and central regions and the ventral forelimbs. In the females they are totally absent. The location and distribution of such glands correspond to the areas of close contact between male and female during amplexus. The histochemical results, highly positive to bromophenol blue and PAS, indicate that the substance secreted by these glands is mainly composed of protein and neutral polysaccharides. The von Kossa technique showed a continuous thin calcified layer in all regions of the body of both males and females, a fact that can be related to the fossorial habits.

Supported by: CNPq, INCTTOX and PAP/SES

12.05 5th Introductory course to the "PROGRAMA DE APRIMORAMENTO PROFISSIONAL (PAP) DA SECRETARIA DE ESTADO DA SAÚDE NO INSTITUTO BUTANTAN"

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Introduction: The "Programa de Aprimoramento Profissional (PAP)" was created in 1979, aiming to complement the training of people involved in the health area. In the Instituto Butantan (IBu), this program lasts 1 or 2 years and consists of 40 h/week of activities. Since 2007, before the beginning of their laboratory activities, the students have to attend a course organized by a commission composed of researchers from different laboratories. In this course, pertinent themes are administered by scientific researchers, specialists and also 2nd year PAP-students. The course focuses on the integration of the grant-holders in the different areas of the Institute (Production, Development, Research and Museums), and at the end, students have to answer an anonymous questionnaire which gives them the opportunity to express their point of view about this activity. **Objectives:** The aim of this work was to describe and evaluate the planning, organization, and application of the fourth course offered in 2011. **Methods:** The activities lasted 60 h and were divided into participative and theoretical classes distributed into an 8 h day. All divisions of the IBu, Museums, Collections and theoretical classes concerning several topics including routine equipment operation, first aid and laboratory safety, animal care and ethics and preparation of solutions were presented. At the end, a questionnaire was answered to find out the opinion about the course. **Results and Discussion:** In 2011, 35 just-graduated individuals with different trainings (31 biologists, 3 veterinarians, and 1 biomedic) were received by the program, and among them, 75% did not belong to IBu. The questionnaire was answered by 33 students and the average of the course grade was 8.1. The program was considered totally satisfactory by all of them, as well as the content and workload. The helpfulness and attention of teachers and coordination staff were totally satisfactory in their opinion. The main subjects of interest were laboratory techniques, biosafety guidelines, bioterium, routine equipment operation and museums; on the other hand, bioethics and SUS were indicated as areas of moderate interest. The feedback received can play an important role in the organization and improvement of the course in future years. Although the students' satisfaction does not assure the learning process, it certainly stimulates their performance in their laboratory activities.

All the authors equally contributed to this work

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12.06 Transference of serum and secretory antibodies to offspring of AIRmin and AIRmax mice orally inoculated with Enteropathogenic *Escherichia coli* (EPEC)

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) are a major cause of childhood diarrhea, an important public health problem in Brazil and other developing countries. The transfer of maternal antibodies by both the placenta and breastfeeding is critical to the protection of newborn infants. Animal models of experimental EPEC infection are limited and do not reproduce the human disease in all its features, but allow the study of the pathogenesis and the immune response after infection. AIRmin and Airmax mice, selected according to their acute inflammatory response, develop serum and secretory antibody response after oral inoculation with EPEC, without symptoms of diarrhea. **Objectives:** The objective of this study was to evaluate the transfer of serum and secretory antibodies to the offspring of AIRmin and AIRmax mice after oral infection with EPEC. **Methods:** Groups of AIRmin and AIRmax mice, 6 weeks of age, received two oral doses of 10^{10} CFU of EPEC, with a 7-week interval, and control groups received only PBS. The animals were observed for 45 days and the first two weeks they were weighed, and feces collected for detecting the inoculated bacteria. One week after mating, the females received a new bacterial dose as a booster. In the period from 3 to 10 days after birth of the offspring, samples were collected of blood and milk, taken directly from the stomach of the neonates, for the detection of antibodies by ELISA. **Results and Discussion:** The inoculated mice had no diarrhea, and after 48 h, the inoculated bacteria were no longer detected in the feces. There were no significant differences in weight of the animals inoculated with bacteria compared to the controls. The results showed higher levels of serum IgG antibodies in neonates of mothers that received the bacterial inocula compared to the control group, and the antibody levels of AIRmax mice were higher than in AIRmin. The levels of IgG and IgA antibodies in milk were higher in treated AIRmax mice compared to control AIRmax and control or treated AIRmin. This model of oral infection and analysis of the offspring was shown to be suitable to study the immune protection of neonates against EPEC disease.

Supported by: CNPq and PAP/SES

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12.07 Gometoxin-3: antimicrobial peptide from *Acanthoscurria Gomesiana* venon

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Introduction: The interest in AMPs as potential antibiotic pharmaceuticals has always been high because of their rapid-acting and broad-spectrum antimicrobial properties. These peptides were proposed as antimicrobials to treat microbial infections, particularly those caused by antibiotic-resistant bacteria. We have recently isolated five fractions with antimicrobial activity from the venom of *Acanthoscurria gomesiana.* This result showed us the importance of studying the Brazilian fauna. **Objectives:** The objective of this study was the characterization of the toxin called gometoxin-3 from the venom of *Acanthoscurria gomesiana*. **Methods:** The venom was obtained from venom glands of *A. gomesiana* spider, which were macerated with water and centrifuged, and the soluble part was concentrated by vacuum centrifugation and reconstituted with acidified water (TFA - 0.05% trifluoracetic acid). The soluble part was applied to HPLC reversed-phase chromatography with a C18 column. Elution was performed with a linear gradient of ACN/TFA 0.05%. The presence of antimicrobial 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 this molecule was analyzed by mass spectrometry. **Results and Discussion:** Based on our results, we focused on the molecule called gometoxin-3 isolated from the venom *A. gomesiana* with a molecular weight of 1658.9 Da and with anti-*M. luteus* activity. This molecule was reduced, alkylated and cleaved by trypsin and the products submitted to mass spectrometry (MALDI-TOF-MS). The masses of the fragments were analyzed in the database (PROTEIN BLAST and MASCOT) with similarity being found with a fragment neurotoxin 23 of the Chinese scorpion *Lychas mucronatus* (family Buthidae), as well *Ixodes scapularis* (family Ixodidae), *Latrodectus mactans* (family Theridiidae), *Loxosceles arizonica* (family Sicariidae), *Lycosa singoriensis* (family Lycosidae) and *Ornithodoros coriaceus* (family Argasidae). Gometoxin-3 will be characterized by Edman degradation.

Supported by: FAPESP and PAP/SES

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12.08 Histopathological evaluation of toxicity of two cianobacteria extracts in mice

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Introduction: Cyanobacteria are photosynthetic unicellular, colonial or filamentous organisms that are able to produce substances with interesting biological activities and even toxicity. Some of their toxic metabolites are well known for causing poisoning and death in humans and animals and include the microcystins (hepatotoxins), saxitoxin and anatoxin (neurotoxins). We have screened the Cyanobacteria Bank of the Center for Research in the Institute of Botany, and some cyanobacterial extracts were found to cause intoxication in mice injected intraperitoneally, with signs and lesions different than that in animals poisoned by known cyanotoxins. **Objectives:** To aim of this work was to submit the extracts of cyanobacterial strains *Phormidium sp* CCIBt 3280 and *Synechocystis aquatilis* CCIBt 3276 to toxicological studies in mice, observing its effects on vital organs by macroscopic analysis at necropsy and histopathology. **Methods:** The biomass of the strains CCIBt 3280 and CCIBt 3276, obtained in culture, were filtered, dried and subjected to extraction (4x) with 0.1 M acetic acid (AE) or methanol/water (75:25; v/v) (ME), by ultrasonication (4 x 10 s, 50 W). The extracts were centrifuged, freeze-dried (concentrated) and administered (i.p.) to male Swiss-Webster mice (19-21g). The animals were kept under observation for 7 days, after which they were euthanized and subjected to *post-mortem* examination, and their organs (liver, kidneys and lungs) were removed for histological analysis. **Results and Discussion:** The AE of the CCIBt 3280 and ME of the CCIBt 3276 had no effects in animals. One of the mice that received ME of the strain CCIBt 3280 died in 48 h, and the other two were euthanized after seven days, and showed macroscopic changes in the liver (dark spots). Under the microscope, the lung showed collapse of the alveoli and the presence of large number of macrophages, suggesting the presence of granulomatous inflammatory reaction type, and presence of liver necrosis. The mice that received AE of the strain CCIBt 3276 showed abdominal pain, paralysis and dyspnea after administration, but there were no deaths. After the necropsy they showed enlarged liver with white spots. In the histological study, there was necrosis of the liver, the hepatocytes had pyknotic nuclei, and some areas of the lungs showed excessive bleeding and macrophages. Histochemical analysis of tissue samples are ongoing to better understand the changes observed.

Supported by: PAP/SES, FAPESP and CNPq

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12.09 Purification and characterization of a hypothermic component from the venom of the spider "*Phoneutria nigriventer*"

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Introduction: Arthropod venoms contain potent toxins responsible for a significant number of non-lethal human envenomations, but may be severe in children and elderly. The spider *Phoneutria nigriventer* toxins cause several systemic manifestations including vomiting, sweating and hyperthermic or hypothermic state. The influence of the venom on thermoregulation is poorly studied. **Objectives:** The aim of this study was to characterize the fractions from the venom of *Phoneutria nigriventer,* obtained by purification, and to determine their effects on thermoregulation in rats. **Material:** The crude venom was purified by gel filtration chromatography (Sephadex G-50), and the "pool II" obtained was separated by reverse phase HPLC and used for the tests. Pool II was administered intraperitoneally in rats, anesthetized with a solution of ketamine hydrochloride (25 mg/kg)/xylazine hydrochloride (10 mg/kg). Body temperature was recorded by a thermal sensor (ML 309 Thermistor Pod - ºC Scale - AdInstruments®) inserted subcutaneously in the dorsal portion of the animal, connected to a software (Lab Chart Powerlab – Windows) for 3 h of experimentation. The peaks of the pools were obtained by reverse phase HPLC, (Merck - Hitachi) with a LiChroCART column 250-4 RP-18 (5 μm), detected with Septech Variable Wave Length monitor at a wavelength of 214 nm. **Results and Discussion:** The results obtained from the pool II showed a drop in body temperature of the animals during the last two hours of the assay. In the third hour, the body temperature fell by even 1 to 2.5ºC. An important point is that in one of these animals, body temperature fell nearly 5ºC, and then it died. However, in the first hour the test, there has no significant differences between control animals and those administered pool II. The data obtained from pool II indicate a hypothermic state in rats that involves a neurotoxin activity on the thermoregulation homeostasis probably without the involvement of inflammatory components.

Supported by: PAP/SES

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12.10 Antiviral effect of the egg wax extract of *Amblyomma cajennense* (Acari: Ixodidae) against H1N1 and EMC viruses in insect and mammalian cells systems

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Introduction: The control of viral infections, especially those caused by influenza viruses, is of great interest in public health. Bioprospecting has shown the presence of active principles in the hemolymph of arthropods as well as in the salivary glands of ticks. Some of these are of interest for the development of new pharmacological drugs. Ticks lay their eggs in the environment, and to protect them from desiccation and microbial attack, they cover the eggs in a wax layer produced by an organ known as Gené's Organ. *Amblyomma cajennense* is a species of tick maintained in a colony in our laboratory for different studies. This species is the most important vector of *Rickettsia rickettsii* in the Neotropical region. **Objectives:** The objective of this study was to evaluate the antiviral effect of the crude wax that surrounds the eggs of this tick species. **Methods:** The wax was extracted using chloroform/methanol (2:1) or ice-cold phosphate buffer. Influenza (H_1N_1) and picornavirus (EMC- *encephalomiocardite virus*) viruses were used to determine the antiviral activity of the tick eggs' wax. MDCK cells were infected with influenza viruses and L929 cells were infected with picornavirus (EMC). The cell infection was performed on the 3rd day post cell culture, started with 100 $TCID_{50}$ of picornavirus or with 128 UHA (hemagglutination unit) of influenza virus. **Results and Discussion:** The first issue to be addressed was the examination of possible cytotoxicity and genotoxicity of the crude egg wax extract, and for that purpose VERO cell lines were used. Concentrations equal to or less than 2 mg/ml were not toxic to VERO cells, and high percentages of viable cells were observed throughout the three experiments. MDCK cells were also used to determine the antiviral effect of egg wax, and the cytotoxicity of the egg wax on L929 cell growth was also determined. As observed in VERO cells, no adverse effect in terms of viability or morphological alteration was observed when a concentration of 2 mg/ml of egg wax was used. For enteroviruses, no cytopathic effect in Vero cell cultures was observed for at least 48 h, and the cell morphology was preserved, suggesting a very interesting potential antiviral activity. This result is in accordance with that similarly obtained for the hemolymph of *Lonomia obliqua* and for other insect hemolymph, such as that of *Calliphora vicina.*

Supported by: FAPESP, PAP/SES and CNPq

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12.11 Inventory and comparison of spider fauna (Araneae, Arachnida) using modified arboreal traps to capture spiders migrating on tree trunks

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Introduction: Trees comprise a large part of the forest biomass. Their trunks are used by various animals for shelter, food and as an important route between the soil and the forest canopy. **Objectives:** The aim of this study was to inventory the spiders during one year in two fragments of the Atlantic Forest in the state of São Paulo: Parque Ecológico do Estoril, São Bernardo do Campo and Instituto Butantan, São Paulo. **Methods:** The spiders were collected using arboreal pitfall-traps made out of 2 L and 250 ml pet bottles to analyze upward (soil-canopy) and downward (canopy-soil) migration. The traps are easy to transport and inexpensive to make, allowing the installation of a large number and thus increasing the sampling effort. We selected trees with diameters of approximately 70 cm, and the traps were placed five feet above the soil. Sixty traps (30 for upward and 30 for downward migration) were placed monthly in each area, for seven days. For a similarity analysis of species found in soil and trunks, we used three pitfall-traps placed around each tree for five days every three months. **Results and Discussion:** Preliminary results show that the upward traps from the Parque Estoril had an average of 0.35 spiders per sample (25.53% adults and 74.47% juveniles), with higher abundance of Corinnidae (36.4%) and Theridiidae (15.15%). In the Institute Butantan, the average was 0.44 (25.83% adults and 74.17% juveniles), with higher abundance of Miturgidae (19.35%) and Oonopidae (16.13%). The downward traps obtained an average of 0.95 spiders per sample (42.8% adults and 48.2% juveniles) in the Parque Estoril, with a greater abundance of Theridiidae (43.70%) and Linyphiidae (35.45%). In Butantan the average was 0.62 (32.54% adults and 67.46% juveniles) with greater abundance of Theridiidae (33.34%) and Oonopidae (16.70%).

Supported by: PAP/SES

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12.12 Clinical management and case reports of centipedes of the genus *Scolopendra sp*. (Linnaeus, 1758) kept in captivity in the Arthropod Laboratory of Instituto Butantan

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Introduction: Centipedes are arthropods from the class Chilopoda. These animals have long and flattened bodies divided into segments with a pair of legs on each one. Animals from the genus Scolopendra have 21 pairs of legs, and are 16 to 28 cm in length. The feeding habits of *Scolopendra sp.* involve mainly invertebrates, but they prey also on small rodents, lizards, snakes, birds, fruits and rotted organic material. Like most of vertebrates, a big part of the health problems in centipedes is due an incorrect husbandry. That is the reason why maintaining a good hygiene, quality food, optimum moisture and temperature for the species are the key to disease prevention. A quarantine period is extremely important to maintain new acquired animals before introducing them to the collection animals, avoiding the spread of infectious diseases and parasites to healthy centipedes. Invertebrates are poorly studied in veterinary medicine, where it is hard to determine what exactly the problem with an ill animal is, and to define the right protocol to follow. **Objectives:** The main objective of this study was to report health alteration in centipedes from the genus *Scolopendra* kept in the Arthropod Laboratory of Instituto Butantan, and the protocol adopted in the different situations. Preventive examinations such as fecal samples to identify parasites and necropsy procedures of dead animals are reported as well. **Methods:** The animals kept in the laboratory were observed daily for behavioral alterations, exoskeleton discolorations or wounds, feces appearance, food ingestion, and other signs. The centipedes in treatment were sedated by anoxia for handler's and animal's safely. **Results and Discussion:** The centipedes in treatment showed total recovery. Septicemia was observed in all necropsied animals. For being very poorly studied in the veterinary medicine, many studies must be performed to stipulate what is normal and what are illness signs in centipedes. Which drugs can be used in the different cases, their respective doses, frequencies and administration routes must be established as well.

Supported by: PAP/SES

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12.13 Informatization, identification and revision of the collection of Opiliones deposited at the Butantan Institute

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Introduction: The order Opiliones is the third largest in Arachnida, surpassed in number of species only by Araneae and Acari. They are characterized by having the body divided into prosoma and opisthosoma, the second pair of legs long and slender, with sensory function and repugantory glands. Systematically, they are divided into four suborders: Cyphophthalmi (6 families, 130 species); Eupnoi (6 families, 1,780 species); Dyspnoi (7 families, 290 species); and Laniatores (26 families, ~3,750 species). Due to the fact that they are morphologically similar to spiders, the Butantan Institute started receiving many of these animals from the general population and thus, around 1935, the collection of Opiliones was initiated. Until 2010, the collection harbored more than 30,000 specimens. Nevertheless, in May of that same year a fire destroyed the building in which the collection was housed, destroying part of this material. The saved samples were removed and are being kept temporarily in a shed. The collection harbors a large number of type specimens and thus, curatorial work should not be taken lightly. However, the Institute has never had a specialist in harvestmen taxonomy and this has led to very poor identification of the material and the necessity of a thorough revision. In addition, all information on this material was recorded solely in collection books and the computerization of this data became imperative to evaluate the loss of material. **Objectives:** The aims of this work were: to create a computerized database of the harvestmen material, based on the information of collection books and literature; to verify what was lost during the fire; and to identify and reorganize the remaining material in order to attain a real understanding of what is still available. **Methods:** The data from the collection books was compiled in Excel worksheets divided into 14 columns: number, sex, suborder, family, genus, species, author and description year, locality, municipality, state, country, coordinates, collection date and collector. The material that was not located of not on loan was indicated as destroyed. The remaining material was identified using identification keys for South American harvestmen and taxonomic revisions. **Results and Discussion:** The data from the collection books was completely computerized. According to the database, the collection included approximately 10,500 lots with over 37,000 specimens. Of these, 865 lots were identified to the family level, 220 to genus level and only 192 to species level. All remaining material was identified only as "Opiliones" and is currently being revised and identified.

Supported by: PAP/SES

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12.14 Study of the presence of the *sat* gene (secreted autotransporter toxin) in atypical enteropathogenic *Escherichia coli* samples of different origin

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Introduction: The term atypical EPEC (aEPEC) was created in 1995 to identify diarrheagenic *E. coli* samples that differed from the typical EPEC (tEPEC) for not showing the EAF plasmid and from entero-hemorrhagic *E.coli* (EHEC) for not producing the Stx toxin. Presently, it is known that this sub-category is among the most common diarrheagenic *E. coli* in epidemiologic studies performed in developing countries. The search for virulence factors in aEPEC has demonstrated a high heterogeneity, with the common finding of virulence factor genes previously thought to be specific to other *E. coli* pathotypes. Three aspects were important in the definition of this work: i) toxin Sat, a protein from the autotransporter serine protease family of the *Enterobacteriaceae* (SPATEs), is important in the pathogenicity of the uropathogenic pathotypes *E. coli* (UPEC) and *E. coli* with a diffuse adhesion pattern (DAEC); ii) aEPEC shows a high virulence factor heterogeneity; iii) and the toxin secretion profile, in aEPEC, is practically unknown. **Objectives:** The aim of this study was to investigate the presence of the *sat* gene in aEPEC samples of different origins. **Methods:** A total of 98 samples from different countries were submitted to PCR using specific primers (GenBank: AF 289092.1). Y1 cells incubated for 5 h with *sat* + isolates were washed and stained with trypan blue for cell viability and cell displacement analysis or fixed and stained with Giemsa for morphology and cell displacement analysis. Samples FB 114 (DAEC) and C600 (non-pathogenic) were used as positive and negative controls, respectively. **Results and Discussion:** Only samples CB5304 and CB3338, both of the O125ac:H6 serotype and sample CV323/77, serotype O125ac:H21, had the *sat* gene amplified. The results from cytotoxic assays showed suggestive changes in the action of the toxin, such as rounding and partial cell displacement. On the other hand, no changes were observed, under these conditions, in cell viability. Although the *sat* gene has been found in only 3.6% of the samples, it is interesting that the positive samples are from different continents (Australia and Brazil). The apparent expression of the Sat toxin in aEPEC samples is in agreement with the high heterogeneity of the group. Considering that toxin expression is an important factor for the pathogenicity of different microorganisms, these samples will be further studied in order to better understand the pathogenic mechanism of aEPEC.

Supported by: FAPESP and PAP/SES

12.15 Effect of crotoxin on secretion of cytokines by peritoneal macrophages co-cultivated with tumor cells

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Introduction: Crotoxin (CTX) inhibits tumor growth and modulates the function of macrophages. Despite this evidence, the contribution of macrophage inhibition to the decrease in tumor growth, caused by CTX, has not yet been determined. Macrophages provide a defense mechanism against tumor cells and two distinct polarization states, M1 and M2, have been described for these cells. In the beginning of tumor progression, M1 macrophages release reactive nitrogen/oxygen intermediates and the cytokines TNF-α, IL-1β and IL-6. In contrast, during tumor development, the release of these mediators by tumor-associated macrophages (M2 cells) is inhibited, contributing to tumor development. **Objectives:** In the present study, the effect of CTX on the secretion of TNF-α, IL-1β and IL-6 by macrophages and macrophage co-cultivated with LLC WRC 256 tumor cells was evaluated. **Methods:** Macrophages were obtained from the peritoneal cavity and cells ($5x10^5$) were incubated with CTX (0.3 μg/mL) for 2 h at 37°C. Afterwards, the macrophages were co-cultivated in the presence of LLC WRC 256 tumor cells ($5x10^4$), previously plated in 24-well culture dishes. After 12 h, the levels of IL-1β, IL-6 and TNF-α in the culture supernatants were determined by ELISA using kits from R&D Systems. **Results and Discussion:** The results showed that macrophages previously incubated in the presence of CTX increased cytokine secretion (IL-6:136%; IL-1B: 1.54x and TNF-alpha: 35%). Macrophages pre-incubated with CTX and co-cultivated with LLC WRC 256 tumor cells showed secretion decrease of IL-6 (19%) and increase of secretion of IL-1B (61%) and TNF-alpha (31%). Taken together, the results indicate that CTX modulates the cytokine secretion of macrophages, which may contribute to the inhibitory action of the toxin on tumor growth. These data reinforce the actions of CTX on defense mechanisms and provide new perspectives for the development of a new substance with therapeutic properties.

Supported by: FAPESP, PAP/SES and INCTTOX

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12.16 Anatomical topography and ultrasound imaging of the coelomic structures in the boidae snake *Boa constrictor*

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Introduction: The anatomical topography of snakes, guided by ultrasound examination, is used as a tool in determining the sex of monomorphic species, assessing reproductive status, detecting and monitoring pregnancy, aiding disease diagnosis and obtaining biopsies of organs or lesions. *Boa constrictor* belongs to the family Boidae. In Brazil, it is found in several types of environments, from tropical rainforests to caatinga, where there are two subspecies: *B. c. constrictor*, a large yellowish subspecies of slightly aggressive nature that is distributed in the Amazon region and northeastern Brazil; and *B. c. amarali*, a smaller, dark gray subspecies of more aggressive nature that is distributed from the central-western to southern regions of Brazil. This study aimed to locate all the organs of these snakes in relation to their snout-vent length (SVL), in percentage terms, and also to describe the ultrasound appearance of coelomic structures in this species, in order to facilitate the work of veterinarians dealing with diagnostic imaging when faced with these snakes, which are commonly raised as pets in many parts of the world. **Objectives:** The aim of this study was to determine the anatomical topography and ultrasound imaging of the coelomic structures in the boidae snake *B. constrictor*. **Methods:** Adult specimens of *B. constrictor* (10 males and 6 females) were used for the anatomical topography study, where they had died of natural causes. Before dissecting, ultrasound examination was performed on other healthy adult animals (10 males and 6 females). **Results and Discussion:** The three-chambered heart was located at 30% of the SVL from the head and its muscle was hyperechoic in relation to the liver. The liver took up more than half of the third middle of the snake's body (on average, it began at 33% of the SVL from the head and ended at 55%), had a homogeneous echo texture, moderate echogenicity and sharp edges. The gallbladder was located at 65% of the SVL from the head, had anechogenic content and thin and well-defined walls, and could be used as a reference point to locate the other internal organs. The biometric data on these snakes corroborated the findings described by Gomes et al. (1993), in which the females had greater SVL and weight than the males did. There were no statistical differences in the total numbers of ventral scales found in males and females, but the number of caudal scales in the males was significantly greater than in the females. There were also no statistical differences in the position of organs between the sexes.

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

XIII Reunião Científica Anual do Instituto Butantan
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12.17 Morphological study of the skin of a new caecilian (*Microcaecilia* sp.) from Pará (Brazil)

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Introduction: Microcaecilia is a genus within gymnophionan amphibians (or caecilians) composed of small thin species of violaceous color. Because of their fossorial habits, dimensions and coloration they are easily confused with earthworms, making it quite difficult to identify them in the field, at the moment of excavation. Microcaecilians have a large number of rings along the body (98-123), and possess many secondary rings in the third caudal portion. In this region the skin has scales inserted in transversal folds forming pockets with a large number of cutaneous glands. During three expeditions to the municipality of Belterra (PA), on the shores of the Tapajós River, 20 specimens of a non-identified microcaecilian were collected and are awaiting taxonomic description. **Objectives:** We aimed to describe the skin morphology of a new genus of *Microcaecilia*, comparing the results with those already obtained for other gymnophionans. **Methods:** Five rings of skin along the body were taken from specimens fixed in Karnowsky fixative. The head and the cloacal (terminal) region were completely decalcified in order to obtain sagittal sections. The samples were embedded in glycol methacrylate and the sections were stained with toluidine-fuchsin and submitted to PAS, alcian blue, pH 7.2, and bromophenol blue histochemical reactions. **Results and Discussion:** Except for the presence of scales in the caudal region, the skin is very similar to the other caecilians already studied (*Siphonops annulatus* and *Boulengerula taitanus*). There are three types of glands, two mucous glands, and a granular gland. The mucous gland type 1 is acinar and had a wide lumen. Mucous gland type 2 shows a narrow lumen and is full of a secretion with a flocculent appearance. The granular glands are much larger, and are composed of very large cells with a basal nucleus and a great number of tiny granules. The scales are very well organized inside the skin pockets and seem to be covered by a very thin layer of living cells. Study along the body revealed that while the head is covered with large type 2 mucous glands the predominant gland type along the body gradually changes in the direction of the caudal region, culminating with the predominance of very large granular glands completely covering the cloacal region. The placement of the cutaneous glands along the body is also similar and seems to be related to the fossorial behavior, allowing the animal to be able "dive" into the soil. On the other hand, the presence of a massive group of granular glands on the caudal tip are probably used for defense, when the animal is attacked by the tail, the only part of the body which is permanently exposed inside the tunnels where the animal live.

Supported by: CNPq, PAP/SES and INCTTOX

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12.18 Evaluation of inoculum concentration in diphtheria toxin production

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Introduction: Butantan Institute produces diphtheria toxin by the fermentation process of *Corynebacterium diphtheriae*, Park-Williams 8 strain in a bioreactor (500 L) containing IB culture medium for diphtheria toxin production. After cultivation, diphtheria toxin is separated from bacterial cells by tangential flow filtration and is concentrated by molecular ultrafiltration through 30 kDa membranes. Concentrated diphtheria toxin is detoxified by formaldehyde solution with subsequent filter sterilization through 0.22-µm membranes. The inoculum preparation is an important step to get an optimum fermentation process and consequently high levels of diphtheria toxin production. The inoculum concentration used in the industrial production of diphtheria toxin is 0.6% in relation to the total culture volume. **Objective:** The aim of this study was to evaluate three different concentrations of inoculum for diphtheria toxin production. **Methods:** *C. diphtheriae* Park Williams 8 strain was cultivated in Erlenmeyers containing IB culture medium for diphtheria toxin production with rotary shaker to prepare the inoculum. Three different inoculum volumes corresponding to 0.6%, 6% and 10% of the total volume of the production culture were used to simulate the production culture. The cultures were incubated at 36°C with constant agitation for 64 h. Samples were collected at 24, 48 and 64 h of culture and optical density (O.D.) was evaluated at 530 nm. The measurement of diphtheria toxin titer was performed by determination of flocculation limit expressed in Lf/mL. The flocculation limit test was performed using Ramon assay in which the flocculation value (Lf/mL) of a toxin was determined by the number of units of antitoxin which, when mixed the sample produces an optimal flocculating mixture. Visible flocculation was formed as the result of the formation of the antigen-antibody complex. **Results and Discussion:** The culture O.D. values after 64 h with inoculum concentration of 0.6%, 6% 10% were respectively 0.468, 0.556 and 0.710 at a 1:10 dilution. The results of flocculation limit in 24 h were 40, 70 and 100 Lf/ml, representing a yield increase of 75% and 150% in the production of diphtheria toxin by comparing the inoculum concentration of 6% and 10% in relation to 0.6%. After 48 h of cultivation the titers of toxins were 60, 70 and 120 Lf/mL and at 64 h, they were 80, 100 and 160 Lf/mL using 0.6%, 6% 10% inoculum concentration. The results suggest that high titers of diphtheria toxin can be achieved using higher concentration of inoculum and the time of fermentation can be reduced maintaining the usual titers obtained in industrial production.

Supported by: Fundação Butantan and PAP/SES

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12.19 Analgesic effect of crotalphine in experimental autoimmune encephalomyelitis, a model of multiple sclerosis

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Introduction: Multiple sclerosis (MS) is a chronic neurodegenerative disease that causes widespread CNS inflammation, inducing CNS demyelization and locomotor impairments, and it affects approximately 2 million people worldwide. Included in the alterations induced by MS, chronic pain is an important symptom, affecting between 50 and 80% of MS patients, interfering with many aspects of their lives. It is important to point out that MS has no cure, where therapeutic approaches focus on stopping disease progression and cumulative neurological disability. Despite the importance of pain symptoms to MS patients, only recently experimental studies were developed in order to characterize this phenomenon since motor dysfunctions make pain evaluation difficult. These studies demonstrated that in the MOG_{35-55}-induced experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, hypernociception appears before the onset of motor disability, allowing these two phenomena to be studied separately. **Objectives:** Therefore, the aim of this work was to evaluate the analgesic effect of crotalphine, a 14-amino acid peptide that induces potent and long-lasting analgesic effect in acute and chronic pain models, in the pain observed in the experimental autoimmune encephalomyelitis model. **Methods:** EAE was induced by subcutaneous immunization of C57BL/6J mice (18-20 g) with 200 µl of an emulsion containing 150 µg of MOG_{35-55} peptide and 400 µg of *Mycobacterium tuberculosis* extract in incomplete Freund's adjuvant oil. In addition, animals received 150 ng of pertussis toxin i.p. on day 0 and day 2. Pain threshold was assessed daily using an electronic pressure-meter test for mice, until the onset of clinical signs or motor alterations. Motor activity was evaluated using the rota rod test. Clinical signs of EAE were assessed according to the following score: 0, no signs of disease; 1, loss of tone in the tail; 2, hindlimb paresis; 3, hindlimb paralysis; 4, tetraplegia; 5, moribund. **Results and Discussion:** The pain threshold of the animals decreased on day 4, while the first sign of disease appeared on days 11–12, coinciding with the onset of motor abnormalities observed in the rota rod. Crotalphine administered by the oral route 5 days after immunization (1 day after onset of pain threshold alteration) induced long-lasting antinociception. These results indicate that crotalphine is able to interfere with the pain observed in the experimental model of EAE.

Supported by: INCTTOX and PAP/SES

12.20 Project: Training agents to disseminate science - INCTTOX

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Introduction: Science is more present in people's everyday life by reason of increasing access to information. Scientific dissemination expanded over the last century by channels that facilitated conversation between science and society. The purpose of the project *Formando Divulgadores da Ciência* is to establish a communication route between universities, research institutes and public schools, in order to promote discussion about production processes and means of dissemination of science in society. **Objectives:** The main objective of the project was to bring high school students closer to scientific culture. In other words, students have contact with different ways to publish science and produce materials that could be made available to the general public. The aim was to strengthen the school/research relation, facilitating the dialogue between public schools and the research institutes involved. **Methods:** In weekly meetings of two and a half hours from September to December of 2010, five high school students from *Escola Estadual Professor Flávio José Osório Negrini* (São Paulo – SP) participated in workshops and lectures and visited museums and laboratories. The activities were arranged by members from *Instituto Butantan*, *Faculdade de Educação da Universidade de São Paulo* (FE-USP) and from the participating school. The students had access to conceptual, cultural and educational help, so they could produce material to promote science. **Results and Discussion:** Activities that triggered students' initiative and desire to learn were carried out. The students showed noticeable interest in the topics presented, interacting with the activities and with the questions presented. The materials produced were a blog (http://divulgadoresdaciencia.blogspot.com), containing descriptions and analyses of the activities, and a research concerning popular knowledge about toxins, and graphs are also in the blog mentioned. It is important to mention that we faced some challenges during the development of the project: taking into consideration that the students were volunteers and the activities were not part of the school program; three students abandoned the group because of financial matters. To avoid this situation, the group of 2011 had support from FEUSP by the *Programa Pré-iniciação Científica da Universidade de São Paulo*. Apart from the challenges just mentioned, it is also important to emphasize the innovative traits of this proposal that place the students in a process of critical thinking about scientific knowledge and its relations with society.

Supported by: PAP/SES

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12.21 Molecular characterization of atypical Enteropathogenic *Escherichia coli* (aEPEC) isolated from children with diarrhea in Ribeirão Preto, São Paulo, Brazil

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Introduction: Diarrheagenic *Escherichia coli* (DEC) can be classified into six pathotypes according to their virulence factors, serotypes, epidemiologic aspects and interactions with epithelial cells "in vitro". Enteropathogenic *E. coli* (EPEC) is one of the leading causes of diarrhea among children in developing countries. EPEC strains cause a characteristic lesion to the intestinal epithelium called 'attaching and effacing' (A/E) and can be classified as typical (tEPEC) or atypical (aEPEC). The main difference between these two groups is the EAF plasmid, which contains the genes encoding bundle forming pilus (BFP), present only in tEPEC. In addition, aEPEC may show additional virulence genes described in other DEC pathotypes. **Objectives:** The aim of this study was to characterize 32 strains of aEPEC isolated from children with diarrhea in Ribeirão Preto, São Paulo, Brazil, regarding the presence of characteristic genes of different DEC pathotypes. **Methods:** All samples were grown in Lauria-Bertani (LB) broth and incubated overnight for genomic DNA extraction by CTAB method. The plasmid DNA was obtained using plasmid miniprep (alkaline lysis method). The presence of the genes *ast*A, *hly*A, *efa*1, *tox*B, *pic*, *pet*, *ehx*A and *she*A was analyzed by PCR and agarose gel electrophoresis. **Results and Discussion:** None of the strains showed all virulence genes tested. Genes *pet*, *pic* and *tox*B were not detected in any strain analyzed. The gene *she*A was found in 13 (42%) strains, *hly*A in 4 (12.5%), *ast*A in 3 (9%) and *ehx*A in 2 (6%). Unlike tEPEC, which comprises a homogeneous group, where the LEE region encodes most of the virulence genes, aEPEC can have a variety of characteristic genes of other DEC pathotypes and do not have a specific virulence gene, common to all strains. The results obtained in this study corroborate this statement and clearly demonstrate the heterogeneity regarding the virulence genes present in aEPEC.

Supported by: PAP/SES

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12.22 Angiotensin-degrading serine protease: a new chymotrypsin-like activity in *Bothrops jararaca* venom, partially blocked by the commercial antiserum

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Introduction: Snakebite envenomation is considered a highly relevant public health hazard in South America, having an impact in terms of mortality and morbidity. In Brazil, *Bothrops* spp poisoning is responsible for 90% of snakebites, and in patients treated at the Hospital Vital Brazil, *B. jararaca* poisoning is as high as 97.5%. **Objectives:** The aims of this study were to analyze more specifically the quality of the antibothropic serum produced by Instituto Butantan, to neutralize metallo- and serine proteases, known as the major toxins present in *B. jararaca* venom, and also, to find new mechanisms of the envenomation through the hydrolysis of bioactive peptides. **Methods:** A library of FRET peptides was studied using the *B. jararaca* venom (BjV) and site-directed inhibitors PMSF, EDTA and 1,10-phenanthroline. BjV was also incubated with the bioactive peptides angiotensin I and dynorphin1-13 using the inhibitors described above. Hydrolysis was detected by HPLC and the fragments were submitted to mass spectrometry analysis. Hydrolysis of both FRETs and bioactive peptides were submitted to neutralization by antibothropic serum. To determine the presence of antibodies against serine proteases in serum, BjV was incubated with it, centrifuged and the supernatant was submitted to fluorimetric assay. **Results and Discussion:** Two substrates were obtained to be used as specific tools for studies with metalloproteinases, Abz-FASSAQ-EDDnp, and the serine proteases, Abz-RPPGFSPFRQ-EDDnp. In disagreement with the literature, the use of both substrates and the antibothropic serum showed a weak neutralization of serine protease(s) present in this venom and a strong neutralization of metalloproteinase(s). Immunodepletion showed that the lack of antibodies against serine proteases is most likely the cause of this phenomenon. In the course of investigating possible mechanisms of action that have not yet been described for serine proteases from BjV, the present work showed an angiotensin-degrading serine protease activity. The cleavage was between Tyr and Ile, showing a new tyrosine-specific chymotrypsin-like activity in BjV. At a lesser degree of hydrolysis, dynorphin1-13 had at least two cleavage points, but unlike angiotensin I, both classes of proteases were responsible for this hydrolysis. In both cases, hydrolysis was partially blocked by the commercial antiserum produced by Instituto Butantan. To our knowledge, this is the first report on a chymotrypsin-like activity in BjV partially blocked by the antiserum, which still deserves better characterization in the future.

Supported by: PAP/SES

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12.23 Study of macrophage activation and tissue regeneration in mice selected for high or low antibody production

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Introduction: Two mouse lines were produced by bidirectional selection according to 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 study was to analyze the phenotypic differences of macrophage activation and the capacity of tissue regeneration in mice selected for high or low antibody production. **Methods:** Adherent peritoneal cells harvested 4 days after thioglycollate i.p. (Thio) injection were cultured for 48 h with and without LPS. NO was measured with Griess reagent. H_2O_2 production was determined in total peritoneal cells stimulated with PMA using phenol red solution. In order to evaluate tissue regeneration, a 2-mm diameter hole was made in each animal's ear and measured periodically with a digital caliper. **Results and Discussion:** Analyzing peritoneal cell activation, we found differences between males and females. These differences were observed in High strain mice. NO and H_2O_2 production were higher in cells of male mice, which received Thio and were stimulated with LPS, compared to females and Low mice. However, in Low strain, males and females showed similar profiles. In fact, cells from H mice released two times more NO and five times more H_2O_2 than did cells from L mice and no significant levels of NO were produced in control cells. H and L mice did not show tissue regeneration after the ear punch; however, in response to stimulus, L mice exhibited more intense and local inflammation than did H mice, with edema on the third day after ear puncture. Despite being selected for High or Low antibody production, these mice showed differences in some phenotypes of the inflammatory response.

Supported by: PAP/SES, FAPESP and CNPq

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12.24 Standardization of primary cultures of embryonic cells of argasid ticks

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Introduction: Considering the emergence of diseases transmitted by ticks, and the difficulty of diagnosis and isolation of some bioagents transmitted by them, the establishment of tick cell lines from embryonic egg masses has become a significant tool for a wide variety of studies of casual agents of diseases, including the isolation and diagnosis of microorganisms. **Objectives:** The objective of this study was to isolate and to standardize primary cultures of embryonic cells of two argasid ticks. **Methods:** The species *Ornithodoros mimon* and *Ornithodoros rostratus* are maintained in colonies in our laboratory for different studies. The egg masses 8 and 11 days old, from both tick species, respectively, were washed using ethanol and antibiotic/antimycotic agents. They were then crushed in L-15 modified medium with addition of nonessential amino acids, vitamins and minerals and adjusted to pH 6.4 and pH 7.2. The suspension of embryonic tissues was transferred to 5-mL flasks containing 1-2 ml of supplemented L15B medium. They were incubated at 31°C, and the medium was replaced weekly. **Results and Discussion:** The ideal age of egg masses to obtain embryonic cells of these argasid ticks was not defined. However, due to these species having a short period of egg incubation, we chose eggs with exactly half the incubation period. Cells adhered to the tissue culture flask after 24 h showed several types. Although we obtained primary cultures from eggs with ages of 8 and 11 days, different ages of eggs (5, 10, 12 and 15 days old) still need be tested in order to establish the best. Once protocol of primary culture is standardized, the tick cell lines will become very important as a tool for studies *in vivo* of certain diseases and also as a model for functional genomics of ticks, which can be used effectively to study the isolation and spread of various pathogens.

Supported by: FAPESP, CNPq and PAP/SES

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.25 Natural history and morphology of *Holoaden luederwaldti* (Amphibia, Anura, Strabomantidae)

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Introduction: *Holoaden luederwaldti* was described in 1905, with type locality in Campos do Jordão. Until 2005, it was believed that the species had been extinct since the last record of occurrence was in 1956, performed by Bertha Lutz. The distribution of this frog is restricted to the Atlantic forest of high elevation (1,500 to 2,200 m) of Campos do Jordão (SP) and to the National Park of Itatiaia (RJ and MG). The biology and natural history of this species is virtually unknown. Morphologically, the large amount of protuberances in the skin calls attention; it is characterized by the accumulation of granular glands (or poison glands). These accumulations, associated with its dark color, gives *H. luederwaldti* the appearance of a blackberry. **Objectives:** The aim of this study was to determine the skin morphology of *Holoaden luederwaldti,* using the results obtained to make inferences about the biology of the species, particularly about its passive chemical defense. **Methods:** The field work was conducted within the State Park of Campos do Jordão at an average altitude of 1,400 m. Histological studies were performed with six specimens, collected using pitfalls. Fragments of dorsal, dorso-lateral, ventral and inguinal skin were removed, fixed in Karnovsky, stained with toluidine blue-fuchsin and submitted to bromophenol blue, PAS, alcian blue histochemical methods. **Results and Discussion**: *H.luederwaldti* lives associated with litter, a habitat where there is a wide variety of potential predators for anurans. However, the presence of large amounts of glands covering the whole body of the frog may be an indication that it invests a high amount of energy in the production of toxic secretions used in defense against predators. Histological analysis showed that the poison glands are large and are arranged side by side in the dermis. The histochemistry revealed that these glands are filled with spherical granules with a high affinity for bromophenol blue, indicating a high protein content and low affinity for alcian blue, indicating the presence of small amounts of acidic polysaccharides. The distribution of the skin poison glands indicates that *H. luederwaldti* is adapted to ensure an effective chemical defense throughout the dorsal and dorso-lateral body. The morphology of the ventral skin confirms that the animal spends most of its time in moist litter, since *verruca hydrophilica*, specialized in the uptake of water, are practically absent.

Supported by FAPESP, CNPq, INCTTOX and PAP/SES

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.26 Standardization of a new method for neurotransmitter measurement in rat hippocampal slices

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Introduction: The development of preparations with cerebral tissue slices is an important landmark for advances in neuroscience since they are metabolically viable maintaining the neurons' excitability. Therefore, this methodology has advantages over experiments in vivo, such as lack of maintenance of physiological functions of the animal, the easy manipulation of the external environment allowing the observation of the behavior of neurons in response to different stimuli, and the ability to acquire multiple slices of a single animal, allowing the animal to be experimental and control in the procedure. **Objectives:** The aim of this work was to standardize a method based on slices of hippocampus after application of toxins to check for alterations in intracerebral neurotransmitter levels. **Methods:** Male Wistar rats (230-250g) were anesthetized in a CO_2 chamber and decapitated. The brains were quickly removed and placed in a cold and oxygenated (95% O_2 and 5% CO_2) standard buffer. The buffer consists of 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 11.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 11 mM glucose and 225 mM sucrose. The hippocampus was dissected and sliced in sagittal sections (thickness of 400 μm) using a vibratome. Some slices were kept at room temperature and others in chilled buffer. They were maintained at rest for at least 60 min to equilibrate before any experimental manipulation. Afterwards, 2 slices were placed in each incubation chamber, at room temperature or in chilled buffer, with 200 μl of standard buffer, continuously oxygenated. Aliquots of 100 μl were taken every 15 min to determine baseline levels of mediators and the same volume of medium was replaced. After collection of two samples, 10 μl of kainic acid were added, and four samples were collected for determination of changes in levels of mediators. All aliquots were kept frozen (-80ºC) until assay. The determination of cerebral levels of excitatory amino acids was performed by high performance liquid chromatography (HPLC) with fluorimetric detection. **Results and Discussion:** Amino acid levels obtained before and after exposure of the slice to kainic acid were compared but they did not show significant differences. However, the data showed that there were detectable levels of neurotransmitters, indicating that this new method is feasible.

Supported by: PAP/SES

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.27 Preliminary data on geographic distribution of *Imantodes cenchoa cenchoa* (Linnaeus, 1758) in Brazil

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Introduction: The blunt-headed vine snake *Imantodes cenchoa* has a wide geographic distribution in the Americas, from Istmo de Tehuantepec, in Mexico, to the south of Argentina. Generally it is found in forested areas, has an arboreal habit and may go to the ground to look for food. The subspecies *I. cenchoa cenchoa* has the largest geographical distribution among the three subspecies, which extends from Panama to the southern part of South America, including Trinidad and Tobago. Detailed studies about the occurrence and geographical distribution of snake species are very important because they can be used as a source for studies about the inter- or intraspecific variations, taxonomy and natural history, and also for decisions in conservation strategies. **Objectives:** This study aimed at preliminary analyses of the geographic distribution of *I. cenchoa cenchoa* in Brazil, in order to attain a greater understanding of its geographic distribution through scientific collection data, complementing what is already available in literature. This is also the first step in the taxonomic approach that is intended to be developed. **Methods:** Records at the Herpetological collections at Butantan Institute (IBSP), the Zoology Museum USP (MZUSP) and Campinas University (ZUEC) were reviewed. The Diva-Gis program was used for data georeferencing. **Results and Discussion:** A total of 107 Brazilian locations were obtained in the collections. The literature data contributed 60 locations, totaling 147 locations, excluding the duplications. A total of 88 new possible recordings were collected, but this number must lower because access to publications was still not obtained. In the state of São Paulo, about 60% of the locations found in the analyzed collections are in areas recently defined as intermediate knowledge or low herpetofauna state. These areas account for 37 new recordings of specimens in the collections, which are added to 10 recording from the literature with control specimens in the examined collections in the state of São Paulo, except the Alphonse Richard Hoge Herpetological collection (IBSP), where information about specimens rescued after the fire in May, 2010 is not yet available. The occurrence of *Imantodes cenchoa* in the Cerrado and Caatinga areas in the Southeast, Central-West and Northeast regions of Brazil is probably a result of the heterogeneity of vegetation formations in South America, which makes it possible to find forest enclaves, gallery forests and marshes, suitable environments for the propagation of the species, propitiating microclimates and important catches for its maintenance.

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12.28 Characterization of the cellular immune response in horses immunized with Crotalus venom

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Introduction: The Health Ministry reported that 7.2% of 75,500 cases of snakebites in the period between 2007 and 2009 were caused by *Crotalus* rattlesnakes. The venom of *Crotalus durissus* causes neurotoxic and myotoxic systemic coagulation disorders. The antivenom is specific and effective treatment in cases of accidents. Currently, Butantan Institute is responsible for 60% of the anti-crotalic horse serum produced in Brazil. Horses are used for the production of anti-venom because of their large size, resistance to toxins, and production of large amounts of plasma containing effective IgG(T) antibody titers. Despite the well-established efficacy of rattlesnake antivenom horse serum, there is no data in the literature about the specific cellular immune response to the venom. **Objectives**: The aim of this study was to evaluate the profile of the specific cellular immune response to *Crotalus* venom and compare with the specific IgG antibody titer in the peripheral blood of horses immunized with the same antigen. **Methods**: Male horses used for anti-crotalic serum production were immunized according to a standard protocol consisting of different immunization cycles. Heparinized blood samples were collected at different times after immunization as well as serum samples. A group of non-immunized animals was used as control. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradient separation. Proliferation assays with PBMC stimulated with *Crotalus durissus* venom were performed by [^{3}H] thymidine incorporation, after 5 days of culture. ELISA was performed on horse serum to determine venom specific antibody titers. **Results and Discussion**: We observed a significant proliferation in the different PBMC samples, compared to the control group. In addition, increased proliferation correlated with the number of antigen immunizations. There was also a quantitative variation in proliferation among animals of the same group. At the moment, it is still not possible to correlate the proliferative response with production of venom specific IgG(T) antibodies. Nevertheless, new samples will be analyzed in this group as well as a new group currently being evaluated. The evaluation of other aspects of the cellular immune response of immunized horses compared to antibody response may contribute to the improvement of the immunization protocol for horses for the production of sera with higher antibody titers.

Supported by: Instituto Butantan

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12.29 Public's reaction to the traveling exhibition of the Butantan Institute *The Pathway of Scientific Dissemination*

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Introduction: The traveling exhibition of Butantan Institute *The Pathway of Scientific Dissemination* was conceived by means of a CNPq Project, where its focus was to disseminate the institute policy in relation to public health issues. The promotion of science is an institutional responsibility, and therefore, the main objective of the traveling exhibition was to present different activities of the institute to the public: production, research and cultural dissemination. The exhibition is composed of six display case modules with support and a touchscreen computer. **Objectives:** The aim of this study was to examine the behavior of the visitors and assess their interaction. The information gathered would be used to improve the exhibition and maximize its impact. **Methods:** Collection of data occurred on March 18th and 19th of 2011 in Vila de Paranapiacaba (Santo André/São Paulo), during this period the traveling exhibition was open to the public. Microbiology and Biology Museum Guides and the Center for the Dissemination of Knowledge (Núcleo de Difusão do Conhecimento) of the Butantan Institute welcomed the visitors, offered guidance in the use of the instruments and provided information when requested. The behavior of 18 randomly chosen visitors was observed. Their behavior was recorded on an observation form covering five categories of interaction: *observation*, *reading*, *handling*, *interaction*, *visitor-visitor* and *visitor-guide*. The retention time of the public in each exhibition module, the detailed description of the visitor's behavior and characteristics such as age, sex and number of accompanying people were also recorded. **Results and Discussion:** The most frequent characteristics, from those related to visitor's behavior, were *interaction visitor-monitor* and *visitor-visitor*, recorded for 14 visitors. Next, the categories *manipulation* occurred with 14 visitors and *reading* with 7. In relation to the time spent observing, the module *Table* showed the highest average retention time, 2 min 49 s, followed by the module *Brazilian Savanna* (2 min 49 s), *Atlantic Forest* (1 min 51 s), *Prism* (43 s), *Vital Brazil* (16 s) and the modules *Surfactant* and *Photos* (12 s each). Some factors observed are important and corroborate the results. The first one is the human mediation; the visitors were welcomed in the museum entrance by the guides, establishing thus an initial interaction. The arrangement of the modules was a factor that had an influence on the retention time. *Table* and the diorama *Brazilian Savanna* were near the entrance and obtained more retention time. Finally, it is important to mention that this research presents important information to enhance the exhibition and its impact. We intend to implement this research as a routine to all exits of the exhibit.

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12.30 Prevalence of *perABC* operon in atypical enteropathogenic *Escherichia coli*

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Introduction: Enteropathogenic *E. coli* (EPEC) are currently subdivided into typical (tEPEC) and atypical (aEPEC). Both are devoid of genes encoding Shiga toxins (Stx) and have in common the ability to produce the attaching-effacing lesions (A/E) on the intestinal mucosa. The main difference between the two groups is the presence of EPEC adherence factor plasmid (pEAF), which occurs only in tEPEC. pEAF harbors the operon that encodes the bundle forming pillus (BFP), as well as the plasmid encoded regulator (*per*) operon, composed of the *perA*, *perB* and *perC* genes. This operon encodes proteins that regulate the expression of BFP and the chromosomal pathogenicity island termed LEE. Although aEPEC does not carry the EAF plasmid, some investigators have described aEPEC strains that are *perA* gene positive but do not hybridize with the EAF fragment probe (EAF-/*perA*+). **Objectives:** The aim of this study was to evaluate the presence of the genes composing the *per* operon among aEPEC strains. **Methods:** Seventy-two aEPEC strains isolated from cases of acute diarrhea in children in Salvador (BA) were selected. We first carried out the extraction and quantification of genomic DNA from each strain, and the *perA*, *perB* and *perC* genes were then detected by dot-blot and PCR. For both experiments, the genes were searched using specific primers based on the respective gene sequences of tEPEC E2348/69 (GenBank accession number: DQ388534.1). Also, the *per*-positive strains were searched by PCR for the presence of the EAF probe fragment and the *bfpA* gene using specific primers. **Results and Discussion:** Only 8 (11.1%) strains were positive for all three genes, while 4 (5.6%) were positive for *perA* and *perB* and 2 (2.8%) for *perB* only. These 14 strains were analyzed for other characteristics of the EAF plasmid. The EAF probe fragment was not found in any of them and the *bfpA* gene was detected only in three strains harboring *perA*, *perB* and *perC*. Subsequently, the *bfpA*+ strains were evaluated for expression of BFP fimbriae, which was not detected by immunofluorescence. These data indicate that some aEPEC may harbor the *per* operon, intact or not, without BFP expression and without the probe EAF fragment, suggesting that in these strains *perABC* is located in another plasmid or even in the chromosome. Therefore, our data confirm that the evaluation of BFP expression is more suitable than the detection of pEAF genetic markers (*perABC*, EAF probe or *bfpA*) for the classification of EPEC as typical or atypical.

Supported by: PAP/SES and FAPESP

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12.31 Characterization of the biological activities of the venom of the snake *Bothrops atrox* from Santarém region, Pará, Brazil

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Introduction: *Bothrops atrox* (Ba) snakes are responsible for most of the bites in the Brazilian Amazon region. People bitten by Ba snakes usually show local and systemic effects. Regarding the systemic effects, the occurrence of hemostatic disturbances, caused mainly by the procoagulant enzymes of the venom, is very common. It is also known that the biochemical composition and the related biological activities of the snake venoms may vary, not only between species, but also inside a single species. This variation may be related to the geographic origin, habitat, size and ontogenetic stage of the snake. **Objectives:** The aim of this study was to characterize some biological activities of the *Bothrops atrox* snake venom (Bav) from the Santarém region, in Pará State. **Methods:** Lyophilized crude venom from Ba snakes (n=8), males and females, captured in the National Forest from Tapajós (FLONA-Tapajós) was used for *in vitro* assays, to evaluate the minimum coagulant doses in fibrinogen (MCD-F) and bovine plasma (MCD-P), the phospholipase activity on soybean lecithin, the coagulant activity on factors II and X from blood coagulation, using the respective chromogenic substrates S-2238 and S-2765 (0.1 mM), and the neutralization of the coagulant activity by the *Bothrops* antivenin (BA) from Butantan Institute, considering 2 times the MCD-P. To compare the results obtained in the assays, we used a pool of crude *Bothrops jararaca* snake venom (Bjv) from Butantan Institute. **Results and Discussion:** The low values of the MCD-P and MCD-F from Bav (15.1 ±0.81 and 24.5 ±3.27 µg venom/mL, respectively) compared to that from Bjv (37.0 ±0.32 and 70.4 µg venom/mL, respectively) indicate that Bav from the Santarém region is more coagulant than that from Bjv. Bav also showed a higher phospholipase activity (1117 U/mg of venom) compared to Bjv (60.17 U/mg of venom). Regarding the activation of the procoagulant factors by Bav, a higher factor II activity (459.3 ±32.2 µmol *p*-nitroaniline/min/mg of venom) was observed in comparison to that for factor X (131.4 ±6.1 µmol *p*-nitroaniline/min/mg of venom). BA was neutralized Bav (626.8 µL of antivenin/mg of venom) and also Bjv (1400 µL of antivenin/mg of venom), for 2 times the MCD-P. In conclusion, the data indicate that Bav from snakes of the Santarém region has a higher potential to cause fibrinogen hydrolysis (thrombin-like) and activate procoagulant factors (II and X), and also a very high phospholipase activity, when compared to Bjv.

Supported by: INCTTOX, CNPq, SES-SP and PAP/SES

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12.32 Revision of the Brazilian species of the genus *Pycnothele* Chamberlin (Araneae, Nemesiidae) and notes on the species from Uruguay and Argentina

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Introduction: The family Nemesiidae comprises 42 genera and 353 species with worldwide distribution. It is subdivided into six subfamilies, of which only two occur in Brazil, namely Anaminae with three genera and Pycnothelinae with eight genera, the latter including *Pycnothele* Chamberlin, 1917, the subject of our study. The genus is represented by five species, three of which are native to Brazil, the type species *P. perdita* Chamberlin, 1917, *P. singularis* (Mello-Leitão, 1934) and *P. piracicabensis* (Piza, 1938). *Pycnothele auronitens* (Keyserlin, 1891) occurs in Brazil and Uruguay and *P. modesta* (Schiapelli & Gerschmann, 1942) in Argentina and Uruguay. **Objectives:** Although the genus was previously revised by several authors, none of them ever addressed all known species. Thus, the aim of this study was to revise *Pycnothele* including all currently known species. **Methods**: We examined the holotypes of *P. perdita*, *P. singularis*, *P. modesta* and material belonging to the collections of six museums. The specimens were examined using a Leica MZ 12.5 stereomicroscope with attached camera lucida. Female spermathecae were dissected and cleared in clove oil for observation of internal structures. **Results and Discussion:** In this study, we present an extended diagnosis for the genus, mainly in what concerns the females: males have large bulbs, which rest on a broad and deep excavation of the tibia of the palp; the females differ from the other Nemesiidae by the presence of a spermathecal chamber as observed in *Neostothis* Vellard, 1925, but sclerotized throughout its entire area (in *Neostothis* only the central region is sclerotized) the lobes of the *Pycnothele* spermathecae are three or four times narrower; and the tarsal scopula III is divided. The females of *P. singularis* and *P. auronitens* are newly described; *P. auripila* (Mello-Leitão, 1946), previously considered a junior synonym of *P. auronitens* is revalidated; *P. piracicabensis* is transferred to *Rachias* Simon, 1892; new species are described. In addition, the female of *P. auronitens*, as described by Goloboff, is considered to be in fact the female of *P. auripila*. A distribution map for all *Pycnothele* species is presented.

Supported by: PAP/SES

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12.33 Evaluation of SFV-GFP produced by different lipid-based transfection protocols

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Introduction: SFV based systems are increasingly the virus vector systems of choice for production of recombinant proteins for research, vaccination, therapy and diagnosis. In combination with the GFP reporter, SFV vectors allow the delivery of genes to be quantified. The production of recombinant SFV particles is performed *in vitro* by transfection of expression and helper RNAs transcribed *in vitro*. **Objectives:** The aim of this study was to establish and standardize the molecular biology methods for the production of high titers of recombinant SFV by using a SFV-GFP construction. **Methods:** Different lots of SFV-GFP were obtained by transfection of BHK-21 cells with the TransMessenger® QIAGEN kit, changing the amount of *in vitro* transcribed RNA and the ratio of RNA to lipid concentration. For analysis of virus lots, BHK-21 cells ($7x10^5$ cells/well) were plated in 6-well culture plates with α-MEM and infected the next day when they reached 80% confluence: the supernatant was discarded and cultures were infected with 25 µl or 100 µl of lots A, B or C of SFV-GFP in 2 ml of culture medium, and incubated at 37ºC with 5% CO_2. After 24 h, development of fluorescence was measured by flow cytometry (FACS) and visualized by fluorescence microscopy. **Results and Discussion:** The GFP-positive cells were visualized by fluorescence microscopy, showing few but highly fluorescent cells. The quantitative analysis of the amount of infected cells by FACS showed that cultures infected with 25 µl or 100 µl of lot A reached 14.5% or 4.57% fluorescent cells, respectively. Upon infection, virus lots B and C produced higher and similar amounts of GFP-positive cells: 15.3% and 10.7% with 25 µl of virus and 24.0% and 28.5% with 100 µl, respectively. These results pointed to the path to improve protocols for obtaining SFV-GFP by the lipid-based transfection method. Based on the results, we chose protocol C for future optimization because it was more affordable by using more RNA but less expensive lipid complex per transfection. The transfection by the less expensive and more aggressive electroporation method is under study.

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12.34 Effect of crotoxin isolated from *Crotalus durissus terrificus* on dendritic cells

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Introduction: Dendritic cells (DCs) are potent mediators for the development of effective immunity to several pathogenic agents. These cells are considered as a principal antigen-presenting cell (APC) involved in T cell priming. As a mechanism of escape of the immune system, some pathogens are able to modulate the functional activity of the DCs, with consequent generation of antigen-specific cellular immunity. The *Crotalus durissus terrificus* venom and its main fraction, crotoxin (CTX), have the ability to suppress the immune system. Our results show that CTX is able to down-regulate the humoral and cellular responses of mice immunized with ovalbumin (OVA). We also found that CTX down-modulates the expression of MHC class II and costimulatory molecule expression on APCs in mice. **Objectives:** The aim of this study was to evaluate the effect of CTX in the functional activity of DCs as the cytokine expression/secretion and the ability to induce T cells proliferation. **Methods:** Immature DCs (iDC) were derived from BALB/c mouse bone marrow in RPMI medium containing GM-CSF/IL-4. On day 7, iDCs were incubated with OVA (200 μg/mL), CTX (5 μg/mL) or OVA+CTX for 18 h. Afterwards, the DCs were centrifuged and $0.3x10^5$ cells incubated with $TCD3^+$ cells ($1x10^5$) purified from BALB/c immunized with OVA (200 μg/animal) 7 days before. After 48h, the proliferative response was analyzed. In another experiment, iDCs were incubated with OVA (200 μg/mL), CTX (5 μg/mL), OVA+CTX, LPS (1 μg/mL) or LPS+CTX for 18 h. At this point, the supernatants were collected to quantify the TNF-α secretion, and the cells were used to obtain mRNA for the analysis of cytokine gene expression by real-time PCR. **Results and Discussion:** DCs previously incubated with OVA induced high proliferation of T cell when compared to DCs maintained with medium or incubated only with CTX. In contrast, low proliferative response was observed in cultures of T lymphocytes and DCs incubated with OVA+CTX. The quantitative real-time PCR analysis showed that LPS upregulated the expression of IL-6 and downregulated the expression of TGF-β in DCs when compared to control DCs. In contrast, CTX promoted an enhancement of TGF-β expression. Moreover, CTX inhibited the expression of IFN-γ and increased the expression of IL-10 and TGF-β LPS-induced. CTX also inhibited the expression OVA-induced of IFN-γ and IL-6 in DCs. The secretion of TNF-α was also inhibited in DCs cultured with OVA+CTX or LPS+CTX when compared to DCs incubated with OVA or LPS. The results suggest that CTX exerts a direct effect on DCs.

Supported by: CNPq and FAPESP

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12.35 Comparison of proteins sets of the venom of three different species of Brazilian *Tityus* scorpions

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Introduction: Scorpions of the genus *Tityus* are widely distributed all over Brazil. The scorpion venoms are constituted by a complex mixture of toxins: proteins of high molecular mass (MW > 10 kDa), peptides (MW 3-10 kDa) and low molecular mass compounds (MW <1 kDa). Usually proteomic studies focus on neurotoxins, with low molecular mass basic peptides. These toxins act on sodium, potassium, chloride or calcium channels on neuronal terminals, leading to the release of neurotransmitters. However, studies with proteins of high molecular weight have been neglected. Only two proteins have been detected in *Tityus* venom: hyaluronidase and an enzyme with gelatinolytic activity. **Objectives:** The aim of this study was to compare the protein (MW> 14 kDa) profile of the venom from three different species of the genus *Tityus*: *T. serrulatus* (*Ts*), *T. bahiensis* (*Tb*) and *T. obscurus* (*To*). **Methods:** Venom from *Ts*, *Tb* and *To* were obtained from the Laboratory of Arthropod, Instituto Butantan. The proteins of the venom from *Ts*, *Tb* and *To* (30 μg) were separated by SDS-PAGE (10%) and were stained with Coomassie Brilliant Blue. The gel was scanned and the density of the bands was quantified with Quantity One software (Bio-Rad). Western blotting was performed to determine whether scorpion antivenom produced by Instituto Butantan can recognize the proteins of these venoms. **Results and Discussion:** Analyses of the protein profile of the venom from three species of *Tityus* scorpions showed that 3 bands of around 74, 60 and 46 kDa are present in all three venoms. Two bands of approximately 36 and 16 kDa are present in *Ts* and *Tb* venoms. Two bands of approximately 63 and 41 kDa are exclusively in *Ts* venom and 3 bands of approximately 37, 33 and 22 kDa appear only in *To* venom. Scorpion antivenom recognizes most of the proteins of the venoms. Only the 16- and 22-kDa proteins from *Tb* and *To*, respectively, were not recognized. Our data showed marked differences between the venoms analyzed, suggesting that these proteins could have a significant role in the toxicity of the venom of scorpions, which could contribute to the pathology of the envenoming.

Supported by: CAPES

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12.36 Sexual dichromatism in neonate of snakes as a criterion for sex determination of neonate *Bothrops atrox* (SERPENTES: VIPERIDAE)

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Introduction: In snakes, sexual dimorphism occurs in several traits such as body size, tail length and head shape. Sex differences in color pattern are referred to as sexual dichromatism and are widely reported in the literature for adult individuals. However, in neonates, these data are neglected, but sexual dichromatism may appear in body color, tail color (for caudal luring) and mental scales. For neonate Crotalinae, sexual dichromatism was previously reported in color tail (for *Bothrops asper*, *Bothrops atrox* and *Bothropoides neuwiedii*) and in mental scales (for *Bothrops moojeni* and *Bothrops jararacussu*). **Objectives:** The aim of this work was to describe sexual dichromatism in neonates of *Bothrops atrox* and propose criteria for aiding sex determination of neonate *Bothrops atrox* from Acre, Brazil. **Methods:** We analyzed 13 neonates (eight males and five females) from the Federal University of Acre Herpetological Collection (UFAC). The following data were taken from each specimen: snout-vent length (SVL), tail length (TL), head length (HL) and mass. In addition, we observed the color pattern of the mental scales and tail to characterize sexual dichromatism in this species. Sex confirmation was performed by dissection of the caudal region and exposure (or not) of the hemipenis. **Results and Discussion:** None of the morphological traits analyzed (SVL, TL, HL and mass) varied between sexes. However, we observed an evident sexual dichromatism in the tip of the tail. Males showed a clearer pattern (yellowish/pale brown) of the tail tip, whereas females showed a dark pattern (dark brown). Absence of morphological sexual dimorphism and occurrence of sexual dichromatism at birth was previously reported for a population of *Bothrops atrox* from Peru and other species of the *atrox* group such as *B. asper* and *B. leucurus*. This pattern differs from most of the genus *Bothrops* in which tail tip coloration is the same for males and females. Interestingly, we observed a marked variation between sexes in the mental scales. All neonates showed a consistent pattern of pigmentation with females showing a clearer pattern than males. Sexual dichromatism in mental scales was previously reported for *Bothrops jararacussu* and *Bothrops moojeni* (member of the *atrox* complex). In field work, sexing in snakes is commonly performed by eversion of the hemipenis or visual comparison of tail length. However, due to the absence of sexual dimorphism in tail length in neonate *Bothrops atrox*, we propose the use of this sexual dichromatism as an easy and reliable way to sex neonates.

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12.37 Cloning of genomic oligonucleotides encoding key peptides of the jararhagin toxin from *Bothrops jararaca*

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Introduction: Venom toxins not only cause morbidity and mortality, but also may act as potential therapeutic drugs for a large number of diseases such as cancer. The anticancer activity of different toxins from various organisms has long been reported. Being a rich source of enzymes, venom proteins and peptides work on different targets. Jararhagin and bothropasin metalloproteinases from *Bothrops jararaca* venom contain an ECD motif in the disintegrin-like domain, which is supposed to be an integrin ligand on the surface of tumor cells. Blocking these receptors results in the inhibition of tumor cell migration, thus preventing the establishment of metastases. **Objectives:** The aim of this study was the isolation, cloning and sequencing of the genomic DNA tract encoding the ECD motif of jararhagin and bothropasin from *B. jararaca*. **Methods:** DNA was extracted from blood cells of one specimen of *B. jararaca* by the current phenol: chloroform: isoamyl alcohol procedure. PCR primers were designed for the region containing the ECD motif. Two PCR products were cloned and sequenced in order to confirm the orientation and specificity of the inserts. **Results and Discussion:** Two PCR products, DY7R-B2 and DY12R-A1, 65 bp and 135 bp long, respectively, were obtained by the use of two alternative reverse primers: *AGCAAATCAGGAACAGAATG*CCGGGC (Forward) ATCAATGAGTGAATGTGACCC*GGCTGAACACTGCACTGG* (Reverse 1) CCAA TCCTCTGAGTGTCCTGCAGATGTCTTCCATAAGAATGGACAACCATG*CCTAG ATAACTACGGTTAC* (Reverse 2) encoding the oligopeptides SKSGTECRAS MS**ECD**PAEHCT/GQSSECPADVFHKNGQPCLDNYGY. These sequences are included in the published bothropasin and jararhagin cDNA sequences. Both inserts have been purified and will be inserted into the pCDNA3.1 expression vector to obtain peptides to be used in the clinical oncology field.

Supported by: PAP/SES and FAPESP

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12.38 Topographic anatomy and ultrasound imaging of the internal organs of the pitviper (*Bothropoides jararaca*) and the rattlesnake (*Caudisona durissa*)

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Introduction: Knowledge of the topographic anatomy of a species is important to understand the functioning of the body and its diseases, and is essential in the clinical examination and interpretation of ultrasound images. Ultrasonography is becoming a strong ally for wild-animal clinicians, and it is a safe and noninvasive exam that provides information about the architecture and dimensions of internal organs, vascular structures and aids in reproductive studies. **Objectives**: The aim of this study was to contribute to the existing information regarding the visceral topography of Brazilian snakes, comparing the arrangement of the organs between *B. jararaca* and *C. durissa,* and to describe the ultrasound imaging of normal internal organs. **Methods**: For the topographic anatomy, ten adult males and ten adult females of both species, dead of natural causes, from various cities of São Paulo state were used. The animals were dissected and 25 internal structures examined for their position in cm (from the rostrum to the beginning and end of each organ), the percentage in relation to the snout vent length (SVL), and on the number of ventral scales (NVS). For the description of the ultrasound imaging of the organs, five adult males and six adult females, born in captivity and in good health were used. **Results and Discussion**: Although these two genera of snakes show differences in their external morphology and size of the scales, there is almost no difference in the position of the internal organs in relation to their SVL. The data of the relationship between the positioning of the organs and the ventral scales obtained in this study corroborate other studies. In relation to the ultrasound imaging, the ventricle muscle of the heart shows a homogeneous echotexture, with an echogenicity similar to that of the liver. The liver has a moderate echogenicity and homogeneous echotexture. The gallbladder has an anechogenic content and is easily located in the middle portion of the snake's body, serving as a reference point to locate the other organs. The pancreas is hypoechogenic in relation to the spleen. The testes have an ellipsoid shape and are hypoechogenic in relation to adjacent tissues, while the ovarian parenchyma is hyperechogenic. The ovarian follicles in stages I and II are anechogenic and follicles in stages III and IV are more hyperechogenic. The kidneys have the cortical region hyperechogenic compared to the medullary region. The coelomic fat is hyperechogenic and makes the visualization of certain organs such as kidneys and testis difficult.

Supported by: INCTTOX / FAPESP and INCTTOX / CNPq

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.39 Investigation on polymorphisms in exon 15 of the *PTCH1* gene in multiple basal cell carcinomas

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Introduction: Basal cell carcinoma (BCC) is the most common type of cancer among Caucasian populations. BCC occurs mostly sporadically influenced by sun exposure and/or aging, although its incidence is significantly increased in some rare genetic disorders. Several single nucleotide polymorphisms (SNPs) may be found in human genes. Mutations in the Hedgehog receptor Patched 1 (Ptch1), encoded by the *PTCH1* gene, have been linked to both familial and sporadic forms of BCC. So far, 750 SNPs have been reported in *PTCH1,* including some cases of non-familial MBCC. Several polymorphisms were found in exon 15, for instance, the missense mutation c.2320G>A G774R, which has an effect of amino acid change in the protein formation. **Objectives:** This study aimed to detect polymorphisms in the exon 15 of the *PTCH1* gene in Brazilian patients bearing multiple BCC and to associate each polymorphism with the susceptibility to develop this cancer. **Methods:** DNA from normal and tumor tissues from 10 patients was extracted by current methods. The search of polymorphisms was performed by PCR using intronic primers and direct sequencing (Applied Biosystems) of products. Putative point mutations were confirmed by cloning (pGEM T-easy vector, Prodimol) and sequencing the insert using T7 and SP6 plasmid primers. The sequences were compared to the published database (http://www.ncbi.nlm.nih.gov/gene/5727) for *PTCH1* and the electropherograms were analyzed. **Results and Discussion:** Seventeen putative polymorphisms, besides 11 mutations, were identified in samples of normal and, in some cases, in both BCC and normal tissues. Some of these mutations were common to several samples, suggesting that particular sites of exon 15 of the *PTCH1* gene are susceptible to alterations. The experiments are being repeated through direct genomic DNA sequencing as well as increased number of clones in order to confirm the polymorphisms in each case.

Supported by: PAP/SES

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.40 Recognition and neutralization of venom of *Bothrops atrox* snakes from Santarém - Pará using commercial antivenom produced by Instituto Butantan

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Introduction: The Santarém region has one of the highest incidences of snakebites in Brazil. These accidents are mainly caused by *B. atrox* and treated by the administration of commercial antivenom. Considering that *B. atrox* venom is not included in the pool of immunization for obtaining the antivenoms, it is particularly important to evaluate antivenom effectiveness in the treatment of envenoming in Santarém. **Objectives:** The aim of this study was to characterize the reactivity of commercial antivenom (SAB), produced by Butantan Institute, with venoms from specimens of *B. atrox* collected in the region of Santarém. **Methods:** The pool of *B. atrox* venoms used was obtained from venoms extracted from eight adult specimens captured in the Tapajos National Forest and *Bothrops jararaca* venom was obtained from Laboratório de Herpetologia, Instituto Butantan. The electrophoretic profile of the venoms was determined in a polyacrylamide gel (SDS-PAGE) and recognition of their antigens by SAB was evaluated by ELISA and Western blotting. The neutralization of venom lethality was assessed by *i.p.* injection of 3DL50 venoms pre-incubated with increasing volumes of SAB, and the neutralization of hemorrhagic activity was estimated by measuring the hemorrhagic area in the dorsal skin of mice, 3 h after injection *i.d.* of 10 µg of venom pre-incubated with different volumes of SAB. **Results and Discussion:** SAB recognized the venom of *B. atrox* with an antibody titer of 320,000, slightly below that of 640,000 obtained against the venom of *B. jararaca*. The electrophoretic profile of the venom of *B. atrox* was similar to *B. jararaca* showing a predominance of proteins with molecular mass above 30 kDa, and a minor fraction with lower molecular mass compatible with phospholipases. The SAB strongly recognized the antigens of the venoms of *B. atrox* and *B. jararaca* in a non-reduced condition and weakly in the reduced form. Neutralization tests also showed satisfactory neutralization of lethality and hemorrhage induced by the venom of *B. atrox*, however, with doses slightly higher than that recommended by the manufacturer. These results indicate the effectiveness of SAB in the recognition and neutralization of antigens contained in the venom of *B. atrox*, even though it is not part of the pool of immunization for obtaining SAB.

Supported by: INCTTOX (MCT/CNPq/FAPESP)

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.41 Comparative analysis of protein composition and reactivity with anti Bothropic serum of venoms from snakes of different genera, according to a new classification

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Introduction: Viper venoms have been recently subjected to wide taxonomical revisions. The genus *Bothrops*, involved in approximately 90% of snakebites reported in Brazil, underwent a new classification in 2009 giving rise to three genera, *Bothropoides, Bothrops* and *Rhinocerophis*, together with *Bothropoides* and *Bothrocophias* genera, split from *Bothrops* in a previous taxonomic revision. Snake phylogeny may be represented in venom composition with implications in the production of antivenoms and treatment of envenoming (Fenwick et al., 2009). **Objectives:** The aim of this study was to analyze the chromatographic profile of venoms according to the new classification and their reactivity with anti-bothropic antivenom produced in Instituto Butantan. **Methods:** For phenotyping, 5 mg of venoms of *Bothropoides jararaca, Bothropoides neuwiedi, Bothrops atrox, Bothrops jararacussu, Rhinocerophis cotiara, Rhinocerophis alternatus, Bothriopsis bilineta* and *Bothrocophias hyoprora* were submitted to reverse-phase chromatography in HPLC C18 column. Identity of the fractions was inferred by the column retention time according to previous reports, ELISA and dot-blot were performed to check the reactivity of the venoms and their fractions with the antivenom. **Results and Discussion:** Venoms showed distinct chromatographic profiles, with no correlation with phylogeny. For instance, *Bothropoides jararaca/neuwiedi* venoms had a diverse chromatogram as well as *Bothrops atrox/jararacussu* venoms. In *Bothrops* genus, *B. atrox* showed the greatest abundance of snake venom metalloproteinases (SVMPs), while phospholipases were more abundant in the venom of *B. jararacussu. B. neuwiedi* showed the highest diversity of SVMP isoforms. In opposition, venoms of the genus *Rhinocerophis (alternatus* and *cotiara*) showed relatively similar profiles. *B. bilineta* and *B. hyoprora* venoms were the least complex. The antivenom recognized venoms of different genera with similar antibody titers (256,000), with a single dilution difference when titered against *B. atrox, B. bilineta* and *B. hyoprora.* For all venoms tested, antivenoms strongly recognized fractions that correspond to class P-III SVMPs. Although the number of venoms tested is still limited, our data indicate that there was no apparent correlation between the new proposed phylogeny with venom composition based on chromatographic profiles of venoms. Moreover, the commercial antivenom tested recognized similarly the venoms of all genera, with predominance of SVMPs, which are key toxins for venom-induced pathology in cases of snake bite.

Supported by: INCTTOX (MCT/CNPq/FAPESP)

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30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.42 *Ascaris suum* experimental infection modulates acute inflammation via CD4+CD25+FoxP3+ T cells

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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 downregulates pulmonary allergic inflammation and LPS-induced acute inflammation. Furthermore, the regulatory cytokine IL-10 was implicated as an effector molecule of *Ascaris suum*-mediated suppression. **Objectives:** In the present study we investigated the expansion of T cell clones by *Ascaris suum* infection and the effect of these cells on LPS-induced acute inflammation. **Methods:** BALB/c mice were infected by the intragastric route with 2500 *Ascaris suum* eggs. A week later, the mesenteric lymph node cells were recovered, and the expression of CD4+CD25+FoxP3+ markers from non-infected and Ascaris-infected mice was detected using PE-Cy5.5-anti-CD25, FITC-anti-CD4 antibodies and PE-anti-FoxP3 antibodies. All samples were acquired using FACSCanto and analyzed by FlowJo. The TCD4+CD25- and TCD4+CD25+ cell populations from infected or non-infected mice were purified from lymph node cells by magnetic activated-cell sorting (MACS). These cells were adoptively transferred to recipient mice by the intravenous route, and 3 days later, the air pouches induced by sterile air injections on the back of the recipient mice were LPS-injected. Three hours after the stimulation, the air pouch exudates were recovered for the measurement of cytokine levels by ELISA and quantification of cellular migration. **Results and Discussion:** FACS analyses showed that the total CD4+ T cell numbers did not differ in *Ascaris suum*-primed and non-primed total cells. The number of CD25+FoxP3+ cells gated on CD4+ cell population presented a 4 fold increase in Ascaris-primed total cells compared to non-primed cells. Furthermore, CD4+CD25-FoxP3+ cell numbers were also higher in Ascaris-primed total cells (14.1%) compared to non-primed cells (4.8%). Regarding the suppressive effect of these cell clones, our results demonstrated that adoptive transfer of $CD4^{+}CD25^{+}$T cells from infected mice resulted in a decrease in leukocyte recruitment (3.5-fold) and inflammatory cytokine levels (IL-1β, IL-6 and TNF-α), but increase in IL-10 and TGF-β cytokines. On the contrary, transfer of $TCD4^{+}CD25^{-}$ cells or $TCD4^{+}$CD25+ from non-infected mice had no effect on leukocyte influx or cytokine levels compared to the positive control group. In our previous studies, the regulatory cytokine IL-10 has been implicated in the down-regulatory mechanisms induced by *Ascaris suum*. The results herein indicate that the source of these cytokines may be $CD4^{+}CD25^{+}$ T cells. Thus, when these cells, but not other T cell types, were obtained from lymph nodes of *Ascaris*-infected mice, they prevented the inflammatory response induced by LPS.

Supported by: CNPq

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12.43 Daily activity and substrate use in captive *Dipsas bucephala*

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Introduction: The genus *Dipsas* is widely distributed throughout the Neotropical region. Diet is highly specialized focusing on slugs and snails, and habits are considered semi-arboreal and activity as nocturnal. However, the duration of the daily activity had not yet been investigated, nor had the differences in the substrate used for activity or resting. **Objectives:** The present work aimed to characterize the daily activity patterns, as well as substrate use, in captive *Dipsas bucephala*. Moreover, we determined the influence of food availability on the duration of the activity. **Methods:** Three individuals of *D. bucephala* were kept in a glass terrarium measuring 50 x 25 × 35 cm, with a water dish, branches, natural litter and natural photoperiod. For characterizing the daily activity and the substrate use, snakes were continuously filmed for 20 days (10 days with available food (slugs and snails) and 10 days without food. Activity records were computed using spreadsheets, composed of 48 intervals of half-hour each. The schedules of greater activity and the most used substrate during this period were analyzed, for the whole study period. To characterize the use of resting substrate, two daily observations were made (in the morning and in the afternoon), during continuous 10 days, for each of the three individuals. In such observations, the place where the animal was found while resting was recorded, where it was differentiated as branches or ground. **Results and Discussion:** Activity in *D. bucephala* was recorded between 5:30 pm and 6:00 am, that is, almost all the nocturnal period. There were no considerable differences in the duration of the activity with or without availability of food. With food available, activity lasted 13 h (from 5:30 pm until 6:30 am), whereas without food available, activity lasted 11 h 30 min (from 6 pm until 5:30 am). The activity peak was higher with food present, (8 h 30 min) than without available food (6 h 30 min). Thus, food availability seems to influence directly the activity peak. Regarding the use of the substrate, the branches (61%, n = 2836) were significantly more used than the ground (39%, n = 1784) during the activity period. During the resting period, there were no differences on the selected substrate (ground = 60% *vs.* twigs = 40%; P = 0.12; n = 60). However, snakes did not use resting substrate randomly. In 75% of the instances, animals were hidden under the leaves instead of resting on the litter. Such analyses corroborate the semi-arboreal habits for the genus. Nevertheless, they suggest differences in the pattern of substrate use by *D. bucephala* in relation to the displayed activity (foraging or rest).

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12.44 Anatomical topography of the genus *Micrurus*

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Introduction: The family Elapidae snakes is composed the species that possess a pair of fixed proteroglyph fangs at the front of the upper jaw. The vast majority of species has the typical coloration of coral snakes, with rings around the body, and contrasting colors, red, yellow (or white) and black, in typical arrangements, such as black rings around singly or arranged in triads. This species occurs south of the Amazon Basin in Brazil, the habitat is tropical and subtropical deciduous forest, and littoral areas. They have fossorial habits. In Brazil, some *Micrurus* species are extremely rare and little-known, sometimes confined to very restricted geographical areas. **Objectives:** The aim of this study was stipulate the anatomical topography of the coelomic structures in the snake genus *Micrurus*. **Methods:** Adult specimens of *Micrurus sp.* (5 males and 7 females) that they had died of natural causes were used for the anatomical topography study. Before dissection, the biometric data, such as weight, snout-vent length (SVL), total length (TL), caudal length (CL) and the count of ventral scales and flow rates were recorded. The ventral scales were marked with 10 in 10 projector pen, considering the first ventral scale as the one that has a length greater than the width and the last preceding the anal scale. The dissection process was initiated through an incision in the middle portion of the body, which remained in cranial and caudal cut in the midline of the ventral scales. Once cut, the skin was folded back and secured with pins laterally on a dissecting table, starting with the visual recognition of each body, determining the beginning and end of the same in centimeters and correlating them with the number of ventral scales. With CRC snake equivalent to 100% (from head to vent), the beginning and end of each organ were located, in percentage, the body of the snake. **Results and Discussion:** The three-chambered heart was located at 23% of the SVL from the head. The liver took up more than half of the third middle of the snake's body (on average, it began at 30% of the SVL from the head and ended at 60%). The gallbladder was located at 76% of the SVL from the head. The esophagus in the genus *Micrurus* occupies more than 50% of the animal's body, because this genus feeds on other snakes. Because of their eating habits, i.e., they have a diet composed of small snakes and other serpentine reptiles, the esophagus is longer in proportion to its body compared to other snakes, demonstrating a strong anatomical adaptation of the genus.

Supported by: INCTTOX/FAPESP and INCTTOX/CNPq

XIII Reunião Científica Anual do Instituto Butantan
30 de Novembro a 02 de Dezembro de 2011, São Paulo, SP

12.45 Natural history of *Rhinella granulosa* from Caatinga especially regarding the adaptations against desiccation

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Introduction: The toads from the group *Rhinella granulosa* have a wide distribution in South America, inhabiting from humid forests (Amazonian and Atlantic) to the Brazilian semi-arid region (Caatinga), always associated with open areas. They are small but robust, with a very dry and rough skin and, during the day, remain hidden in the ground inside cracks among rocks, tree trunks or breaks in the soil, probably as an adaptation against desiccation. **Objectives:** The aim of this study was to describe the natural history of a population of *Rhinella granulosa* from the Caatinga, making correlations with skin morphology and its adaptations against water loss. **Methods:** Skin samples from dorsal, ventral, inguinal, chin and parotoid regions were fixed in Bouin solution and embedded in paraffin or glycol methacrylate. Sections of 5 μm were stained with HE or toluidine blue-fuchsin, and submitted to PAS, alcian blue, pH 7.2, bromophenol blue and von Kossa histochemical reactions. **Results and Discussion:** The dorsal skin has a typical relief, with many cornified peaks. The ventral skin has a smooth surface, with many folds, especially in the inguinal region, which has an important role in water uptake from the substrate. The whole skin bears two glandular types: the mucous glands, small and with two types of cells, and the granular glands, much larger, especially in the parotoid macroglands. Granular glands do not have a lumen and are composed of a syncytium with nuclei in the periphery and filled by spherical granules immersed in a homogeneous matrix. The calcified dermal layer, positive to the von Kossa method, is continuous in the dorsum and thin and discontinuous in the ventral skin. The compact dermal layer is constituted by well-arranged collagen fibers. The *lamina propria* is rich in blood vessels, which in the ventral skin can reach more superficial layers, extending to the epidermis. The cutaneous ornamentation of *R. granulosa* from the Caatinga can be related to water collecting from the environment. Also, the dorsal thick calcified dermal layer may prevent water loss while the ventral thin calcified layer, together with the large number of superficial vessels may help water uptake from the substrate. The skin characteristics within the group *Rhinella granulosa* added to the versatility of these animals in occupying both humid and dry environments may confer to these animals a large ability for dispersion which results in a widespread distribution. Broader comparative studies encompassing other species in the group are needed for a better understanding of the adaptive capabilities of these anurans to different types of environment.

Supported by: CNPq, INCTTOX and PAP/SES