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EDITORIAL

The 67th volume of "Memórias do Instituto Butantan" presents the proceedings of the XII Annual Scientific Meeting of Butantan Institute, organized by the Division for Scientific Development. The theme of the 12th edition of the Instituto Butantan's meeting is "Biotechnology and Innovation in Public Health: Perspectives for the Next Decade."

The scientific program addresses important issues regarding new biotechnology approaches for the solution of public health problems and development of human resources. Three theme sessions and a conference were planned in order to discuss these topics.

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 its Scientific Collections, and a tribute to Prof. Henriques Moisés Canter.

The 67th 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 Diffusion; Others; Scientific Initiation (PIBIC) and PAP program. The impressive number of submitted posters indicates the involvement of our scientific community with the Annual Scientific Meeting. These abstracts are representative of the scientific research that was carried out at Instituto Butantan in the last year. Therefore, the "Memórias do Instituto Butantan" electronic issue is an important source of scientific information and knowledge dissemination.

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XII Annual Scientific Meeting of Instituto Butantan

December 01-03, 2010

Biotechnology and Innovation in Public Health: Perspectives for the Next Decade

Scientific Program

Tuesday 11/30/2010

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

Coordinator: Norma Yamanouye

14:00 – 15:00 **Conference**

Peptide toxins that affect the gating of sodium channels

Paulo Sérgio Lacerda Beirão – Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

15:00 – 16:45 **Mini-Conferences**

15:00 – 15:30 **Mechanisms involved in the hemorrhagic action of metalloproteases from snake venoms: tissue distribution and *in situ* hydrolysis**

Cristiani Baldo – Laboratório de Imunopatologia, Instituto Butantan

15:30 – 15:45 **Coffee break**

15:45 – 16:15 **Formation of lipid bodies induced by a phospholipase A2 from snake venom: mechanisms involved in this effect**

	Elbio Leiguez Junior – Laboratório de Farmacologia, Instituto Butantan
16:15 – 16:45	**Proteomics and transcriptomics applied to the study of the variability of *Bothrops jararaca* venom**
	André Zelanis – Laboratório Especial de Toxinologia Aplicada, Instituto Butantan
16:45 – 17:15	**Oral Presentation**
	Detection of proteins in the microvesicles present in the venom of *Crotalus durissus terrificus* snake
	Andréia de Souza, Sylvia M. Carneiro, Fernanda Sakai, Sávio S. Sant´Anna, Wilson Fernandes, Norma Yamanouye

Wednesday 12/01/2010

09:00 - 09:30	**Opening Session**
09:30 - 12:30	**Thematic Session I: Creating an environment for innovation**
	Coordinator: Ivo Lebrun – Instituto Butantan
09:30 - 10:10	**Role of the technological parks in the stimulation of innovation in public health**
	Pedro Primo Bombonato – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo
10:10 - 10:50	**From genomic sequencing to the biotechnology industry. The ALELLYX experience**
	Paulo Arruda – Instituto de Biologia, Universidade Estadual de Campinas
10:50 - 11:10	**Coffee break**
11:10 – 11:50	**Evaluation and perspectives of the Graduate Program Interdisciplinary Units in Biotechnology USP/IPT/Instituto Butantan: contribution of Instituto Butantan**

Ana Clara Guerrini Schenberg – Instituto de Ciências Biomédicas, Universidade de São Paulo

11:50 - 12:30 **Research, development and production**
Hernan Chaimovich Guralnik – Fundação Butantan

12:30 – 13:00 **Interval**

13:00 – 15:00 **Poster Session I**
1. Venoms and Envenomations
2. Biochemistry
3. Pharmacology
11. Scientific Initiation (PIBIC)

15:00 – 17:30 **Young Scientist Award - Scientific Initiation and PAP Program**

Award Committee:
Rodrigo Hirata Willemart – Escola de Artes, Ciências e Humanidades, Universidade de São Paulo
Wagner Ricardo Montor – Departamento de Ciências Fisiológicas, Faculdade de Ciências Médicas da Santa Casa de São Paulo
Zulma Felisbina da Silva Ferreira - Instituto de Biociências, Universidade de São Paulo

15:00 – 16:00 **Scientific Initiation Award**
Coordinator: Yara Cury – Instituto Butantan

16:00 – 16:30 **Coffee break**

16:30 - 17:30 **PAP Program Award**
Coordinator: Isabel de Fátima Correia Batista – Instituto Butantan

Thursday 12/02/2010

09:00 – 12:00	**Thematic Session II: Pathways to the development of drugs** Coordenadora: Ana Marisa Chudzinski-Tavassi – Instituto Butantan
09:00 - 09:40	**Strategies of biopharmaceutical production in Brazil** Spartaco Astolfi Filho – Universidade Federal do Amazonas / Cristália
09:40 - 10:20	**National Institute of Science and Technology of Drugs and Medications (INCT-INOFAR) and the pharmaceutical technology innovation** Eliezer Jesus de Lacerda Barreiro – Faculdade de Farmácia, Universidade Federal do Rio de Janeiro
10:20 – 10:40	**Coffee break**
10:40 - 11:20	**Speeding up drug discovery with phenotypic HTS** Fernando Dossin – Institut Pasteur Korea
11:20 – 12:00	**Nanotechnologies: development, opportunities and challenges** Oswaldo Luiz Alves – Instituto de Química, Universidade Estadual de Campinas
12:00 – 13:00	**Interval**
13:00 – 15:00	**Poster Session II** 7. Cellular Biology and Genetics 8. Animal Biology 10. Others 12. PAP Program
15:00 – 17:30	**Young Scientist Award – Master and Doctoral Degrees** **Award Committee:**

Carlos Alberto da Silva – Centro de Ciências Naturais e Humanas, Universidade Federal do ABC

Rosana Camarini – Instituto de Ciências Biomédicas, Universidade de São Paulo

Sônia Godoy Bueno Carvalho Lopes - Instituto de Biociências, Universidade de São Paulo

15:00 – 16:00 **Master Degree Award**

Coordinator: Norma Yamanouye – Instituto Butantan

16:00 – 16:30 **Coffee break**

16:30 - 17:30 **Doctoral Degree Award**

Coordinator: Ana Maria Moura da Silva - Instituto Butantan

Friday 12/03/2010

09:00 – 11:00 **Thematic Session III:** Therapy and vaccines: challenges and perspectives

Coordenador: Hugo Aguirre Armelin – Instituto Butantan

09:00 - 09:40 **Immunotherapy and opportunities for innovation: anticancer vaccines and humanized antibodies**

Fernando Thomé Kreutz – FK Biotecnologia

09:40 - 10:20 **Viral vaccines**

Ricardo Galler – Biomanguinhos, Fundação Oswaldo Cruz

10:20 - 11:00 **The importance of angiogenesis and vascular heterogeneity in the development of new therapies**

Ricardo José Giordano – Instituto de Química, Universidade de São Paulo

11:00 - 11:20 **Coffee break**

11:20 - 12:15	**Conference** **What synthetic biology can do for public health?** Jack D. Newman – Amyris, Canadá
12:00 – 13:00	**Interval**
13:00 – 15:00	**Poster Session III** 4. Immunology and Vaccines 5. Microorganisms 6. Biotechnology 9. History, Education and Scientific Dissemination
15:00 – 15:30	**Instituto Butantan Flashbacks** **From the cabinets of curiosities to the scientific collections of Instituto Butantan** Camilla Carvalho, Fan Hui Wen, Roberto Henrique Pinto Moraes and Sávio Stefanini Sant´Anna - Instituto Butantan
15:30 – 15:50	**Special Session** **In honor of Prof. Henrique Moisés Canter** Giuseppe Puorto – Instituto Butantan
15:50 – 16:30	**Young Scientist Award**
16:30 – 17:00	**Closing Session**

1. Venoms and Envenomations

1.01 Comparison of Bothropoides jararaca bites with and without envenoming treated at Hospital Vital Brazil of Instituto Butantan, São Paulo, Brazil

Nicoleti AF[1,2], Medeiros CR[2], Duarte MR[3], França FOS[1,2]
[1]Departamento de Moléstias Infecciosas e Parasitárias, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil; [2]Hospital Vital Brazil, [3]Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: In Brazil, 90% of the venomous snake bites are caused by *Bothrops, Bothropoides, Bothriopsis, Bothrocophias* and *Rhinocerophis*, predominantly in hot and rainy months. *Bothropoides jararaca* is widespread in south, southeastern and part of northeastern Brazil. Due to the fact they have a great adaptive capacity, it is the predominate species in São Paulo City and neighborhood. **Objectives:** The objective of this study was to examine the cases of all bites (including dry bites) caused by Bothropoides jararaca treated at the Hospital Vital Brazil do Instituto Butantan, São Paulo, Brazil (HVB), only when the snake was brought by the victim, examining epidemiological, clinical and laboratory aspects. **Methods:** A retrospective study was made in patients bitten by *Bothropoides jararaca* (n= 792) between 1990 to 2004 in Hospital Vital Brazil, São Paulo, Brazil. The data were obtained from medical records. **Results and Discussion:** The majority of the cases in this study were caused by female and juvenile snakes. This could be explained by the fact that *Bothropoides jararaca* females grow to greater lengths than males. Due to this fact as well as to pregnancy, females have higher food requirements and forage more actively, which demands more thermal control, and consequently, they become more susceptible to human encounters. Juvenile snakes probably also cause more bites because visualization of the snake is more difficult. No stomach contents were found in 93.4% of the snake specimens. There was no statistical difference between the occurrence of dry bites and maturity or sex of the snake. We observed that necrosis was more common in the digits of the feet and hands (4.8%) compared to the other body regions (1.8%). It is possible that there is a likelihood of developing compartment syndrome of the lower limbs is greater than in other regions of the limbs. A significant difference was found between severity and time interval greater than 6 hours between the bite and hospital admission. We found a significant association between gingival bleeding and abnormal blood coagulability. It was observed that in accidents caused by adult snakes, necrosis was more frequent (7.2%) when compared to accidents caused by juvenile snakes (1%). In this work, we highlight the association between some epidemiological data and biological parameters evolved in the clinical picture of *Bothrops*-like accidents, contributing to the improvement of snake bite care.

1.02 Unusual bites by Bothrops-like species treated at Santarém Municipal Hospital, Pará, northern Brazil

Torrez PPQ[1], Nicoleti AF[2], França FOS[2], Duarte MR[3]
[1]Núcleo de Extensão em Medicina Tropical - Convênio do Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina da Universidade de São Paulo, SP, e Secretaria Municipal de Saúde de Santarém, PA, Brasil; [2]Hospital Vital Brazil, [3]Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: Ninety percent of venomous snake bites in Brazil are caused by *Bothrops, Bothropoides, Bothriopsis, Bothrocophias* and *Rhinocerophis*, mainly in warm and rainy months. In 2008, there were reports of 26,654 snakebites with a fatality rate of 0.44%. The *Bothrops*-like snakes were responsible for 86.9%. The venom has local acute inflammatory, coagulant and hemorrhagic effects. The most common signs and symptoms include pain, swelling and bleeding, occurring in the first six hours after envenomation. The municipality of Santarém, State of Pará, is characterized by a significant number of accidents by venomous animals, mainly *Bothrops*-like snakes. **Objectives:** Retrospective study of data obtained from medical records. Case 1: A 43-year-old man was bitten on the forehead, by a green snake that was on a tree, when he was fishing. He denied any symptoms. Physical examination revealed only one perforating mark. Clotting time (CT), hematologic parameters and renal function were normal. The accident was considered a dry bite. Case 2: A 30-year-old female was bitten on the head by a green snake, while working in the field. She presented with pain, swelling and bleeding at the bite site, nine hours after the accident when she was admitted to the hospital. The accident was classified as mild and she received five vials of antivenom. CT at admission was normal. Case 3: A 12 years old female student was bitten in medial right thigh, where she presented an extensive edema and pain. She sought medical care four hours after the accident. The initial CT was incoagulable and the accident was classified as moderate. She received eight vials of antivenom. Case 4: A 43-year-old farmer was bitten on the head by a snake, when he was hunting. He denied any symptomatology and his physical examination was normal. The accident was classified as a dry bite. CT, blood parameters, and renal function were normal. **Methods:** Retrospective study of data obtained from medical records. **Results and Discussion:** Although the snakebites in the centripetal body are rare, this localization has variable severity. Some cases develop with local complication such as necrosis and infection, systemic complications such as airway obstruction due intense edema and uncommonly death, while others develop without any manifestations (dry bite). In general, these accidents occur in particular epidemiological situations: large and/or arboreal snakes, patient is lying down or sitting on the ground or the snake is in a ravine. Conclusion: Although this unusual bite site is rare, it is important to point out that this accident could be fatal and that preventive actions are very difficult.

Supported by: INCTTOX Program - CNPq, FAPESP

1.03 Effect of DM43 and venom peptides on the proteolytic activity of metalloproteinases of *Bothrops jararaca* venom

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[1]Laboratório Especial de Toxinologia Aplicada/CAT-cepid, Instituto Butantan, SP, Brasil; [2]Laboratório de Toxinologia de Manguinhos, Instituto Oswaldo Cruz, RJ, Brasil

Introduction: Snake venom metalloproteinases (SVMPs) play a significant role in envenomation, causing local hemorrhage and necrosis. SVMPs isolated from *B. jararaca* show different proteolytic and hemorrhagic activities: HF3 (P-III class) is highly glycosylated and is an extremely hemorrhagic SVMP; bothropasin (P-III class), which has a minor carbohydrate moiety, is a highly proteolytic protease; and BJ-PI (P-I class) is a potent proteolytic enzyme but is not hemorrhagic. Previous studies have shown that the protein DM43, isolated from *Didelphis marsupialis* serum, inhibited *B. jararaca* crude venom hydrolysis of casein, fibronectin and fibrinogen and the hemorrhagic effect of jararhagin, a P-III SVMP from *B. jararaca*. Bradykinin-potentiating peptides (BPPs) are involved in the symptoms of hypotension observed upon envenoming by *Bothrops* species. The venom tri-peptides pEQW and pEKW are known to inhibit SVMPs by occupying the S^{-1} pocket of the proteinase with the tryptophan residue. Recently, it was demonstrated that the venom from *Echis ocellatus* contains an unusual pHpG peptide which inhibits the venom hemorrhagic activity. **Objectives:** To analyze the effects of the venom peptides BPP10c, BPP5a, pEKW and pHpG1 and the protein DM43 on the proteolytic activity of HF3, bothropasin and BJ-PI. **Methods:** HF3, bothropasin and BJ-PI were preincubated with 5 mM of each peptide for 30 min at room temperature and incubated with casein, fibrinogen and collagen VI for 1 h at 37°C. Native and N-deglycosylated HF3 and native bothropasin were preincubated with DM43 (1:1 and 1:2, enzyme-to-inhibitor ratio) for 40 min at room temperature and incubated with fibrinogen for 1 h at 37°C. Proteins were incubated without enzyme under identical conditions. The reactions were stopped by adding the Laemmli sample buffer and submitted to SDS-PAGE (12% polyacrylamide gels). Gels were silver stained. **Results and Discussion:** BPP10c did not affect the degradation of fibrinogen and collagen VI by all SVMPs but enhanced the caseinolytic activity of BJ-PI. BPP5a altered the hydrolysis pattern of all SVMPs on fibrinogen. Moreover, it enhanced the caseinolytic activity of BJ-PI but did not affect the degradation of collagen VI by SVMPs. pHpG1 affected the degradation of collagen VI and fibrinogen but did not affect the caseinolytic activity of the SVMPs. DM43 inhibited the fibrinogenolytic activity of bothropasin but had no effect on native and N-deglycosylated HF3. These data indicate that the SVMPs are differently affected by the peptides and DM43 probably due to their diverse tertiary structures.

Supported by: FAPESP

1.04 Effects of pre-natal exposure to *Tityus bahiensis* scorpion venom on the reproductive development of pregnant dams and on the development of pups

Dorce ALC[1,2], Dorce, VAC[1], Nencioni, ALA[1]
[1]Laboratório de Farmacologia, Instituto Butantan, SP, Brasil; [2]Programa de Pós-Graduação em Ciências, Centro de Controle de Doenças, Ministério da Saúde, SP, Brasil

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 literature about the toxic effects of this venom. **Objectives:** The objective of this work was to determine possible toxicological effects of the *Tityus bahiensis* scorpion venom on maternal reproductive development and on the development of pups, when administered to pregnant dams. **Methods:** The dose of the venom used was 2.5 mg/Kg. The venom was administered to two groups on the 5th (GD5, n=5) or on the 10th (GD10, n=10) gestational day. The control group (C, n=10) received 1.46% NaCl on both days. On the 21st gestational day, the pups were taken out through a laparotomy and divided into three groups that received specific treatments for skeletal, visceral and histological analyses. On the following day after laparotomy, the pups selected for skeletal analysis had their organs removed and weighted. **Results and Discussion:** No changes in maternal weight were observed during the gestational period. There were no alterations in the number of implantations (C=7.1±0.4; GD5=6.0±0.3; GD10=6.5±0.3), reabsorptions (C=0.4±0.1; GD5=0.2±0.1; GD10=0.1±0.06), corpus luteum (C=7.4±0.4; GD5=7.0±0.4; GD10=7.1±0.3), on the number of pups (C=6.6±0.3; GD5=5.8±0.4; GD10=6.4±0.4), or in the number of live pups (C=6.6±0.3; GD5=5.7±0.5; GD10=6.3±0.4). There were no changes in uterus weight (C=99.5±4.3; GD5=95.2±6.6; GD10=94.8±3.6). Also there were no changes to the weight of the pups (C=5.1±0.1; GD5=5.7±0.04; GD10=5.8±0.4). There was an alteration in the weight of the placentas in GD5 and in GD10 (C=0.49±0.01; GD5=0.54±0*; GD10=0.53±0.01*). In relation to the development of the pups, some changes were observed, such as in heart weight (C=0.04±0.01; GD5=0.06±0.0*1; GD10=0.05±0.01*) and lung weight (C=0.14±0.01; GD5=0.15±0.01*; GD10=0.15±0.01*) on GD5 and GD10, and in liver weight (C=0.47±0.01*; GD10=0.58±0.01*) on GD10. The moderate envenomation by *Tityus bahiensis* scorpion venom causes subtle changes in maternal reproductive development and in fetal development.

Supported by: CAPES, Fundação Butantan

1.05 Detection of proteins in the microvesicles present in *Crotalus durissus terrificus* snake venom

Souza A[1], Carneiro SM[2], Sakai F[1], Sant'anna SS[3], Fernandes W[3], Yamanouye N[1]
[1]Laboratório de Farmacologia, [2]Laboratório de Biologia Celular, [3]Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: Microvesicles are known structures that shed from the surface of many cells. These small specific structures have proteases or specific inhibitors that can modulate the activation of zymogen and can therefore play an important physiological and pathological role. Numerous electron-dense microvesicles (40 – 80 nm in diameter) are observed on the luminal side of secretory cells of the venom gland and in the venom of *Crotalus durissus terrificus.* Microvesicles found in the *Crotalus durissus terrificus* venom originate from microvilli by fragmentation or membrane budding and have intramembranous particles on the cytoplasmic leaftet, suggesting the presence of transmembrane proteins. **Objectives:** The aim of this study was to determine the presence of proteins in the microvesicles present in *Crotalus durissus terrificus* venom. **Methods:** The venom used was manually extracted from *Crotalus durissus terrificus* maintained in the Laboratory of Herpetology at Instituto Butantan. A volume of 5.0 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 was ultracentrifuged at 200,000 g for 60 min at 4°C, and the pellet was resuspended in 20 ml of cold PBS and ultracentrifuged again under the same conditions. The resulting pellet was processed for morphological analysis using transmission electron microscopy or for protein analysis by one-dimensional (1-DE) and two-dimensional (2-DE) electrophoresis. The density of the spots in 2-DE was quantified using ImageMaster 2D Platinum 7. **Results and Discussion:** Morphological analysis showed that after ultracentrifugations the microvesicles kept their morphology. Analysis of protein profile of microvesicle extracts in 1-DE showed s band of approximately 66 kDa and bands between 116 and 200 kDa which were not found in the protein profile of the venom. Analysis of the protein spots in the 2-DE images showed many stained spots that are present in microvesicles extract but not in the venom. Only some spots are present in both microvesicles extract and venom. The proteins detected only in microvesicles are: 1) approximately 180 kDa with PI around 4; 2) ranging from 60 to 70 kDa with PI ranging from 7 to 9; 3) 11 kDa with PI 4; 4) approximately 10 kDa with PI ranging from 5 to 7 or 9 to 10. Our data showed that the microvesicles have proteins that differ from the venom proteins, suggesting that these proteins could have important role such as regulating the activity of toxins from the venom, or even having a biological activity that contributes to the pathology of the envenoming.

Supported by: Fundação Butantan, FAPESP

1.06 Expression of adhesion molecules by different metalloproteases isolated from Bothrops: role of different domains

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Introduction: Snake venom metalloproteinases (SVMP) are major toxins involved in inflammatory reactions at the site of the bite in human envenoming. Depending on the domain composition, SVMP can be classified as P1 to P4. Three toxins were used: Jararhagin (JAR) and JAR-C, isolated from *B. jararaca* venom, and BnP1, isolated from B. neuwiedi venom. JAR, a P3 SVMP with a strong hemorrhagic activity, comprises catalytic, disintegrin-like, and cysteine-rich domains. JAR-C is a degraded form of JAR devoid only of the catalytic domain, with no hemorrhagic activity. BnP1, a weakly hemorrhagic P1 SVMP, has only the catalytic domain. **Objectives:** In this study, the participation of different domains of SVMP on alterations of leukocyte-endothelium interactions (LEI) in the microcirculation of the cremaster muscle of mice was evaluated as well as the expression of the adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31), responsible for adhesion and cell migration, respectively. **Methods:** JAR, JAR-C or BnP1 (0.5µg) or PBS (100µL) were injected into the scrotal sac of mice, and the microcirculation of cremaster was analyzed by intravital microscopy 2 or 24 h after the injections. A 100-µm segment of a post-capillary venule was observed for 5 min, and leukocytes rolling, adhered and emigrated were counted. The cremaster muscle was isolated and incubated with antibodies against CD54 and CD31 tagged with fluorescence, 2, 4 or 24 h after the toxin injections to evaluate the expression of integrins. **Results and Discussion:** A significant decrease in leukocyte rolling was observed 2 h after toxin injection. Adhered and emigrated leukocytes were increased at all times studied. There was a high increase in expression of CD54 in the first hours (2 and 4 h) analyzed and a decrease 24 h after the injection. Related to the CD31 immunostaining table, we observed a progressive increase related to time, when compared to the controls, with a similar distribution in the microcirculation in the period studied. It is suggested that the alterations observed in the microcirculation occur in the expression of the adhesion molecules CD54 and CD31.Despite differences in hemorrhagic activities and domain compositions of the three toxins used, the dose of toxins used induced alterations in leukocyte-endothelium interaction of similar magnitude. In conclusion, our results suggest that catalytic, disintegrin-like, and cysteine-rich domains of these *Bothrops* SVMP can induce alterations in leukocyte-endothelium interactions mediated by CD54 or CD31.

Supported by: FAPESP

1.07 Analysis of differential gene expression of venom gland of *Bothrops jararaca*

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Introduction: The venom gland of the Brazilian venomous snake *Bothrops jararaca* (Crotalinae, Viperidae) is an exocrine tissue related to the salivary gland. When the venom is released, the production of new venom is triggered by the activation of noradrenaline on both α1- and β-adrenoceptors. However, the genes involved and the regulation of venom production cycle are poorly known. Here we describe the construction and first analysis of nylon cDNA arrays aiming to identify the most differentially expressed genes of *Bothrops jararaca* venom glands. **Objectives:** The aim of the study was to identify the gene expression pattern of venom gland of *Bothrops jararaca*. **Methods:** The cDNAs arrays were constructed with 4608 clones from male and female B. jararaca cDNA libraries, using the methodology that consists of bacterial clones grown on a nylon membrane. The venom gland was removed from snakes in which venom was not manually extracted (0 days), and from snakes in which venom was extracted 1, 2, 4 and 15 days before sacrifice. We used 3 snakes, all adult males, for each experimental condition. RNA was extracted and converted into cDNA which was used as the probe for hybridization of cDNA arrays. For probe labeling and array hybridization, we used Direct Labeling and Detection System with CDP-Star (GE Healthcare).The image obtained was scanned and analyzed using Bzscan 2 software. All statistical analyses were performed using R language and the package LIMMA3 from the Bioconductor project. Clones with FDR values <0.05 and B-statistics >0 were deemed differentially expressed and selected for the next analysis. Gene expression patterns at the investigated times of venom gland cycle were identified using the Short Time-Series Expression Miner (STEM)4 tool. **Results and Discussion:** In the gene expression pattern, high level of transcripts were found from 1 to 2 days after manual extraction of venom, results which differ from previous data showing higher levels of transcripts from 4 to 8 days. More analyses are necessary to confirm or refute our results.

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1.08 Action of sphingomyelinase D in loxosceles spider venom in human kidney cells

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Introduction: The *Loxosceles* spider (brown recluse) can be found worldwide, although its distribution is heavily concentrated particularly in the tropical urban regions of South America. Brown spider bites can cause dermonecrotic lesions and systemic reactions known as loxoscelism. Systemic manifestations include intravascular hemolysis, disseminated intravascular coagulation and acute renal failure. The venom factor responsible for both local and systemic manifestations is a phospholipase D, with sphingomyelin substrate specificity, called sphingomyelinase D (SMase D). **Objectives:** The aim of this study was to investigate the effects of *Loxosceles* spider venom and the SMase D toxin on the Complement (C) regulators expression and the C-resistance of kidney human cells (HK-2), as well as the possible involvement of endogenous metalloproteinases in the pathogenesis. **Methods:** Cells were incubated with *Loxosceles* venom or SMase D and the expression of complement regulators was assessed by flow cytometry; cell viability was analyzed by the MTT assay, and supernatants of these cells were also analyzed by zymography to verify the expression of endogenous metalloproteinases. **Results and Discussion:** A reduced expression of membrane co-factor protein (MCP) was observed, while expression of decay-accelerating factor (DAF) and CD59 was not affected. Analysis of other cell-surface molecules showed a reduced expression of the major histocompatibility complex I (MHCI), epithelial growth factor receptor (EGFR) and endothelial protein C receptor (EPCR). Removal of MCP, caused by HK-2 cell endogenous metalloproteinase activation, allows complement to be activated on the cell surface, as evidenced by increased deposition of C3 and C4, resulting in cell death, in the presence of normal human serum as source of C-components. Increased deposition of factor H (fH) and properdin, but not of C4-binding protein (C4BP), was observed on these cells after venom/toxin treatments, followed by incubation with normal serum. Zymography assays showed increased expression of MMP-9 after 24 and 48 h of the treatment; after 72 h, an increase in MMP-2 expression was also detected. Our data showed that *Loxosceles* venom and its SMase D are toxic to human renal cells, causing increased expression of endogenous metalloproteinases, which may contribute to cell death. Moreover, they suggest that a failure of complement regulation, allowing complement activation, may play a role in the pathogenesis of kidney failure, present in the systemic form of the human loxoscelism.

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1.09 Isolation of progenitor/stem cells from canine amniotic and allantoic fluids

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Introduction: Pet applied stem cell therapy is a rapidly growing market, which requires constant innovation and developing new technologies in order to expand the list of currently treated diseases. The dog is an excellent preclinical model for the study of diseases, pharmacological tests and new therapies for future application in humans. Thus, the canine model is a excellent model for isolation of (PS) cells from amniotic and allantoic liquids **Objectives:** We aimed at isolation and comparative characterization of (PS) cells from amniotic and allantoic liquids **Methods:** Therefore, ovarian hysterectomy technique was performed during company castration in order to isolate progenitor/stem (PS) cells from canine amniotic and allantoic fluids (CAFs). **Results and Discussion:** We showed that efficient CAF-PS cell harvesting occurs at 50 days of gestation. Different culture media were used and optimal conditions for culturing of these cells were established. CAF-PS cells expressed vimentin, nestin and cytokeratin-18 proteins, which were negative for Oct-4, a marker of pluripotent stem cells. PS-cells from canine amniotic fluid were able to undergo osteogenic and chondrogenic differentiation, while potential of PS cells from allantoic fluid was limited to chondrogenic differentiation. Our data suggest that CAF-PS cells are a source of PS cells with restricted differentiation, but these cells can be useful in the emerging field of regenerative veterinary medicine due to their low rejection following heterologous transplantation.

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1.10 Transcriptomic and proteomic analysis of Duvernoy's glands of the colubrid snake *Phalotris mertensi*

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Introduction: The venom from members of the Colubridae family has been neglected for a long time, and currently little is known about this heterogeneous and diverse family. Many colubrid snakes have Duvernoy's glands, which are homologous to the venom gland, and this species can have opisthoglyphous or aglyphous dentition. *Phalotris mertensi,* a colubrid *(*Xenodontinae subfamily), popularly known as false coral occurs in the central region of Brazil and possesses Duvernoy's gland and opisthoglyphous teeth. In vitro assays with the venom of P. mertensi indicated that it acts on casein, fibrinogen and gelatin, and contains some enzymes. **Objectives:** This work aimed to characterize the transcriptome of Duvernoy's gland of the snake *P mertensii* and to use the ESTs generated database as a tool for the identification of proteins present in the venom by mass spectrometry. **Methods:** The transcriptome was determined by extraction of Duvernoy's glands of a snake followed by total RNA extraction with Trizol reagent (Invitrogen) and mRNA purification through oligo dT cellulose. The cDNA library was constructed with 5 μg mRNA using the Superscript Plasmid System for cDNA Synthesis and Cloning kit (Invitrogen), directionally cloned in the pSPORT-1 plasmid and transformed in Escherichia coli DH5 alpha cells. Plasmid DNA was isolated using alkaline lysis from randomly chosen clones and DNA was sequenced on an ABI 3100 sequencer using BigDye2 kit (Applied Biosystems) with standard 5' primer. After bioinformatics treatment, the ESTs were grouped into clusters using the program Cap3 and were annotated with Blast2Go program. **Results and Discussion:** As a result, we obtained 1540 ESTs, grouped into 777 clusters, 131 contigs and 646 singlets. Toxins represented about 38% of gene expression in Duvernoy's glands of this colubrid. Metalloproteases (SVMPs) were the most abundant toxins, accounting for 54% of the total toxins, followed by protease inhibitors and C-type lectins. The profile of toxin transcripts suggests the presence of a new abundant toxin (3% of all clones) similar to vertebrate lysosomal lipase A. Another 21% of gene expression was not identified, indicating that many components present in venoms of these animals have not yet been studied. The database generated was also used to identify venom components analyzed by mass spectrometry for spots obtained in two-dimensional electrophoresis of venom P mertensi. Mass spectrometry allowed the identification of most toxins found in the transcriptome. Further analyses are being carried out aiming to a better match the transcriptome and proteome sets.

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1.11 Crotoxin inhibits the secretion of pro-inflammatory cytokines: effect on cell migration

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Introduction: *Crotalus durissus terrificus* snake venom (CdtV) modulates the inflammatory response, and the long-lasting anti-inflammatory effect of CdtV on inflammatory response induced by carrageenan (Cg) has been demonstrated. Other results showed that CdtV inhibits cytokine secretion, such as IL-α and IL-1β, by macrophages stimulated by phagocytosis or lipopolysaccharide (LPS). Recent data demonstrated that crotoxin (CTX), the main toxin of CdtV is responsible for this long-lasting anti-inflammatory effect. **Objectives:** The aim of this study was to evaluate histologically the inhibitory effect of CTX on the migration of cells to the subcutaneous tissue of footpads injected with Cg, and to investigate if this inhibitory effect involves the action of CTX on the secretion of pro-inflammatory cytokines. **Methods:** A single dose of CTX (0.89 μg/50 μL s.c.) or saline (50 μL) was administered 1 h before Cg injection in male Swiss mice (18-22g) (n=6). For histopathological analysis, the footpad was removed after 6 h of intraplantar injection of Cg (300 μg/50 μL) or saline (50 μL). The tissue was processed for light microscopy. Sections were obtained from paraffin embedded pieces (5 mm thick), stained with hematoxylin and eosin (H/E) and observed under Zeiss microscope (Axiolab) with pickup AxioCam MRC (Zeiss). To evaluate the effect of CTX on cytokine secretion, after 4 h of intraperitoneal injection of Cg (300 μg/200 μL) or saline (200 μL), peritoneal exudate was collected and the cytokines IL-1β, TNF-α and IL-6 were determined by ELISA, using reagent kits (R&D System). **Results and Discussion:** Histological analysis confirmed the inhibitory effect of CTX on cell migration. Tissue sections from animals treated with CTX 1 h before intraplantar injection of Cg showed significantly fewer areas of edema and consequent reduction of inflammatory infiltration (polymorphonuclear cells) compared to untreated animals (Saline+Cg). The peritoneal exudate of animals pretreated with CTX showed a significant decrease in the secretion of all pro-inflammatory cytokines evaluated, IL-1β, TNF-α and IL-6, when compared to untreated animals. The decrease in cytokine secretion observed in exudates from animals pretreated with CTX was 71%, 51% and 35% for IL-1β, TNF-α and IL-6, respectively. Our results showed for the first time, by histological analysis, the inhibitory effect of CTX on cell migration. We suggest that this inhibitory effect involves the action of CTX on the secretion of important pro-inflammatory cytokines that participate in the inflammatory response induced by Cg.

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1.12 South American rattlesnake bite with soft-tissue infection in Santarém, Pará, Brazil

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Introduction: According to SINAN - Information System for Notification of Diseases - the number of snake bites in Brazil was 29,374 in 2006. During 2001 to 2006, Brazil reported 155,973 cases, and in Pará State's 22,886 cases, rattlesnake bites were responsible for 10,462 cases. The majority of snake bites in Brazil were caused by *Bothrops* (90.5%), followed by *Crotalus* (7.7%), *Lachesis* (1.4%) and *Micrurus* (0.4%). Despite the frequency and severity of rattlesnake bites, which are considered potentially more serious than *Bothrops* (lethality of 0.3% to 1.3%, respectively, in 2006), local complications are very rare with South American rattlesnake. **Objectives:** We report a case of a rattlesnake bite with secondary soft-tissue infection **Methods:** A retrospective study of data obtained from medical record was carried out. A 17-year-old patient, male, previously healthy, was bitten in the medial portion of the left foot by a rattlesnake in June, while working in the field. He did not use a tourniquet or placed substances on the site of the bite; he just washed the bite with water. After 120 min, he developed intense myalgia associated with change in visual acuity (in fact, bilateral ptosis) and on the injury site there was only local erythema, without bruising or bleeding. He reached the Santarém Municipal Hospital seven hours later and at admission he had bilateral ptosis and complained about decreased urine output, besides darkened urine. The initial laboratory results were: leukocytosis and left shift, CPK = 123,000, coagulation time normal, urea = 32 and creatinine = 3.2 mg/dl. The accident was classified as moderate and the patient received 10 ampules of anticrotalic antivenom. The patient was transferred to the intensive care unit for monitoring and for support treatment. After 1 day of admission, he had fever, progression of left lower limb edema and blister at the site of the bite. He was prescribed 2 g of chloramphenicol/day for the treatment of secondary soft-tissue infection. He remained hospitalized for 15 days and needed two sessions of hemodialysis. **Results and Discussion:** Patients bitten by a rattlesnake in South America usually do not show local changes. In contrast to what occurs with North American rattlesnake bites, widely described in literature, it is not common for patients bitten by South American rattlesnakes to develop soft-tissue infection. Soft-tissue infection is a widely known complication of *Bothrops* bites, probably because of the local acute inflammatory action and the oral fauna of snakes. Even in South American rattlesnakes, it is important to note the local clinical picture.

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1.13 Juruin: an antimicrobial peptide from *Avicularia juruensis* venom

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Introduction: Antimicrobial peptides (AMPs) are the key elements of the innate immunity against bacteria and fungi in both the animal and plant kingdoms. AMPs are an extremely diverse group of small proteins that are considered together because of their native antimicrobial activity, and their function is essential to the animal immune response. Natural animal venoms are good sources of potential antimicrobial substances, and their venoms contain a large number of diverse biologically active components of various chemical structures, such as proteins, polypeptides and amines. Cystine knot toxins (CKTs) in spider venoms represent a rich source of novel ligands for various ion channels, and are among the most extensively studied constituents of spider venoms. They are small, compact molecules cross-linked by three to five disulfide bonds, ensuring greater stability in the conformation of the molecule that contains it, and therefore offers a high potential for applications in engineering proteins. They occur in a variety of peptides and proteins and are relatively common in small cysteine-rich toxins and small peptides. Toxins containing the cystine knot feature a range of biological activities, such as antimicrobial activities, anti-HIV potential and blocking of ion channels. CKTs have molecular masses ranging from 3.5 to 7 kDa. **Objectives:** The aim of the study was to identify antimicrobial peptides from *Avicularia juruensis* venom and its potential to become a new antibiotic. **Methods:** The venom was purified by reversed phase HPLC and the fractions obtained were submitted to antimicrobial activity assay (determined by a liquid growth inhibition assay against Gram-negative and -positive bacteria *Escherichia coli SBS363* and *Micrococcus luteus A270* and yeast *Candida albicans*). The fractions that showed antimicrobial activity were submitted to MALDI-TOF mass spectrometry to determine purity. The pure ones were submitted to Q-TOF mass spectrometry for sequencing. **Results and Discussion:** Juruin has a mass of 4,005 Da and showed antimicrobial activity against all the microorganisms tested, and was sequenced by Q-TOF mass spectrometry and "De Novo" sequencing. The probable sequence obtained is the 38 amino acid sequence FTCALSCNLKVNKGPCKGTNEGKCSGGWSCKFNVCVKT. BLAST analysis showed 71% similarity with cystine knot toxin from the Chinese bird spider *Chilobrachys jingzhao*.

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1.14 Effects of Micrurus snake venoms on the human complement system

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Introduction: The family Elapidae is represented in the Americas by three genera of coral snakes: *Micruroides*, *Leptomicrurus* and *Micrurus*, the latter being the most abundant and diversified group. The genus *Micrurus* (Serpentes, Elapidae) comprises more than 120 species and subspecies distributed from the southern United States to the southern part of South America. *Micrurus* bites can cause death by muscle paralysis and further respiratory arrest a few hours after envenoming. Clinical observations show mainly neurotoxic symptoms, although other biological activities have also been experimentally observed, including cardiotoxicity, myotoxicity, hemolysis and edema. **Objectives:** The aim of this study was to analyze the action of venoms from 8 *Micrurus* species (*M. ibiboboca, M. lemniscatus, M. altirostris, M. spixii, M. surinamensis, M. corallinus, M. frontalis* e *M. hemprichii*) on the complement system (C) in *in vitro* tests. **Methods:** Samples of normal human serum, as source of complement components, were incubated with the snakes' venoms and the remaining complement activity was measured in conditions to develop classical, alternative or lectin pathways. **Results and Discussion:** All venoms were able to activate the classical but not the alternative complement pathway. This activation was in part associated with the cleavage of C1-INH by proteases present in these venoms, which disrupts complement activation control. To determine if the observed alteration in C-activity was caused by C-inhibition or C-activation/consumption, the generation of C-activation products C3a, C4a and C5a was measured. Moreover, C3a was generated in human serum treated with the venoms, not only through C-activation, but also through the direct cleavage of the C3 component, as determined using purified C3. These results suggest that *Micrurus* spp venoms can activate the complement system, generating large amounts of anaphylatoxins, which can play a role in the pathogenesis of human coral envenomation and may also contribute to the spread of other venom components, due to their vasodilatory effects.

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1.15 *Echinometra lucunter* (rock-boring urchin) accident: much more than a mechanical incident

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Introduction: The rock-boring sea urchin *Echinometra lucunter* can be found along the Western Central Atlantic shores, from the USA to most of Brazil. 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, however, have been treated mostly for the (swollen) mechanical consequences of the accident. The symptoms, on the other hand, usually surpass trauma and may be pathologically varied and last from spontaneous healing in a few days to painful consequences for weeks (for the worst untreated cases). Moreover, injuries may be complicated due to secondary infections or the development of a chronic inflammatory response, with granuloma formation. Based on these data, we mimicked the sea urchin accident by administering an aqueous extract of the spine into mice and rats (CEUAIB 438/07) and evaluated the main symptoms observed in the victims. **Objectives:** The aim of this study was to characterize the sea urchin accident by means of evaluating the main physiopathological symptoms. **Methods:** An aqueous extract of *E. lucunter* spines (10 μg) was injected in mice and after 2, 4, 24 and 48 h, the alterations in the microcirculation (rolling, adhered and emigrated leukocytes) were observed. Mouse paw edema formation was evaluated with a plethysmometer ¼, ½, 1, 2 and 3 h after injection (10 and 20 μg doses). In order to determine whether the extract would cause hyperalgesia, the pain threshold in rats, was measured before and after the administration of 10 or 20 μg, at times 1, 2 and 4 h, using a Ugo Basile® pressure apparatus. Hemorrhagic activity was determined by injection of the extract (10 and 20 μg) in the abdominal skin of mice, followed by exposure of the skin three hours after treatment in order to verify the extent of the hemorrhagic spots. The extract was also analyzed by SDS-PAGE and RP-HPLC, as an initial biochemical characterization. **Results and Discussion:** Our data clearly indicate that the sea urchin accident is indeed a pro-inflammatory event, triggered by toxins present in the spine which can cause edema and alterations in the microcirculation. Moreover, the extract demonstrated a hyperalgesic effect. The extract is also rich in proteins, as observed by SDS-PAGE, but also contains other molecules that can be analyzed by RP-HPLC. Altogether, these effects corroborate that an *E. lucunter* encounter is an accident and not an incident, as frequently reported by the victims.

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1.16 Effectiveness of serotherapy in pregnant mice after experimental evenomation by *Bothrops jararaca*

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Introduction: Snakebite accidents are considered a rare event among pregnant women, but serotharapy is indicated even when envenomation is not severe. However, antivenom can cause maternal adverse reactions, and consequently fetal death. Experimental *Bothrops jararaca* (Bj) envenomation can provoke marked morphological alterations in the antimesometrial (AM) region of uterus in pregnant mice which can culminate in the end of gestation. **Objectives:** This investigation aimed to verify whether *Bothrops* antivenom (BAV) could restore the normal morphology of the murine uterus after *Bothrops jararaca* (Bj) envenomation. **Methods:** On the morning of day 8 of pregnancy, animals received Bj venom (0.24 mg Bj venom/kg body weight) i.m., and after 3 h they were treated with BAV (Bj+BAV). Control groups received saline and were treated with BAV (Sal+BAV) or Bj venom (Bj). On day 9, uterine morphology was analyzed, especially at the maternal-fetal interface in the antimesometrial region. Plasma fibrinogen (Fg) was assayed in plasma samples of pregnant animals. Aiming to study the external appearance and the skeletal morphology of fetuses, as well as the incidence of fetal resorptions, another group of animals on day 8 received the same treatments mentioned above and was sacrificed on day 19. **Results and Discussion:** Histological analysis of most dams of the Bj+BAV group revealed the maternal and fetal tissues organized, similar to the uteri of dams of the Sal+BAV group. However, sometimes the antimesometrial region was not preserved in some implantation sites, showing hemorrhagic areas and a prominent inflammatory infiltrate at the maternal-fetal interface. Additionally, decidual cells (maternal) and trophoblastic giant cells (fetal) exhibited evident signs of necrosis. These findings were similar to the Bj group, indicating the possibly that BAV does not reach all implantations sites equally. Analyses of the external appearance and skeletal morphology of fetuses on day 19 showed no difference between groups; however, the dams that received Bj venom and were not treated with ABS showed smaller fetuses and a higher incidence of fetal resorption. Plasma Fg levels of the Bj+BAV group were similar to those of Sal+BAV group. However, experimental Bj envenomation showed lower plasma Fg levels. These findings show that although Bj venom induces a characteristic Fg consumption, BAV could re-establish plasma Fg levels, which might have contributed to the maintenance of the pregnancy.

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1.17 K49-PLA2 homologue in *Bothrops jararaca* venom

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Introduction: Endocrine effects of reptile venoms are in vogue and that from the lizard *Heloderma suspectum* is the most representative. This venom contains exendin-4 (EX-4), a peptide that gave rise to a new class of antidiabetic drugs with agonist activity on the glucagon-like peptide-1 receptors. Considering the phylogenetic proximity between snakes and lizards, we are prospecting for EX-4-like peptides in *Bothrops jararaca* venom (*vBj*). **Objectives:** The aim of this study was to describe a phospholipase A2 (PLA2) detected in this search. **Methods:** The supernatant of 10 mg *vBj*/2 mL 2 M HAc (centrifuged at 3,000Xg, 5 min, 4ºC) was chromatographed in G-50 column (46x1.5 cm, Vo=40.5 mL, flow rate=1.5 mL/min). The pooled fractions 31-45 (interval=15 min) was dried, resuspended in 1 mL (concentrated 1.5 times) and submitted to HPLC (C18 column, 4.6x250 mm, 5 μm particles) with linear gradient elution (0-100% solution B) formed from solution A (0.05/99.50 [v/v] TFA/water) to solution B (0.05/19.95/80.00 [v/v/v] TFA/water/acetonitrile) (240 min, flow rate=1 mL/min, Abs 214nm). Six peaks eluted near the retention time (RT) of EX-4 were dried, resuspended in EIA buffer and then analyzed by monoclonal anti-exendin-4 ELISA. ELISA was positive for all these peaks. These peaks pooled in 500 μL (concentrated 3 times) were then administered via ip, at a dose equivalent to 12 μg EX-4 /kg body wt in a 120-min oral glucose tolerance test (OGTT) in a rat model of streptozotocin-*diabetes mellitus*. As a result, the animals that received the pool had lower glycemia (monitored by Accu-Chek , Roche at 15, 30 and 60 min) when compared to controls. The peaks collected from the first HPLC were rechromatographed separately in the same column with a gradient of 0-40% of B for 18 min followed by 40-60% of B for 61.5 min (flow rate=1 mL/min). Five peaks (P1-P5) were then obtained and the first (P1) was analyzed by mass spectrometry (MS) including peptide mass fingerprint (PMF) search against database nrNCBI using MASCOT software. **Results and Discussion:** The linear mode MS analysis of P1 detected polypeptides with mw (Da) 13672.01 (+1), 6835.51 (+2) and 4548.05. The ion 6835.51 is probably the double charge of 13672.01. P1 was further reduced, alkylated and digested by trypsin resulting in 12 peptide ions. Half of these peptide ions covered 43% (MASCOT score of 433) of the amino acid sequence of K49-PLA2 from *B. pirajai* (chain A, piratoxin-II), which had not yet been reported in *vBj*. It is known that the catalytic mechanism of PLA2 involves the binding of Ca(2+) to Asp49, that the substitution Asp49Lys reduces the K49-PLA2 catalytic activity (Biochemistry 40; 28-36, 2001), and that the stimulation of insulin secretion is one of PLA2 activities not affected by catalytic inhibitors (Toxicon 54; 413-420, 2009). Thus, K49-PLA2 of *vBj* could be responsible for the lowering of glycemia observed in the OGTT test, but the existence of EX-4 cannot be discarded in the peak P1 containing K49-PLA2 homologue, as well as in the other four purified peaks of *vBj* with positive ELISA and hypoglycemic activity.

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1.18 Specific antibodies and anti-inflammatory drugs decrease edema and nociception induced by *Potamotrygon motoro* stingray venom in a murine model

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Introduction: When stingrays are stepped on, they whip the tail to the stimulated dorsum site, as a defensive behavior, and insert the stinger into the victim's limb. The stinger is a mineralized structure covered by glandular and integumental tissues, where toxins (venom) are produced. Besides mechanical trauma, stingray venom causes an intense pain followed by edema, erythema and usually necrosis. There is so far no specific therapy for stingray envenomation, and treatment consists of the administration of analgesic, antipyretic and anti-inflammatory drugs. **Objectives:** The aim of this work was to determine the ability of specific antibodies and pharmacological inhibitors to decrease nociception and edema induced by *P. motoro* venom. **Methods:** Samples containing 25 μL of rabbit anti-*P. motoro* serum or its IgG purified (Protein A Sepharose) were incubated with 4, 8 and 16 μg of *P. motoro* venom (in 5 μL) for 30 min at 37 °C. After incubation with antibodies, the mixture was centrifuged and the supernatant injected into mouse footpad to evaluate nociception and edema formation. Normal rabbit serum and its purified IgG were used as controls. In order to assess pharmacological inhibition of toxic activities, dipyrone (200 mg/kg), indomethacin (4 mg/kg), etoriboxib (10 mg/kg) or PBS (control) were injected (100 μL) i.p. 30 min before injection of venom into the mouse footpad. Edema was measured at 0.5, 1, 2, 4, 24 and 48 h after venom injection by plethysmography. Nocipeptive activity was determined by recording the time of licking the injected paw by experimental animals over 30 min. **Results and Discussion:** Antibodies reduced nociception (in at least 50 %) at all doses tested. Purified IgG also partially diminished edema formation at all doses and time periods tested. All drugs neutralized nociception, but not totally, and dipyrone could decrease it at all doses tested reaching values as high as 90 % of reduction of nociception when using 16 μg of venom. These drugs also partially neutralized edema formation at early time periods. These results show the involvement of eicosanoids in stingray envenomation, which mediate local inflammatory reaction. In addition, specific antibodies neutralized edema and nociception, providing new perspectives for therapy.

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1.19 Effect of Piperaceae amide on *Biomphalaria glabrata* and *Schistosoma mansoni* stages

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Introduction: Schistosomiasis is an endemic parasitic disease. It occurs in 54 countries, mainly in South America, the Caribbean, Africa and east of the Mediterranean. In Brazil, it affects over 8 million people and about 30 million live in risk areas due to the presence of infected snails. One of the most efficient methods to control this disease is the application of molluscicides that eliminates or reduces the intermediate host population. The high cost of production, environmental pollution and resistance of snails to synthetic molluscicides have stimulated the study of molluscicides of plant origin. The species from the Piperaceae family have a diversity of chemical compounds, some with bioactive properties such as essential oils, unsaturated amides, pyrones, flavonoids, monoterpenes, sesquiterpenes, arylpropanoids and lignoids. **Objectives:** In the present study, the molluscicide and schistosomicidal actions of an amide from the genus *Piper* (Piperaceae) were evaluated on adult and embryos stages of *Biomphalaria glabrata*, miracidium and cercaria, the free-living larval stages of *Schistosoma mansoni.* **Methods:** The Piperaceae amide was evaluated at concentrations lower than 10 ppm to obtain LC_{90} (concentration producing 90% mortality) **Results and Discussion:** The amide was active at concentrations (LC_{90} of 7.18 ppm and 0.99 ppm for adult and embryo, respectively) lower than that recommended by OMS. However, at the same concentrations, the amide was not active on miracidia and cercaria. The molecular structure of the amide is being studied and modified in order to increase the molluscicide and schistosomicidal activities.

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1.20 Inflammatory effects of patagonfibrase, a metalloproteinase isolated from the venom of Philodryas patagoniensis (Serpentes: Dipsadidae)

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Introduction: Patagonfibrase is a 57.5-kDa hemorrhagic metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis*. Local inflammatory reactions are conspicuous signs of snakebites inflicted by this species. **Objectives:** Taking into consideration that most snake venom metalloproteinases, besides inducing hemorrhage and myonecrosis, play a relevant role in the complex and multifactorial inflammatory response characteristic of envenomation, this study dealt with the pro-inflammatory effects evoked by patagonfibrase. **Methods:** Male *Swiss* mice were intradermally injected into the right hind paw with patagonfibrase (0.1 μg/50 μL). The contralateral paw received the same volume of vehicle (50 mM Tris-HCl buffer, pH 7.4, containing 1 mM $CaCl_2$). Paw edema was determined by measuring paw thickness using a caliper at 0 (time before intraplantar injection), 45 min, and 1, 2, 4, 6 and 24 h after injection. Results were calculated as the difference in thickness of both paws, and edema was expressed as the percentage increase in paw thickness. In order to investigate, by intravital microscopy, the effects of patagonfibrase on the leukocyte-endothelium interactions in the microcirculation of the cremaster muscle, the enzyme (0.1 μg/100 μL) was injected s.c. into the scrotal sac of mice. At 2 (T2), 4 (T4) or 24 h (T24) after the injection, the cremaster was exposed. Ten minutes after the microcirculation exposure, a portion of 100 μm of a post-capillary vessel (20-40 μm diameter) was evaluated for 1 min, and rolling, adherent and emigrated leukocytes were counted. **Results and Discussion:** Patagonfibrase caused a time-dependent edema, which was accompanied by hemorrhage. The peak of edema was noticed as early as 45 min after injection. The enzyme induced cell recruitment with a significant decrease in leukocyte rolling (at all tested times after injection), a significant increase in cell adhesion to the endothelium surface (at T2 and T4), and cell migration to extravascular tissue (mainly at T4 and T24). The presence of 1 mM o-phenanthroline, which chelates metal ions, significantly inhibited the pro-inflammatory effects induced by patagonfibrase. Taken together, these results imply that patagonfibrase is an important contributor to local inflammation elicited by *Philodryas patagoniensis* envenomation.

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1.21 Disintegrin-like/cysteine-rich domains of the reprolysin HF3: site-directed mutagenesis reveals essential role of specific residues

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Introduction: HF3, a highly glycosylated protein, is the most potent hemorrhagic toxin isolated from *Bothrops jararaca* venom. The recombinant protein composed of its disintegrin-like/cysteine-rich domains (DC) inhibited collagen-induced platelet-aggregation. Moreover, native HF3 and DC activated alphaMbeta2-mediated phagocytosis of opsonized-zymosan particles by macrophages. The ECD sequence of the disintegrin-like (D) domain has been assigned as the disintegrin motif, and the hyper-variable region (HVR) of the cysteine-rich (C) domain was suggested to constitute a potential protein–protein adhesive interface. **Objectives:** The aims of this study were i) to evaluate the effect of recombinant D and C domains of HF3 as well as three peptides resembling its HVR on platelet aggregation, and ii) to investigate the role of specific residues of the putative ECD disintegrin motif and of the HVR of HF3 by site-directed mutagenesis. **Methods:** Recombinant (wild-type and mutant) DC, D16 or D18, and C proteins were obtained in fusion with GST in *E. coli* BL21 (DE3). Peptides based on the HVR of HF3 were synthesized by the solid phase peptide synthesis FMOC strategy. Charged residues of the disintegrin loop and of the HVR of HF3 were individually mutated to Ala to identify residues essential for the functionality of the DC domains. The recombinant proteins of non-catalytic domain of HF3 and synthetic peptides were tested for their ability to affect platelets. A suspension of washed platelets was preincubated with these proteins and peptides for 3 min and incubated with collagen I at 37°C for 8 min using an aggregometer. Alterations in the microcirculation were analyzed using intravital microscopy by transillumination of mouse cremaster muscle after topical application of GST-DC-D469A at 5 mM in PBS. **Results and Discussion:** The recombinant proteins GST-DC, GST-DC-D469A, GST-D16, GST-D18 and GST-C were purified by affinity chromatography and recognized by an anti-HF3 antibody. Recombinant D and C domains of HF3, expressed together or individually, and the HVR synthetic peptides inhibited collagen-induced platelet-aggregation. The mutation of the Asp residue of the ECD motif caused loss of the ability of the DC domains to affect platelet aggregation and to promote leukocyte rolling in the microcirculation. Moreover, the C domain and its HVR were demonstrated to be critical for HF3 to affect platelets and leukocytes; however, the disintegrin loop may be important for the functionality of the D domain in the context of the C domain.

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1.22 Africanized honey bee (*Apis mellifera*) venom profiling: seasonal variation of melittin and phospholipase A2 levels

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Introduction: The venom of the European honey bee, Apis mellifera, is composed basically of melittin, phospholipase A2, histamine, hyaluronidase, catecholamine and serotonin. While some of these components have been associated with allergic reactions, among several other symptoms, and sometimes leading to anaphylactic shock, mass stinging by the Africanized honey bee (AHB) causes serious toxic effects often leading to death, through a massive injection of venom. As AHB spreads through Brazil and the Americas, the number of mass stinging cases is rising, becoming a serious public health issue. The development of efficient serum-therapies has, therefore, become an urgent necessity. **Objectives:** The aim of this study was to carry out a biochemical characterization of the seasonal variation of the major components of the AHB venom, by analyzing the pooled venom composition of individuals pertaining to one specific hive over one year. **Methods:** The venom was collected by manual and electrical stimulation of bees from one specific hive at the Apiary of Botucatu School of Veterinary Medicine and Animal Husbandry (UNESP). The venom profiling and component purification was performed by RP-HPLC with an acetonitrile gradient in a C8 column. Mass spectrometry of the samples was performed in an ESI mass spectrometer (LCQDuo™, ThermoFinnigan, USA), equipped with a nanospray source and connected to nanoHPLC system. The venom profiling was correlated with climatic parameters obtained in Botucatu. Complete or N-terminal sequencing of purified peptides was performed by Edman degradation using a Shimadzu PPSQ-21 automated protein sequencer. **Results and Discussion:** It was possible to detect a seasonal variation on the venom contents of melittin and phospholipase A(2). Moreover, both compounds showed a synchronized variation of their levels, with an increased production in the same months. This variation does not correlate or synchronize with any climatic parameter. Data on the variation of the AHB venom composition is necessary to guide future intra- and interspecies studies. The production of specific AHB antivenom should take into account the possible regional variability of the venom composition due to climatic, seasonal and feeding factors. These variations could be either quantitative or qualitative. The compounds analyzed in this work showed a quantitative variation, but a closer inspection can reveal peaks that undergo qualitative variation through the year. The possible qualitative variations still need to be investigated compared with other bee colonies, and other regions.

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1.23 N-acetyl-l-cysteine (NAC) affects renal function, aminopeptidases and oxidative stress in *Bothrops jararaca* envenomation in mice

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Introduction: NAC is a thiol antioxidant for which there are few reports of side effects. NAC has been reported to be effective in the prevention of acute renal failure (ARF). **Objectives:** This study aimed to contribute to the understanding of the mechanisms and consequences of the nephrotoxic effect of *Bothrops jararaca* venom (*vBj*) and to evaluate the possibility of introducing NAC as a coadjuvant in *Bothrops* antivenom therapy. **Methods:** The effects of NAC on hematocrit, protein, classical parameters of renal function, aminopeptidase activities and redox status were measured in mice with ARF induced by LD50 of *vBj* (protocol approved by the Ethics Committee of the Instituto Butantan, 492/08). **Results and Discussion:** NAC affected oxidative stress (GSSG/GSH index), uricemia, proteinemia and creatinuria (full restoration) and uremia (ameliorated) in envenomed animals. Alone or combined with *vBj*, NAC caused the reduction of protein content of membrane fractions of the cortex and renal medulla, increased proteinuria and reduced urinary excretion of urea. However, urinary hyperosmolality, also typical of *Bothrops* envenomation, was aggravated by NAC. Remarkable is the ability of NAC to affect the levels of aminopeptidase activity in renal tissue. In the soluble fraction of the renal cortex and medulla of envenomed animals NAC restored normal levels of APB, APN and PIP, but increased the DPPIV. In the membrane fraction of renal cortex, NAC restored normal levels of APN and PIP, but increased CAP, PAP and DPPIV of envenomed animals. In the membrane fraction of renal medulla, NAC restored normal levels of APA, APN and PIP, ameliorated the decrease of CAP, but increased PAP and DPPIV.
These data allow us to outline the pattern of action of NAC on renal function, aminopeptidase activity and renal oxidative stress in normal and *vBj* envenomed mice, showing significant beneficial effects and suggesting the convenience of the clinical evaluation of the association of this agent with the serotherapy of this envenomation.

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1.24 Effects of allopurinol and probenecid on lethality and acute renal failure induced by *Crotalus durissus terrificus* envenomation in mice

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Introduction: Acute renal failure (ARF) is one of the most serious complications of rattlesnake envenomation. The venom of *Crotalus durissus terrificus* (*vCdt*) at a dose of 80% LD50 produces oxidative stress and 100% incidence of hyperuricemia and urinary hypo-osmolality, suggesting that the latter two can be early signs of direct nephrotoxic action (without myotoxicity) of *vCdt* (Toxicon 52:445-54, 2008). **Objectives:** This study aimed to evaluate the relevance of these alterations as mechanisms of induction of ARF by *vCdt*. **Methods:** The effects of uricostatic (allopurinol) and uricosuric (probenecid) drugs on hematocrit, protein, renal function parameters (osmolality, creatinine, uric acid and urea) and oxidative stress (GSSG/GSH) were measured in mice envenomed with 80% LD50 of *vCdt* (protocol 717/10 approved by the Ethics Committee of the Instituto Butantan). **Results and Discussion:** Allopurinol restored uricemia, osmolality, and the redox status of renal tissue and, above all, surprisingly completely abolished the lethality of 80%LD50 of v*Cdt* in mice without treatment with antivenom. In turn, probenecid also restored the level of uric acid in plasma and its excretion in urine and the renal redox status, but simultaneously caused hypercreatinemia, hypocreatinuria and urinary hypo-osmolality in envenomed mice.
Hyperuricemia and urinary hypo-osmolality seem to be the two main causes of mortality of ARF induced by the direct nephrotoxic action of v*Cdt.* Hyperuricemia in envenomed mice should be predominantly generated by increased production of uric acid, since allopurinol is uricostatic and showed higher therapeutic efficacy than that due to the uricosuric action of probenecid. However, it is also possible that the effectiveness of probenecid in *vCdt* envenomation is compromised only due to the hypercreatinemia, hypocreatinuria and urinary hypo-osmolality caused by this drug. Consequently, these data allow us to recommend a clinical evaluation of the use of allopurinol in rattlesnake envenomation, which seems to be an important complementary approach to antivenom therapy.

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1.25 *Bothrops jararaca* envenomation elicits low uterine fibrinogen

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Introduction: Besides the classic function in hemostasis, fibrinogen (Fg) is also involved in the maintenance of gestation. *Bothrops* snakebites usually cause plasma fibrinogen consumption, but it is not clear whether hypofibrinogenemia causes gestational interruption. **Objectives:** This investigation aimed to evaluate the presence of Fg in the uterus of pregnant mice submitted to *Bothrops jararaca* (Bj) envenomation. **Methods:** On the morning of day 8 of pregnancy, animals were submitted to a single i.m. injection of 0.48 mg Bj venom/kg body weight. The control group received saline (i.m.). On day 9, implantation sites were: (a) isolated and immediately immersed in Bouin fixative solution for morphological evaluation; (b) immersed in 4% PFA in phosphate buffer for evaluation of Fg expression in uterine tissue; and (c) homogenized in solution to evaluate Fg expression by immunoblotting. Plasma fibrinogen was also assayed in plasma samples of pregnant animals. **Results and Discussion:** At the antimesometrial region, uterine analysis of control dams showed trophoblastic and mature decidual cells with morphological characteristics similar to those of animals submitted to no treatment. However, among some animals in the Bj venom group, the maternal and embryonic tissues were disorganized, showing trophoblastic and mature decidual cells with clear signs of cell death, hemorrhagic areas and inflammatory infiltrate. Immunohistochemistry revealed that the antimesometrial area showed positive reaction to Fg, which was scattered over the extracellular matrix; however, the reaction was more intense in the saline group than in the Bj group. Imunoblotting analysis showed that Fg bands in the Bj group were fainter than in the saline group, and a correlation between these results and those obtained with the immunohistochemistry was observed. These findings show that Bj venom evokes a decrease in fibrinogen in plasma and in maternal uterine tissue.

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1.26 Ontogeny in the venom proteins of neonates of *Crotalus durissus terrificus* and *Crotalus durissus collilineatus*

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Introduction: Venomous snakes are widespread in tropical and subtropical regions of the world. Snake venoms are mixtures of biologically active substances, most of which are enzymes or non-enzymatic polypeptides. There is reason to believe that envenomation behavior may vary with ontogeny, the composition and properties of which vary with age and the supply of available venom increases exponentially with growth. Individuals from different families, genera and species even from the same species differ in the constituents of their venoms. The intraspecific variations are present in relation to season, habitat, age of the specimen, sexual dimorphism and diet. Some researchers have conducted comparative studies on the venom constituents of males and females of the same species, some female snakes have shown similarity in the production of a component (crotamine) in the venom, which is absent in males of the same species. **Objectives:** The objective of this research was to determine whether there is any variability in the venom composition of *Crotalus durissus terrificus* and *Crotalus durissus collilineatus* neonates and if it is related to factors associated with sex, ontogenic development, geographical origin and/or individual variation. **Methods:** Twenty *Crotalus durissus terrificus* from three different litters (1st litter= 3 males and 4 females; 2nd litter = 5 males and 4 females; 3rd litter= 4 females); and seventeen *Crotalus durissus collilineatus* all from the same litter (6 males and 11 females) were used in this experiment. The first extraction was performed when the rattlesnakes were 24 months old and the 7th at 42 months. Protein levels were quantified by a biochemical method (Biuret) and then submitted to electrophoresis. The proteins were separated by SDS-PAGE (T=10.-20.0%) and stained with 0.25% Coomassie solution to analyze the proteins present in the gels. **Results and Discussion:** In the venom of the genus *Crotalus durissus,* there is variation between crotamine-positive and crotamine-negative protein, depending on the snake's geographic distribution. Investigating the litter's venoms in the two subspecies at different ages, the results showed that both subspecies of *Crotalus durissus* possess a variety of crotamine-positive and -negative proteins, present in males and females. The patterns of crotamine-positive and -negative protein are constant in the litters, independent of the animal age.

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1.27 Antibody modeling and expression of human anti-crotoxin single-chain fragment variable (ScFv)

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Introduction: Antibody molecules bind antigens with high affinity and specificity by synergistically using multiple noncovalent forces. The combining site (paratope), whose shape is complementary to the epitope on the antigen, is made up of the hypervariable regions, also called complementarity determining regions (CDRs). The CDRs in the light and in the heavy chains fold into structures that are stabilized by the b-sheet framework of the variable domains. scFv contains the variable domain of heavy (VH) and light (VL) chains linked by a flexible polypeptide $(G_4S)_3$ and may be useful as auxiliary therapy to envenoming by snake bite. Due to the difficulty in obtaining crystals suitable for the structural elucidation of antibody fragments in complex with proteins, other information about the key residues involved in the interaction are very useful. The human neutralizing recombinant anti-crotoxin scFv-6 was isolated by phage display technology from a naive library of more than 10^{10} scFv clones. **Objectives:** The aim of this study was to analyze the structure of anti-crotoxin scFv-6 and to express it in the periplasm of bacteria in order to obtain this molecule in its soluble and functional form. **Methods:** With computer-aided homology modeling using Modeller 9v5 program, the structural/functional relevant regions of heavy and light chains, CDRs, were defined. In each step of modeling, about a hundred models were generated, and the one with the best energy was selected. scFv-6 coding sequence was cloned into pET20b+ vector and the construction was used to transform C43 bacteria. The production of scFv was accomplished using 0.5 mM IPTG and growth condition at 37°C for 4 h. After expression, soluble scFv was recovered from the periplasm by osmotic shock and further purified with nickel resin. ELISA, SDS-PAGE and Western blotting were used to evaluate the purity of the sample. **Results and Discussion:** The modeled structure of ScFv showed the common features of a classical antibody. Its antigen binding surface exhibits electropositive and electronegative potentials that can be related to crotoxin recognition. The yield of expressed scFv-6 was 2.3 mg/L. The purified protein showed an expected band around 30 kDa, soluble and specific for crotoxin. Advantageous mutations of scFv will be generated by site-directed mutagenesis. Original and mutants scFv will then be biochemically characterized regarding their affinity and ability to neutralize crotoxin and venom toxic activities.

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1.28 Crotoxin modifies intracellular signaling involved in phagocytosis by neutrophils

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Introduction: Previous studies showed that *Crotalus durissus terrificus* venom (CdtV) inhibits the phagocytic activity of macrophages and neutrophils and that crotoxin (CTX), the main component of CdtV, is responsible for this effect. In macrophages, CTX causes reorganization of the actin cytoskeleton and inhibition of phosphotyrosine. **Objectives:** Considering that the signaling pathways for phagocytosis both in macrophages and neutrophils have some differences and that the mechanisms involved in the inhibitory effect of CTX on phagocytosis by neutrophils is still unknown, the aim of this study was to investigate the in vitro effect of CTX on tyrosine phosphorylation and actin polymerization on nascent and mature phagosomes. **Methods:** Neutrophils were obtained from the peritoneal cavity of male Wistar rats (CEUAIB 705/10) 4 h after the intraperitoneal administration of carrageenan (4.5 mg/kg). Neutrophils ($1x10^6$ cells/mL) were incubated (1 h) with CdtV (0.5 µg/mL) or CTX (0.08 µg/mL) and then submitted to phagocytosis of opsonized zymosan for 5 or 15 min. Next, neutrophils were fixed and permeabilized. Incubation with primary antibody against phosphotyrosine was performed overnight. The cells were then incubated with the secondary antibody FITC-labeled and stained with rhodamine-phalloidin. Nuclei were stained with DAPI. Slides were mounted and observed by confocal microscopy. **Results and Discussion:** The incubation of neutrophils with CTX induced a marked reduction in staining of phosphotyrosine (97%) and F-actin (73%) in neutrophils during phagocytosis at 5 min, when compared to the controls. Similarly, when phagocytosis was performed for 15 min, CTX reduced the content of F-actin (86%) in relation to controls. The same effect was observed when neutrophils were incubated with crude CdtV. During phagocytosis, the engulfment of the particle begins with nascent phagosome formation, which occurs at 5 min and is complete in approximately 15 min with maturation of the phagosome. In nascent phagosomes, an increase in tyrosine phosphorylation and actin polymerization is observed; this polymerization leads to phagosome maturation. Unlike what occurs in macrophages, our results demonstrate that CTX inhibits tyrosine phosphorylation and consequently actin polymerization. The results presented herein may contribute to explaining the inhibitory effect of CdtV, particularly CTX, on phagocytosis by neutrophils. Furthermore, taking into account the importance of these phagocytes in the inflammatory response, these results contribute to the elucidation of the mechanisms involved in the anti-inflammatory effect of CTX, which has been reported in the literature.

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1.29 Hybrid offspring from *Bothrops erythromelas* and *Bothrops neuwiedi* snakes: characterization and biological activities of their venoms during development

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Introduction: Hybridization between species is a natural process, which can lead to the merging of taxa, to the reinforcement of behavioral barriers to mating, or even to the emergence of new hybrid species. In fact, interspecific hybridization has been shown to play a major role in the formation of new species. Natural hybridization between *Bothrops* spp has been already reported, but such investigations were restricted to morphological analyses of hybrids. Although grouped in the same taxonomical category, the snakes *B. erythromelas*, distributed in northeastern Brazil, and *B. neuwiedi*, occurring in southern states in Brazil, have striking differences in their venom composition, particularly regarding the absence of thrombin-like enzymes in *B. erythromelas* venom. **Objectives:** The aim of this study was to characterize the biological activities of venoms from hybrid snakes between *B. erythromelas* and *B. neuwiedi* with regard to their development. **Methods:** A male *B. neuwiedi* was mated with a female *B. erythromelas* on June 8, 2006, and 15 hybrids were born on February 22, 2007. At three-month intervals, venom was milked from parents and hybrids, lyophilized and used for analyses. The following tests were carried out in venom samples from hybrids and parents: protein assay (bicinchoninic acid method); determination of minimum coagulant dose (MCD) in bovine plasma and fibrinogen; and assays of collagenolytic (using azocoll) and amidolytic activity (using BAPNA). **Results and Discussion:** Protein concentration increased in hybrid venoms over time, reaching values similar to those of the father when hybrids were 2 years old (yo). Amidolytic and proteolytic activities were reduced in newborn venom samples, and progressively increased as snakes grew, reaching values similar to those of the father when hybrids were 1 yo and 6 months old, respectively. Using bovine plasma, lower MCD values were noticed in newborn venoms, close to *B. erythromelas* venom, and reached values similar to those of the father venom when they were 1 yo. The assay of thrombin-like activity showed that hybrid venoms do not exhibit such activity during the first 6 months after birth, beginning to exhibit it when they are 1 yo; similar thrombin-like activity as in the father was noticed when hybrids were 3 yo. Our findings show that there are important changes in the composition and activity of hybrid venoms over their development. Hybrid venoms imitate *B. erythromelas* venom when the offspring is young, and become similar to *B. neuwiedi* venom as hybrids grow older. In addition, our results show that *B. erythromelas* and *B. neuwiedi* are not well-differentiated species.

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2. Biochemistry

2.01 Isolation of bioactive peptides by the action of serine proteases from the venom of *Bothrops jararaca* on endogenous substrates and their actions in cell culture

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Introduction: Venoms are a rich source of proteolytic enzymes. In *Bothrops jararaca* venom, serine proteases and metalloproteases are the main enzymes that act on various tissues and proteins present in the victim. Besides the action on tissues, these proteases could generate some compounds that could have specific actions in cells or display other mechanisms towards the generation of bioactive peptides. Protein precursors of bioactive peptides are generally the target, but nowadays there is new class of proteins that in some conditions may generate bioactive peptides, namely cripteins. **Objectives:** The objectives were to a) identify the bioactive peptides resulting from the action of the serine proteases, trypsin and those from the venom of *Bothrops jararaca* on endogenous substrates, and b) to biochemically isolate and characterize the peptides obtained and determine the possible effects and biological properties of these peptides through several biological tests in vitro and in vivo. **Methods:** A serine protease from *Bothrops jararaca* was separated from the whole venom using an HPLC column for molecular exclusion. The endogenous substrates were also incubated with trypsin and the serine protease from *Bothrops jararaca*. After incubation, proteins and other products were observed by gel electrophoresis with silver nitrate staining and also by means of HPLC profile. The pools of the hydrolysates were tested on cultured fibroblasts and then the purified peaks of these pools. After testing of the peaks on cultured cells, the active peptides were sequenced and synthesized for further testing. **Results and Discussion:** Peaks obtained by HPLC from the hydrolysis of substrates induced cell proliferation in tests with FN-1 cells (fibroblasts) and caused positive membrane lipid peroxidation, and picrosirius red staining showed low stimulation of collagen. These peptides were also found in the proteins that are observed on the cell surface. Therefore, this study suggests that serine proteases are able to generate important biologically active peptides, especially in the interaction of snake venom with human macromolecules.

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2.02 Purification and identification of microRNAs from *Bothrops jararaca* venom glands

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Introduction: The snake venom gland probably evolved from salivary glands of non-venom ancestral species. This epithelium is very specialized in toxin production and is subject to various kinds of regulations, with protein synthesis being initiated by different factors such as reduction of the volume of lumen secretion and other mechanisms dependent on adrenoreceptors. MicroRNAs are small RNAs of 21-23 nt that function as post-transcriptional regulators of gene expression. MicroRNAs have essential roles in development, cell differentiation, cell proliferation, cell death and other functions in plants and animals. **Objectives:** Our aim in this work was to purify and identify microRNAs involved in the regulation of the production and secretion of proteins present in the venom glands of snakes. **Methods:** Our snake model was *Bothrops jararaca*, a Brazilian species belonging to the family Viperidae. In this study, microRNAs were isolated and enriched from total RNA of *B. jararaca* venom glands. After the ligation of suitable clone linkers (mirCAT kit - IDT) the microRNAs, converted into cDNAs were cloned, sequenced, and analyzed by bioinformatics. **Results and Discussion:** The bioinformatics analysis revealed the identity of different small RNAs, with a size range of 13 to 26 nt. Seventeen cloned sequences showed the following sizes: 13 nt (1/17; 6%); 16 nt (4/17; 24%); 18 nt (1/17; 6%); 19 nt (2/17; 12%); 21 nt (1/17; 6%); 22 nt (2/17; 12%); 23 nt (2/17; 12%); 24% (1/17; 6%); 25% (1/17; 6%); 26% (2/17; 12%). The 21-23 nt cloned sequences showed similarity to known microRNAs, such as eca-miR-99a and xtr-miR-99, found in different organisms. We also found significant similarity to piRNAs, a different class of small RNAs, in our 24-26 nt samples. The functions of small RNAs will be analyzed in order to better understand gene regulation in the venom gland. microRNAs represent a new boundary in the knowledge of gene regulation in animals and plants, but the snake microRNA transcriptome set has not yet been studied. We found the first microRNAs and piRNAS in reptiles. Since Brazil is an important repository of this fauna, it is very important to ensure primacy in the study of microRNAs from this taxon.

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2.03 Effects of rLosac on coagulation parameters and comparative studies with RVV-X in the activation of factor X

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Introduction: Losac is the first factor X (FX) activator purified from *Lonomia obliqua* bristles. The mechanism of cleavage is still unknown. On the other hand, the mechanism of RVV-X, a FX activator from *Daboia Russelli,* is well known. RVV-X activates FX through the recognition of the Gla domain, and after this the cleavage of the FX heavy chain occurs through the RVV-X metalloprotease domain. **Objectives:** The aim of the study was to obtain recombinant Losac with enzymatic activity and to compare its activity with RVV-X. **Methods:** The *E. coli* BL21 (DE3) strain was transformed with the recombinant plasmid (pAE-Losac). This system is designed for expression of rLosac with a tail of 6x-His at the N-terminal end. We evaluated the activation of human FX (345 nM) using different concentrations of rLosac and RVV-X in chromogenic assays (0.2 mM S-2765) and also based on the degradation of normal and deglycosylated FX by SDS-PAGE. The specificity of Losac activity for FX was evaluated assaying its activity with several other substrates, including prothrombin (chromogenic assays), fibrinogen and fibrin (SDS-PAGE and fibrin plates, respectively). The capacity of rLosac to activate factor X in plasma was assayed by plasma recalcification time, TTPA and TP. **Results and Discussion:** rLosac and RVV-X activated FX in a dose-dependent manner. rLosac was able to activate deglycosylated FX, although this activity was lower than that with normal FX. Apparently, the protein is specific for FX, since it does not activate prothrombin and does not cleave fibrinogen or fibrin. The normal plasma recalcification time (195.6±37.67s) was shortened after 2 min of incubation with rLosac (70.08 ± 4.80 s) or RVV-X (59.4 ± 3.48 s). Moreover, when we incubated in plasma for 10 min, rLosac, using similar molar concentrations of rLosac or RVV-X, induced a more significant reduction of recalcification time - rLosac:(11.64 ± 4.27.s), RVV-X:(54.06 ± 6.15.s). The TTPA and TP times were reduced when incubated for 2 min with rLosac in comparison with the control - control TTPA:(196.05 ± 2.52), rLosac TTPA:(162.9 ± 5.14), control TP:(16, 6 ± 0.52), rLosac TP:(13.1 ± 0.52). These results demonstrate that the activation of FX by rLosac is different from the one produced by RVV-X. The exact mechanism is not yet known. We are working to understand the mechanism by which rLosac recognizes FX.

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2.04 Characterization of keratinocyte responses to FGF1, FGF2 and FGF7

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Introduction: Keratinocytes, the main epidermal cells, are responsible for the formation of an impermeable barrier that protects the body against injuries and attachment of microorganisms. Therapeutic agents that favor wound healing are of great interest to medicine and have been extensively sought. Studies have suggested the participation of FGF (fibroblast growth factor) family members in wound healing. It is known that these factors promote growth (FGF7 and FGF10, also known as keratinocyte growth factor 1 and 2, respectively) and stimulate the migration of keratinocytes, but their molecular mechanisms and biological role in these cells are not totally established. **Objectives:** The aim of this study was to characterize keratinocyte responses to FGF1, FGF2 and KGF and the expression of endogenous FGFs and FGF receptors (FGFRs). **Methods:** Responses of the HaCaT cell line and keratinocytes immortalized with E6 and E7 (HPV oncogenes) to FGFs were monitored by growth curves, mitotic nuclei counting, MTT assay, migration assay and Western blotting for ERK and Akt phosphorylation. The expression of FGFRs (fibroblast growth factor receptors) and endogenous FGFs were determined by quantitative PCR and PCR, respectively. **Results and Discussion:** All three growth factors, namely, FGF1, 2 and 7, activated the ERK pathway, but only FGF7 promoted keratinocyte proliferation, whereas FGF1 and 2 were more important in triggering migration. Keratinocytes expressed FGFR2, FGFR3 and FGFR4, the last being detected for the first time in this cell type. These cells also expressed endogenous FGF2, which can have a role in paracrine stimulation of dermal fibroblasts and an autocrine role stimulating its own migration. These results suggest that different FGFs trigger different responses in keratinocytes and probably are very important in wound healing, promoting keratinocyte proliferation and migration and, in addition, the stimulation of dermal cells.

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2.05 Antimicrobial activity in whole extracts of two species of Chilopoda

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Introduction: One of the main components of the defense mechanism in vertebrates and invertebrates are the peptides with immune functions. The defensive role of a variety of antibiotic peptides and different types of molecules in multicellular organisms is increasingly recognized. However, the characterization of the molecules with antimicrobial function from chilopods had not begun until recently. For these reasons, it is important to study this subject, not only to understand the success of these factors in the defense mechanisms of these animals, but also to find alternatives to fight the infectious and also parasitic diseases that affect humans. **Objectives:** The aim of this study was the analysis of different molecules in order to identify the antimicrobial factors in the extract of the body of the Brazilian myriapods *Otostigmus* sp. and *Escolopendra viridicornis.* **Methods:** The bodies of the animals were first subjected to maceration and acid extraction and then fractionated in two steps. First, using C18 Sep Pak column cartridge, the hydrophilic and hydrophobic fractions were separated. The hydrophilic fraction was purified in two more steps, first by ion exchange chromatography and then by gel filtration chromatography. The hydrophobic fraction was loaded onto a semi-preparative C18 Jupiter column using a linear gradient of ACN in 0.05% TFA for the second purification step. The column effluent was monitored by absorbance at 225 nm and the antiparasitic and antimicrobial activities were determined by liquid growth inhibition assay. First, characterization was by mass spectrometry (MALDI-TOF). **Results and Discussion:** After HPLC separation of the highly hydrophilic compounds, antimicrobial activity against *Escherichia coli* and *M. luteus* was detected in three fractions (one from *Otostigmus* sp. and two from *E. viridicornis*). These fractions also showed anti-parasite activity against *Leshmania* spp. and *T. brucei.* The analysis with mass spectrometry showed that these fractions have a low molecular weight. In the hydrophobic fraction, seven fractions were detected with antimicrobial activity against *M luteus* (3 fractions) and *E. coli* (4 fractions). Four were from *Otostigmus* sp. and three from *E. viridicornis*. The characterization of these fractions is still in progress.

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2.06 Characterization of major cathepsin L-like proteases from hepatopancreas of the scorpion *Tityus serrulatus*

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Introduction: Cathepsin-L is widely distributed 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 the digestion of proteins ingested. Our group had found for the first time that the major endopeptidase responsible for protein digestion in the scorpion *Tityus serrulatus* is a cathepsin-L-like cysteine peptidase. **Objectives:** The aim of this study was the characterization of the major cathepsin-L-like component present in the hepatopancreas of the scorpion *Tityus serrulatus*. **Methods:** In order to characterize this enzyme, *Tityus serrulatus* females were fed *Gryllus sp*, dissected and the isolated hepatopancreas was homogenized in a solution of 1 mM MMTS, a reversible inhibitor of cysteine peptidases, dissolved in Milli-Q water. Enzyme source was obtained from crude homogenized samples or partially purified on a HiTrap S column equilibrated in 50 mM citrate-phosphate buffer, pH 5.0, where the elution was obtained with a gradient of 0 – 1 M NaCl in the same buffer. Activity was measured using Z-FR-MCA or Abz-FRQ-EDDnp as substrate in different buffers, all containing 3 mM cysteine and EDTA. **Results and Discussion:** Assays using fluorescent or quenched fluorescent substrates indicated that the enzyme is a cathepsin-L-like hydrolyzing preferentially substrates with a Phe residue at P2. This enzyme showed characteristics similar to another arthropod's digestive cathepsins L, with a pH optimum of 5.5, a molecular weight of 44 kDa (proenzyme) and 26 kDa (active form) determined by Western blotting using an antibody specific for insect digestive cathepsin-L. It was mainly stable in acidic conditions (pH 2.5 to 6.5) at 4°C and 30°C, with thermal stabity showing a half-life of 8 min at 65°C. The isoeletric point calculated for the proenzyme was 5.8 and for the active form 6.5. Also, the K_m values were calculated for two different substrates: Z-FR-MCA (24 μM) and Abz-FRQ-EDDnp (30 nM).

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2.07 Antibiotics from spider eggs

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Introduction: The proliferation of infectious diseases is an important human health problem. Inappropriate use of antibiotics brings about the appearance of bacterial strains resistant to conventional drugs, which means that research is necessary to identify new molecules as alternatives to the currently used drugs. The study of antimicrobial peptides may be advantageous for human use, because its action is faster than conventional antibiotics. Many studies have identified antimicrobial peptides in several species. One example is gomesin, an antimicrobial peptide isolated from the hemocytes of the spider *Acanthoscurria gomesiana,* which possesses a high spectrum of activity against many microorganisms. Lysozymes, 14kDa molecules with antimicrobial activity against Gram-positive bacteria and yeast, have been identified in eggs of some species. Studies on spider silk pointed to the possibility of antimicrobial activity, but no research reported antimicrobial activity from spider eggs. **Objectives:** Our objective was to purify and characterize antimicrobial factors from eggs of the *Phoneutria nigriventer* spider. **Methods:** In order to do so, we used reversed phase high performance liquid chromatography (RP-HPLC) to isolate proteins and peptides, electrophoresis and mass spectrometry techniques to characterize them, and Edman degradation for sequencing **Results and Discussion:** As results, we isolated two antimicrobial peptides from these eggs. The peptides were active against the Gram-positive bacteria *Micrococcus luteus*. Two other fractions, most likely lysozymes, were purified and were active against the yeast *Candida albicans* and the bacterium *M. luteus*. This is the first report of antimicrobial factors from spider eggs.

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2.08 Production of a recombinant platelet aggregation inhibitor from the leech *Haementeria depressa* in *Pichia pastoris*

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Introduction: The saliva of hematophagous animals has substances that maintain blood fluidity during the feeding process, including molecules affecting hemostatic processes. Our group has identified several compounds and determined the profile of transcripts and proteins from the *H. depressa* leech salivary complex through biochemical, transcriptomic and proteomic analysis. In this tissue were detected some clones similar to an inhibitor of platelet aggregation from *H. officinallis* leech named LAPP. LAPP is around 14 kDa and has a pI of 4.0, and it inhibits platelet aggregation by collagen. **Objectives:** The aim of this work was to clone, in an expression vector (pPIC9K), a transcript from the *H.depressa* salivary complex cDNA library which showed identity to LAPP, and to define the best method of expression and purification of this clone for future comparative studies with LAPP. **Methods:** The H06A09_pGEM11Zf was chosen for this study, and after amplification by PCR with specific primers, the product was cloned in pPIC9K vector between *EcoRI* and *NotI* cloning sites. The sequence of the H06A09_pPIC9K clone was confirmed, and it was linearized using *Sac*I digestion. The clone was transformed in *Pichia pastoris* (GS115) and expressed in different conditions. The recombinant protein expressed was submitted to some different purification methods [ultra filtration (Amicon / Millipore 5 and 30kDa); gel filtration (Superdex 75 / GE); anionic exchange (Mono-Q / GE) and reverse phase (C18)]. The different expression and purification steps were analyzed by SDS-PAGE. The platelet-aggregation inhibition assays were performed using collagen as agonist with protein of some purification steps, performed in whole blood. **Results and Discussion:** The H06A09_pPIC9K sequencing showed 93% similarity with LAPP, where all Cys residues were conserved. 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 for recombinant purification was submitting the culture supernatant to dialysis and concentration by ultra filtration in Amicon 5kDa and then to gel filtration in Superdex 75, followed by reversed phase chromatography on a C18-column in an HPLC system. The recombinant protein was expressed and showed about 20 kDa (SDS-PAGE). Until now, only a few tests of platelet aggregation inhibition with the semi-purified recombinant molecule could be performed, and it showed a low inhibition using collagen as agonist. However, we are purifying a greater amount of protein for the characterization of this inhibitor to be better evaluated.

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2.09 Processing of SVMPs: isolation of the recombinant pro-domain for antibody production

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Introduction: Snake venom metalloproteinases (SVMPs) are abundant enzymes in *Bothrops* venoms responsible for local and systemic symptoms of human poisoning. These enzymes hydrolyze components of the extracellular matrix and modulate the activation of platelets, endothelial cells and the inflammatory system. SVMPs are synthesized as zymogens, and it is believed that enzyme activation is regulated by removing the pro-domain, as occurs in MMPs. However, it is not known exactly how or where the processing of the pro-domain and the activation of SVMPs occur. **Objectives:** In this work we aimed to clone and express the pro-domain of a P-III class SVMP, jararhagin, and produce specific antibodies. These will be used as a tool in immunohistochemistry experiments to identify the exact location where the activation occurs. **Methods:** The sequence of the pro-domain was amplified by PCR from cDNA extracted from venom glands of *Bothrops jararaca.* The gene obtained was cloned into the pAE vector in fusion with 6 histidines. The production of recombinant protein was induced in *Escherichia coli* (BL21 (DE3) star plys S) for 4 h at 37° C by the addition of isopropyl b-D-thiogalactoside (IPTG) to a final concentration of 1 mM. The recombinant pro-domain was purified with immobilized metal affinity chromatography (IMAC), and its size, integrity and purity evaluated by SDS-PAGE. The recombinant protein was injected into mice and rabbits to produce polyclonal antibodies. **Results and Discussion:** Based on the sequences of jararhagin and bothropasin pro-domains, oligonucleotides were synthesized and used to amplify the pro-domain cDNA by PCR. The PCR product was a single band with an estimated size of 500 bp. This band was purified, digested with restriction enzymes and then cloned into the pAE vector. After transfection, colonies were screened by PCR using the vector forward and reverse primers and a positive clone, with a ~500 bp amplification band was found. The insert was sequenced and showed 98% homology with the pro-domain of jararhagin and 97% with the pro-domain of bothropasin. Thus, *E. coli* (BL21 (DE3) star plys S) was transformed with this clone and expression induced by IPTG, resulting in a major protein with a molecular mass of approximately 20 kDa in the soluble extract. The protein was purified by IMAC and injected into rabbits and mice to obtain polyclonal antibodies, which may help to better understand the biosynthesis of venom metalloproteinases and their importance in poisoning.

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2.10 Enzymatic characterization of *Bothriechis schlegelii* snake venom from Colombia and Costa Rica

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Introduction: The *Bothriechis schlegelii* snake (eyelash pit viper, also known as "boracacá") is a relatively small animal (rarely exceeding 75 cm in length) found in the mesic forest in Mexico, Central America and northwest of South America. Patients bitten by *B. schlegelii* show at the bite site pain, edema, ecchymoses and necrosis. Hematuria, gingival bleeding, hipovolemic shock and oliguria are the systemic signs of envenomation. **Objectives:** The aim of this work was to characterize enzymatically *B. schlegelii* snake venom from Colombia (BsCo) and Costa Rica (BsCR). **Methods:** SDS-PAGE (12%) was used to evaluate the protein profile of BsCo and BsCR venoms (5 µg). To analyze the enzymatic activity, zymography was employed using gelatin (2 mg/mL), casein (2 mg/mL), fibrinogen (0.5 mg/mL) and hyaluronic acid (170 µg/mL) as substrate in a polyacrylamide gel (12%). **Results and Discussion:** After SDS-PAGE, under non-reducing conditions, many components with similar molecular masses (150 – 22.5 kDa) were noticed in BsCo and BsCR venoms. However, some bands around 62.5, 33.2, 25.7 and 22.5 kDa were observed exclusively in BsCo venom. No hyaluronidase activity was detected in BsCo and BsCR venoms. Weak fibrinogenolytic activity was observed. BsCo venom showed three bands with MW around 46.8, 42.1 and 30.5 kDa, and BsCR venom displayed components with fibrinogenolytic activity with approximately 43.4 kDa. Different profile for gelatinolytic activity was also observed in BsCR and BsCo venoms. BsCR venom showed bands with intense gelatinolytic activity around 43.1 and 40 kDa. However, BsCo venom showed several components with weak activity around 54, 46.7, 38.1, 31.5 and 25.1 kDa. BsCR and BsCo venoms showed similar profiles for caseinolytic activity located between 50.8 and 23.6 kDa. Both venoms contained an enzyme with higher level of caseinolytic activity around 34.8 kDa. Our results demonstrate that geographic distribution can have an influence on venom composition, which may modify the local and systemic symptoms observed in human envenoming.

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2.11 Anti-mitogenic effects of FGF2 in human cells transformed by the RasV12 oncogene

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Introduction: Our group recently reported that fibroblast growth factor 2 (FGF2) causes cell cycle arrest and induces senescence-associated β-galactosidase in Ras-dependent malignant mouse cells. Aiming to uncover the mechanisms underlying this anti-mitogenic action, we studied the effects FGF2 in human embryonic kidney cells (HEK293 line) stably infected with the construction ER:rasV12, whose product is 4-hydroxy-tamoxifen (4-OHT)-binding domain of the estrogen receptor fused to the oncoprotein H-RasV12. This approach propitiates a molecular switch to turn H-RasV12 on/off, respectively with or without 4-OHT, allowing us to analyze FGF2 effects on HEK293 cell cycle under both normal and malignant phenotypes. **Objectives:** The aim of the study was to determine the effects of FGF2 on HEK293 cells displaying an inducible Ras-dependent malignant phenotype. **Methods:** *Generation of cell sublines.* HEK 293 cells were infected with the pBabe-Neo-ER:rasV12 viral vector and selected for resistance to geneticin (800 μg/ml). Stable poly- and monoclonal sublines were analyzed for integration and inducible expression of H-rasV12 by Western blotting. *Phenotype characterization.* HEK293 sublines were analyzed with respect to growth curves in monolayer cultures (day 0: 3000 cells/cm^2 in 35-mm dishes) and clonogenic assays in suspension cultures of soft-agar (10^3 cells/well in 24-well plates). Cells were grown in 10% FBS-DMEM and treated with 10 ng/ml FGF2 and/or 200 μM 4-OHT. **Results and Discussion:** In 10% FBS-DME, HEK293 sublines displayed a "normal" phenotype growing regularly in monolayer, but not in suspension cultures. FGF2 mitogenically stimulated HEK293 sublines in both monolayer and suspension cultures. Under 4-OHT treatment, HEK293 sublines exhibited morphological transformation in growing monolayers and developed colonies in suspension cultures. These last results have shown that HEK293 cells are not prone to oncogene stress, undergoing malignant-like transformation when submitted to H-RasV12 activation. On the other hand, the proliferation of HEK293 sublines treated with both FGF2 and 4-OHT was drastically inhibited in monolayer and suspension cultures. Thus, like mouse cells, HEK293 sublines could not cope with both FGF2 treatment and H-RasV12 oncogene activation, resulting in severe stress.

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2.12 Effect of FGF2 on human keratinocytes expressing H-RasV12

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Introduction: FGF2 (fibroblast growth factor 2) was initially described as a fibroblast-specific growth factor, but today, it is known for acting in different cell types, triggering signaling pathways involved in proliferation, surveillance, migration and others. Many studies relate FGFs and its receptors to tumorigenesis. Recently, our laboratory reported that FGF2 restores tumor defense mechanisms, inhibiting proliferation and inducing senescence in malignant mouse cells, expressing Ras oncoprotein, but not in immortalized nontumorigenic cell lines. **Objectives:** The aim of this study was to determine if FGF2 also induces antiproliferative effects in human keratinocytes expressing H-RasV12 and if p53 is involved in this phenomenon. **Methods:** HaCaT cells, a human immortalized keratinocyte cell line without functional p53, were infected (retroviral vector) with a construct with the ligand-binding domain of estrogen receptor fused to H-RasV12 (ER:RasV12). This fusion protein allows the activation of H-RasV12 oncoprotein when 4-hydroxy-tamoxifen (4OHT), an estrogen receptor agonist, is added to the culture. **Results and Discussion:** HaCaT sublines responded mitogenically to FGF2; in addition, these sublines displayed traces of malignant phenotype when H-RasV12 was activated with 4OHT. These results imply that HaCaT keratinocytes are not susceptible to oncogene stress under H-RasV12 activation. On the other hand, HaCaT sublines exhibited a severe stress when treated with both FGF2 and 4OHT. Thus, HaCat, like mouse cells, cannot stand FGF2 treatment plus H-RasV12 activation. Furthermore, the stress triggered by FGF2 in Ras-driven human malignant keratinocytes seems to be independent of the tumor suppressor protein p53.

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2.13 Analysis of antithrombin variants from *Bothrops jararaca* snake plasma

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Introduction: Antithrombin, a plasma glycoprotein, is the major inhibitor of the coagulation serine proteinases in mammals and plays a crucial role in the maintenance of normal hemostasis. Fish antithrombins have three polysaccharide chains while mammalian antithrombins show two forms, α-antithrombin, which contains four polysaccharide chains, and β-antithrombin, which lacks one carbohydrate chain situated near the heparin binding site, leading to a higher heparin affinity than the α-form. **Objectives:** The aim of this work was to investigate the presence of glycosylation variants of antithrombin in *Bothrops jararaca* plasma. **Methods:** Antithrombin was isolated from snake plasma with a HiTrap Heparin column using a step-wise gradient with 2 M NaCl. Purified antithrombin was applied to the same column and eluted by a linear gradient from 0.5 to 2 M NaCl. Antithrombin variants would be separated using a HiTrap Heparin column according to their heparin affinity. **Results and Discussion:** Our SDS-PAGE results suggest that glycosylation variants of antithrombin may be present in *Bothrops jararaca* plasma. Further experiments will be carried out to deglycosylate the purified antithrombin from *B. jararaca*. The perspective for this work is to clone the DNA fragment, which encodes for *B. jararaca* antithrombin.

Supported by: FAPESP

2.14 Rondonin: an antifungal peptide isolated from the plasma of the spider *Acanthoscurria rondoniae*

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Introduction: A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. We found an antimicrobial peptide in the plasma of *Acanthoscurria rondoniae* with antifungal activity, and this molecule was characterized by mass spectrometry as a single molecule of 1,236 Da. This peptide has been submitted to "de novo" sequencing, elucidating its primary structure: IIIQYEGHKH, which showed similarity with a hemocyanin fragment and was named rondonin. It has become increasingly clear that, due to the continuous use of antibiotics, the emergence of multi-resistant bacterial strains has occurred all over the world. As expected, antibacterial and antifungal peptides have attracted attention in recent years, in order to find new therapeutic agents. **Objectives:** The objective of this study was to determine the spectrum of activity of rondonin and its analogue: IIIQYEGKH. **Methods:** Synthetic peptide was obtained in an automated bench-top simultaneous multiple solid-phase synthesizer (PSSM 8 system from Shimadzu Co.) using solid phase peptide synthesis by the Fmoc [rocedure. The peptide was purified by reversed-phase chromatography (Shim-pack Prep-ODS, 5μ, 20 mm × 250 mm Shimadzu Co.) semi-preparative HPLC, and the purity and identity of the peptide confirmed by MALDI-TOF mass spectrometry and by analytical HPLC, under the same conditions described above. The range of activity was determined by a liquid growth inhibition assay against three Gram-negative bacteria, three Gram-positive bacteria, two fungi and seven yeasts. The time-kill curve for rondonin was performed using twice the MIC (67 μM) against *Candida albicans* MDM8. The hemolytic activity of these peptides was tested against human red blood cells at an initial concentration of twice the MIC for both peptides. Hemolysis was determined by reading the absorbance at 595 nm of each well in Victor[3] (1420 Multilabel Counter/Victor[3] – Perkin Elmer). **Results and Discussion:** We found that rondonin was active against all yeasts tested and one fungus in a concentration range of from 67 μM to 1.1 μM and its analogue showed antimicrobial activity only against *Pseudomonas aeruginosa* ATCC 27853 with a concentration range from 67 μM to 33.5 μM. The time-kill curve showed us that rondonin is a fungicidal peptide and compared to conventional antifungals, it is more effective. These two peptides have no hemolytic activity at the concentration tested. These results suggest that rondonin could be the first step in the development of new antifungal drugs.

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2.15 Weaving health: the weaving of antimicrobial substances from the ootheca of the spider *Phoneutria nigriventer*

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Introduction: Increasingly, conventional antibiotics are losing their ability to heal, and infectious diseases are responsible for 20 million deaths per year worldwide, where they are among the most common causes of death in the human population. Researchers have demonstrated that bacteria are acquiring resistance to conventional antibiotics rapidly and widely, mainly due to the slow activity of these chemical substances. The conclusion of these facts is that in the future these antibiotics will not be effective and the number of deaths from infectious diseases will just grow. **Objectives:** The objective of this research was to find new antibiotics that drastically reduce the chances of bacteria acquiring resistance. Based on this idea, the hypothesis was proposed that it would be possible to find these antibiotic substances in the *ootheca* (structure made of silk that surrounds the eggs) of the spider *Phoneutria nigriventer*, since it is used to protect the eggs, also from predators, not just physically but also from infectious agents. Besides that, early Brazilian cultures used web silk as a healing substance for deep cuts. **Methods:** With an elaborated hypothesis, and using laboratory methods, we could begin the process of indentifying and characterizing the antimicrobial substances. The samples were purified by high performance liquid chromatography (HPLC) which fractionated the molecules of the ootheca with spectrophotometric monitoring. **Results and Discussion:** The results of the antimicrobial tests showed 8 fractions with antimicrobial and also antifungal activity. Beyond that, two of these molecules were characterized, one with molecular weight of 1338 Da and the other 1194 Da; these two fractions showed antimicrobial activity against *E. coli*. MIC assays based on molar amounts are in process, as well as the sequencing of these molecules.

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

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Introduction: It is common in *Phoneutria* sp envenomation that there are clinical manifestations with local events. Systemic envenomation is considered severe when causing more severe cardiovascular changes, especially in children. The occurrence of changes in body temperature, mainly hypothermia is related to hemodynamic changes, suggesting that the signs and symptoms occur by the release of norepinephrine at sympathetic nerve terminals. Another possibility is the action on the CNS, due to neurotoxic polypeptides present in the venom. **Objectives:** The aim of the study was the biochemical characterization of fractions obtained from the venom of *Phoneutria nigriventer* and the determination of their biological action on the regulation of body temperature in young rats. **Methods:** The crude venom was purified using gel filtration (Sephadex G-50) and reverse phase HPLC. The body temperature of young rats (140-160 g) was recorded by a subcutaneous probe acquisition system (ML 309 Thermistor Pod - ºC Scale - AdInstruments®) and recorded using the software Powerlab (AdInstruments®) every 0.5 min for 3 h. **Results and Discussion:** Fractionation of the crude venom of *Phoneutria nigriventer* revealed four pools. Pool I caused no changes in body temperature, and the partial results of pools III and IV revealed oscillations, and these pools need to be purified again. Partial results of pool II showed that fraction III causes a very significant fall in body temperature of young rats.The results suggest that the hypothermic effect involves a neurotoxin activity on the thermoregulation of young rats. Purification of the other pools and fractions are underway to determine their activity on the thermoregulation of young rats.

Supported by: INCTTOX Program - CNPq, FAPESP, Fundação Butantan

2.17 Factor X activation mechanism by recombinant Losac (a hemolin from *Lonomia obliqua*): potential inducer of cell proliferation and cell survival

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Introduction: Losac is the first, and so far only, factor X (FX) activator purified from a Lepidoptera secretion. We have shown that Losac is capable of inducing the expression of hemostatic mediators and cell proliferation in HUVECs. However, little is known about the mechanism and structural features implicated in both activities. **Objectives:** The aim of the study was to improve heterologous expression of recombinant Losac (rLosac), to study the FX activation mechanism, and to study the mechanism of cell proliferation and survival. **Methods:** Several *E. coli* strains and methodologies for expression and refolding were applied. Characterization of rLosac: chromogenic assays, SDS-PAGE, fibrin plate and immunoblotting. Cultured cell lines: HUVECs and fibroblast. Cell viability: MTT method. Cell cycle: flow cytometry. Cell signaling pathways: Western blotting. Gene expression: RT-PCR. **Results and Discussion:** rLosac was expressed in *E. coli* BL21(DE3) as inclusion bodies and a molecular mass of 48.6 kDa (containing a His_6-tag N-terminus). rLosac was able to shorten the plasma recalcification time and to activate FX but had no effect on prothrombin, fibrin or fibrinogen, indicating its specificity for FX. rLosac was recognized by antilonomic serum suggesting its role in the envenomation processes. The FX cleavage pattern induced by Losac was similar to that of RVV-X (a well-known factor X activator) and inhibited by PMSF. When a Gla-domainless FX was used the activation was markedly diminished, indicating that the Gla-domain is important for Losac's activity. In an attempt to understand the interaction between Losac and FX, a three-dimensional structure model of Losac was built. Structure comparison analysis identified in Losac's model a probable interaction surface and electrostatic potential quite similar to the Gla-domain FX/RVV-X binding region. Our model of the mechanism of FX predicted that Losac's binding region in domain 2 could be a good candidate for the interaction with the Gla-domain FX followed by the activation through the Losac's catalytic site predicted in domain 1 (Asp^{62}, His^{94} and Ser^{112}). We also determined that rLosac is able to induce proliferation and antiapoptotic effects in HUVECs and fibroblasts submitted to starvation. An increase in the cell cycle (S phase, 48 h) was also observed. Moreover, a significant release of NO and t-PA were modulated by rLosac. Cell signaling studies revealed the activation of survival pathways, and the expression of antiapoptotic genes was confirmed by RT-PCR. Taken together, these results contribute to extending the scope of the biological functions of hemolins.

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2.18 Amblyomin-X induces cell apoptosis and microenvironment alterations by proteasome and NF kappa B modulation

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Introduction: A recombinant protein, with inhibitory effects on factor-X, and characterized as a Kunitz type inhibitor, was obtained from the cDNA library of the salivary gland of the *Amblyomma cajennense* tick. When tested in different cell lines, the protein showed apoptotic activity in tumor cells (Sk Mel-28, Mia Paca-2). Amblyomin-X does not show cytotoxic activity in fibroblasts and melanocytes. **Objectives:** The aim of the study was to evaluate the influence of Amblyomin-X on cell viability and on the microenvironment of cancer cells after treatment. **Methods:** Cell death was evaluated by the MTT assay and flow cytometry. The release of uPA, PAI and tPA/PAI was evaluated by ELISA. The protesomal activity was assessed by fluorimetry, and NF*kappa*B was accessed by Western blotting. **Results and Discussion:** The data obtained showed that Amblyomin-X induces apoptosis in cancer cells but not in normal cells. The cancer cell microenvironment showed changes in uPA and PAI levels. Amblyomin-X also inhibited tryspsin- and chemotrypsin-like activity of proteasomes. Amblyomin-X induced apoptosis in cancer cells but not in normal cells. Furthermore, the treatment with Amblyomin-X decreased the release of uPA and PAI. Our hypothesis is that Amblyomin-X inhibits the activity of the proteasome, thus indirectly inhibiting NF*kappa*B. This disrupts cell cycle control and prevents the transcription of anti-apoptotic proteins, thereby killing cancer cells.

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2.19 Antimicrobial activity of synthetic mygalin and analogues

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Introduction: Mygalin is an acylpolyamine of 417 Da found in blood cells of the mygalomorph spider *Acanthoscurria gomesiana*. Mygalin was identified as bis-acylpolyamine N 1,N 8-bis(2,5-dihydroxybenzoyl)spermidine, in which the primary amino groups of spermidine are acylated with the carboxyl group of 2,5-dihydroxybenzoic acid (gentisic acid). Native mygalin was active against *Escherichia coli* at 85 μM, this activity being completely inhibited by catalase. Therefore, the antibacterial activity of mygalin was attributed to its production of hydrogen peroxide (H_2O_2). **Objectives:** The aim of the study was to synthesize and evaluate the antimicrobial and hemolytic activity of the acylpolyamine mygalin and analogues. **Methods:** Mygalin and its analogues were synthesized using the technique for peptide synthesis. The product of synthesis was analyzed by reversed-phase HPLC on a semi-preparative Jupiter C18 column. The antibacterial activity was determined by a liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Molecular weight and purity of the molecules were analyzed by mass spectrometry (MALDI-TOF). **Results and Discussion:** During the synthesis of mygalin, we obtained three molecules, one of mygalin itself and two analogues. The mygalin showed two gentisic acids on each end of spermidine. The first analogue showed only one gentisic acid and the other analogue had three gentisic acids. Synthetic mygalin was active against *E. coli* at 14 μM, six times more active than the native form. The synthetic molecule and its analogues presented different antimicrobial activities. Synthetic mygalin was active against *E. coli* and *P. aeruginosa* and did not show hemolytic activity. The analogue with one gentisic acid and mass of 281.4 Da, was active against *E. coli* and showed hemolytic activity. The other analogue with three gentisic acids and mass of 553.5 Da, was active against *E. coli* and *Candida albicans*. The determination of the activity spectrum of these three molecules is in progress.

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2.20 Interaction of the antimicrobial peptide longipin with LUV's

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Introduction: Longipin is an antimicrobial peptide (AMP) composed of 18 amino acid residues isolated from the plasma of the harvestman *Acutisoma longipes*. This molecule showed *in vitro* activity against *Micrococus luteus* (Gram-positive bacterium) and *Candida albicans* (yeast). The most common target of AMPs is the lipid membrane. They can form pores or act in a "detergent-like" manner. Otherwise, they can have an internal target. Therefore, in both cases, the antimicrobial peptides must interact with the lipid membrane to exert their actions. **Objectives:** The aim of the study was to investigate the interaction of longipin with large unilamellar vesicles (LUVs) by spectroscopic techniques. **Methods:** The LUVs were extracted through 10 freeze-thaw cycles and were composed of different ratios of palmitoyl-oleoyl phosphatidylglycerol (POPG) and palmitoyl-oleoyl phosphatidylcoline (POPC). LUVs were used in three different assays: (i) binding with different peptide:lipid vesicle ratios (monitoring the fluorescence of tyrosine residues), (ii) dye leakage (using carboxyfluorescein loaded vesicles) to investigate the membrane permeabilization, and (iii) insertion into the bilayer (acrylamide was used as a quencher). The conformational changes of the molecule in the presence of different POPC:POPG ratios in D_2O were analyzed by Fourier transformed infrared spectroscopy. **Results and Discussion:** Longipin showed preferential interaction with POPG-containing vesicles, increasing their permeability. There was a change in the secondary structure of the peptide in contact with POPG:POPC (1:1) when compared to POPC vesicles and the peptide in buffer (10 mM NaCl in D2O). Longipin showed no conformational differences between the POPC vesicles and the buffer systems. Selective activity of antimicrobial peptides can be explained by their preferential binding to the more negatively charged membranes from microorganisms instead of the zwitterionic ones from mammals. Our results elucidate the preferential binding of longipin to negatively charged vesicles (POPG-containing vesicles), increasing their permeability, when compared to zwitterionic vesicles.

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2.21 Purification of Amblyomin-X expressed in *Pichia pastoris*

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Introduction: A cDNA library had been previously constructed with the *Amblyomma cajennense* salivary glands and a clone was chosen to be expressed. The recombinant protein, named Amblyomin-X, inhibits FXa and causes tumor remission in mice implanted with melanoma cells, without, however, affecting the normal cells. The inhibitor was then expressed in the yeast system *Pichia pastoris* using the pPIC9K vector. **Objectives:** The aim of this study was to purify, sequence and obtain parameters of an inhibitor of FXa, obtained from Amblyomin-X. **Methods:** The recombinant protein was separated by distinct processes. Centrifuged materials were first partially purified by ion-exchange chromatography in Source Q (System ÄKTA purifier - GE) and affinity heparin Sepharose. Chromatography (Source Q15 and Heparin Sepharose) used the equilibrium buffer 20 mM Tris- HCl, pH 8.0. Elution buffer was 0 – 500 mM NaCl in 20 mM Tris- HCl, pH 8.0. Flow rate was 10 mL/min. Fractions of 10 mL were collected. Protein profile was monitored at 280 and 214 nm. **Results and Discussion:** Both materials had been partially purified by an ion-exchange chromatography in Source Q (System ÄKTA purifier - GE). The yeast-expressed Amblyomin-X inhibited FXa amidolytic activity using the chromogenic substrate S-2765. Amblyomin-X induced cytotoxicity in both cell lines analyzed, causing morphological changes. The responses were found to be dose- and time- dependent. Therefore, Amblyomin-X is a potential drug for preventing thrombosis and intravascular coagulation in cancer patients.

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2.22 Evaluation of expression in *Pichia pastoris* and purification of Amblyomin-X produced on shaker

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Introduction: A cDNA library had been previously constructed with the *Amblyomma cajennense* salivary glands and a clone was chosen to be expressed. The recombinant protein, named Amblyomin-X, characterized as a Kunitz-type inhibitor, inhibits FXa and causes tumor remission in mice implanted with melanoma cells, but without affecting normal cells. **Objectives:** The aim of this work was to evaluate the expression and purification of Amblyomin-X in *Pichia pastoris*, due to its potential to be of great pharmacological interest. **Methods:** The inhibitor was then expressed in a yeast system *P. pastoris* using the pPIC9K vector. This system offers the advantage of producing an inhibitor to be up-streamed, 8.5 liters of inhibitor had been produced in a shaker. The recombinant protein was separated from the culture medium by centrifugation, filtrations and membrane clarification/concentration (UFP-5-C-4X2MA). The material was partially purified by an ion-exchange chromatography in Source Q (System ÄKTA purifier-GE). The purifications steps were analyzed by 10% SDS-PAGE, Western blotting, and the Schiff method. Also evaluated for specific activities, the cytotoxic activity of FXa against the tumor cell lines Mia-PaCa-2 and Sk-Mel-28. **Results and Discussion:** Amblyomin-X produced in a shaker was about 35 kDa by SDS-PAGE, was identified by anti-Amblyomin-X, inhibited the FXa amidolytic activity using the chromogenic substrate S-2765 (Ki = 0.6 nM), and showed cytotoxic activity in cell lines Mia-PaCa-2 and Sk-Mel-28. Therefore, Amblyomin-X has the potential to prevent thrombosis and intravascular coagulation in cancer patients.

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2.23 FGF2 targets an "Achilles' heel" of Ras-driven mouse malignant cells

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Introduction: Fibroblast growth factor 2 (FGF2) has ordinarily been reported as a mitotic, pro-survivor and sometimes oncogenic signal which stimulates cell growth and proliferation. However, some groups, including ours, have reported that this factor can also selectively trigger unexpected antiproliferative effects in malignant cells. Here, we report that the FGF2/FGFR signaling system targets an "Achilles heel" of a robust K-Ras-dependent malignant cell line. **Objectives:** We aimed to elucidate the cell and molecular mechanisms underlying the antiproliferative stress response triggered by FGF2 in K-Ras-dependent Y1D1 mouse malignant adrenocortical cells. **Methods:** Cell cycle flow cytometry analyses following DNA content and BrdU pulse-labeling, together with a number of other molecular biology techniques, were employed to address the dynamics of FGF2 stress response. **Results and Discussion:** FGF2, in spite of stimulating G0>G1>S transition, selectively caused in malignant cells, a delay in FCS-stimulated DNA synthesis followed by a strong block of the G2/M transition, uncoupling cell growth from cell division. In addition, FGF2 induced specific markers of the DNA damage response in late G1 phase. Furthermore, polyploidization, giant cellsformation and cell death were also late effects of FGF2 treatment. Altogether, these results thus show that FGF2 can uncover a cancer-specific "Achilles heel" in K-Ras-dependent malignant cells, once considered to be highly resistant to cell death.

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3. Pharmacology

3.01 Crotalphine and opioid receptor agonists hyper-activate opioid receptors underperipheral sensitization

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Introduction: Several data have shown that the peripheral efficacy of opioid drugs is enhanced in the presence of tissue injury, but the mechanisms involved in this phenomenon are not well known. Previous data of our group showed that, in rats, prostaglandin E_2 (PGE_2, intraplantar/i.pl.) and chronic constriction injury (CCI) of the sciatic nerve increase the peripheral analgesic efficacy of opioid agonists and of crotalphine (CRP), a peptide obtained from *Crotalus durissus terrificus* snake venom. CRP induces peripheral analgesia mediated by the activation of κ- opioid receptors in PGE_2-induced hyperalgesia or κ- and δ- opioid receptors in the CCI model. We have recently demonstrated that opioid receptor expression is distinctly regulated by the presence of acute or chronic injury in nerve paw (NP) and dorsal root ganglia (DRG) of rats, which may explain the increased efficacy of CRP and opioids. **Objectives:** This study aimed to further characterize some of the mechanisms involved in the increase of the analgesic efficacy of opioids caused by inflammation/tissue injury. **Methods:** For this purpose, the effect of PGE_2-induced hyperalgesia and CCI on opioid receptor activation in DRG and NP of male Wistar rats was evaluated. Activation of opioid receptors was assessed by ELISA assays in slices of NP or DRG, using antibodies to regions within the N-terminus of activated opioid receptors. This assay was performed 1 h after intraplantar injection of DAMGO (5 μg/paw), U-50488 (10 μg/paw), DPDPE (20 μg/paw), μ-, κ- and δ-opioid receptor agonists, respectively, or CRP (0.6 ng/paw) in naïve rats or in rats 3 h after i.pl. injection of PGE_2 (100 ng/paw) or 14 days after CCI. **Results and Discussion:** PGE_2 or CCI, *per se*, did not cause receptor conformational changes in opioid receptors in NP or DRG. Activation of opioid receptors was observed after treatment with CRP or opioid agonist. PGE_2 enhances the μ-opioid activation caused by DAMGO (22%) and κ opioid receptor activation induced by CRP or U50,488 (16 and 20%, respectively). In contrast, δ–opioid activation caused by DPDPE was not altered by previous sensitization. CCI enhances the κ– and δ–opiod activation caused by CRP (26 and 15%), but not by the selective agonists. These results indicate that acute and chronic sensitization increases opioid receptor activation. These alterations can contribute to the higher efficacy of CRP and opioid agonists peripherally administrated.

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3.02 Fluorescent analogues of crotalphine: antinociceptive effect and mechanism of action

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Introduction: Crotalphine (*CRP*) is a 14-mer peptide isolated from the venom of *C. durissus terrificus* that triggers long-lasting antinociception (3-5 days) mediated by κ– and/or δ-opioid receptor activation in experimental animal models of acute and chronic pain when administered *p.o.* and *i.v.* or *i.p.* injection. In spite of this, we do not know how *CRP* elicits its antinociceptive effect. Thus, the use of fluorescent analogues of *CRP* could allow us to understand its mechanism of action. Fluorescent ligands have been used for studies of processes triggered by ligand-receptor interaction due to their advantages on radioligands. However, the addition of fluorescent moieties to macromolecules can affect both their receptor selectivity as well as their biological activity. **Objectives:** The aim of this work was to synthesize *CRP* and *CRP*-fluorescent, functionally active analogues by the solid phase method and to investigate the mechanism of action of *CRP* on sensory neurons isolated from adult rat dorsal root ganglia. **Methods:** The peptides were synthesized at 60°C on a Cys(Trt)-Wang resin by Fmoc strategy. Carboxyfluorescein (CF) was introduced in the peptide-resin. Disulfide bond formation was achieved by air oxidation. The crude peptides were purified by RP-HPLC and characterized by amino acid analyses and LC/ESI-MS. Antinociceptive activity was evaluated through the paw pressure test in rats with hyperalgesia induced by prostaglandin E_2 (PGE_2, 100 ng/paw in 50 μL) treated or untreated with *CRP* or *CRP*-fluorescent analogues. The effect of *CRP*-fluorescent analogues on sensory neurons was evaluated by confocal microscopy in cell cultures pretreated with 1 μM PGE_2 and 10 μM bradykinin (BK) for 15-120 min. **Results and Discussion:** *CRP* and *CRP*-fluorescent analogues were successfully synthesized and their overall purities were higher than 96%. CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* did not induce an antinociceptive effect as did *CRP* and [Glu^1]-*CRP* when administered *p.o.* (0.25-5 μg/kg) in PGE_2-induced hyperalgesia. However, the administration of CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* by *i.p.* injection (0.5 μg/kg) induced antinociceptive effects equal to *CRP* and [Glu^1]-*CRP* in PGE_2-induced hyperalgesia. These results suggest that the large fluorescent moiety prevented the absorption of CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* by *p.o.*, but did not affect the pharmacophore structure require to trigger antinociception. Confocal microscopy analysis showed that CF-[Gln^1]-*CRP* internalize in sensory neurons pretreated with BK. On the other hand, sensory neurons pretreated with PGE_2 or without any pretreatment showed low fluorescence, indicating that these cells require a pretreatment or priming stimulus with BK for functional competence *in vivo*.

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3.03 A phospholipase A_2 isolated from snake venom up-regulates ADRP expression in macrophages

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Introduction: ADRP (adipocyte differentiation-related protein) is a member of the PAT protein family that is involved in the transport and storage of neutral lipids in multiple cell types. This protein is highly expressed in macrophages differentiated into foam cells from atherosclerotic lesions, where it is found on the surface of lipid bodies, which are important organelles in the inflammatory process. Recently, we showed that MT-III, a phospholipase A_2 (PLA_2) isolated from *Bothrops asper* snake venom, increased the number of ADRP-enriched lipid bodies in cultured macrophages. **Objectives:** The aims of this study were to evaluate the ADRP gene and protein expression in macrophages stimulated by MT-III, and to correlate these parameters with the number of lipid bodies. **Methods:** Thioglycolate-elicited macrophages from male Swiss mice were incubated with MT-III (0.4 μM) or culture medium (control) from 1 to 24 h, and gene and protein expression of ADRP was determined by Western blotting and real-time PCR, respectively. Lipid bodies were quantified by both the fluorescence method and staining with osmium tetroxide (1%), followed by analysis under phase contrast microscopy. **Results and Discussion:** Incubation of macrophages with MT-III significantly increased ADRP mRNA at 1 h of incubation and ADRP protein expression from 6 up to 12 h. Moreover, a significant increase in the number of LB was detected from 1 up to 24 h of stimulation with MT-III, with a maximum between 12 and 24 h of stimulation with MT-III. MT-III is able to up-regulate the ADRP gene and protein expression and lipid body formation in macrophages. Since maximal levels of LB followed ADRP protein expression, MT-III-induced ADRP expression may be relevant to the late increase in lipid body numbers induced by this venom PLA_2.

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3.04 Crotalphine reduces peripheral sensitization evoked by activation of TRPV1 receptor in mice

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Introduction: Crotalphine (CRP), a peptide first identified and isolated from *Crotalus durissus terrificus* snake venom, produces a potent and long-lasting analgesic effect mediated by activation of *kappa* and *delta* 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. **Objectives:** In order to further characterize the role of previous sensitization in the action of this peptide, the aim of the present work was to evaluate the influence of peripheral sensitization induced by activation of TRPV1 receptors on the antinociception induced by CRP. **Methods:** All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 742/10). Male swiss mice (30-40 g) received an intraplantar (i.pl., 20 μl) injection of capsaicin (CPS; 0.03 or 1 nmol/paw) or prostaglandin E_2 (PGE_2; 0.01 nmol/paw) or the corresponding vehicles. In the experiments of previous sensitization, mice received the i.pl. injection of PGE_2 (0.01 nmol/paw) 3 h prior CPS administration (0.03 nmol/paw, i.pl., sub-threshold dose). Immediately after the treatments, the overt nociception [licking time (s)] was recorded for 5 min. Three hours after treatments, the development of allodynia was also evaluated, in the same animals, using the von Frey filaments (VFF). In this experiment, 0.6 g VFF was applied (10 applications with a duration of 3 s each) to the plantar surface of one of the hind paws of the mice and the % of withdrawal response frequency determined. **Results and Discussion:** Intraplantar administration of CPS induced overt nociception and mechanical allodynia as compared to controls. CRP (50-200 μg/kg), administered p.o. 1 h before CPS (1 nmol/paw), reduced, in a dose-dependent manner, the mechanical allodynia (52%, for 200 μg/kg), without interfering with overt nociception induced by the algogenic agent. The injection of PGE_2 3 h prior to CPS (sub-threshold dose) significantly increased overt nociception (CPS: 18±3 s; PGE_2: 16±4 s; CPS+PGE_2: 59±7 s) and mechanical allodynia caused by CPS. The PGE_2-induced potentiation of CPS-induced nociceptive phenomena was reduced (63% and 50%, respectively) by pre-treatment with CRP (200 μg/kg). These data confirm and extend previous findings from our group which demonstrated that the antinociceptive effect of CRP depends on prior sensitization. These results confirm the clinical importance of crotalphine as an analgesic agent, since inflammation is a component present in a great diversity of pathophysiological conditions.

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3.05 A new potential animal model of Parkinson's disease: The use of mouse strains selected for acute inflammatory response

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Introduction: One of the possible causes for the neuronal loss that leads to Parkinson's disease (PD) is a neuro-inflammatory response to chemical agents. Strains of animals disposed to produce intense or weak inflammatory responses can be used to determine the involvement of inflammation in the genesis of parkinsonian lesions. **Objectives:** We investigated the susceptibilities of two strains of inbred mice selected for high (AIRmax) or low (AIRmin) inflammatory response to stimuli such as Biogel. **Methods:** Groups of male animals (six months of age; BALBc mice) were used as controls in animal models of PD induced by treatment with 1-methyl-4-phenyl-1 ,2,3,6-tetrahydropyridine (MPTP) (5 x 20mg/kg i.p in 8 h) and rotenone (continuous infusion of 6 mg/kg/day, Alzet osmotic pump for 28 days). The animals were subjected to motor coordination assessment by Rotarod using a paradigm of rotation from 5 to 50 rpm over 5 min, measuring the time spent on the rotating bar. The lesion was quantified by immunohistochemistry for tyrosine hydroxylase in sections of the striatum and substantia nigra. **Results and Discussion:** The results showed that all strains of mice were resistant to MPTP or rotenone. The AIRmin strain showed immunohistochemistry suggestive of injury observed in substantia nigra by rotenone. Strains showed no significant motor impairment when evaluated by the Rotarod test. We suggest that the strains differ in peripheral inflammatory response, but not in neuro-inflammatory mechanisms. Still, we think the BALBc controls may not be ideal for this evaluation since the strain most often employed for Parkinson animal models is the C57BL/6. The BALBc are traditionally used as controls for AIRmax and AIRmin strains.

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3.06 Effect of gender on pain sensitivity and on the analgesic action of crotalphine, a peptide obtained from *Crotalus durissus terrificus* snake venom

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

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3.07 Effects of methotrexate and bee venom on plasma aminopeptidases

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Introduction: In the last years, the search for selective inhibitors for neutral (APN) and basic (APB) aminopeptidases and dipeptidyl peptidase IV (DPPIV) has increased. It is known that the inhibition of the above aminopeptidases leads to immunosuppression, due to their involvement in various immune mechanisms. Their roles include post-translational modifications of chemokines, antigenic processing, angiogenesis, molecular signaling and others. One drug commonly used for treating autoimmune diseases and cancer is methotrexate, which diminishes cell proliferation, chemotaxis and cytokine production; however, its mechanisms of action are not completely understood. In alternative therapy, bee venom is also applied for autoimmune inflammation; it contains some components to which are ascribed anti-inflammatory and antinociceptive actions. **Objectives:** The goal of the present study was to investigate the direct effects of methotrexate and bee venom on APN, APB and DPPIV activities in plasma of normal rats. **Methods:** The blood of six healthy male Wistar rats was collected with heparin and centrifuged at 200 x *g* for 10 min in order to obtain plasma. Methotrexate or bee venom at concentrations of 50, 500 and 5000 µg/mL in distilled water was incubated with plasma for 20 min at 37ºC. Subsequently, synthetic naphthylamide substrates in appropriate buffers were added to the reaction mixture. This mixture was allowed to react for 30 min at 37ºC. Aminopeptidase activities were measured fluorometrically. Samples without methotrexate or bee venom were considered controls (100%), and results were expressed as relative percentage of control±SEM. **Results and Discussion:** Bee venom caused a decrease of 60±2% of APN activity at concentration of 5000 µg/mL, did not alter APB activity, and increased DPPIV activity by 47±9% at a concentration of 50 µg/mL, 259±10% at a concentration of 500 µg/mL and 803±22% at a concentration of 5000 µg/mL. Methotrexate at a concentration of 500 µg/mL inhibited 60±9% of APN, 57±9% of APB and 55±10% of DPPIV activities, and at a concentration of 5000 µg/mL caused total inhibition of all aminopeptidases under study. The increase in DPPIV in samples incubated with bee venom corroborates data that describes DPPIV activity in this venom. Moreover, bee venom may contain a component capable of inhibiting APN activity, by which it could present its anti-inflammatory properties. APB activity is not altered by bee venom. Methotrexate may be an aminopeptidase inhibitor, which could be a novel mechanism of action of this drug.

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3.08 New cytolytic peptides from the venoms of solitary Eumenine wasps

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Introduction: In our continuing survey of biologically active substances in solitary wasp venoms, we have isolated four new cytolytic peptides from two species of Eumenine wasps, *Eumenes rubrofemoratus* (Eu) and *E. fraterculus* (Ef). Two of them, named Eu-5 (LNLKGLIKKVASLLN) and Ef-10 (LNLKGLFKKVASLLT), are quite similar to Eumenitin (LNLKGIFKKVASLLT), whereas the other two, named Eu-6 (FDIMGLIKKVAGAL-NH_2) and Ef-11 (FDVMGIIKKIASAL-NH_2), can be grouped in the Mastoparan (INLKALAALAKKIK-NH_2) class. **Objectives:** The aim of this study was to investigate the biological activity profile of these new Eumenine peptides and their structural and pore-forming activity in asolectin lipid bilayers. **Methods:** The peptides were isolated from the venom sacs by HPLC, sequenced in MALDI-TOF-TOF, and synthesized. The synthetic peptides were used in bioassays for antimicrobial, hemolytic, and mast cell degranulation activities. The pore-forming properties of the peptides were evaluated in mimetic lipid bilayers and circular dichroism experiments. **Results and Discussion:** The peptide Eu-5 was the most effective in the antimicrobial assay, showing the lowest MIC values against both gram-positive and gram-negative strains. The solitary wasp peptides displayed low (Eu-5 and Ef-10) to moderate (Eu-6 and Ef-11) hemolytic activity against mouse erythrocytes in a dose-dependent manner, and were able to induce mild mast cell degranulation with equivalent potencies and dose-dependent action. The peptides induced ion channel-like incorporation into lipid bilayers formed from GUVs of asolectin under positive and negative voltage pulses, within a 10-min incubation time, but the peptides Eu-5 and Ef-10 showed higher conductance levels. These helical peptides possess an amphipatic structure as foreseen from their helical wheel projections, and insert into the lipid membranes forming channel-like pores. Based on the results, it was shown that Eu-5 shows the highest potential as a leading compound in drug development. It was associated with an average net charge and low hydrophobicity, which resulted in improved antimicrobial activity with minimum hemolytic effect.

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3.09 Macrophage prolyl dipeptidyl aminopeptidase IV (DPPIV/CD26) and neutral aminopeptidase (APN/CD13) activities are affected by phospholipases A_2 (PLA_{2S}) isolated from *Bothrops jararacussu* and *Crotalus durissus terrificus* venom

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Introduction: Phospholipases A_2 from snake venoms are able to activate macrophages (Mϕs) to release inflammatory mediators. Mϕs are the main effector cells of the immune system. Once activated, these cells undergo morphological, biochemical and functional changes. Aminopeptidases (APs) are enzymes involved in leukocyte activation and migration. DPPIV/CD26 and APN/CD13 are expressed in monocytes/macrophages and regulate biological processes relevant to the immune response. **Objectives:** In this study, we evaluated the *in vitro* effect of both bothropstoxin II (BhTX-II), a myotoxic PLA_2 from *Bothrops jararacussu* snake venom and the crotoxin B (CB), a neurotoxic PLA_2 from *Crotalus durissus terrificus* venom, on the activities of soluble (S) and membrane-bound (M) DPPIV/CD26 and APN/CD13 in Mϕs. **Methods:** Resident Mϕs were collected from the cavities of male Swiss mice and incubated with non-toxic concentrations of either BhTX-II or CB (3.5 μg/mL) for selected periods of time (30 min, and 1 and 3 h). DPPIV/CD26 and APN/CD13 activities were quantified by a fluorimetric assay. **Results and Discussion:** Data, represented as UP/mg of protein, showed that BhTX-II increased DPPIV/CD26 activity (3416.9±27.2) after 3 h incubation and APN/CD13 activity (104.2±2.7) at 1 h in the M fraction, as compared with controls (2522.2±14.5; 51.9±1.2, respectively). CB decreased APN/CD13 activity (29.5±1.2) at 30 min in the M fraction, as compared with control (111.5±2.6), but did not affect DPPIV activity for any period of incubation tested. S fraction activity of both enzymes was not altered by either of these venom $PLAs_2$. BhTX-II and CB were able to modify the activities of membrane-bound peptidases (APN/CD13 and DPPIV/CD26). However, these PLA_2s showed distinct actions on the aminopeptidases, BhTX-II being stimulatory and CB inhibitory. Such a difference in effects may be related to the distinct roles of the two PLA_2s on inflammatory and immunological processes.

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3.10 Opioid receptors are involved in the antinociceptive effect of crotalphine in a bone cancer pain model

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Introduction: Crotalphine, a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, induces analgesia mediated by the activation of δ- and κ-opioid receptors. **Objectives:** The aim of this work was to characterize the analgesic effect of crotalphine in a new rat model of bone cancer pain induced by inoculation of Walker 256 carcinoma cells ($4x10^6$) into the rat femoral cavity. **Methods:** The presence of bone metabolic alterations was determined by scintigraphy, using ^{99m}Tc-MDP, which is significantly concentrated in areas of osteogenesis. Femoral images were obtained before and 7, 14 and 21 days after tumor cell inoculation. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, determined using the rat paw pressure test or von Frey filaments, respectively. **Results and Discussion:** Incorporation ^{99m}Tc-MDP was significant 7, 14, 21 days after tumor cell injection, suggesting the development of tumor in femoral cavity. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after cell inoculation. Interestingly, we observed that paw withdrawal threshold in 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). Crotalphine (8 µg/kg, p.o) administered on day 21, blocked hyperalgesia and allodynia. The analgesic effect was detected for up to 2 days after peptide administration. This effect was mediated by *delta* and *kappa* opioid receptors. These results indicate that intrafemoral injection of Walker 256 cells causes bone cancer and pain. Crotalphine induces a potent, long-lasting and opioid-mediated antinociception in this model of cancer pain.

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3.11 Cnidaria venom as pharmacological tool for studying pain and analgesia

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Introduction: Animal toxins are directed against a wide variety of pharmacological targets, making them an invaluable source of ligands for studying the signaling pathways of pain and its control. Sea anemone (cnidaria) venoms contain many biologically active compounds such as cytolysins (18–20 kDa) and ion channel modulators (3–5 kDa). In addition, low molecular-weight compounds have been isolated and identified in these venoms; however, few studies have been carried out in order to determine the biological activity of such compounds. BDS 391 is a low molecular-weight, non-peptide compound purified from venom of the Brazilian sea anemone *Bunodosoma cangicum*. Studies on the structure of BDS 391 have demonstrated that this compound is composed of a bromoindole group connected to histidine. Our recent data have indicated that BDS 391 administered by the intraplantar route into the rat hind paw induces potent peripheral analgesia in models of acute and chronic pain. Initial results indicate that peripheral 5-HT receptors and K_V channels mediate the analgesic action of this compound. **Objectives:** The aim of the present work was to further characterize the analgesic action of BDS 391 and its mechanisms, determining the type of 5-HT receptor involved in this effect, the presence of these receptors in the inflamed tissue and the ability of BDS 391 to directly activate K_V channels. **Methods:** Male Wistar rats and Swiss mice were used. The effect of BDS 391 was evaluated in the rat paw pressure test, before and 3 h after injection of prostaglandin E_2 (PGE_2, 100 ng/paw) and against nociception induced by 1% formalin solution in mice. Spiroxatrine, ketanserin or ondansetron (6 mM/paw, antagonists of $5\text{-}HT_{1a}$, $5\text{-}HT_2$ and $5\text{-}HT_3$ receptors, respectively), were used to characterize the type of serotonin receptors involved the analgesic effect. Expression of 5-HT receptors in the paw tissue was evaluated by immunoblotting assays. In voltage clamp studies, BDS 391, was screened in 9 cloned Kv channels. **Results and Discussion:** BDS 391 (0.15 - 1.5 μM) inhibited PGE_2-induced hyperalgesia and nociceptive response induced by formalin. Ondansetron but not spiroxatrine and ketanserin was able to totally reverse the antinociceptive effect induced by BDS 391. These pharmacological data indicated that peripheral $5\text{-}HT_3$, but not $5\text{-}HT_{1a}$ and $5\text{-}HT_2$ receptors, mediate the action of BDS 391. The immunoblotting data showed that 5-HT receptors are expressed in nerve paw and that PGE_2-induced hyperalgesia increases (15 – 20%) the expression of these receptors. BDS 391 did not modify the peak or shape of ionic potassium current. These data indicate that peripheral $5\text{-}HT_3$ receptors are involved in the analgesic effect of BDS 391 and demonstrate for the first time, that inflammation induces up-regulation of 5-HT receptors. The opening of Kv channels induced by BDS 391 does not result from a direct action of the compound, but could be due to activation of $5\text{-}HT_3$ receptors (a channel activated by ligands). These results also contribute to the better characterization of the role of $5\text{-}HT_3$ receptors in pain control.

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4. Immunology and Vaccines

4.01 Technological innovation in the process of obtaining vaccine against bacillary hemoglobinuria

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Introduction: Toxoid-composed vaccines have a higher degree of purity, consequently inducing more specific immunological responses and less adverse reactions. Bacillary hemoglobinuria is caused by the toxin produced by *Clostridium haemolyticum*, a gram-positive, strict anaerobic bacterium, which is also spore-forming. Objectives: In this study, two processes of bacterial fermentation were evaluated, static cultivation and cultivation under stirring conditions (vibromixer and bubbling with N_2), for a highly pathogenic *C. haemolyticum* sample (ATCC no. 9650). **Methods:** The bacterial suspension titers (minimum lethal dose) showed no significant difference; however, the toxin produced by the fermentation process under stirring was 20 times greater than the one produced in static fermentation. A batch of toxoid was prepared from the toxin produced by fermentation under stirring conditions. Eight guinea pigs were vaccinated with 1/5 the bovine dose (1.0 ml) on days 0 and 21, and five guinea pigs were inoculated with the same volume of saline solution. After 14 days after the second dose, all animals were challenged by inoculation of *C. haemolyticum* spore suspensions with 100 LD50. **Results and Discussion:** The group of animals vaccinated with toxoid showed 100% protection to the challenge (8/8), while the control group showed 100% lethality, given the requirements of test. According to the results, the efficiency of the toxoid produced under stirring conditions was proven, inducing an immunological response above of the minimum required for vaccine approval, according to the Federal Code Regulations (9CFR113.107). The requirements for a veterinary vaccine are different from those for a human vaccine, which needs greater refinement. This study describes a differentiated methodology applied in the process of veterinary vaccine production, demonstrating that this toxoid reduces the need for downstream processes, which are responsible for 50-70% of the cost of the final product.

4.02 Detection of antibodies (IgG) against hantavirus in human population of Amazon region and Brazilian southwest (rain florest), using recombinant antigen of Araraquara virus

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Introduction: The genus *Hantavirus* of the family *Bunyaviridae* includes a large number of rodent-borne viruses that are distributed worldwide. The occurrence is due mainly to ecological disturbances and it is transmitted to the humans through inhalation of virus particles contained in the excreta of wild rodents. Two different human diseases known to be caused by *Hantavirus* are hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). The diagnosis of the infection is accomplished mainly by serology (detection of antibodies) against the *Sin nombre* and/or *Andes* virus. These viruses are found mainly in the US and in Latin America (except for Brazil). The nucleoprotein is the major antigenic protein of the hantavirus. The nucleoprotein is the most appropriate target for use in the diagnosis of hantavirus infection. For accurate diagnosis, early during the course of the disease, it is essential to show an ELISA reaction for Brazilian Hantavirus. **Objectives:** The main objective of this study was to use and to evaluate the recombinant protein (antigen) of the *Araraquara* virus expressed in *Escherichia coli* (kindly donated by Professor Luiz Tadeu Moraes Figueiredo - USP, and Professor Marcos Lázaro Moreli -UESC), in populations of Amazonia and rural workers of the state of Sao Paulo, who live in contact with wild rodents. **Methods:** ELISA, imunoblotting, focus reduction neutralization test (FRNT), and strip immunoblot assay (SIA). **Results and Discussion:** Serum samples from 1308 individuals were analyzed by ELISA for antibodies against *Hantavirus* (IgG), and they were later confirmed by Western blotting. The cross reaction between the *Araraquara* virus and other *Hantavirus* and *Arbovirus* also needs to be carefully studied. Of the incoming sera from the Amazonian area, in the years of 2003 and 2005, 59 (5%) positive sera were found. From the city of Machadinho do Oeste - RO, 633 sera were analyzed, where 20 were found to be positive (4.5%). On the Machado River (RO), 435 sera of the river-dwelling population were analyzed, where 39 (5%) positive sera were found. After the analysis was accomplished for 151 human sera coming from the Vale do Ribeira - SP, in 2007, and 84 from the Pontal do Paranapanema - SP, in 2008, 14 (9%) and 6 (7%) of the samples were observed to be positive, respectively. A possible explanation is that the incidence found is approximately 8% for antibodies of the IgG class for *Hantavirus* in the sub-tropical (Atlantic forest of the state of São Paulo) area and approximately 5% in the tropical area (western Amazônia in the state of Rondônia), an endemic among the rural workers that live together with wild rodents.

4.03 Standardization of an in vitro test for determination of beta toxin titer of *Clostridium haemolyticum*

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Introduction: Clostridia are gram positive, spore-forming bacilli and anaerobic. *C. haemolyticum* causes bacillary hemoglobinuria in cattle and occasionally sheep. In bacillary hemoglobinuria, beta toxin destroys circulating red cells, resulting in the excretion of hemoglobin in the urine; simultaneously, there is the presence of blood in the intestine due to destruction of the endothelium capillary. **Objectives:** The aim of this study was the standardization of an *in vitro* test for determination of the beta toxin titer of *Clostridium haemolyticum* as an alternative to *in vivo* method for process control in the production of clostridial vaccines for veterinary use **Methods:** The strain of *Clostridium haemolyticum* ATCC 9650 was grown statically in medium recommended by WHO. The beta toxin was concentrated and purified by molecular ultrafiltration. Its toxicity was observed in Swiss mice weighing 18 to 22 g; the mice were inoculated with 0.5 mL, intraperitoneally. Cytotoxicity assays were performed according to ISO 10993-5. **Results and Discussion:** The beta toxin was highly toxic in vivo causing 100% mortality in calves in less than 24 h, confirming the in vitro tests where the L929 cells treated with beta toxin and quantified by the method of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction showed no viability in 24 h until a dilution of 1:64. The morphological changes in cells treated with beta toxin can be visually verified by optical microscopy when compared with control L929 cells.

4.04 Two loci interact with Slc11a1 gene to regulate the sensitivity to LPS-induced endotoxic shock

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Introduction: AIRmax (high inflammation) and AIRmin mice (low inflammation) differ in their resistance to LPS-induced endotoxic shock. *Slc11a1 R* and *S* alleles are involved in this phenotype. To study the *Slc11a1* gene interaction with acute inflammatory reaction loci (AIR-QTL), AIRmax*RR*, AIRmax*SS*, AIRmin*RR* and AIRmin*SS* sublines were produced. AIRmax*RR* mice are extremely susceptible to LPS-induced shock, while AIRmin*SS* are the most resistant. **Objectives:** The objective of this work was to identify loci that interact with *Slc11a1* alleles to modulate LPS shock. **Methods:** Mice were injected i.p. with 20 ug LPS and mRNA from bone marrow (BM), and liver cells were isolated. Global gene expression analysis was performed on Codelink bioarrays (36 k- genes) using RNA pools (n=4) of LPS-treated or control BM cells from AIRmax*RR*, AIRmax*SS*, AIRmin*RR* and AIRmin*SS* mice. Serum levels of inflammatory cytokines were determined by ELISA. In parallel, genome wide association (GWA) studies with SNPs (Illumina bead arrays) were performed to demonstrate LPS-resistance QTL in the F2 (AIRmax x AIRmin) population. **Results and Discussion:** The highest number of differentially expressed genes ($P<0.001$) after LPS injection was found in AIRmin*SS* mice. AIRmax*RR* had higher serum levels (2- to 5-fold) of inflammatory cytokines and higher expression of *Tnf*, *Il6* and *IL1b* genes in liver and BM cells. *Il10* expression was higher in AIRmin*RR* mice (2-fold) than in the other lines. GWA analysis revealed two significant QTL on chromosomes 4 and 11 (LOD=3.6) for resistance to LPS shock. These results suggest that *Slc11a1* alleles may interact with these loci to modulate the activation of inflammatory genes during LPS shock.

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4.05 Effect of temperature on the immunoreactivity of murine monoclonal antibodies against Shiga toxin (Stx1) from *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) has been identified as an important food- and water-borne pathogen worldwide, causing from uncomplicated diarrhea up to hemolytic uremic syndrome. For STEC the production of enterotoxins (Shiga toxin 1 and/or 2) is considered the principal virulence factor and responsible for the serious complications. The Bacteriology Laboratory of the Butantan Institute has been dedicated to developing polyclonal and monoclonal antibodies (MAbs) against these toxins aiming to detect their expression in bacterial isolates. Murine MAbs against Shiga toxin 1(Stx1) have been developed and well characterized by L.B. Rocha. To expand the application of these MAbs antibodies as reagents in the detection of this toxin, these antibodies must be stable. **Objectives:** The aim of this study was to purify murine MAbs against Stx1 and to investigate the effects of temperature on the immunoreactivity of these MAbs. **Methods:** The hybridoma was cultured in RPMI supplemented with 10% fetal bovine serum. The supernatants (1.5 liter) were collected and filtered (0.45μm), and MAbs were purified using a Protein-A column. The MAbs were eluted with 0.1 M glycine-HCl, pH 3.0 and the pH was neutralized with 1 M Trisma base. Afterward, it was dialyzed against PBS, pH 7.4, overnight. The Pierce BCA assay kit was used for protein determinations and bovine serum albumin as the standard. The immunoreactivity of the MAbs was measured by ELISA. Briefly, 96-well ELISA plates were coated for 2 h at 37º C with Stx1 purified toxin at 1 μg/mL in PBS, pH 7.4. After blocking, the plates were incubated with MAbs dilutions. After incubation with HRP-goat anti-mouse IgG, the reaction was revealed by the addition of O-phenylenediamine (OPD) plus hydrogen peroxide. The reaction was stopped with 1M HCl. The absorbance was measured at 492 nm in a Multiskan EX ELISA reader. All samples were tested in duplicate. A heat inactivation curve was determined by the incubation of purified MAbs at 25, 37, 50, 60, 70, 80, 90 or 100º C for 10 min. Following the heat treatment, the MAbs were tested for immunoreactivity by ELISA. A time course for the loss of reactivity was determined by incubating the MAbs for 1, 2, 4, 5, 6, 8 or 10 min. **Results and Discussion:** A total of 4.6 mg of purified murine MAbs against Stx1 were obtained. Retention of immunoreactivity was observed up to 50º C. Heating at 80º C caused a total loss after one minute, and partial loss of immunoreactivity was observed between 60º C and 70º C. The evaluation of the stability of the murine MAbs available against Stx1 will contribute to the assurance of the methodology employed.

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4.06 Immunomodulatory effect of high-molecular weight components from *Ascaris suum* extract in TLR2- and TLR4-deficient mice

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Introduction: High MW components (PI) from *Ascaris suum* extract have immunosuppressive effects and down-modulate the ability of antigen-presenting cells (APCs) to activate OVA-specific T cells. APCs, as dendritic cells (DCs) when recognizing pathogens through distinct receptors, such as the toll like receptors (TLRs), acquire the capacity to induce a specific cellular response. **Objectives:** The aim of this study was to determine the involvement of TLR2 or 4 in the suppressive effect of PI on anti-OVA humoral and cellular responses. Furthermore, the role of these receptors in the ability of PI to down-modulate DC maturation induced by agonists of TLRs was investigated. **Methods:** For this, WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ C57BL/6 mice were immunized with OVA (200 µg/animal) or OVA+PI (200 µg/each antigen/animal) in CFA. After 8 days, the mice were challenged with aggregated OVA in the footpad, and the DTH reaction measured after 24 h. All groups were also bled and anti-OVA IgG1 and IgG2a production evaluated by ELISA. The effect of PI on the expression of the molecules involved in the antigenic presentation was evaluated in cells obtained from these groups of mice immunized with OVA or OVA+PI 5 days before and stained with anti-MHC-II, anti-CD80 and CD86 mAbs labeled with FITC or PE by flow cytometry. In another experiment, immature DCs derived from WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ mouse bone marrow were induced to differentiate in RPMI medium plus GM-CSF/IL-4. On day 7, these cells were incubated *in vitro* with LPS (1 µg/mL), PI (200 µg/mL), LPS+PI (1 µg+200 µg/mL), pam3 (3 µg/mL) or pam3+PI (3 µg+200 µg/mL), Poly I:C (10 µg/mL) or Poly I:C+PI (10 µg+200 µg/mL) for 18 h. Afterward, the supernatants were collected for cytokine detection by ELISA. **Results and Discussion:** PI was able to inhibit both anti-OVA DTH reaction and antibody production as well as in WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ OVA+PI-immunized mice when compared with OVA-immunized groups. Lower expression of CD80, CD86 and MHC-II molecules was also seen in cells obtained from these groups of OVA+PI-immunized mice when compared with those observed in cells from OVA-immunized groups. High production of IL-12, IL-6 and IL-1 was obtained in cultures of DCs from WT, TLR4$^{-/-}$ or TLR2$^{-/-}$ mice stimulated with different TLR agonists. In contrast, PI down-modulated the secretion of these cytokines when added to the DC cultures stimulated with the TLR agonists. The results indicate that TLR2 and 4 are not involved in the suppressive effect of PI on DC activity.

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4.07 Population dynamics of myeloid-derived suppressor cells during the course of tumorigenesis in Airmax and Airmin mice treated with urethane

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Introduction: Predisposition to neoplastic process is due to both genetic and environmental factors that determine the resistance or susceptibility phenotypes. The suppressor activity of myeloid-derived suppressor cells (MDSC) during tumorigenesis is an important factor that determines the susceptibility phenotypes. These cells are produced in the bone marrow compartment and migrate to the tumorigenesis site or to the secondary organs, interfering with the antitumoral activity of the T cells. In mice, these cells can be identified by co-expression of the surface molecules that determine the $CD11b^{+}/Gr1^{+}$ phenotype. Resistance to tumorigenesis in mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory reaction is influenced by regulatory genes, which determine respectively the resistance or susceptibility to urethane action. Moreover, these lines of mice show important differences concerning the bone marrow activity related to myeloid cell maturation. **Objectives:** The aim was to study in AIRmax and AIRmin mice the dynamics of MDSCs after lung tumorigenesis induced by urethane by analyzing myeloid and B and T cell populations in the lung, spleen and bone marrow tissues. **Methods:** Mice were treated by two i.p. injections of urethane (1000 mg/kg body weight) at a 48-h interval for tumorigenesis induction and observed for 200 days after treatment. The number of tumor lesions was determined by macroscopic observation and the cellularity was evaluated by both specific antibodies to GR1/CD11b (myeloid cells), CD4/CD8 (T cells) and B220 (B cells) molecules and morphology analysis. **Results and Discussion:** At 10 days of urethane treatment, we observed a significant increase in cell number in the lung parenchyma, especially in AIRmax, indicating an inflammatory process, followed by a decline cell number. Forty days after urethane injection, we observed in AIRmin mice, a second cell increase in the $CD11b^{+}/Gr1^{+}$ population consisting of macrophages and mature and immature neutrophils, which were revealed by different molecular expression. At the same time, the CD4 and CD8 T cell populations showed a significant decrease in the lung and spleen tissues. This cellular alteration is related to the high tumor multiplicity in AIRmin mice, indicating a possible modulation by MDSC on T cell action. These preliminary results indicate that MDSC playd a role in the susceptibility of the AIRmin line during tumorigenesis provoked by urethane.

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4.08 Biologic effects of *Propionibacterium acnes* on mouse peritoneal B1b lymphocytes

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Introduction: B1 lymphocytes differ from conventional B cells on the basis of their main localization on peritoneal and pleural cavities, surface phenotype and functional features, such as auto-renewing, natural IgM and auto-antibodies production and their role in immune response regulation by IL-10 synthesis. In the mouse peritoneal cavity, B1 lymphocytes are divided into B1a ($CD11b^+$ $CD5^+$), B1b ($CD11b^+$ $CD5^-$) and B1c ($CD11b^-$ $CD5^+$), but B1c are a differentiation stage of B1a and B1b. *In vitro*, B1b lymphocytes can be obtained from adherent cells of mouse peritoneal exudates, and, when re-cultured for 5 to 10 days, they differentiate into macrophage-like phagocytes. However, B1 response to antigens or adjuvants is still poorly understood. An important biological adjuvant is *Propionibacterium acnes* (*P. acnes*), whose effects, as killed bacterial suspension, include activation of macrophage phagocytic and tumoricidal activities, adjuvant effect on antibody response, resistance to infections and induction of pro-inflammatory cytokine synthesis. A bacterial compound of great importance is its soluble polysaccharide (PS), with effects similar to those of the whole bacterium. **Objectives:** Herein, we studied *P. acnes* and PS effects on peritoneal B1b cell functions, differentiated or not into phagocytes. **Methods:** Mice were treated with one intraperitoneal injection of saline (control), heat-killed *P. acnes* or PS. After 24 h, peritoneal exudate cells were analyzed by flow cytometry or cultured for 2 or 5 days to obtain a B1b lymphocyte-enriched population, and these were re-cultured for 24 h for phagocyte differentiation. Proliferation and cytokine release were evaluated in B1b cells and phagocytes, as well as phagocytic activity in phagocytes. **Results and Discussion:** We observed higher numbers of B1b and macrophages in the peritoneal cavity of *P. acnes*-treated mice, compared to the control group, as well as an *in vitro* increase in the percentage of phagocytic cells. We also found that B1b cells proliferated *in vitro*, on days 2 and 5. When re-cultured on 2nd day, *P. acnes* cells proliferated less than control, but, when re-cultured on the 5th day, the proliferation was higher in bacteria-treated group. Besides, there was a decrease in IL-10 levels in *P. acnes* and PS phagocyte supernatants, compared to saline, and increase in IL-12 in the *P. acnes* group. These data indicate that *P. acnes* can stimulate early differentiation of B1b cells into phagocytes, in just 24 h of re-cultivation, as shown by increased phagocytic cell percentage and IL-12 release. The polysaccharide compound does not seem to be responsible for this effect.

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4.09 Investigation of human CD59 orthologs in the *Schistosoma mansoni* genome

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Introduction: *S. mansoni* is the predominant parasite responsible for schistosomiasis, which affects 200 million individuals in several countries. The treatment is based on praziquantel, but chemotherapy does not prevent re-infection, emphasizing the need for a more effective approach. With the outcomes of the *S. mansoni* genome, it was possible to investigate *in silico* new vaccine candidates as human CD59 orthologs. CD59 is an important inhibitor of the membrane attack complex (MAC) by the complement system. **Objectives:** The aim was the investigation of human CD59 orthologs in the *S. mansoni* genome and characterization of one member of this family (Ly-6.1 gene). **Methods:** The alignment and the phylogenetic analysis of family members were performed. The mRNA expression levels of the genes across the life cycle stages were evaluated by real-time RT-PCR. The Ly-6.1 gene was cloned into the vector and transformed into *Escherichia coli.* The protein was purified by affinity chromatography, and polyclonal antibodies were obtained. Western blot and immunolocalization assays were performed to characterize the protein. Molecular shaving assays were carried out by incubating adult worms with the PiPLC enzyme that cleaves membrane-binding proteins by GPI anchor. **Results and Discussion:** In *S. mansoni,* the Ly-6 family is composed of six genes (Ly-6.1, Ly-6.2, Ly-6.3, Ly-6.4, Ly-6.5, Ly-6.6) with 25-30% identity to human CD59. They contain Upar/Ly-6 domains, signal peptides, transmembrane domains and GPI anchors. Most of them show increased gene expression in schistosomulum stage by real-time RT-PCR, except the Ly-6.3 gene, which shows increased expression in eggs and Ly-6.4 increased expression in adult worms. These results were corroborated by microarray analysis. One of the genes, Ly-6.1, was cloned and Western blot analysis revealed high expression levels of the Ly-6.1 protein in schistosomula, some in cercariae and adult worms and none in eggs and miracidia. The protein was immunolocalized to the tegument of 3-h, and 7- and 21day-old schistosomula by confocal microscopy. Localization of the Ly-6.5 protein was also determined on surface of adult worms. The characteristic of these proteins to be membrane binding by GPI-anchor was confirmed for Ly-6.1 and Ly-6.5 by the molecular shaving technique. In conclusion, most of the genes of the Ly-6 family are up-regulated in the schistosomulum stage, and the protein is associated with the tegument and possesses similarity with human CD59, which inhibits the complement system. These results indicate that the family should be investigated as potential vaccine candidates. Immunization and challenge assays, the characterization of other members of the family, and functional assays are underway.

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4.10 Selection of a family 1 PspA capable of inducing broad-ranging cross-reactivity by complement deposition and opsonophagocytosis by murine peritoneal cells

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Introduction: *S. pneumoniae* is a major cause of pneumonia, meningitis and sepsis. Among the vaccine candidates against this pathogen is pneumococcal surface protein A, an exposed and protective protein. Due to its structural diversity, an effective PspA-based vaccine should include at least one fragment from each of the two major families (1 and 2). Also, it has been shown that PspAs from different clades show variable degrees of cross-reactivity. In the present work, we investigated the level of cross-reaction among different PspA molecules within family 1, in order to determine the best candidate to be included in a PspA-based vaccine. Since PspA inhibits complement deposition onto pneumococci, therefore avoiding phagocytic clearance by the immune system, we evaluated the ability of the antibodies produced to abrogate PspA's function, enhancing complement deposition onto pneumococci and promoting opsonophagocytosis. **Objectives:** The aim of this study was to determine, from a panel of Brazilian pneumococcal isolates, which is able to induce the higher level of cross-reactivity within family 1. **Methods:** We have produced recombinant PspA fragments from 10 family 1 pneumococci (5 of each clade), containing the whole N-terminal half of the protein. These fragments were used to immunize BALB/c mice, and the sera were tested for their ability to recognize diverse pneumococcal strains bearing PspAs of clades 1 and 2 by Western blotting. The most cross-reactive antibodies were tested for their ability to enhance complement deposition on pneumococci. Also, the two antisera selected were tested for their ability to promote the opsonophagocytosis of different pneumococcal strains by peritoneal cells. **Results and Discussion:** The analysis of serum cross-reactivity among PspA fragments from clades 1 and 2 revealed a significant variation in the level of recognition. Four sera able to recognize bacteria from both clades were tested for their ability to increase complement deposition on the pneumococcal surface. Of these, two led to an increase in complement deposition on strains bearing PspAs from both clades, in FACS analysis. The opsnophagocytic assay indicated that both anti-PspA antisera tested were able to promote opsonophagocytosis, leading to a minimum reduction of 30% in the number of pneumococci recovered, suggesting a possible protective effect. We therefore suggest that the inclusion of either one of the two PspA fragments in a PspA-based anti-pneumococcal vaccine could induce broad protection against family 1 strains.

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4.11 Evaluation of DNA vaccines expressing N-terminal fragments of pneumococcal surface protein from clade 4 (PspA4) against an intranasal lethal challenge model in mice

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Introduction: *Streptococcus pneumoniae* causes serious diseases such as meningitis and pneumonia, besides common infections of the respiratory tract. The vaccine composed of different capsular polysaccharides (PS) purified from pneumococci has low efficacy in children and the elderly, besides not being able to induce immunological memory. Although the development of vaccines composed of PS conjugated to carrier proteins was an advance, their production cost is still a major barrier for their use by public health systems. A proposal to increase vaccine coverage at a low cost consists in the identification of an antigen common to the majority of strains. Several proteins have been investigated as alternatives for a cost-effective vaccine against *Streptococcus pneumoniae*. PspA (pneumococcal surface protein A) is one of the most promising candidate antigens. Our group has recently shown that PspA from clade 4 (PspA4) induces antibodies with broad cross-reactivity with pneumococcal isolates. **Objectives:** The proposal of the present work was to evaluate two different fragments of PspA4 - PspA4Pro encompasses the complete N-terminal alpha-helical region plus the proline-rich region and PspA4A has only the first half of the alpha-helical region - in an intranasal lethal challenge model as recombinant protein adjuvanted with alum or as DNA vaccine. **Methods:** BALB/c mice were immunized subcutaneously with the recombinant proteins using alum as adjuvant or intramuscularly with the DNA vaccines. Immunizations were performed in 3 doses with intervals of two weeks. The animals were bled after the last immunization for the analysis of serum antibodies by ELISA. The capacity of the antibodies to bind to intact pneumococci and to mediate deposition of C3 was evaluated by FACS using anti-mouse IgG antibodies or anti-mouse C3 antibodies conjugated with FITC, respectively. Mice were then challenged intranasally for the analysis of survival. **Results and Discussion:** High antibody concentrations were elicited by both PspA4A and PspA4Pro protein and DNA immunizations. Antiserum to PspA4A showed reduced capacity to bind and to mediate C3 deposition onto intact pneumococci as analyzed by FACS. Furthermore, binding of antibodies to PspA4Pro was not blocked by the addition of recombinant PspA4A. Only animals injected with PspA4Pro, either as recombinant protein or DNA vaccine, showed significant higher survival. Our results indicate that the response elicited against the complete alpha-helical region in PspA4Pro is essential for the immune response and for protection against pneumococcal infection.

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4.12 Peritoneal inoculation of pristane induces distinct leukocyte infiltration kinetics in experimental arthritis resistant (HIII) and susceptible (LIII) mice

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Introduction: Mice selected for low (LIII) or high (HIII) antibody production against *Salmonella* flagellar antigens are respectively susceptible and resistant to pristane-induced arthritis (PIA). In the first 2 weeks after pristane injection, more splenic cells from LIII mice produced IL-1β, TNF-α and IL-12, while more cells from HIII mice produced IL-4. IL-6, considered as an important cytokine in PIA pathogenesis, which is produced in similar high amounts by peritoneal cells of both lines 72 h post-pristane injection. These results suggest that the differences between HIII and LIII mice are expressed in the early phase of PIA induction, influencing the late-phase of arthritis development. However, little is known about the cellular and molecular events that occur in the peritoneal cavity during this phase. **Objectives:** The aim of this study was to evaluate the kinetics of the cell populations in the peritoneal cavity after pristane injection in HIII and LIII mice. **Methods:** HIII and LIII mice were i.p. injected with 0.5 mL pristane and evaluated after distinct time points (48 h; 4, 7, 17 and 32 days). Peritoneal cells were harvested and counted in Malassez hemocytometer chambers. Giemsa staining of cytospin cell preparations was used for morphological analysis and differential counts. **Results and Discussion:** Total lymphocyte numbers did not show significant alterations between lines or treatments. Low numbers of mast cells were detected in control HIII and LIII mice, but these cells were absent in the peritoneal exudates of the respective pristane-treated animals. Eosinophil numbers were slightly increased in both lines up to day 7. On the other hand, the kinetics of monocytes/macrophages and neutrophils was dramatically different in LIII as compared to HIII mice. The number of inflammatory infiltrate cells was highest in LIII mice at day 7, while HIII mice showed a modest increase in neutrophils and decreased numbers of macrophages. At day 32, inflammatory cell numbers had decreased in LIII mice, while in HIII neutrophils increased and macrophages returned to control levels. This differential pristane-induced inflammation kinetics may be related to the divergent cytokine profile observed in the spleen, although more data on the molecules expressed/secreted by these and other cell populations are needed to support this hypothesis.

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4.13 Adjuvant activity of *Salmonella* FliCi flagellin in the development of a subunit vaccine against leptospirosis

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Introduction: Leptospirosis is a global zoonotic disease caused by pathogenic leptospires that colonize the renal tubules of wild and domestic animals. Commercially available leptospiral vaccines, consisting of killed whole cells, suffer from several limitations such as short-term immunity and adverse reactions. The development of a subunit vaccine could be a promising strategy against this disease. Various outer membrane proteins (OMPs) have been evaluated as potential vaccine candidates; one of these, the *Leptospira* immunoglobulin-like protein A (LigA), was able to induce immunoprotection against leptospirosis. However, immunization with LigA did not confer sterilizing immunity. Adjuvants are necessary to increase the immunogenicity and efficacy of purified antigens. Flagellin, a highly conserved bacterial protein that elicits TLR5-dependent responses, has been successfully used as a vaccine adjuvant. **Objectives:** In the present study, we evaluated the adjuvant activity of *Salmonella* FliCi flagellin in the protective immunity of LigA and of six other novel recombinant leptospiral OMPs against lethal challenge with *L. interrogans* in hamsters. **Methods:** The recombinant 6xHis-tagged proteins expressed in *E.coli* were purified by nickel affinity chromatography. Native *S.Typhimurium* FliCi was purified from the attenuated SL3201 strain. Hamsters were immunized subcutaneously with a cocktail of six purified recombinant OMPs with or without LigA as well as in combination with FliCi or alum. All animals were bled to evaluate the antibody response against each antigen and the expression levels of Th1/Th2 cytokine mRNA in the peripheral blood mononuclear cells. **Results and Discussion:** Immunization of hamsters with LigA or LigA coadministered with OMPs cocktail, either with FliCi or alum, induced robust antibody responses against recombinant proteins, as detected by ELISA and immunoblot, and conferred imunoprotection after challenge (80-100%). Animals inoculated with OMPs cocktail with alum or FliCi survived (30-70%) after challenge. Control animals vaccinated with PBS with alum or FliCi died with symptoms of leptospirosis, and hamsters vaccinated with commercial vaccine survived (100%) after challenge. Moreover, only groups inoculated with commercial vaccine or LigA coadministered with OMPs cocktail and FliCi as adjuvant showed reduced bacterial load in kidneys (> 90% negative culture) with significant enhancement of both Th1 and Th2 cytokine levels. Taken together, the data of this study suggest a new formulation for the development of a subunit vaccine for leptospirosis.

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4.14 Treatment of experimental melanoma with imiquimod

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Introduction: Aldara® (imiquimod 5%, 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine) is an immunomodulator mostly used for the treatment of genital warts and actinic keratosis. It is an agonist of the toll-like receptor 7 and induces dentritic cell migration and maturation, thus upregulating some of the cytokines that mediate Th1 immunity. **Objectives:** The aim of this study was to evaluate tumor growth and metastasis formation and to describe the immune system activation following topical application of 5% imiquimod in an experimental murine melanoma model. **Methods:** The tumor was induced by injecting $5x10^4$ B16F10 melanoma cells subcutaneously in two months old males C57BL/6J mice. The experimental group was treated with 5% imiquimod applied topically once a day at the inoculation site, from day one until death. Weight and tumor volume (using a caliper) were evaluated every two days and the tumor volume was defined by the formula: (length) x $(width)^2$ x (π/6). In order to describe the development of the treatment and establishment of the tumor growth, 4 treated and 2 control animals were euthanized on days 11, 18, 26 and 33 and submitted to necropsy for macroscopic evaluation; samples of normal tissues and tumors were fixed in 10% formalin and embedded in paraffin for further histological (HE) and immunohistochemistry analysis (S-100 and HMB-45). Part of the tumors was separated for flow cytometry analysis to qualify and quantify cell death by apoptosis and/or necrosis using FITC-labeled caspase-3 and annexin V. **Results and Discussion:** We observed a significant reduction ($p<0.001$) in tumor volume in treated animals, as well as an increase in the spleen and lymph node sizes, suggesting a systemic activation of the immune system. There was some neovascularization observed upon necropsy, which was proportional to the size of the tumor, with evident fibrinous exudates all around. Flow cytometry revealed that the treated animals had less necrotic cells when compared to the control group ($p<0.001$). The treated animals also showed a higher percentage of apoptotic cells on days 11 and 18 ($p<0.01$) and of cells in late apoptosis on days 26 and 33 ($p<0.001$). The presence of dendritic cells in the lymph nodes was evident using S-100 antibody, revealing an increased number of dendritic cells in the treated animals in comparison to control group. Metastases were visualized by HMB-45 antibody, revealing an increased number of melanocytes in the lymph nodes and some internal organs of the control group, in a time-dependent fashion. Imiquimod 5% in this therapeutic protocol delayed melanoma development, probably through the stimulation of Th1-mediated immunity by inducing tumor necrosis and/or apoptosis.

4.15 Influence of rHsp65 passive administration on survival, antibody production and histopathological changes in aged H responder mice

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Introduction: The Hsp60 are phylogeneticaly conserved molecules involved in protein folding, refolding and translocation across cell membranes, having a major role in cell growth and differentiation and being associated with chronic-degenerative processes. **Objectives:** The present study evaluated the actions of *M. leprae* rHsp65 wild type [WT] and its mutant $K^{409}A$ in the aging process and immunoresponse of mice. **Methods:** Measures of mean survival time [MST], titration of IgG1 and IgG2a isotypes, avidity tests of anti-Hsp65 IgG antibodies and histopathological analyses were performed in mice genetically selected for High [H_{III}] and Low [L_{III}] antibody production, inoculated intraperitoneally at 120 [adult] or 270 [aged] days of life with 2.5 µg/mL (200 mL of PBS) of recombinant proteins **Results and Discussion:** There was a decrease of 42% of the MST in aged H_{III} females inoculated with the WT protein ($p \leq 0.01$) compared to control and mutant groups. Adult H_{III} females receiving the WT molecule showed also a reduction of MST ($p \leq 0.05$) in relation to control and $K^{409}A$-treated group, but the interval between the treatment and first death was fourteen times higher (247 days) in relation to the aged ones (18 days). WT molecule administration in aged L_{III} females resulted in increased ($p \leq 0.01$) MST compared to the control and $K^{409}A$ groups. Aged male mice from both lines showed no difference in their mean survival time. There were no marked changes in the production of IgG1 and IgG2a anti-Hsp in all groups, but the IgG avidity was lower ($p \leq 0.05$) in old H_{III} female treated with wild type protein. Despite the occurrence of hepatitis in all groups, perhaps a normal process in the senescence of these strains, the histopathological analyses showed a worse phenotype of nephritis and nephrosis, with the presence of inflammatory influx and intraluminal eosinophilic material in the kidneys of aged H_{III} females treated with WT protein. The results presented revealed the interference of WT rHsp65 in the immunity of aged females from the H_{III} line during senescence and are discussed and related to those obtained previously on the effect of rHsp65 in autoimmune processes.

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4.16 Comparison of detoxification process of tetanus toxin by addition of glycine or lysine

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Introduction: Tetanus toxin is produced at Butantan Institute by fermentation of *Clostridium tetani* that releases the toxin during the process into the culture medium. The toxin is recovered by tangential flow filtration, concentrated by molecular ultrafiltration (30KDa) and converted into tetanus anatoxin by the addition of formaldehyde, glycine and sodium bicarbonate and incubation at 37ºC for 30 days. Tetanus anatoxin is purified by chromatography and after approval in the quality control test is designated Tetanus anatoxin final bulk. This product is one component of the associated vaccines (dT, DT, DTP and DTP-Hib) and is used as antigen to produce anti-tetanus serum for human. **Objectives:** The goal of this study was to evaluate lysine instead glycine in the detoxification process of tetanus toxins, analyzing efficiency and reprodutibility. **Methods:** Nine consecutive batches of tetanus toxin were detoxified with formaldehyde and lysine, and another nine with formaldehyde and glycine. After the detoxification process, each of three batches of tetanus anatoxin detoxified using lysine were mixed and purified by gel filtration chromatography. The same procedure was performed with tetanus anatoxin using glycine. Before purification, a sample of tetanus anatoxin was submitted to flocculation limit test, protein nitrogen quantity and antigenic purity test. After purification, the following process controls were analyzed: flocculation limit test, protein nitrogen quantity, antigenic purity test, potency, electrophoretic profile (SDS-PAGE) and chromatographic profile by gel filtration chromatography with Superdex 200® (GE Healthcare) resin. **Results and Discussion:** The flocculation limit average of tetanus anatoxin using glycine in the detoxification process was 783.33 Lf/mL and with lysine 700 Lf/mL. In relation to the tetanus anatoxin final bulk, the average of flocculation limit was 2666 Lf/mL and 2133.33 Lf/mL using glycine and lysine, respectively. All results obtained in the antigenic purity were above 1000 Lf/mgPN, and potency was higher than 2 IU/mL, in accordance with national and WHO requirements. The electrophoretic profile showed one band of approximately 75 KDa to 250 KDa in samples using lysine in the detoxification process and one band of approximately 65 KDa to 250 KDa using glycine. It was observed that the chromatographic profile was very similar in all samples, demonstrating the reproducibility of the process. The detoxification process with lysine and glycine showed the same efficiency, and therefore, lysine can be used as an alternative in the production.

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4.17 Production of monoclonal antibody to protein L2 of bovine papillomavirus type 2

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Introduction: Bovine papillomatosis is an infectious disease of worldwide occurrence, without any effective control. Several attempts have been undertaken with prophylactic or therapeutic purposes, but without effective results. Our group is involved in a project of national relevance (Renorbio) whose purpose is to develop vaccine products aimed at bovine papillomavirus (BPV). This study is part of this initiative to establish a comprehensive quantitative and descriptive framework on the prevalence of BPV in the national herd. The production and characterization of monoclonal antibodies against L2 protein of BPV-2 is an essential tool for the development of diagnostic methods and vaccines against this virus. **Objectives:** The aim of this study was to produce monoclonal antibodies against recombinant L2 protein of BPV-2 using the technique of cell fusion and hybridoma production, in order to allow its application in the development of diagnostic methods and evaluation of the effectiveness of prophylactic and therapeutic vaccine tests. **Methods:** As antigen, we used the recombinant protein of BPV-2 L2 cloned into expression vector pGEX, transformed and expressed in BL21 *E. coli.* Affinity chromatography column was selected for purification with a yield of about 530 ng/ul. Immunization was performed with L2-2 suspensions injected intraperitoneally into Balb/C mice (4 weeks of age), in a protocol repeated in the 7th, 10th and 13th weeks of life. **Results and Discussion:** The immunization protocol did not induce unexpected reactions and may be continued in the production of monoclonal antibody.

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4.18 Recombinant SmNPP-5 induces antibodies that partially inhibit surface enzymatic activity but fail to protect against challenge with *Schistosoma mansoni*

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Introduction: Schistosomiasis affects 200 million individuals in several countries, including Brazil; its treatment is based on praziquantel, but chemotherapy does not prevent re-infection, emphasizing the need for a more effective approach. Recent proteomic characterization of the *S. mansoni* tegument, the major parasite-host interface, identified a putative nucleotide pyrophosphatase/phosphosdiesterase (SmNPP) 5, as a plasma membrane-associated protein. NPPs are ubiquitous membrane-associated or secreted ecto-enzymes that act by regulating the metabolism of extracellular nucleotides, consequently having a role in purinergic signaling, which affects diverse biological processes such as platelet aggregation, apoptosis, cell proliferation, differentiation and motility. **Objectives:** We evaluated the potential of this protein as a vaccine candidate. **Methods:** The gene was cloned by RT-PCR from *S. mansoni* RNA, heterologous expression was obtained in *E. coli,* and the protein was purified by nickel affinity chromatography. The protein was formulated with Freund's adjuvant to immunize mice subcutaneously. The immune response profile was characterized by ELISA and ELISPOT to measure the levels of antibody isotypes and cytokines, respectively. The protective potential of this protein was evaluated by the challenge of immunized animals with cercariae followed by perfusion 45 days later. **Results and Discussion:** The protein was expressed as inclusion bodies, solubilized with 8 M urea and purified under denaturing conditions. The refolding was performed by slow dialysis, but the protein precipitated. The protein surface-exposed localization at the parasite-host interface was confirmed by immunoblotting and immunolocalization; the antibodies induced by the immunization with recombinant protein were able to partially inhibit the enzymatic activity in *ex vivo* live adult worms (~60%). The humoral immune response was characterized by high levels of anti-SmNPP-5 IgG1 and low levels of IgG2 antibodies; the cytokine profile revealed low levels of IFN-g and high levels of IL-10 and IL-5, suggesting a more Th2 drift immune response. Despite the induction of a specific immune response, no reduction in worm burden was observed in the immunized animals. It is possible that it will be necessary to obtain the recombinant protein in its native form to exert a stronger specific immune response capable of complete inhibition of the worm enzyme, and thereby obtain better protection levels. To achieve this goal, we will try to express this protein in a eukaryotic expression system, *Pichia pastoris*, and different routes of immunization with different adjuvants must be tested as well.

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4.19 Comparative study between the flocculation limit technique and total combining power (TCP) for quality control of clostridial vaccines

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Introduction: Enterotoxemy is considered the disease most important for ruminant livestock in the world. Due to the characteristics of the development of the disease, vaccination is the preventive measure with the greatest impact in the control of this illness. In the last years, many scientists have been looking for the development of alternative *in vitro* methods with the objective of reduceing or eliminating the use of *in vivo* methods, which involves a series of bioethical questions. Besides, they show lower sensitivity and specificity and greater economic cost when compared with *in vitro* methods. **Objectives:** This work was carried out to assess and standardize the *in vitro* limit of flocculation test to be used in the quality control of vaccine against enterotoxemy. **Methods:** This study used experimental vaccines against enterotoxemy and toxicoid produced from the epsilon toxin of *Clostridium perfringens* type D, previously evaluated by serum neutralization in mice and the TCP test. The limit of the flocculation test was performed by mixing different amounts of antitoxin and a fixed amount of toxicoid in each tube. It was proposed that the first tube to flocculate would indicate unit of flocculation (Fl) of the sample tested. **Results and Discussion:** The main results indicated that the lower degree of antitoxin detectable both in rabbit and in sheep serum by the limit of flocculation technique is equivalent to 10 IU/mL. The TCP and limit of flocculation correlation ratio was 99.97%. In conclusion, the limit of flocculation method is suitable for replacing *in vivo* methods for the analysis of epsilon toxin. Nevertheless, this technique is less effective when used for the potency test of vaccines against enterotoxemy in substitution of serum neutralization in mice.

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4.20 Action of disintegrin in expression of pro-inflammatory mediators by endothelial cells

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Introduction: Studies involving SVMPs (snake venom metalloproteinases) carried out by our group have shown the interesting role of these toxins as a cell activator, triggering responses such as apoptosis and inflammation, where complex signaling cell pathways are involved. However, it is important to understand the involvement of different structural domains of SVMPs in this process. The disintegrin domain of class P-II SVMPs is an integrin ligand that interferes with responses to extracellular signals and cell migration. Thus, we aimed to examine the role of a recombinant disintegrin, cloned from *Bothrops Insularis* venom and combined with a fusion protein (glutatione-S-tranferase, GST), on the activation of pro-inflammatory genes in endothelial cells. **Objectives:** The specific aim was to characterize the effects of a disintegrin (GST-INS) on cell viability and adhesion of endothelial cells, checking the expression of genes involved in the process of inflammatory responses, transcribed after the stimulus. **Methods:** Initially we used a Detoxi Gel TH Endotoxin Removing Gel (polymyxin B) column to remove LPS from the recombinant samples. The efficiency of the treatment was verified by LAL and SDS-PAGE methods. In experiments of cell viability and cell adhesion to the substrate, the endothelial cells (HUVECs) were treated with 200 nM GST-INS, GST or cell culture medium (as a control) and evaluated by the MTT method. In experiments for gene expression, cDNAs were transcribed from mRNA obtained from the HUVECs treated as described below and the fold change in expression of inflammatory genes was detected by real-time PCR. **Results and Discussion:** The recombinant samples were completely free of endotoxins and suitable for use in cell cultures. The cells treated with GST-INS showed a significant decrease in adhesion to substrate after stimulus. Cell viability was preserved even after the cells were detached. Of the primers analyzed, IL-6 and MMP-10 showed a small decrease in their expression while I-CAM 1 showed an increase in its expression. Disintegrins from snake venoms play a role as antagonists of cell adhesion of endothelial cells and interfere with the expression of genes coding for pro-inflammatory mediators.

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4.21 Evaluation of serological method for potency testing of whole cell pertussis vaccine

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Introduction: The potency of whole cell pertussis (wP) vaccines for human use needs to be checked and must comply with the requirements of the Brazilian Pharmacopeia. The vaccines need to have a potency of at least 4 IU per dose, (with a lower limit of the 95 % confidence interval of at least 2 IU) as determined in the Kendrick test (MPT). In this test, the dose necessary to protect 50 % of mice (ED50) against the effect of a lethal dose of *B. pertussis* administered intracerebrally is compared with the dose of a reference vaccine needed to give the same protection. The test inflicts severe pain and distress to the animals, requires highly experienced operators, is sensitive to small technical changes resulting in a high variability and requires frequent repeats due to invalid test results. Therefore, there is an urgent need of a viable alternative to MPT. **Objectives:** The aim of this study was to investigate whether serological potency testing in guinea pigs, using an ELISA based on plates coated with wP bacteria, is a possible alternative to the current MPT to the pertussis component potency assessment in DTP vaccines. **Methods:** Sera from guinea pigs immunized with DTP vaccines that are normally used to determine diphtheria and tetanus potency were tested by a direct ELISA. Plates were coated with *B. pertussis* suspension at 0.8 OpU/mL (opacity units/mL). As reference, a serum of guinea pigs immunized with reference vaccine is used. The titer was determined by comparison of the sample response and the reference by the four parameter analysis method (sigmoidal curve) using Combistats. To compare MPT to ELISA, correlation statistical assay followed by a paired non parametric t-test was used. **Results and Discussion:** Comparing MPT and ELISA results, the high variability of MPT was evident, resulting in confidence intervals of up to 240 %. Observing both, even with quite fair correlation, possibly due to the MPT variability, the differences as determined by t-test are not that different (p=0.353). In conclusion, the ELISA assay to assess pertussis potency in DTP vaccines can be considered viable, especially due to its qualities, namely rapidity and reproducibility, and most importantly, due to the fact that its use would reduce animal suffering. However, it demands some refinement in order to achieve a higher correlation with MPT.

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4.22 Comparative analysis of acute inflammatory processes in the subcutaneous tissue and exudate of mice genetically selected for maximal or minimal acute inflammatory response

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Introduction: The inflammatory response is a physiological process that aims to restore homeostasis and tissue integrity after an injury, whether triggered by physical, chemical or biological agents. AIRmax and AIRmin mouse lines were selected according to their maximal or minimal inflammatory response to a subcutaneous injection of polyacrylamide beads (Biogel). The cellular influx and protein concentration in exudates were the inflammatory phenotypes measured 24 h after the Biogel injection. Despite the cellular and protein concentration being well established in the exudate, nothing is known about the inflammatory mechanisms acting in subcutaneous tissue. **Objectives:** Here, we investigated the cellular and molecular mechanisms involved in the acute inflammatory process in the subcutaneous tissue of AIRmax and AIRmin mice. **Methods:** AIRmax and AIRmin mice were injected with Biogel-P100 in the subcutaneous dorsal region; 48 h later, the local tissue was excised and the RNA was extracted. Real-time PCR analysis for several genes involved in the inflammatory response was performed. A histological study was also carried out. **Results and Discussion:** AIRmax and AIRmin mice differ in cell counts and protein concentration in inflammatory exudates, which are respectively about 25- and 2.5-fold higher in AIRmax than AIRmin mice, with neutrophils predominating. AIRmax animals also had a significantly ($P<0.05$) higher expression of *Il6* (5-fold), *Cxcl2* (19-fold) and *Mmp9* (21-fold) genes than AIRmin mice. These molecules are known to favor leukocyte accumulation at the inflammatory site as well as stimulating their motility through the blood vessels to reach the inflamed tissue. Together, these results show that the largest number of cells and protein concentration in exudates may result from an increased local expression of inflammatory genes overexpressed in AIRmax mice.

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4.23 Oral immunization for hepatitis B vaccine: the promising use of mesoporous SBA-15 silica adjuvant

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Introduction: Mesoporous SBA-15 silica is an inorganic material with ordered channels of uniform hexagonal nanostructured pores measuring approximately 10 nm in diameter. These particles of silicon oxide are able to interact with atoms, ions and molecules, and due to their physicochemical properties, this material shows great potential as vehicle/adjuvant. **Objectives:** The applicability of SBA-15 silica as an oral adjuvant in immunizations with the recombinant vaccine for hepatitis B that contains particles purified of the surface antigen [HBsAg] produced by the Butantan Institute was experimentally assayed. **Methods:** Female isogenic BALB/c mice, 8 to 12 weeks old, [n= 5 per group] received subcutaneously or orally, 0.5 µg of HBsAg adsorbed/encapsulated on SBA-15 or adsorbed on $Al(OH)_3$ in a final volume of 0.25 mL PBS, mixed in a ratio of 1:10 antigen:SBA-15 or 1:20 antigen:$Al(OH)_3$ [v/v]. The mixtures were kept at 4°C for 24 h before immunizations. To assess the secondary immune response, following the same procedures, a booster was administered 30 days after the first immunization. Individual serum and pooled fecal samples of each group were periodically collected for titration of specific antibodies by ELISA. **Results and Discussion:** Analysis of secretory IgA [s-IgA] showed that mice orally immunized with HBsAg adsorbed on SBA-15 had increased levels of specific antibodies at day 14 post first immunization [4.5 $\log_2$] and 7 days after booster [5 $\log_2$]. Animals immunized by the subcutaneous route had undetectable s-IgA levels. Specific serum IgA in HBsAg:SBA-15 orally immunized mice reached 5 $\log_2$ at days 7 and 14 after booster. When $Al(OH)_3$ was used as adjuvant in subcutaneous immunizations, specific anti-HBsAg titers were detected only at day 7 post booster [4.5 $\log_2$]. The overall analysis of the results indicates the promising use of SBA-15 silica as an adjuvant to be used in oral immunizations. Proteins usually fail to produce detectable mucosal immune response because nearly all antigens are degraded along the gastrointestinal tract. It is believed that this nanoparticle acts on the physical protection of antigens and protective epitopes, providing its slow release, and in the efficient activation of the immune system.

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4.24 Investigation of venom allergen-like proteins (VALs) from *Schistosoma mansoni*

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Introduction: In mining the database generated from the *S. mansoni* transcriptome using Gene Ontology categorization, potentially surface-exposed or exported proteins were identified. Among the antigens tested as DNA vaccines, a gene with similarity to venom-allergen-like proteins (SmVAL5) conferred a worm burden reduction of 31 %. Further searches for similar domains in the *S. mansoni* genomic databank revealed 29 paralogs from this new gene family. SmVALs are members of a protein superfamily containing a conserved SCP/TAPS (sperm-coating protein/Tpx-1/Ag5/PR-1/Sc7) domain, which may be important in host-pathogen interactions. **Objectives:** Based on the transcriptome profile, proteomics and microarray data available for these molecules, we selected four members (SmVALs 4, 5, 7 and 26) to be investigated as vaccine candidates. We also evaluated the allergenic potential of these proteins by exploiting the murine model for allergenic airway inflammation. **Methods:** Native or codon optimized versions of the selected SmVALs genes were expressed in *P. pastoris*, and the recombinant proteins purified by nickel affinity chromatography. To evaluate the protective potential of these molecules, C57BL/6 mice were inoculated subcutaneously (sc) with 3 doses of 25 µg rSmVALs formulated with Freund's adjuvant. Six weeks after challenge infection, worms were collected and counted from the hepatic portal vein. The immune response profile was characterized by ELISA and ELISPOT. To evaluate allergenic airway inflammation, BALB/c mice were sensitized by 3 doses (sc) of 10 µg rSmVALs formulated with alum, and challenged by 2 intranasal doses of 10 µg rSmVALs. One day after the last challenge, the cells present in the bronchoalveolar lavage (BAL) were analyzed and the supernatants collected for cytokine measurements. **Results and Discussion:** Preliminary immunization and challenge assays revealed that rSmVAL5 induced a 40% reduction in worm burden, confirming the previous protective potential for this molecule. The humoral immune response was characterized by high levels of IgG1 and low levels of IgG2 antibodies; the cytokine profile revealed low levels of IFN-g and high levels of IL-4, suggesting a more Th2-driven immune response. The allergenic airway inflammation data revealed that rSmVAL-4 caused eosinophil (45.9%) and macrophage (37.5%) recruitment into the lungs of mice after sensitization and challenge. This effect is characterized by a systemic increase in specific IgE (serum) and by a local augment of IL-5 (BAL). Our results suggest the potential of this class of molecules as vaccine candidates, but also point out that allergenic effects should be considered in the design of a schistosomiasis vaccine.

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4.25 Evaluation of anti-EspA and anti-EspB polyclonal sera for diagnosis of enteropathogenic and enterohemorrhagic *Escherichia coli*

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Introduction: Among the six categories of diarrheagenic *Escherichia coli*, enteropathogenic *E. coli* (EPEC) is responsible for 30-40% of diarrhea episodes, and enterohemorrhagic *E. coli* (EHEC) is the principal cause of hemolytic uremic syndrome. EPEC and EHEC have in common a pathogenicity island called locus for enterocyte effacement (LEE) which encodes the proteins involved in the type three secretion system (SST3). EspA and EspB proteins are secreted by SST3, and are therefore excellent targets for the diagnosis of EPEC and EHEC. **Objectives:** In this study, we evaluated the use of anti-EspA and anti-EspB polyclonal sera for diagnosis of EPEC and EHEC. **Methods:** Recombinant proteins EspA and EspB purified by affinity chromatography column containing nickel were used to immunize rabbits. 100 µg of purified EspA or EspB plus aluminum hydroxide were intramuscularly injected into New Zealand rabbits, and a booster dose was administered after 15 days of immunization. Serum anti-EspA and anti-EspB obtained were absorbed and used in immunoblotting assays to evaluate the expression of EspA and EspB. For this, isolates of EPEC and EHEC were cultivated in different media and collected at different stages of growth. Subsequently, ELISA and immuno-dot assays were used for detection of EspB using the polyclonal anti-EspB. **Results and Discussion:** High titers of antibodies against the proteins EspA and EspB were observed. By immunoblotting, the serum anti-EspA was specific only for strain E2348/69, precluding the evaluation of EspA expression in other isolates. Furthermore, serum anti-EspB recognized all EPEC and EHEC isolates tested. Most isolates tested secreted EspB in higher amounts when cultured in Dulbeco´s MEM (DMEM) than in other media. These results point out the use of anti-EspB serum as a tool for the detection of EHEC and EPEC; since EspA has antigenic polymorphism, serum obtained with the protein of EPEC E2348/69 was unable to recognize other strains of EPEC and EHEC. Regarding EspB secretion, the use of DMEM promoted its increase in bacterial logarithmic growth phase, and immuno-dot assays can be used for qualitative detection and ELISA for quantitative detection of EspB.

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4.26 Production and use of EspB monoclonal antibody for the diagnosis of enteropathogenic *Escherichia coli* and enterohemorrhagic *Escherichia coli*

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Introduction: EPEC and EHEC are two important diarrheagenic *Escherichia coli* pathotypes; they are responsible for high rates of morbidity and mortality worldwide. *E. coli* secreted protein B (EspB) is among the virulence factors involved in their pathogenesis and is therefore a potential target for detection. Diagnosis is an essential tool either for the treatment of disease or to prevent further outbreaks. In Brazil, there are no commercial kits for detection of EPEC and EHEC, and thus, their developments are extremely important. **Objectives:** The present study aimed at the production and use of anti-EspB monoclonal antibodies for the diagnosis of EPEC and EHEC. **Methods:** Balb/c mice were immunized with 20 μg of purified EspB. The mouse with the highest antibody titer was boosted with the same amount of purified EspB without adjuvant four days prior to cell fusion, and then sacrificed by cervical dislocation. Popliteal lymph nodes were removed aseptically and B lymphocytes were fused with myeloma cells in the presence of polyethylene glycol. Ten days after fusion, hybridomas were screened for antibody production by ELISA. The specificity of the hybridomas was evaluated by immuno-dot assay using supernatant of positive and negative bacterial isolates. Two hybridomas were selected and subjected to limited dilution, and the selected clone was cultivated on a large scale in order to collect the supernatant. The IgG isotype was characterized by ELISA and purified by affinity chromatography column containing protein A. The purified monoclonal antibody was titrated by ELISA using 1.5 μg/mL EspB, and the dissociation constant was calculated. After the characterization of the monoclonal antibody, detection of EspB was standardized by ELISA using supernatants of bacterial isolates cultivated in Dulbeco's MEM. **Results and Discussion:** We obtained more than 300 anti-EspB-secreting hybridomas, and by immuno-dot we observed a high specificity of the 4D9 clone. This clone was characterized as IgG2a and its dissociation constant was 2×10^{-9} M, and only 1 μg/ml was needed for detection of EspB in the supernatant of the isolates. These results indicate that this monoclonal anti-EspB is a promising tool for the detection of EPEC and EHEC.

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4.27 SBA-15 silica adjuvant and the expression of co–stimulatory molecules

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Introduction: Adjuvants are compounds that can enhance the immune response to vaccine antigens by amplifying any of the signals involved in the process for eliciting an efficient immune response. The achievement of protective immunity through vaccination includes the recognition of relevant epitopes and activation of the antigen–presenting cells [APCs] such as macrophages [Mf] and dendritic cells [DC], either by the vaccine antigens themselves and/or by the adjuvant's physicochemical nature. The ordered nanostructured silica SBA-15 is an adjuvant that due to its physical and structural properties is capable of carrying, protecting and delivering antigens [International Patent WO 07/030901]. **Objectives:** The main goal of the present study was to evaluate the capacity of SBA-15 in inducing the expression of MHC class II and co-stimulatory molecules during the immune response against human g–globulin [HGG]. **Methods:** Isogenic BALB/c mice were immunized by the subcutaneous route with 10 μg/animal of HGG adsorbed or not on SBA-15 or $Al(OH)_3$. At different times after immunization, the expression of CD11c, CD11b, CD4, CD8, B220, CD80, CD86, CD40 and MHC-II in the lymph node cells and purified $CD11c^+$ cells were analyzed by flow cytometry. **Results and Discussion:** In the SBA-15 immunized mice, there was a significant increase in the expression of CD40, CD80 and CD86 in lymph nodes and purified $CD11c^+$ cells when compared to $Al(OH)_3$; nevertheless, these nanoparticles did not modulate MHC-II expression. No difference in recruiting of CD11c, CD11b, CD4, CD8, B220 cells was observed. These results agree with the ability of SBA-15 to induce successful immunity, recruiting and activating specific immune cells to the site of immunization. Thus, this new adjuvant is a candidate for immune modulation and nanovaccine design.

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4.28 The human complement regulators FH and C4BP interact with the leptospiral surface protein LcpA

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Introduction: Leptospirosis, an emerging global infectious disease, is caused by spirochetes belonging to different pathogenic species of the genus *Leptospira*. After penetrating the host, *Leptospira* have the ability to spread and to trigger a specific immune response. Like other pathogens, they have evolved strategies to evade innate immune defense systems, thereby causing severe disease. One strategy adopted by pathogenic *Leptospira* to resist hosts' innate immunity is their potential to acquire fluid phase complement regulators on their surfaces, particularly those of the alternative and the classical complement pathways such as factor H (FH), and C4b-binding protein (C4BP). Recently, we have shown that C4BP bound to *Leptospira* retains its cofactor activity, indicating that acquisition of this complement regulator may contribute to leptospiral serum resistance. **Objectives:** In this study, we screened a number of putative leptospiral membrane proteins for their capacity to interact with human complement regulators. **Methods:** The genes coding for putative outer membrane proteins were cloned and the proteins were expressed in *E. coli*. The binding of the purified recombinant proteins to FH and C4BP was assessed by Western blot overlay and ELISA. Surface exposure was assessed by immunoelectron microscopy and also by a proteinase K accessibility assay. **Results and Discussion:** We found that a predicted membrane lipoprotein of 20 kDa, named LcpA (leptospiral complement regulator-acquiring protein A), bound to both C4BP and FH. LcpA was shown to be surface-exposed, and the gene coding for this 20-kDa lipoprotein is conserved among pathogenic leptospiral species. Moreover, *Leptospira* strains that resist, at least to a certain degree, complement-dependent killing by normal human serum express LcpA, whereas the serum-sensitive strain Patoc does not. To our knowledge, this is the first description of a *Leptospira* protein that binds both C4BP and FH.

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4.29 Inflammatory and genetic mechanisms involved in ear tissue regeneration in mice selected for high or low acute inflammatory response

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Introduction: Mice selected for high (AIRmax) or low (AIRmin) acute inflammatory response were used in tissue regeneration experiments. It was observed that AIRmax mice show faster ear tissue regeneration than do AIRmin mice, suggesting the involvement of common regulatory loci for both inflammation and ear tissue regeneration phenotypes. **Objectives:** In this study, we investigated some inflammatory phenotypes and global gene expression profiles in AIRmax and AIRmin mice during the initial phase of ear tissue regeneration. **Methods:** Histology, ear edema thickness and MPO levels were investigated. Global gene expression analysis was performed with CodeLink bioarrays in both control and experimental 48-h ear punched mice, and to validate this research real-time PCR was used. **Results and Discussion:** The histological analyses showed that AIRmax regeneration was not only complete (with no sign of the original opposing epithelial surfaces), but cartilage islands and sebaceous glands were formed in the middle of the regenerated area. AIRmin mice displayed some regeneration but never closure. Ear edema thickness and MPO levels were higher in AIRmax than AIRmin mice ($P < 0.001$). Global expression analysis showed 794 activated and 528 repressed genes in AIRmax, while 1086 activated and 1145 repressed genes were observed in AIRmin mice 48 h after injury. AIRmax and AIRmin mice showed up-regulated genes over-represented in inflammatory response, cell adhesion and chemotaxis biological themes (gene ontology). However, down-modulated genes were significantly over-represented for transportation in AIRmax and for taxis, muscle contraction and ubiquitin cycle in AIRmin mice. In the QTL regions previously detected on chromosome 1 differentially-expressed *Stat1, Casp8* and *Hspe1* genes were found, while *Lect1, Fndc3 and Egr3* were detected on chromosome 14. qPCR experiments showed high expression of *Il-1β* and *Il-8rb* in AIRmax and *Cxcl2*, *Tnfa*, *Tgfb1* and *Mmp9* in AIRmin mice. AIRmax and AIRmin mice displayed several (some on chromosomes 1 and 14) differentially-expressed inflammatory genes which could be involved in the acute inflammatory response and ear tissue regeneration phenotypes.

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4.30 Expression profile of inflammatory genes in mice selected for high acute inflammatory response, bearing distinct Slc11a1 alleles after pristane-induced arthritis protocol

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Introduction: Rheumatoid arthritis is a chronic inflammatory autoimmune disease, and the main symptoms are chronic synovitis, joint erosion and several immune abnormalities. 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 the early phase of PIA. **Methods:** Mice received 0.5 mL i.p. pristane injection, and the peritoneal macrophages were isolated at day seven. Macrophage mRNA transcript levels of several inflammatory related genes (*Il1b, Tnfa, Il6, Il8rb, Il18, Cxcl2*) and *Tgfb* were measured by quantitative real-time PCR. Nitric oxide (NO) production was detected in culture supernatants after 48 h LPS stimulation. **Results and Discussion:** Results showed that pristane treatment significantly decreased the total cell number in the $AIRmax^{RR}$ and $AIRmax^{SS}$ peritoneal cavity. Distinct *Tnfa*, *Il1b*, *Il6* and *Cxcl2* gene expressions between $AIRmax^{RR}$ and $AIRmax^{SS}$ macrophages were observed. *Tnfa* and *Il1b* expression was higher in $AIRmax^{RR}$ than $AIRmax^{SS}$ macrophages ($p<0.001$). However, *Il6* and *Cxcl2* RNA expression was lower in $AIRmax^{RR}$ after pristane treatment (3-fold and 5-fold, respectively). *Il8rb, Il18* and *Tgfb* did not show significant differences among all the groups. Significant ($p<0.001$) NO production was observed in all mice only after LPS stimulation *in vitro*. These data suggest that *Slc11a1* alleles modulate inflammation-related gene expressions in peritoneal macrophages, which could influence susceptibility to pristane-induced arthritis in AIRmax mice.

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4.31 PAS-1 released by *Ascaris suum* in early larval stages suppresses LPS-induced acute inflammation

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Introduction: Helminth infections have been associated with an important immunomodulatory effect on the host immune system, impairing immune response to heterologous antigen, allografts, and infections. In our laboratory, we have demonstrated that the immunessuppression induced by *Ascaris suum* whole extract is due to a protein called PAS-1. **Objectives:** In this study, we evaluated the suppressive effect of PAS-1 secreted by the earlier larval stages of *Ascaris suum* cultured *in vitro*. The effect of PAS-1 on LPS-induced inflammation was compared to that with the *Ascaris suum* adult worm extract (ASC) and the body fluid (BF). **Methods:** Air pouches were induced on the shaved back of BALB/c mice with 2.5 mL of sterile air on days 0 and 3. The air pouches were then stimulated with 1 mg LPS alone or mixed with 300 mg PAS-1 obtained from the supernatant, whole extract (ASC) or adult worm body fluid (BF). Control groups were injected only with LPS. Three hours later, the exsudates were recovered after washing with PBS, and the magnitude of inflammation was evaluated by cell migration and cytokine levels. Pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and regulatory cytokines (IL-10 and TGF-β) were measured by ELISA. **Results and Discussion:** Our results demonstrate that mice injected with PAS-1, ASC or BF showed a significant suppression on the LPS-stimulated leukocyte infiltrate (mononuclear cells and neutrophils) into air pouches and production of TNF-α, IL-1β and IL-6 in comparison with the control group that received only LPS. In contrast, PAS-1, ASC or BF induced high levels of IL-10 and TGF-β in relation to control mice. On the other hand, no significant differences were seen between the experimental groups (PAS-1, ASC or BF). These data demonstrate that *Ascaris suum* products, including PAS-1 released by earlier larval stages, modulate the LPS inflammation in air pouches by suppressing cell influx and pro-inflammatory cytokines. The mechanism evoked in this suppressive activity is mediated by the regulatory cytokines IL-10 and TGF-β.

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4.32 Investigation of skin carcinogenesis in mice selected for maximal or minimal acute inflammatory response, homozygous for R and S alleles of Slc11a1 gene

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Introduction: Mice selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response differ in susceptibility to carcinogenesis. AIRmin mice are significantly more susceptible to skin carcinogenesis than AIRmax mice due their genetic background and a polymorphism of aryl hydrocarbon receptor (Ahr) gene. They also differ in susceptibility to *Salmonella enterica* serotype *Typhimurium* infection, arthritis and wound healing. The *Slc11a1* gene (formerly *Nramp1*) polymorphism modulates macrophage activity and the susceptibility to infections and autoimmune diseases. To study the interaction of resistant (R) or susceptible (S) *Slc11a1* alleles with acute inflammatory reaction loci found in AIRmax and AIRmin mice, homozygous sublines for these alleles were produced. These mice were designated $AIRmax^{RR}$, $AIRmax^{SS}$, $AIRmin^{RR}$ and $AIRmin^{SS}$. **Objectives:** The objective of this study was to investigate the skin carcinogenesis induced by DMBA in $AIRmax^{RR}$, $AIRmax^{SS}$, $AIRmin^{RR}$ and $AIRmin^{SS}$. **Methods:** To induce carcinogenesis, 50 µg DMBA diluted in 0.1 mL acetone were applied epicutaneously to the shaved dorsal skin of mice for five consecutive days. Gene expression of several inflammatory cytokines was detected in control and experimental mouse skin 48 h after the last DMBA application. This analysis was made by real-time PCR. **Results and Discussion:** All control mice had a basal expression of *Il6, Tnfa, Il1b* and *Cxcl2*. However, in DMBA-treated $AIRmin^{RR}$ mice, mRNA levels of these cytokines were increased 9-, 19-, 30- and 215-fold, respectively. In $AIRmin^{SS}$, these mediators were increased 11-, 4-, 6- and 75-fold in relation to their controls. *Saa3* and *Tgfb1* expression showed no significant differences among all DMBA treated mice. $AIRmin^{SS}$ differed significantly from the other sublines in some cytokines, but $AIRmin^{RR}$ showed the highest number of differentially expressed genes ($P<0.001$) after DMBA application. Preliminary data showed higher susceptibility to skin carcinogenesis in $AIRmin^{RR}$ than in $AIRmin^{SS}$ mice. These results suggest the involvement of on *Slc11a1* gene polymorphism modulating gene expressions to confer higher susceptibility to skin carcinogenesis in $AIRmin^{RR}$ mice.

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4.33 Study of adjuvants for mucosal and systemic vaccines composed of the antigen PspC (pneumococcal surface protein C) in pneumococcal nasal colonization and invasive challenge models in mice

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Introduction: S*treptococcus pneumoniae* (pneumococcus) is a gram-positive bacterium responsible for the majority of pneumonia cases around the world. Available vaccines are based on capsular polysaccharides, but the variability and low cross-reactivity displayed by them require the fermentation of different serotypes for the induction of broad-range coverage immunity. Protein antigens are interesting alternatives for the constitution of a low-cost formulation that can elicit immunity to the different serotypes. PspC is a virulence factor related to pneumococcal adhesion to respiratory epithelial cells and evasion from the immune system. It has already been shown that it is a good candidate for vaccine formulations. **Objectives:** The aim of the present work was to evaluate PspC (a large fragment composed of the N terminal domain plus proline-rich region) and $PspC_{104}$ (a fragment composed of the N-terminal domain, responsible for PspC binding to factor H), in combination with different adjuvants, as vaccines against nasopharyngeal colonization or invasive challenge models in mice. **Methods:** C57BL/6 or BALB/c mice were immunized with the proteins through the nasal route, using whole cell *Bordetella pertussis* vaccine (WCP) or DTP (diphtheria, tetanus and pertussis) vaccine as adjuvants. The formulations were also tested through the subcutaneous route. Induction of mucosal and systemic anti-PspC antibodies was evaluated by ELISA and mice were challenged with different pneumococcal strains. Cross-reactivity of PspC and $PspC_{104}$ sera among the different strains of pneumococcus was tested by Western-blotting. **Results and Discussion:** Nasopharyngeal colonization analysis of immunized mice showed that the vaccines containing PspC proteins and WCP were able to protect mice from pneumococcal carriage. The protection was most effective when the entire N-terminal region was used as antigen instead of the fragment comprising the first 104 amino acids, $PspC_{104}$. The best level of protection correlated with induction of high levels of anti–PspC antibodies in the sera and the secretion of IFN-γ and IL-17 after challenge. On the other hand, the nasal immunization with PspC or $PspC_{104}$ in combination with WCP did not protect mice against the challenge with the invasive strain 6303. Similarly, subcutaneous immunization with PspC in combination with WCP or DTP did not protect the animals against the invasive challenge. These results can be explained by the low reactivity of sera from the animals against the PspC expressed by the 6303 strain, revealed by Western-blotting.

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4.34 The phospholipases A_2 MjTX-I and CB isolated from *Bothrops moojeni* and *Crotalus durissus terrificus* snake venom, respectively, induce mast cell degranulation

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Introduction: Snake venom phospholipases A_2 (PLA_2s) are able to activate immunological responses with activation of leukocytes and release of inflammatory mediators. Among these cells are the mast cells (MC). These cells are central to innate immune responses by releasing a vast array of inflammatory mediators. The literature shows that phospholipases A_2 isolated from snake venoms induce mast cell degranulation; however, this is until now unclear. **Objectives:** The aim of this study was to compare the effects of the two PLA_2s MjTX-I a myotoxin isolated from *Bothrops moojeni* and CB, a neurotoxin from *Crotalus durissus terrificus* snake venom on mast cell viability and degranulation. **Methods:** PT18 lineage mast cells were cultured in tissue culture flasks with RPMI-1640, supplemented with fetal bovine serum (10%), gentamicin (40 mg/mL), L-glutamine (2 mM), 2-mercaptoethanol (2 mM) and IL-3 (5%), and maintained at 37ºC and 5% of CO_2. $2x10^5$ cells/well were incubated with different concentrations of MjTX-I, CB or Tyrode buffer (control) for 1 h at 37ºC and 5% of CO_2. Cell viability was evaluated by counting mast cells after staining with trypan blue solution (1:10). Mast cell degranulation was evaluated by measurement of β-hexosaminidase in the supernadant of cell cultures after incubation with non-cytotoxic concentrations of MjTX-I or CB or Tyrode buffer (control) for 1 h. **Results and Discussion:** Results showed that the viability of mast cells was significantly reduced after incubation of cells with higher concentration of both MjTX-I and CB (3.3 mM). Non-cytotoxic concentrations of MjTX-I (0.06 up to 0.6 mM) significantly increased β-hexosaminidase release in comparison with control. CB also caused a significant increase of β-hexosaminidase release from 0.3 up to 0.6 mM when compared with control. These results demonstrate that despite triggering distinct pathophysiological activities, both snake venom phospholipases A_2 were able to induce mast cell degranulation. The contribution of this action to the final biological effect of each phospholipase studied remains to be determined.

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4.35 Immune evasion of pathogenic *Leptospira*: human complement regulators factor H and C4b-binding protein interact with LigA and LigB proteins

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Introduction: Leptospirosis is a worldwide zoonosis and represents a serious health problem in urban areas of developing countries. Like other pathogens, leptospires have evolved strategies to evade the innate immune system. Binding of the regulators factor H (FH) and C4b-binding protein (C4BP) has been demonstrated for pathogenic strains of *Leptospira*. Leptospiral immunoglobulin-like (Lig) proteins belong to a family of surface-exposed determinants that have Ig-like repeat domains found in virulence factors such as intimin and invasin. The Lig proteins are expressed during host infection, but loss of protein expression occurs upon culture attenuation of pathogenic strains. Lig proteins can bind to a variety of extracellular matrix components, thereby mediating adhesion to host cells. Moreover, LigA has been shown to be the best vaccine candidate against leptospirosis. **Objectives:** Considering that during infection important virulence factors of many pathogens may interact with multiple host proteins, including coagulation cascade molecules, ECM components and complement regulators, we decided to detemine whether Lig proteins contribute to leptospiral immune evasion by interacting with host complement regulators. **Methods:** Both subfragments corresponding to the C- and the N-terminal portions of LigA and LigB genes were cloned and the proteins were expressed in *E. coli*. The binding of the purified recombinant proteins to FH and C4BP was assessed by Western blot overlay and ELISA. **Results and Discussion:** Both LigA and LigB bound serum FH and C4BP in a dose-dependent manner. To date, only two leptospiral proteins (LenA and LenB) have been described to interact with FH, and a single leptospiral protein has been shown to bind C4BP. Considering that leptospires are highly invasive microorganisms, there could be several other bacterial receptors for these host molecules. The identification of these receptors is of great importance, since they may represent targets for immune interference.

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4.36 Crotoxin isolated from *Crotalus durissus terrificus* venom induces mast cell degranulation in vitro

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Introduction: Crotoxin (CTX) is the main toxin from *Crotalus durissus terrificus* (Cdt) snake venom, and it is responsible for the toxicity observed in envenomation. CTX is a presynaptic neurotoxin formed by the complex of two different subunits: the non-toxic and non-hemolytic component, called crotapotin (crotoxin A, CA) and the hemolytic component, the phospholipase A2 (crotoxin B, CB, or Cdt PLA2). CA acts as an inhibitory "chaperone" preventing promiscuous interactions of the phospholipase with phospholipids in membrane surfaces other than its target membrane. CTX is related to important envenomation effects, such as neurological disorders, myotoxicity, and renal failure. Otherwise, the local edema, another feature of snake bite, has been associated with phospholipase A2 activity which induces an increase in microvascular permeability and plasma extravasation. Distinct components from the immune system may be involved in this initial inflammatory reaction, for example, mast cells. **Objectives:** The aim of this work was to evaluate the ability of CTX to induce mast cell degranulation *in vitro* and the capacity of the anti-crotalic horse serum to inhibit this toxin effect. **Methods:** **R**at basophilic leukemia **(**RBL) cell line was incubated for 1 h with different concentrations of CTX (8, 4 or 2 μg), and the ability of the toxin to induce cell degranulation was analyzed by the measurement of the β-hexosaminidase enzyme released in the culture supernatants. To evaluate the capacity of the anti-crotalic serum to neutralize the CTX effect, samples of the toxin (2 μg) were pre-incubated with different concentrations of the anti-crotalic serum (2, 10 or 20 μL) for 30 min and centrifuged, and the supernatants were incubated with RBL cells for 1 h. Afterward, the cellular supernatants were collected and used to estimate the β-hexosaminidase contents. All experimental conditions were tested in quadruplicate. **Results and Discussion:** The results show that CTX was able to induce *in vitro* mast cell degranulation with 48-50% of β-hexosaminidase release even using 8, 4 or 2 μg of the toxin. Furthermore, the anti-crotalic serum was efficient in neutralizing the CTX effect on mast cell degranulation at the proportions of 10 or 20 μL of serum/2 μg of the toxin. In contrast, the non-immunized horse serum did not interfere with the ability of the CTX to induce mast cell degranulation. In conclusion, the CTX exerted a direct effect on mast cell degranulation and the anti-crotalic serum was able to neutralize this toxin activity. Next, we will test if the recombinant single chain variable fragment (scFv) anti-crotoxin is able to inhibit CTX-induced mast cell degranulation.

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4.37 Immunization of mice with *Lactobacillus casei* expressing a β-intimin fragment reduces intestinal colonization by *Citrobacter rodentium*

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Introduction: Diarrheal diseases are common in childhood and can be of particular importance in developing countries, where they are responsible for around 17 % of total mortality in children. Among the pathogens responsible for these infections, enteropathogenic *Escherichia coli* (EPEC) are frequently isolated from infantile diarrhea. Intimin is a 94-kDa adhesin that participates in the local histopathological lesions (attaching and effacing – A/E-lesions), which are characteristic of EPEC infections and are also essential for virulence. A promising approach in vaccine development is the induction of mucosal and systemic immune responses against protective antigens delivered by lactic acid bacteria. **Objectives:** This work aimed to develop a mucosal vaccine against EPEC infections based on lactic acid bacteria. **Methods:** A fragment corresponding to the conserved plus the variable regions of beta-intimin (Int_{cv}) was constitutively expressed in *Lactobacillus casei.* Mice were immunized through the sublingual or oral route with the recombinant *L. casei* (*L. casei*-Int_{cv}), the respective control bacteria carrying the empty vector (*L. casei)* or saline. **Results and Discussion:** Immunization with *L. casei*-Int_{cv} through either route did not induce detectable levels of anti-Intimin IgA or IgG in feces or sera. On the other hand, a significant increase in specific secretion of IL-6 and IFN-gamma was observed in the spleen cells of mice immunized with *L. casei*-Int_{cv}, when compared to the respective control groups. Analysis of feces samples from mice challenged with *C. rodentium*, the mouse model for EPEC infections, showed an average reduction of 100X on colonization, for both groups that received *L. casei*-Int_{cv}. Time course evaluation showed that the reduction was already observed 4 days after infection and maintained for at least 10 days, the endpoint of the experiments. The results show that despite the absence of specific antibodies against intimin, the cytokine responses elicited by *L. casei*-Int_{cv} vaccines seem to correlate with protection against *C. rodentium* colonization.

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4.38 Evaluation of the production of antibodies against botulinum toxins type A and type B and of their neutralizing capacity

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Introduction: *Clostridium botulinum* produces toxins (BT) that have been classified into 7 serotypes (A – G), according to their immunological characteristics. Toxin type A, and sometimes type B, have been used to treat dystonia and various other muscle hyperactivity syndromes in humans. **Objectives:** The aim of this study was to evaluate the production of antibodies to BT type A and type B in hyperimmunizated horses, as well as their neutralization capacity, avidity and the IgG subclass pattern. Moreover, the results obtained in test bleeding and final product were compared. **Methods:** Horses were injected with BT-A and BT-B. These antigens were prepared containing about 0.7 mg/mL of protein. The test bleeding (type A and type B) was obtained and the total quantity of specific antibodies and their subclass was determined by ELISA, and antibody potency was measured by the serum neutralization assay in mice. The serum was purified and concentrated, and the total quantity of specific antibodies was performed by ELISA and their potency measured by the serum neutralization assay in mice. **Results and Discussion:** The total specific antibody titers to type A and type B in test bleeding were similar (1/1024000 to 1/2048000 and 1/512000 to 1/1024000, respectively). However, the potency of type A was 335.8 ± 67.2 IU/mL and the potency of type B was 53200.0 ± 6800.0 IU/mL. The potency of type B was significantly higher ($p<0.01$) than type A. The IgG subclass pattern was similar in the two types, with predominance of IgGT (1/256000 to type A and 1/512000 to type B) followed by IgGa (1/64000 to type A and type B). In the final product, the total specific antibody titers to serum type A and type B were also similar (1/1024000 to 1/2048000 both of them). The potency of serum type A was 414.70 ± 38.2 IU/mL and the potency of serum type B was 28660.0 ± 1337.0 IU/mL. The potency of serum type B (final product) was significantly higher ($p<0.05$) than serum type A. BT-A and BT-B apparently have the same capacity to stimulate antibody production; furthermore, BT-A and BT-B induce the same IgG subclass pattern of antibodies. However, the antibodies derived from BT-B horse immunization offer higher protection in the mouse model.

Supported by: Fundação Butantan

4.39 Characterization of antibodies from horse serum producers, used against *Botulinum* toxins type A and type E

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Introduction: Botulinum toxins are considered the most potent lethal substance known to humans. Human botulism is caused by neurotoxins types A (BT-A), B (BT-B) and E (BT-E). To date, no commercial vaccines have been available, and treatment consists of antitoxic therapy prepared by the hyperimmunization of horses. **Objectives:** The aim of this study was to compare the antibody production to BT type A and type E in horse serum producers, as well as their neutralization capacity, avidity and IgG subclass pattern. **Methods:** Horses were injected with BT-A and BT-E. These antigens were prepared containing about 0.5 mg/mL protein. The serum (type A and type E) was obtained, and the total quantity of specific antibodies and their subclass were determined by ELISA; antibody potency was measured by serum neutralization assay in mice and their avidity was determined by modified ELISA. **Results and Discussion:** The total specific antibody titers in the group of all producer horses (AP) type A and type E were significantly different (1/1,024,000 and 1/256,000, respectively). In the group of best producers (BP), this difference was higher (1/2,048,000 to type A and 1/256,000 to type E). The potency to type A was 335.8 ± 67.2 IU/mL and to type E was 155.9 ± 45.7 IU/mL. This difference was significant ($p<0.01$). Moreover, avidity showed a significant difference when we compared samples from AP (82.3% ± 3.3 to type A and 81.4% ± 2.7 to type E) and BP (96.8% ± 2.8 to type A, $p<0.05$ and 102.3% ± 1.3 to type E, $p<0.01$). The IgG subclass pattern was similar to both types, with predominance of IgGT (1/256,000 to type A and 1/128,000 to type E) followed by IgGa (1/64,000 to type A and 1/16000 to type E). The BT-A had a higher capacity to stimulate antibody production than did BT-E; this difference was higher when we observed the best producers. Apparently, the antibodies of type A have higher capacity of neutralization; however, it could be due to their total quantity. The antibodies derived from the best producers showed higher avidity; furthermore, BT-A and BT-E induce the same IgG subclass pattern of antibodies.

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4.40 Phenotypic characterization of cells during *Lagochilascaris minor* helminth infection

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Introduction: *Lagochilascaris minor* is a helminth that usually affects the cephalic region. The mechanism of infection is still unknown. This infection is also reported in cats, and mice are considered intermediate hosts. We have recently shown that C57BL/6 are more susceptible to *L. minor* than BALB/c mice, having more intense lesions in the lungs, with a greater number of nodules. Both mouse strains have a mixed cytokine pattern. **Objectives:** The aim of this study was to characterize cell populations present in the peripheral blood and spleens and to investigate the presence of lung infiltrating cells during the infection of both BALB/c and C57BL/6 mice with *L. minor.* **Methods:** BALB/c and C57BL/6 mice were orally infected with 10^3 eggs of *L. minor* per animal. After 7, 35, 100 and 250 days of infection, groups of 5 mice were sacrificed. The same numbers of non-infected mice were used as controls. Splenocytes and PBMC were isolated and incubated with FITC-, PE- or PE-Cy5-conjugated antibodies to perform phenotyping of cells. Immunohistochemistry of spleen and lung frozen sections was performed to analyze cell populations and IFNγ production, *in situ.* **Results and Discussion:** There was an increase in B220+ cells in PBMC from infected BALB/c (27.2 ± 5.2 vs $41.8 \pm 5.6\%$, $p < 0.001$) and infected C57BL/6 (32.5 ± 6.2 vs $56.1 \pm 6.6\%$, $p < 0.001$) mice compared to controls, 15 days after infection, with an increase in these cells in the spleen during the whole infection. In addition, there was an increase in CD3+ and CD4+ cells in PBMC from 60 day-infected BALB/c mice with a decrease of these cells in the spleens of these mice compared to controls. We detected more CD4+CD25+ cells in PBMC from infected BALB/c mice ($2.8 \pm 0.4\%$) compared to infected C57BL/6 ($1.3 \pm 0.5\%$), 60 days post-infection ($p < 0.001$). On the other hand, infected C57BL/6 mice showed a higher percentage of CD4+CD25+Foxp3+ spleen cells compared to controls (1.4 ± 0.4 vs $4.9 \pm 2.1\%$), 7 days after infection ($p < 0.05$). Both infected BALB/c and C57BL/6 mice showed an increase in MHC IAIE+ cells that can be correlated to an increase of the F4/80+ macrophage cell population in the spleen in the late period of infection (250 days). CD3+ and CD19+ cells were detected by immunohistochemistry, in the lung of infected C57BL/6 and BALB/c mice. IFNγ+ cells were also detected in the lungs of *L. minor* infected mice but not in controls, suggesting the presence of an inflammatory process. Our results suggest the participation of different T, B and macrophage cell populations in the immune response to *L. minor* infection in attempt to eliminate or control the parasite.

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4.41 Evaluation of the *Slc11a1* gene's role in the activation of macrophages stimulated by thioglycollate or *Mycobacterium bovis* BCG

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Introduction: Mouse lines were genetically selected for maximal and minimal acute inflammatory response (AIR). AIRmax mice are more resistant than AIRmin when infected with *S. typhimurium*. *Slc11a1* (formerly *Nramp1*) gene is involved in resistance to this infection as well as to the control of BCG infection; it interferes with macrophage activation, oxidative burst, inflammatory cytokine production, and nitric oxide (NO) and hydrogen peroxide (H_2O_2) secretion. Mouse lines homozygous for resistance and susceptibility *Slc11a1* gene alleles (AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS}) were produced in our laboratory. These lines show differences in inflammatory capacity, tissue regeneration and carcinogenesis response, phenotypes which are influenced by macrophage (MΦ) activation. **Objectives:** The aim of this work was to evaluate the effect of *SLC11A1* gene polymorphism in the activation of MΦ by thioglycollate (TG) or by *M. bovis* BCG. Systemic infection, cell migration, NO and H_2O_2 secretion and cytokine production were analyzed comparatively in these selected mice. **Methods:** Mice were inoculated ip with PBS (control), TG (48 h) or BCG (14 days). The peritoneal cells were collected and placed in culture. After 2 h, the non adherent cells were discarded and the adherent MΦ stimulated or not with LPS were used in all experiments. Spleens were homogenized and cultured in specific BCG medium and the bacterial colonies were counted. Cytokine levels were determined in culture supernatants by ELISA. **Results and Discussion:** The total numbers of resident cells in the peritoneal cavity of AIRmax^{RR}, AIRmin^{RR} and AIRmax^{SS} were similar but higher than in AIRmin^{SS} mice. TG promoted cell migration and induced low secretion of NO by MΦ in all lines. When LPS was added to the MΦ cultures, higher NO secretion was observed in AIRmax^{RR} followed by AIRmin^{RR}, AIRmax^{SS}, and AIRmin^{SS}. Analyzing the systemic BCG infection by counting the bacterial colonies in the spleen, AIRmax^{RR} mice were the most resistant to infection followed by AIRmin^{RR}, AIRmax^{SS}, and finally AIRmin^{SS} which does not control BCG growth. Cell migration to the peritoneum was induced by BCG ip infection in all lines, especially in AIRmax^{SS}. Higher cytokine production (IL-1β, TNF-α, IL-6 and IL-12p40) by peritoneal MΦ was observed in AIRmax^{RR} than in the other lines which produced basal levels of these cytokines. On the other hand high levels of NO and H_2O_2 were released by AIRmin^{SS} cells. Taken together, our results show that *SLC11A1* ^{RR} Mf is efficiently activated to kill the bacteria and secrete cytokines, and indicate that NO and H_2O_2 play a limited role in the protection against BCG infection.

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4.42 Semliki Forest virus (SFV) as a vector for anti-rabies immunization

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Introduction: The non-replicative SFV system is characterized by the production, in a single round of infection, of a great amount of recombinant mRNA in infected cells, leading to a high expression of the recombinant protein. We have evaluated the antigenicity and immunogenicity of the rabies virus glycoprotein (RVGP) produced *in vivo* by the utilization of a recombinant SFV-RVGP as a vector for vaccination. The SFV system shows several advantages for RVGP presentation to organisms, such as apoptosis-mediated cross-priming, capability to produce high amounts of the antigen, no integration into the genome and stimulation of both humoral and cellular immune responses. **Objectives:** The aim of this study was to describe the characteristics of the immune response directed against the RVGP delivered by a recombinant SFV system (SFV-RVGP). **Methods:** SFV-RVGP suicidal particles were obtained by co-electroporation of expression and helper plasmids in BHK-21 cells. These particles were further titrated by qRT-PCR and used for the immunization of Balb/c mice in a protocol with one immunization and three booster injections within 14 days. Antibodies against RVGP were evaluated by ELISA (Platellia), and neutralizing antibodies by the FAVN method. Cellular immune response was evaluated by cytokine expression profile (ELISA) and by intracellular cytokine staining after splenocyte stimulation with RVGP. **Results and Discussion:** We found that immunization with SFV-RVGP was able to induce the production of satisfactory amounts (> 0.5 IU/mL) of anti-RVGP antibodies after three immunizations and 21 days, attaining very similar levels of positive control group vaccinated with commercial anti-rabies vaccine. The first results on cellular immune response pointed to a preferential proliferation of CD8+ lymphocytes in mice immunized with SFV-RVGP as compared to mice immunized with anti-rabies vaccine.

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4.43 Expression of α2,6-sialyltransferase genes and enzyme activity in hybridomas producing murine anaphylactic and non-anaphylactic IgG1 antibodies

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Introduction: The *N*-linked glycans in the Fc domain of IgG are indispensable for its interaction with Fcγ receptors on effector cells as described for antibody dependent cell-mediated cytotoxicity (ADCC). Recently, we demonstrated that the sialic acid content in the *N*-linked carbohydrate chain of the Fc region of murine IgG1 monoclonal antibodies is essential for their ability to develop anaphylaxis. Therefore, the evaluation of the expression of the sialyltransferase coding genes during the synthesis of anaphylactic and the non-anaphylactic IgG1 antibodies by the hybridomas should show a quantitative difference in the expression of these genes by B cells. **Objectives:** We tested this hypothesis by examining the expression of the sialyltransferase- coding genes and the enzymatic activity of these enzymes in hybridomas that secret the anaphylactic (U7.6 clone) and the non-anaphylactic (H5 clone) IgG1. In addition, we evaluated if the *in vitro* sialylation of the non-anaphylactic IgG1 antibody allows this molecule to induce *in vivo* anaphylactic reaction **Methods:** The expression of the *ST3Gal II-V, ST6GalNac I-IV* and *ST8Sial I-V* genes was analyzed by real-time-PCR in hybridomas secreting theses two types of IgG1 antibodies. Sialyltransferase activity was measured by specific *Sambucus nigra*-binding-ELISA. In the terminal sialylation *in vitro* assay, the non-anaphylactic IgG1 antibody was incubated with sialic acid and the sialyltransferases obtained from the U7.6 hybridoma. After incubation, the IgG1 antibody was purified by *Sambucus nigra* affinity chromatography and its biological activity and the antigenic affinity were tested by PCA reaction and ELISA, respectively. **Results and Discussion:** The expression of *ST3Gal III* and *IV, ST8 Sial III* and *ST6GalNac I*, *II* and *IV* was higher in hybridomas secreting the anaphylactic antibody rather than the non-anaphylactic IgG1. α2,6-ST activity was significantly higher in the anaphylactic IgG1 producing clone when compared to the non-anaphylactic one (1.2 versus 0.5 D.O/mg of protein, $p < 0.001$). These results showed an association between sialyltransferase gene expression and the sialylation grade of the glycan chain *N*-attached to the murine IgG1 antibodies. In addition, we observed that *in vitro* sialylation of the non-anaphylactic IgG1 leads to its ability to induce anaphylactic reaction. The present study explored the influence of the sialic acid residues on the anaphylactic activity of the IgG1 antibodies.

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4.44 Influence of inflammatory response in the determination of 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 their ability to close ear holes completely. AIRmax^{SS} mice showed faster 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 inflammatory response in the determination of distinct phenotypes. **Methods:** Two-millimeter ear holes were punches in mice of each subline and the inflammatory reaction was characterized by measuring histomorphometric analysis, ear thickness and MPO activity. Gene expression profile analysis during the inflammatory stage of regeneration and wound healing was performed by quantitative PCR experiments. **Results and Discussion:** The local inflammatory response was subtly more intense in AIRmin^{SS} than AIRmax^{SS} mice 48 h after ear punch, which was demonstrated by histomorphometric analysis and elevated MPO levels, suggesting the predominance of neutrophils in the ear of AIRmin^{SS} animals. During this period, the expression profile of pro-inflammatory genes showed high transcripts levels of *Il6, Il1b, Tnfa, Cxcl2, Ccl2, Itgb2, Vegfa* and *Mmp9* in both lines, although slightly but significantly higher levels (>3-fold) were observed in AIRmin^{SS} mice ($P<0.05$). An increase in ear thickness was observed in AIRmax^{SS} compared to AIRmin^{SS} mice. The superior initial inflammatory response in the inflamed ear tissue with higher RNA expression of pro-inflammatory cytokines could inhibit epimorphic regeneration in AIRmin^{SS} mice. These results suggest that the degree of inflammatory response in the early events after injury modulates the quality of regeneration or wound healing.

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4.45 Reactivity of serum IgA and IgG anti-rotavirus serotype G3 in ELISA and immunoblotting assay

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Introduction: Rotavirus is well established as the major etiological agent of diarrhea worldwide. The role of serum antibodies in the immune protection against natural infection is not fully understood. Some studies have correlated serum antibodies against rotavirus in adults with protection and lower probability of infection and illness. Anti-rotavirus IgA and IgG are candidate markers for rotavirus immunity. **Objectives:** Our aim was to analyze the reactivity of anti-rotavirus IgA and IgG antibodies in human serum samples from healthy adult blood donors in immunoblotting (IB) assays. **Methods:** ELISA was carried out for detection of IgA and IgG in fifty serum samples using antigens from the SA-11 rotavirus strain propagated in MA-104 cells. Mock-infected cell preparations were used as control. Individual samples were then titrated including a pool of 50 samples as positive control in every assay. ELISA titer was determined as the reciprocal of the dilution giving an absorbance value of 0.5. The final titer was reported as a percentage, considering the pool as 100%. Rotavirus G3 antigen fractionated by gel electrophoresis (SDS-PAGE) was transferred to a nitrocellulose membrane by a semi-dry system. After transfer, the membrane was cut into 4-mm strips. Each strip was blocked for two hours with buffer containing skim milk; the serum sample was added at the appropriate dilutions and incubated overnight. Afterward, the strips were washed and treated with anti-IgA conjugates labeled with alkaline phosphatase at 1:200 dilution or anti-IgG at 1:2000 dilution. After two hours incubation and a cycle of washes, the strips were revealed with NBT/BCIP (5-bromo-4cloro-3-indolyl phosphate/nitro-blue tetrazolium). The reaction was then stopped with water. **Results and Discussion:** The serum samples recognized protein bands in a range from 80 to 20 KDa.The samples with higher IgG ELISA titers showed stronger bands compared to the ones with high IgA titers. Samples with similar IgG and IgA ELISA titers and samples with higher titers of IgG compared to IgA showed a stronger and more diverse IgG recognition. In some samples, we observed a very different recognition pattern when IgA and IgG were detected. The population studied has levels of anti-rotavirus G3 IgG and IgA antibodies varying over a wide range, perhaps due to different levels of exposure to the virus. The differences between the recognition patterns of IgA and IgG by the same sample suggest that the two antibody isotypes may originate by independent mechanisms.

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4.46 Effectiveness of the Brazilian influenza vaccination policy, a systematic review

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Introduction: Since 1999, Brazil has undertaken annual influenza vaccine campaigns targeting the elderly population, other groups vulnerable to influenza complications and health professionals. Flu vaccine has been provided, free of charge, in national campaigns, which usually have been held in late April/early May. In 1999, the campaign targeted the population over 65 years of age. Since 2000, it has targeted the population over 60. Influenza vaccination campaigns have been regarded as largely successful, reaching high vaccine coverage among the elderly, the largest group included in the initiative. According to Ministry of Health (MOH) figures, coverage has reached 70% of the target among the elderly population in all years, except 2000. MOH does not present figures on vaccine coverage in other groups targeted in the flu vaccine campaigns (health care workers, transplant patients, people living with immune deficiencies, and other groups of high risk patients for influenza complications), because of the alleged lack of denominators for the coverage determination. In spite of such apparent success, little is known about the effectiveness of the initiative. **Objectives:** The aim of this study was to assess the effectiveness of the Brazilian influenza vaccine policy. **Methods:** We conducted a systematic review of the literature to evaluate the effectiveness of the initiative. We used the keywords influenza, vaccine, Brazil and effectiveness, to search the main databases. **Results and Discussion:** We found 380 articles that matched our search criteria. We then analyzed the list, excluding the ones that were published before the beginning of the Brazilian influenza vaccination campaigns (1999), those that were not actually related to Brazil (some referred to the Brazil influenza B viral subtype), and a few others that did not address the issues of interest. A total of 51 papers were selected as potentially relevant. Of these, thirty-one studies met our inclusion and exclusion criteria. Influenza vaccine coverage among the elderly is high, although not as high as presented by the official figures. Effectiveness estimates are scarce. The majority of them come from ecologic studies which show a modest reduction in mortality and hospital admissions due to influenza-related causes. Such reduction is not evident in northern and northeastern states of Brazil, a finding that is probably related to the different seasonal pattern of influenza in equatorial and tropical regions. Brazilian epidemiologists still owe the society better designed studies addressing the effectiveness of influenza vaccine campaigns.

4.47 Cloning, expression and characterization of *Leptospira interrogans* serovar copenhageni LIC13435 gene

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Introduction: Leptospirosis is a re-emergent zoonosis characterized by an acute febrile and systemic illness in humans caused by pathogenic spirochetes belonging to the genus *Lepstospira*. It is known that leptospirosis is one of the most common zoonotic infections in the world. In Brazil, leptospirosis is an important economic and public health problem. The complete genomic sequence of *Leptospira interrogans* offered a new strategy for the identification of new antigens that could be vaccine candidates, since environmental control measurements are difficult to implement and because there is no available vaccine for human use. **Objectives:** Secreted and surface exposed molecules are potential targets for inducing protective immune responses in the host. Thus, we selected the gene LIC13435 coding for a predicted outer membrane protein for biological characterization. **Methods:** The sequence of LIC13435 gene was selected from the genome of *Leptospira interrogans* serovar Copenhageni using bioinformatics tools. The sequence was amplified by PCR and the expression of the recombinant protein was tested in *Escherichia coli* strains. Purification of the recombinant protein was done by metal affinity chromatography. The antisera were produced by intraperitoneal immunization of BALB/C mice. **Results and Discussion:** Circular dichroism characterized the secondary structure as being mainly composed of α helix. ELISA test indicated that the recombinant protein is very immunogenic. PCR revealed that the LIC13435 gene is conserved among several serovars of leptospira. Indeed, orthologs of this gene can be identified in sequenced genomes of pathogenic leptospiras but not in the saprophytic *L. biflexa*. Preliminary challenge assay against *Leptospira* in hamster indicates that LIC13435 is not protective. Further characterizations are underway.

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4.48 Pilot 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 still among the major health threats, accounting for more than 500,000 deaths/year from an estimated 350 million individual sufferers due to complications of chronic HBV infection. Current single antigen vaccines that are produced by recombinant DNA technology are highly effective, but 5-10% or more of healthy immunocompetent subjects do not mount an antihepatitis B surface antibody protective response, while others respond poorly (hyporesponders). Instituto Butantan has produced the recombinant hepatitis B vaccine since 1998, using *Hansenula polymorpha* to express the virus surface S-antigen, HBsAg. In order to enhance the immunogenicity relative to that of conventional yeast-derived vaccines, a new construction containing pre-S1, pre-S2 and S surface components was tested. **Objectives:** The aim of this work was to standardize, on a pilot scale, the production of three consecutive lots of a new recombinant hepatitis B vaccine containing the pre-S1, pre-S2, and S antigenic components. **Methods:** The production process began with the fermentation of producing strain, in a 60-L fermentor, followed by cellular disintegration and several purification steps, including chemical precipitation, salt gradient and gel filtration. The fermentation stages were evaluated for biomass, absence of contamination and expression of the S-antigen, by passive hemagglutination assay (PHA). All purification steps were monitored for total and specific proteins, respectively by OD_{280} and PHA. The final product was submitted to quality control, including Lowry method for protein content, purity evaluation by SDS-PAGE and immunobloting for antigen specific, using anti-HBsAg anti-sera, anti-*Hansenula polymorpha* anti-sera, and pre-S1 and pre-S2 Mabs. **Results and Discussion:** The fermentation process of the new strain was slightly different from that of the process of the yeast expressing only HBsAg, with similar productivity index of 0.80 g/L/H on average, after 144 hour of fermentation. The expression of the antigen tested by PHA was satisfactory with 360 UHA/mL on average. After the purification processes, the final product met the requirements of regulators regarding the purity (>95%) and specificity of antigens. The yield was 100,000 doses per fermentation process (25 μg of HBsAg per dose) and 0.4 g of purified antigens per kg of fermented material. The results showed consistency among the lots produced, giving support to process scale-up.

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4.49 The adjuvant effect of DTP_{low} vaccine in combination with the pneumococcal surface protein A (PspA) antigen: proposal of a new vaccine against *Streptococcus pneumoniae*

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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. PspA is a surface-exposed pneumococcal antigen, shown to elicit protection against animal models of pneumococcal infections, in different vaccine formulations. Due to its variability, PspA is classified into 6 different clades. We have previously shown that the whole cell pertussis vaccine (wP) and the new generation wP vaccine that contains low LPS (wP_{low}) are both able to act as adjuvants, when administered to mice in combination with PspA through the nasal route. Both formulations induce high titers of anti-PspA antibodies and protection against models of colonization and invasive disease in mice. **Objectives:** Here, we studied the role of the toll-like receptor 4 (TLR4), the cellular receptor for LPS, in the adjuvant activity elicited by both vaccines. In addition, we tested the adjuvant effect of the wP_{low} formulated in the DTP_{low} vaccine (Diphtheria – Tetanus –Pertussis) in combination with PspA through the subcutaneous route. **Methods:** C3H/HeJ mice (TLR4-) and the parental C3H/HePas mice were vaccinated through the nasal route with PspA from clade 5 (PspA5) in combination with wP or LPS and then submitted to a respiratory pneumococcal lethal challenge. BALB/c mice were vaccinated through the subcutaneous route with PspA5 combined with DT or DTP_{low} and were also submitted to respiratory lethal challenges with two different pneumococcal strains. In both cases, survival was monitored for 10 days. **Results and Discussion:** Co-administration of PspA5 with wP induced higher levels of anti-PspA5 antibodies in C3H/HeJ and C3H/HePas mice. On the other hand, the combination of PspA5 with *B. pertussis* LPS elicited similar levels of anti-PspA5 antibodies when compared to the protein alone. These results indicate that TLR4 expression is not essential and that other *B. pertussis* components besides LPS are important for the adjuvant effect observed. A single subcutaneous administration of PspA5-DTP_{low} in BALB/c mice also elicited significantly higher levels of anti-PspA5 antibodies when compared to PspA5 alone. This formulation elicited 100% protection against a pneumococcal strain that expresses PspA5 and 66% protection against a strain that expresses PspA from clade 2. Our results indicate that a combined vaccine composed of DTP_{low} and PspA has the potential to induce broad-coverage protection against pneumococcal infections.

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4.50 Cleaning validation of molecular ultrafiltration system used in diphtheria toxoid purification process

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Introduction: The cleaning validation of equipment applied in pharmaceutical production process is an integral part of Good Manufacturing Practices (GMP) meeting the national requirements (ANVISA) and WHO recommendations. Cleaning validation procedures are carried out to assure that residues are within acceptable limits after the cleaning process. Diphtheria toxoid, obtained through the detoxification of diphtheria toxin, is purified in the Center for Purification of Bacterial Products at Butantan Institute. In order to obtain a product with high purity the diphtheria toxoid is purified in two steps: the first consists in diafiltration and concentration by molecular ultrafiltration system using Pellicon® equipment, and in the second step the product is precipitated with ammonium sulfate and then diafiltered and concentrated using the same equipment. The purified product after approval in quality control test is denominated Diphtheria Toxoid Final Bulk, which is a component of combined vaccines: dT, DT, DTP and DTP-Hib. **Objectives:** The aim of this study was to validate the cleaning procedures of molecular ultrafiltration system providing documented evidence that the process is consistent. **Methods:** Three consecutive applications of the cleaning process in the molecular ultrafiltration system were perfomed after diafiltration and concentration of diphtheria toxoid. The cleaning process of equipment was conducted using sodium hydroxide, phosphate buffer, pH 6.4, purified water and formaldehyde solution. Samples of critical steps of the cleaning procedure were collected and submitted to the following tests: Bioburden test, pH, conductivity, total organic carbon analysis (TOC), protein determination by Lowry method and residual formaldehyde test. **Results and Discussion:** The results of Bioburden in three tests before and after using equipment were 0 (zero) CFU/50 mL, showing that the cleaning procedures were efficient. The averages of TOC in initial and final samples were 653.67 ppm and 344.00 ppm respectively, indicating a considerable reduction of total organic carbon. All results obtained in other tests were according to the established limits. The cleaning procedure of the molecular ultrafiltration system was demonstrated to be effective and reproducible, allowing the validation of the method. This cleaning validation process will be implemented for other equipment used in the diphtheria toxoid purification process at Butantan Institute.

Supported by: Fundação Butantan

5. Microorganisms

5.01 Validation of sterility test in isolator of *Streptococcus pneumoniae* suspension formulated at Butantan Institute by means of bacteriostatic and fungistatic effect

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Introduction: Sterility of *Streptococcus pneumoniae* suspension formulated at Butantan Institute for pneumococcal vaccine development is determined by direct inoculation in fluid thioglycollate medium (FTM) and soybean-casein digest media (SCM). The current standard requires that all operational procedures used in quality control must be validated according Current Good Laboratory Practice (cGLP). This procedure ensures analysis and final product quality. **Objectives:** The aim of this study was to determine the sensitivity of bacterial and fungal sterility test (by direct inoculation methodology) applied to *S. pneumoniae* suspension formulated at Butantan Institute, in order to validate the technique used in isolators under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of *S. pneumoniae* suspension formulated at Butantan Institute used in pneumococcal vaccine production. These lots were tested according to standard methodology. After direct inoculation of *S. pneumoniae* suspension, a viable inoculum of ATCC microorganisms (10-100 cfu/mL) was used to validate the technique. FTM was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and SCM was challenged 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 determined during an observation period of 5 days. The methodology applied in isolator to bacterial and fungal sterility test of *Streptococcus pneumoniae* suspension formulated at Butantan Institute is effective and demonstrates a high degree of sensitivity to detect low levels of contaminants. We conclude that the methodology can be performed without modification. The formulated product is absolutely safe (sterile) according national and international standards and may be used to vaccinate the public.

Supported by: Fundação Butantan

5.02 The influence of kefir on proliferation of protozoa

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Introduction: Kefir is a beverage derived from the fermentation of milk by a symbiotic association of bacteria and yeast which has the ability to inhibit the proliferation of *Candida albicans* and pathogenic Gram-positive and Gram-negative bacteria. However the influence of kefir on protozoa has never been investigated. **Objectives:** The objective of this work was to determine the influence of kefir on the proliferation and morphology of free-living (*P. caudatum*) and pathogenic (*Giardia lambia*) protozoa. **Methods:** The influence of kefir on proliferation of *P.caudatum* was determined by incubating the protozoa at a concentration of 5 organisms/ml in mineral water with 20 µl/ml of milk in the presence or absence of kefir diluted 1:2, 1:4 and 1:8. After 36 and 96 h of incubation at 30°C, the number of protozoa was determined in a Segewich-Rafter chamber, and their morphology was observed by light microscopy. *Giardia lambia* (10^4/ml) were incubated in YI-S Base medium at 30°C for 24, 48, 72 and 96 h. After incubation, the protozoa were fixed with a saturated solution of $HgCl_2$, stained with bromophenol-blue (0.04%) and counted in a Neubauer chamber. Their morphology was also observed by light microscopy. **Results and Discussion:** The results obtained in this work showed that the 1:2 dilution of kefir killed both protozoa. Morphological visualization by light microscopy showed that at this dilution, kefir disrupted the membrane of *P. caudataum* but not the membrane of *Giardia lambia*. Kefir diluted 1:4 inhibited the proliferation of both *P. caudatum* and *Giargia lambia*, but a 1:8 dilution of kefir had no effect on either the proliferation or morphology of these protozoa. In summary, the results obtained in this work suggest that kefir has the potential to be used as a natural remedy against pathogenic protozoa.

5.03 Preliminary screening for *Leptospira* adhesins through *in vivo* phage display

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Introduction: Leptospirosis is a zoonosis of global importance caused by a pathogenic spirochete. In Brazil, 80% of diagnosed cases are due to *Leptospira interrogans* serovar Copenhageni. Bacterial adhesion to the host is a key event in the infection process. **Objectives:** In attempt to identify the genes coding for adhesins that would bind to some key components of the host extracellular matrix (ECM), we constructed libraries from randomly fragmented bacterial DNA from *L. interrogans* serovar Copenhageni by shotgun for phage display. **Methods:** These libraries were prepared into a phagemid vector and screened in vivo using Golden hamsters. The hamsters were anesthetized, and 200 µL of phage were injected through the left ventricle of the heart and allowed to circulate for 5 min. Animals were perfused with citrate buffer until the perfusate was clear and the organs appeared free of blood. Organs and tissues were placed in DMEM supplemented with protease inhibitors, weighed, homogenized and amplified by infection into bacteria. The phage was titered and the same input phage dose was used for each round of panning. **Results and Discussion:** The phage was titered and the same input phage dose was used for each round of panning. About 100 clones from each of the 4 rounds were sequenced. Candidates with extracellular binding properties were chosen and tested by in vitro binding assays with ECM molecules. This is the first report about screening *L. interrogans* adhesins using in vivo phage display.

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5.04 Application of BacT/ALERT 3D in production process control of diphtheria, tetanus and pertussis vaccine (DTP)

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Introduction: BacT/ALERT 3D (B/A) system is an automatic instrument used for early detection of bacterial and fungal contamination, and the flasks containing culture media provides nutritional and environmental conditions suitable for microorganisms growth. This system uses a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide dissolved in the culture medium, produced as a result of microbial metabolism. The DTP (diphtheria/tetanus/pertussis) vaccine is part of the national immunization schedule used for active immunization of children. Traditional evaluation of bacterial and fungal sterility of this product is carried out by membrane filtration (FM) technique and visual analysis over 14 days. **Objectives:** The aim of this study was to apply the B/A system during the DTP vaccine production process, aiming to reduce retention time and to demonstrate the importance of preservative neutralization (thimerosal) by means of bacteriostatic and fungistatic effect on detection sensitivity of low levels of microorganism contaminants. **Methods:** We used three batches of DTP production previously approved on bacterial and fungal sterility test by traditional methodology. We used BacT/ALERT SN and SA culture media, with a sample volume of 1.0 mL plus 1.0 mL of DNP (Diluent Neutralizing Pharmacopoeic fluid), injected directly into the culture medium and kept under observation for 14 days. Each lot was challenged with a 10-100 CFU/mL inoculum of the following microbial strains: *Aspergillus brasiliensis, Bacillus subtilis, Candida albicans, Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The sensitivity of the culture media was tested with negative control (DNP sterile solution) and positive (ATCC microorganism suspension without the product being tested). **Results and Discussion:** When neutralized by DNP solution, the three lots tested revealed the following recovery times: *A. brasiliensis* 165.4 h; *B. subtilis* 18.9 h; *C. albicans* 72.4 h; *P. aeruginosa* 21.4 h; *S. aureus* 31.4 h and *C. sporogenes* 47.0 h. The negative control showed no microbial growth and the positive controls (pure cultures), recovery times as follow: *A. brasiliensis* 30.0 h; *B. subtilis* 14.6 h; *C. albicans* 17.8 h; *P. aeruginosa* 14.4 h; and *S. aureus* 32.0 h. The results show that the B/A system can only be used in production process control for DTP vaccine when added with a neutralizing preservative solution. We observed that the recovery time of fungal growth in SA medium and *C. sporogenes* in SN medium is greater than in positive controls. The results show that B/A system can be used to control the production process, significantly reducing product retention time. Inhibitory components of microbial growth incorporated on product composition are completely neutralized by DNP and B/A system, allowing low levels of contamination detection.

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5.05 Cloning and analysis of genes that encode proteins identified by mass spectrometry of atypical enteropathogenic *Escherichia coli* of serotype O55:H7

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Introduction: Enteropathogenic *Escherichia coli* (EPEC), one of the six *E. coli* diarrheagenic (DEC) pathotypes produces an adherence factor chromosomally encoded by the *eae* (EPEC attaching and effacing) gene located within the locus for enterocyte effacement (LEE) pathogenicity island and have been categorized into two subgroups termed typical and atypical EPEC. Typical EPEC (tEPEC) strains contain, in addition to *eae*, the EPEC adherence factor (EAF) plasmid, which encodes the bundle-forming pili that mediate localized adherence to epithelial cells. EPEC strains lacking the EAF plasmid and non-expressing BFP have been designated atypical EPEC (aEPEC). **Objectives:** Previous data generated by proteomics analysis, using the technique of peptide mass fingerprint, showed differences between outer membrane proteins (OMPs) from aEPEC serotype O55:H7 and tEPEC serotype O55:H6, Thus, in the present study, four proteins expressed only in aEPEC (spots 114, 135, 192 and 271) were chosen for gene cloning in expression systems and protein characterization. **Methods**: The primers to amplify these genes were designed with the Gene Runner program, and PCR was performed using Platinum Pfx Polymerase (Invitrogen) using standard amplification conditions. The amplified products were purified and cloned into cloning vector pGEM-T Easy vector. These plasmids containing the inserts were transformed into *E. coli* JM109. The confirmation of cloning was performed by restriction analysis with the enzymes HindIII and NdeI. **Results and Discussion:** Among the target proteins, three have identity with proteins of unknown function and one is a mobilization protein. The protein sequences were obtained through its gene identification (gi=in http://blast.ncbi.nlm.nih.gov) and were analyzed by the presence of signal peptide and transmembrane regions using the programs Signal P and DAS transmembrane, respectively. The analysis showed that these sequences have no signal peptide and only the hypothetical Fpla064 protein (spot 114) showed five transmembrane segments. The presence of conserved domains was confirmed with the program Conserved Domains. Thus, the protein 114 showed conserved domains of super family ParBC and SpoOJ; the protein 192 showed conserved domains of mobilization proteins. Oligonucleotides were designed using the gene sequences of each protein, and these oligonucleotides were used for amplification of the respective genes, where only three genes were amplified. One of the generated fragments (spot 135) was cloned in cloning vector pGEM-T Easy vector for later subcloning in expression vectors. The characterization of these proteins should contribute to understanding the virulence mechanism of aEPEC of serotype O55:H7, since these target proteins were identified only in aEPEC and were absent in serotype O55:H6.

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5.06 Construction and analysis of atypical enteropathogenic *Escherichia coli* QseC mutants

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Introduction: Quorum sensing (QS) is a cell signaling mechanism in which bacteria produce and detect extracellular signaling molecules called autoinducers (AI). This mechanism is used by gram-positive and gram-negative bacteria to regulate many of physiological functions. In pathogenic bacteria, QS controls the gene expression of virulence factors. Many QS systems have been identified in different bacterial species. One of these, described in enterohemorrhagic *Escherichia coli* (EHEC), involves the detection of AI-3 and the mammalian hormones epinephrine and norepinephrine. The pathogens use the sensor histidine kinase, QseC (quorum sensing *E. coli* C) to recognize both host-derived signals and the bacterial signal to activate their virulence genes. Upon sensing these molecules, QseC autophosphorylates and phosphorylates the transcription factor QseB, which initiates a regulatory cascade and leads to the transcription of genes such as flagella, adhesins, and the genes of the pathogenic island LEE (locus of enterocyte effacement). The LEE region contains most of the genes involved in the attaching and effacing (AE) lesion, the intestinal histopathology associated with enteropathogenic *E. coli* (EPEC) and EHEC infections. **Objectives:** The purpose of this study was to construct strains of atypical EPEC with a mutation in the *qseC* gene for further studies of its influence on gene regulation of virulence factors encoded on LEE. **Methods:** The *qseC* mutagenesis in two atypical EPEC strains was obtained using homolog recombination technique based on bacteriophage λ Red system. In this technique, the *qseC* gene was replaced by the recombination cassette that contains the *cat* (chloramphenicol acetyl transferase) gene generated by PCR amplification. This allelic exchange was mediated by enzymes that inhibit the degradation and promote recombination of linear DNA target. To confirm the recombination, two PCR amplifications were performed using specific primers: to the *cat* genes and to the flanking region of *qseC* gene. **Results and Discussion:** The recombination generated in mutant strains an amplification of *cat* gene (900 bp), while in the wild type strains, no amplification was detected. When the primers external to the *cat* gene were used in the amplification, the mutant strains showed an amplicon of 1,100 bp, corresponding to the *cat* gene, while the wild type strains showed an amplicon of 1,300 bp corresponding to *qseC* gene. Using adherence assays with cultured epithelial cells, we showed that QseC mutants are capable of forming AE lesions, but not as efficient as wild type strains. Further studies will help us to elucidate the role of QseC in the AE lesion.

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5.07 Development of transcriptional fusions to assess *Leptospira interrogans* promoter activity

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Introduction: Leptospirosis is a zoonotic infectious disease that affects both humans and animals, and has emerged as a major public health problem in developing countries. More than 500,000 cases of severe leptospirosis are reported each year, for which the mortality rate is up to 50%. The genome sequences of pathogenic and saprophytic *Leptospira* revealed the existence of several predicted coding sequences of unknown function. The study of *Leptospira* pathogenesis has been hampered by the lack of appropriate genetic tools. Recent advances have been made regarding the genetic manipulation of *Leptospira*, improving our understanding of the biology of this spirochete, but the need to develop basic molecular systems for the assessment of *Leptospira*–host interactions still remains. **Objectives:** The aim of this study was to develop a novel genetic tool to study leptospiral promoter activity under mammalian host conditions. **Methods:** A series of replicative promoter-probe vectors carrying a reporter gene encoding green fluorescent protein (GFP) fused to the leptospiral promoters of the genes *lipL41*, *ligA* and *sph2* were constructed for use in *L. biflexa*. The *L. biflexa* reporter strains were induced by physiological osmolarity and temperature, urine pH 6.7 and the supplementation with spermine (either individually or in combination). Promoter activity was assessed by fluorescence measurement, RT-PCR and epifluorescence microscopy. The validation of the conditions tested was performed by inducing LigA expression in the pathogenic *L. interrogans* serovar Copenhageni strain L1-130. Native protein expression was assessed by ELISA and RT-PCR. **Results and Discussion:** The *ligA* and *sph2* promoters were the most active promoters, in comparison to the *lipL41* promoter and the non-induced controls. The results obtained are in agreement with LigA expression from *L. interrogans* Fiocruz L1-130 strain, which was cultivated under the same defined growth conditions. The constructed vectors facilitated the *in vitro* measurement of *L. interrogans* promoter activity under defined growth conditions which simulate the mammalian host environment. The fluorescence and RT-PCR data obtained closely reflected transcriptional regulation of the promoters, thus demonstrating the suitability of these vectors for assessing promoter activity in *L. biflexa*.

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5.08 Analysis of biofilm formation by atypical enteropathogenic *Escherichia coli* strains by CFU count, crystal violet assay and confocal fluorescence microscopy

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Introduction: Microorganisms can proliferate as individual cells swimming freely in the environment, or they possess the capacity to adhere, forming biofilms in association with different types of surfaces and interfaces and conferring resistance to some antibiotics. Many models have been described for the study of development of biofilm *in vitro*. The most common procedure used to measure the biofilm formation is counting viable cells on plates; however, several tests for biofilm quantification in microtiter plates, as the method of crystal violet (CV) staining, have been recently described. Another technique often used is confocal laser scanning microscopy (CSLM), as a qualitative method. **Objectives:** The aim of this study was to determine the capacity of biofilm formation by atypical enteropathogenic *E. coli* (EPEC) on an abiotic surface by CFU/cm^2 counting, colorimetric assay of CV and CSLM with fluorescent marker after 24 h of incubation at 37°C. **Methods:** A total of 12 atypical EPEC strains isolated from children with diarrhea were studied after growing in high glucose DMEM. The biofilm formation of the bacterial strains was studied in static conditions by the crystal violet assay (CV) using polystyrene 24-well culture plates and was quantified at 595 nm in an ELISA plate reader. These strains also were tested by CFU/cm^2 counting attached to the biofilm after disrupting with Triton X-100. Serial dilutions were made and plated on Luria-Bertani agar for CFU/cm^2 counting. The strains were also visualized by CSLM, after the staining the bacteria red with propidium iodide. **Results and Discussion:** Six of the 12 strains showed optical density (OD) values above 0.100, and 6 showed OD values below 0.100. Through the analysis of fluorescence confocal microscopy, it was possible to visualize strains that were capable of forming biofilms and others that did not, as well as this biofilm structure. The CLSM and CV methods showed similar results. The bacterial strains showed constant CFU/cm^2 values in the range of 10^8, and compared with the analysis of images obtained with CSLM, there was no agreement between the two methods. The CV assay measured live and dead cells, while these cells may be distinguished by CLSM and CFU/cm^2 counts measure only the viable cell number. The CFU/cm^2 count and CV methods should not be used alone to indicate biofilm formation, but should be used together with a qualitative method such as CLSM, which allows the visualization of biofilm structures and measures biofilm thickness.

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5.09 Expression of TNF-alpha and CXCL-2 mRNAs in different organs of mice infected with pathogenic *Leptospira*

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Introduction: Leptospirosis is a worldwide zoonosis caused by *Leptospira*. The infection in humans is mainly observed in lungs, kidneys and livers. Studies of immune response to leptospira infection can provide information for proper vaccine development and elucidation of the mechanisms of pathogenesis and can contribute to the development of more effective treatment and prevention of the disease. Injury by microbial factors and the production cytokines and chemokines in response to infection have been proposed to be involved in the pathogenesis of leptospirosis. Among the chemokines and cytokines, CXCL-2 (also called macrophage inflammatory protein 2 - MIP-2) and TNF-α are produced in inflamed tissues and they coordinate the migration and accumulation of leukocytes at the inflammatory sites. **Objectives:** In the present study, gene expression of cytokine TNF-α and chemokine CXCL-2 in kidney, liver and lung of mice infected with *Leptospira* was investigated. **Methods:** Mouse strains C3H/HeJ, C3H/HePas and BALB/c were infected i.p. with $2{,}4 \times 10^6$ virulent *Leptospira interrogans* serovar Copenhageni. Three mice of each strain (n=12) were sacrificed on days 0 (uninfected), 1, 3, 5 and 7 after infection. Total RNA was isolated from the tissues and the relative expression of TNF-α and MIP-2 and GAPDH was analyzed by real-time quantitative PCR. Relative expression of the genes was calculated by the $2^{-\Delta\Delta}$Ct method using GAPDH mRNA for normalization. **Results and Discussion:** Our results indicate that the expression of MIP-2 and TNF-α can vary greatly, depending on the tissue, mouse strain, and time of infection. For instance, the expression of TNF-α and MIP-2 rapidly increased on day 3 (40- and 60-fold, respectively) in the liver of BALB/c, returning to baseline levels on day 5. This response can be associated with the resistance phenotype of this mouse strain to leptospirosis.

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5.10 Atypical enteropathogenic *Escherichia coli* strains secrete an inhibitor of phagocytosis

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Introduction: Phagocytosis is important in the establishment of the innate immune defense. Its inhibition is a common escape strategy employed by many pathogens. Typical enteropathogenic *Escherichia coli* (tEPEC) inhibits its own phagocytosis through effector proteins EspF and EspB, translocated into host cells. We have shown that atypical enteropathogenic *E. coli* (aEPEC) O55:H7 is less phagocytized by both lineage and primary culture murine macrophages. **Objectives:** The aim of this study was to investigate the behavior of aEPEC strains 7 and 320 (O55:H7) in J774A1 macrophages. **Methods:** aEPEC and commensal *E. coli* C600 were cultured either in tryptic soy broth or in minimum medium. Macrophages previously incubated with the supernatant from these cultures were infected with tEPEC (E234867). The supernatant from the bacterial cultures was fractionated by solid phase extraction (SPE) in C18 cartridges with 0, 25, 50, 75 and 90% acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA). J774A1 were pre-incubated for 30 min with each lyophilized SPE fraction resuspended in RPMI. Active fractions were analyzed and re-fractionated by reversed-phase HPLC in a C8 column and eluted with a 0 to 90% gradient of ACN or methanol in 0.1% TFA. Alternatively, the bacterial culture supernatants were dialyzed against water using membranes with a 1,000 Da cutoff prior to SPE. Fractions from the HPLC were tested for their capacity to interfere in bacterial and *Saccharomyces cerevisiae* phagocytosis as well as in adhesion of tEPEC to HEp-2 and Caco-2 cells. **Results and Discussion:** Only fractions with molecules smaller than 1,000 Da inhibited phagocytosis. These were then analyzed by HPLC and the sub-fractions were again tested for anti-phagocytic activity. Active fractions also reduced phagocytosis of *S. cerevisiae* and adhesion of intestinal extracellular bacteria to epithelial cells, which is an essential step in the establishment of infection. All HPLC fractions eluted with methanol were inactive. The soluble anti-phagocytic factor secreted by aEPEC O55:H7 is relatively hydrophilic and is smaller than 1,000 Da. It is probably not of peptide nature, since it is inactivated when eluted with methanol in acidic conditions, which is known to promote methylation of glucoside groups. It is the first time that an anti-phagocytic mechanism is described in aEPEC. Furthermore, this mechanism differs from that previously described in that it depends on a secreted factor which is soluble in an aqueous medium. This finding opens the possibility of identifying a factor that prevents bacterial adhesion to professional and non-professional phagocytes, which could provide a means of controlling bacterial colonization and preventing diarrhea.

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5.11 Preliminary identification of secreted proteins by two species of *Leptospira*, one pathogenic and one saprophytic

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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 pathogenesis may be related to the ability of these bacteria to bind to extracellular matrix proteins, to escape host's immune responses and to produce toxins. Proteomic analyses of *L. interrogans* have confirmed the theoretical prediction of many proteins possibly involved in the host-pathogen interactions. Up to now, there are no published data of proteins secreted by leptospires identified using proteomic approaches, despite the existence of homolog genes in *L. interrogans* genome that encode for some transport systems known in other bacteria. **Objectives:** This study aimed to identify secreted proteins of *Leptospira interrogans* serovar Pomona strain Fromm (pathogenic) and *L. biflexa* serovar Patoc strain Patoc I (non-pathogenic) by proteomic analyses. **Methods:** *L. interrogans* serovar Pomona strain Fromm, whose virulence was maintained by passages in hamsters, and *L. biflexa* serovar Patoc strain Patoc I were cultured in EMJH medium at 29°C or 37°C. Secreted proteins were collected by centrifugation for removal of cells and subjected to lyophilization. Protein samples were first resolved by IEF on pH 3 to 10, immobilized pH gradient strips (13 cm). Strips were then processed for the second-dimension separation on 12.5% SDS-polyacrylamide gels. Gels were stained with Coomassie blue R-350 or silver. Spots were detected and analyzed by scanning on Labscan (GE Healthcare). *In silico* analyses were performed using SOSUI-GramN and VirGel V.2.0 programs. **Results and Discussion:** Genome-based signal peptide algorithms predicted 179/251 secreted proteins for *L.interrogans* serovar Copenhageni and 161/326 secreted proteins for *L. biflexa* serovar Patoc I with SOSUI-GramN and VirGel V.2.0 programs, respectively. Our 2D-PAGE analyses successfully detected 67 protein spots from the supernatants of serovar Fromm and 211 protein spots from serovar Patoc 1. Further mass spectrometry analyses of the detected spots will be performed. The identification of these proteins will certainly contribute to the elucidation of the pathogenic mechanisms and development of novel strategies for the treatment and prevention of leptospirosis.

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5.12 Biologically active molecules from the hemolymph and fat body of *Lonomia obliqua*

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Introduction: Several studies have been conducted demonstrating the presence of pharmacologically active substances in the hemolymph with enzymatic, hormonal, antimicrobial and anti-freeze effects. The fat body is the main organ of intermediary metabolism of insects and the main source of components of the hemolymph. It facilitates the process of detoxification, and is involved in the production and secretion of lipids in the hemolymph, and in the recognition of pathogens and secretion of antimicrobial substances. However, few studies have been conducted trying to isolate and characterize the factors involved in these effects. **Objectives:** This study aimed to find new molecules possessing activity for the stimulation or inhibition of microbial growth in the hemolymph and fat body of *Lonomia obliqua*. **Methods:** A total of 70 pupae of *L. obliqua* were selected, 45 of which were challenged with a bacterial mixture of *Escherichia coli* and *Micrococus luteus* and the remaining 25 were not challenged. After 48 h, hemolymph and fat body were extracted and subjected to solid phase extraction using Sep-Pak C18 columns, eluted in two concentrations of acetonitrile (40 and 80%). The rates obtained were subjected to liquid chromatography (HPLC), using a Jupiter C18 semi-preparative column. The fractions were assayed for stimulation or inhibition of microbial growth in liquid medium using microplates. We used for our tests the following microorganisms: *Escherichia coli*, *Micrococus luteus* and *Candida albicans*. The fractions that showed antimicrobial activity and growth were repurified by liquid chromatography using an analytical Shim-pack VP-ODS. The molecules obtained from this step were further analyzed for their biological activity. **Results and Discussion:** Three fractions were found in the hemolymph of animals not challenged, and two fractions in the hemolymph of challenged animals, which showed antimicrobial activity against all three microorganisms. For *M. luteus* and *C. albicans,* two fractions of fat body in challenged animals were found, which showed an increase in growth. We isolated seven molecules in the hemolymph and fat body of the pupae of *L. obliqua*: five of them with antimicrobial activity (hemolymph) and two with microbial growth-stimulating activity (body fat). These molecules are being analyzed by mass spectrometry for subsequent characterization.

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5.13 Identification and characterization of surface-exposed plasminogen-binding proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is a multisystem disease caused by pathogenic strains of the genus *Leptospira*. We have reported that *Leptospira* are able to bind plasminogen (PLG) to generate active plasmin in the presence of an activator, and to degrade purified extracellular matrix fibronectin. **Objectives:** As a follow up to the previous study, we decided to screen recombinant surface exposed leptospiral proteins available in our laboratory as to their ability to bind PLG. **Methods:** We cloned, expressed in *E. coli* and purified 14 leptospiral recombinant proteins. The proteins were confirmed to be surface exposed by immunofluorescence microscopy and were evaluated for their ability to bind PLG. **Results and Discussion:** We identified eight as PLG-binding proteins, including the major outer membrane protein LipL32, the previously published rLIC12730, rLIC10494, Lp29, Lp49, LipL40 and MPL36, and one novel leptospiral protein, rLIC12238. Bound PLG could be converted to plasmin by the addition of urokinase-type PLG activator (uPA), showing specific proteolytic activity, as assessed by its reaction with a specific chromogenic plasmin substrate. The addition of the lysine analog 6-aminocaproic acid (ACA) inhibited the protein-PLG interaction, thus strongly suggesting the involvement of lysine residues in plasminogen binding. The binding of leptospiral surface proteins to PLG was specific, dose-dependent and saturable. PLG and collagen type IV competed with LipL32 protein for the same binding site, whereas separate binding sites were observed for plasma fibronectin. Our results indicate the presence of multiple PLG-binding proteins in *L. interrogans*. PLG-binding and activation through the proteins/receptors on the surface of *Leptospira* could help the bacteria to specifically overcome tissue barriers, facilitating their spread throughout the host.

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5.14 Type IV pilus: the role of PilS and PilV in atypical enteropathogenic *Escherichia coli* in interaction with epithelial cells *in vitro*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) have been identified as etiologic agent of diarrhea in developing and industrialized countries. The virulence of EPEC resides in its ability to promote the attaching-effacing (A/E) lesion. The various proteins involved in A/E lesion formation are encoded by genes located on the pathogenicity island called the locus of enterocyte effacement (LEE). Moreover, EPEC strains may carry a large plasmid known as the EPEC adherence factor plasmid (pEAF), which encodes the bundle-forming pilus (BFP). The EPEC pathotype was subdivided into typical (tEPEC) and atypical (aEPEC) with the basic difference being the respective presence or absence of pEAF and expression or not of BFP. This fimbrial adhesin belongs to a family of type IV bacterial pili and play an important role in the adherence of the bacteria to the enterocyte. In contrast, as aEPEC does not express BFP fimbriae, other fimbrial adhesins could be implicated in its pathogenesis. The pilin PilS and the adhesin PilV, also described as type IV pilus, contribute to plasmid conjugation, epithelial cell adherence, and adherence to abiotic surfaces of enteroaggregative *E. coli* (EAEC) isolates. **Objectives:** In this study, we evaluated the involvement of PilS and PilV proteins in the interaction of aEPEC isolates with epithelial cells in vitro. **Methods:** The presense of *pilS* and *pilV* genes was investigated in aEPEC strains by PCR. The amplified products were purified and cloned into cloning vector pGEM-T. The recombinant plasmids pGEMT-pilS and pGEMT-pilV were digested with *BamHI* and *HindIII* restriction enzymes to release the insert, which was subsequently cloned into expression vector pET28a. HEp-2 epithelial cells were used for adhesion assays, with cell-bacterial interaction of 3 and 6 h, in the presence or absence of mannose. **Results and Discussion:** The *pilS* and *pilV* genes were amplified in isolates BA558, BA956 and BA1244. Adhesion assays showed the following patterns: localized adherence in 6 h for BA558 (LA6h), non adherent for BA956 (NA) and localized-like adhesion for BA1244 (ALL). These results suggested that PilS and PilV proteins are possibly involved in these phenotypes. In the next steps, the PilS and PilV proteins will be purified to produce polyclonal sera to study the role of these proteins in the bacterial-cell interaction.

Supported by: FAPESP

5.15 Validation of sterility test in isolator of influenza vaccine formulated at Butantan Institute by means of bacteriostatic and fungistatic effect

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Introduction: Sterility of influenza A/H1N1 vaccine (inactivated and fragmented) formulated at Butantan Institute is done by filtering the product with a 0.45-µm membrane, rinsing this membrane with a neutralizing fluid and subsequent incubation in fluid thioglycollate medium (FTM) and soybean-casein digest media (SCM). The current standards require that all operational procedures used in quality control be validated according Current Good Laboratory Practices (cGLP). **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied to bacterial and fungal sterility testing in the influenza A/H1N1 vaccine (inactivated and fragmented) in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of influenza A/H1N1 vaccine (inactivated and fragmented) formulated by Butantan Institute, previously evaluated for thimerosal concentration by a spectrophotometric method. These bulks were tested according to standard methodology and membranes were rinsed with Diluent Neutralizing Pharmacopoeic fluid (DNP). After product filtration, an inoculum of viable ATCC microorganisms (10-100 CFU/mL) was added to the final portion of DNP fluid used to rinse the membrane. Fluid thioglycollate medium (FTM) was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and soybean-casein digest medium (SCM) was challenged with *Aspergillus brasiliensis, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for no 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 isolator to test the bacterial and fungal sterility of influenza A/H1N1 vaccine (inactivated and fragmented) is effective and thimerosal present in the product formulation as preservative was completely inactivated rinsing the membrane with DNP fluid. We concluded that the methodology applied detects low levels of microbial contamination, providing the public with a safe product (sterile) according to national and international standards. The sterility test may be carried out without further modifications.

Supported by: Fundação Butantan

5.16 Standardization of single molecule analysis of replicated DNA (SMARD) of *Trypanosoma brucei* chromosome I

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Introduction: DNA replication is a crucial step during the cell cycle, and until now, little is known about the DNA replication of *Trypanosoma brucei*. **Objectives:** Here, we aimed to define how replication occurs on chromosome 1 from *T. brucei* (1.85 and 3.6 Mbp). **Methods:** Single molecule analysis of replicated DNA (SMARD) allows the visualization by fluorescent microscopy of the single molecules of replicated DNA stretched on microscope slides. Using this method, it is possible to determine the numbers of replication origins, the fork direction and the DNA replication rate (Kb/min) of a DNA fragment. **Results and Discussion:** Since the technique limits the molecules analyzed to a maximal length of 500 kbp, two different approaches were developed: the analysis of chromosome 1 (1.85 and 3.6 Mbp) fragments smaller than 500 kbp and the entire chromosome 1 (1.85 Mbp). For the analysis of the fragments, DNA was digested with two different enzymes, FseI and AscI. After pulsed-field gel electrophoresis, the fragments were analyzed using specific probes, and they showed length differences compared to the prediction because the strain sequenced (*T. brucei* TREU 972) was different from that used in this experiment (*T. brucei* 427). Although the fragments were still smaller than 500 kbp, they could be identified on slides by specific probes that are being developed. In order to analyze the entire chromosome 1 (1.85 Mbp), a PFEG program will be developed to separate and extract chromosome 1 from the genome. Probes are also being developed to determine the chromosome 1 orientation (5'–3'). Once all these probes are available, chromosome 1 and the fragments will be analyzed, and then, it will be possible to know how many origins are present on chromosome 1, and also the fork direction and speed.

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5.17 Identification of bovine papillomavirus types in Brazil: co-infection with a new type and a rare variant in a dairy cow

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Introduction: Bovine papillomaviruses (BPVs) are distributed worldwide. These viruses are recognized as causal agents of several benign and malign tumors in cattle, such as cutaneous fibropapillomas, benign fibroplasias in other tissues (teats, genitals), urinary bladder and esophagus cancer. BPVs are related to severe economic losses in meat, milk and leather production. Ten different BPV types have been described and other putative types have been proposed by molecular techniques, such as PCR with generic primers and DNA sequencing. Brazil has a cattle herd of approximately 250 million, and thus the improvement of studies concerning diversity, prevalence and related clinical aspects of BPVs are very relevant. The identification of putative viral types is important for the development of vaccines. **Objectives:** Here, we describe the simultaneous presence of two BPV types in three different warts of a Holstein dairy cow with cutaneous papillomatosis. **Methods:** Wart biopsies were obtained and submitted to histological and molecular techniques. DNA was extracted for viral typing using PCR-RFLP directed to a L1 gene segment (major viral capsid protein) and subsequent sequencing. The L1 gene has taxonomic relevance due to its high degree of conservation. **Results and Discussion:** In all three lesions studied, different restriction patterns were detected which could not be identified among the profiles of any of the ten previously characterized viral types. The sequencing of the amplicons indicated the presence of a rare putative variant (BAPV-3), originally described in Japan. Furthermore, the analysis of the two other samples demonstrated in both a different viral sequence, exceeding 10% of divergence when compared to the other homologous BPVs sequences described in Genbank. It is possible to conclude that this viral sequence represents an entirely new putative type of BPV. The PCR-RFLP/sequencing procedure has been found to be highly effective for BPV identification. This approach provided the first description of BAPV-3 in Brazil and, more importantly, the identification of a possible unreported BPV type, provisionally named 01SP/BR/2009. The relevance of these findings is also related to further vaccine development procedures.

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6. Biotechnology

6.01 Study of the variability of the PspC (pneumococcal surface protein C) in pneumococcal strains isolated in Brazil

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Introduction: Diseases caused by *Streptococcus pneumoniae*, such as pneumonia, meningitis and bacteremia, are a major public health problem. The need to achieve broader serotype coverage at a lower cost has thus stimulated attempts to develop a vaccine based on conserved protein antigens of pneumococci such as PspC (pneumococcal surface protein C). PspC has been described for its role both in colonization of the nasopharynx and in invasive infection. Previous studies have shown that *S. pneumoniae* is able to bind both human factor H (FH), an inhibitor of complement alternative pathway, and secretory IgA (sIgA) via PspC. PspC is highly polymorphic, being divided into 11 groups. Thus, the evaluation of the variability of the antigen in clinical samples is of great importance to determine the ideal vaccine formulation. **Objectives:** The proposal of the present work was to evaluate the variability of *pspC* in Brazilian pneumococcal isolates. **Methods:** Pneumococcal strains were obtained from the University Hospital of the University of São Paulo. Strains were serotyped by PCR and 13 isolates were chosen based on the serotypes present in the new 13-valent conjugate vaccine. The complete *pspC* locus was cloned and the gene sequenced for each isolate. BALB/c mice were immunized with three different recombinant PspC variants for the production of antibodies that were used for Western blot analysis. **Results and Discussion:** Of the 13 pneumococcal isolates analyzed, 6 isolates were found to be from group PspC3, 3 isolates from group PspC6, 1 isolate from group PspC5, 1 isolate from group PspC8 and 1 isolate from group PspC9. A duplication containing *pspC* from group 4 and from group 10 was also found. Three different PspC molecules were expressed in *Escherichia coli* and the purified recombinant proteins were used to immunize BALB/c mice. Antibodies raised against PspC were used to probe pneumococcal whole cell extracts in Western blot analysis. Antiserum raised against PspC3 was able to recognize the majority of pneumococcal extracts, showing a broad cross-reactivity. In contrast, antiserum raised against PspC8 was able to recognize only the isolate expressing PspC8, and the antiserum against PspC5 showed reactivity with half of the isolates. We also evaluated the interaction of FH and sIgA with the pneumococcal extracts by Western blot analysis. Most of the isolates tested showed a strong binding to FH and weaker interaction with sIgA.

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6.02 Production of recombinant protein L1 of human papillomavirus 16 (HPV 16)

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Introduction: Human papillomaviruses (HPV) are responsible for a wide variety of clinical manifestations such as benign warts to cervical cancers. They are classified according to their ability to drive the infection to carcinogenesis: high-risk, probably high-risk and low-risk. Among the types of papillomavirus, HPV-16 is found in 70% of cases of cervical cancers and is classified as a high risk HPV. The L1 protein is the major capsid protein of HPV 16 and is 55 kDa. The L1 protein produced in heterologous expression systems is able to self-assemble in particles similar to virus called VLPs (virus like particles), and shows conformational epitopes that stimulate the production of neutralizing antibodies in animals and humans. **Objectives:** The aim of this study was the production of L1 protein of HPV-16 in *Pichia pastoris*, to develop a Brazilian prophylactic vaccine against HPV-16. **Methods:** The codon optimized sequence of L1 protein was cloned in pPICHOLI episomal vector (MoBiTec). Subsequently, methylotrophic yeast of the genus *Picchia pastoris* was electroporated with pPICHOLI-L1 vector. The expression of protein was induced with 0.5% methanol for 48 h. The purification of L1 protein was performed by affinity chromatography on a heparin-Sepharose column, and the L1 protein was eluted with a NaCl gradient. The analysis of purification procedures was carried out with 12% SDS-PAGE and Western blotting. **Results and Discussion:** The cloning of L1 protein in pPICHOLI vector was confirmed by sequencing. Positive clones were selected based on antibiotic resistance and expression of L1 on a small scale. The purification of L1 protein was analyzed and confirmed by the presence of a 55-kDa band by Western blotting as expected for L1 protein. These results show the feasibility to use *Pichia pastoris* as a platform for the production of L1 for vaccine development against HPV 16.

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6.03 Recombinant mycobacteria expressing *Schistosoma mansoni* surface proteins

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Introduction: Schistosomiasis is a parasitic disease affecting 200-300 million people around the world, where it is endemic in over 70 countries, characterizing it as a serious public health concern. In spite of the effectiveness of praziquantel– the chemotherapeutic agent of choice – it does not protect against reinfections, and periodic mass administrations are needed, especially in endemic areas. This strategy raises the possibility that resistance strains emerge. The consensus is that a sterile immunity induced with a vaccine is not essential, so the development of a vaccine would be very beneficial since it reduces morbidity. The vaccines based on live vectors to present heterologous antigens are an attractive idea because it eliminates the necessity of multiple doses to obtain maximum protection against infection. **Objectives:** This work aimed to investigate the potential of the bacillus Calmette-Guérin (BCG) and *Mycobacterium smegmatis* (Smeg) to express the vaccine candidate SmStoLP-2 from *S. mansoni* identified in studies of functional genomics, capable of a 32% reduction of worm burden when presented as recombinant protein in the murine model. **Methods:** The mRNA of the parasites was extracted and the cDNA obtained by reverse transcription amplified by PCR and cloned in expression vectors from mycobacteria i) in fusion with the whole β-lactamase gene (pLA73), ii) in fusion with the signal sequence of the β-lactamase gene (pLA71) or iii) to a ribosomal binding site (pMIP12), named pKL73-StoLP2, pKL71-StoLP2 and pKL12-StoLP2, respectively. The mycobacterial strains were transfected with these vectors and clones selected by resistance to kanamycin. The expression of SmStoLP-2 was determined by Western blotting using the total protein extract of the clones and anti-SmStoLP2 serum from mice immunized with the recombinant protein previously purified. The subcellular location in mycobacteria of the protein was then determined. **Results and Discussion:** Both BCG- and Smeg-pKL71-StoLP2 showed expression and enrichment of the protein in the cytosol. Both BCG- and Smeg-pKL12-StoLP2 showed expression and enrichment in the cell wall fraction. The Smeg-pKL73-StoLP2 showed expression, while it was not possible to observe it in BCG-pKL73-StoLP2. The intra- or extracellular localization was unexpected since the enriched fraction was not determined by the different plasmid constructs. These preliminary results indicate that recombinant mycobacteria is able to express the vaccine candidate SmStoLP-2 of *S. mansoni* and allows the possibility to determine if this presentation system – with a cytokine Th1 profile – is able to increase protection levels.

Supported by: FAPESP

6.04 Expression of a predicted leptospiral protein LIC10821 in *Escherichia coli*

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Introduction: Leptospirosis is a zoonosis of global distribution caused by infection with pathogenic serovars of *Leptospira* spp. Many species of animals, both domestic and wild, serve as reservoir hosts, resulting in the widespread of the disease. Humans are accidental hosts, with transmission occurring via direct or indirect contact with the urine of infected animals. In Brazil, seasonal outbreaks are observed mainly in the rainy season and about 5,000 cases occur annually. The development of a broad-spectrum vaccine is crucial for the control of leptospirosis. In an attempt to identify new antigens that are conserved among different serovars of *L. interrogans*, current studies have focused on surface proteins due to their potential to induce immune response in animal hosts. **Objectives:** Our aim was to clone and express a gene that encodes a predicted lipoprotein of *L. interrogans* and to evaluate its immunogenic activity in mice. **Methods:** The gene was amplified by PCR from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genomic DNA, using the complementary sequence primers; the insert of DNA was cloned into the *Escherichia coli* expression vector, pAE, at XhoI and HindIII restriction cloning sites. The cloned sequence was confirmed using an automated sequencer and the construction was employed to transform BL21 SI *E. coli* strain with 300 mM NaCl at 30ºC for protein expression. The recombinant protein was expressed with 6 x His-tag at the N-terminus, facilitating protein purification by metal-affinity chromatography. **Results and Discussion:** The chosen protein was predicted to be an outer membrane protein (PSORT program) and a lipoprotein (LipoP program). The gene was amplified, without the signal peptide sequence, and the DNA insert cloned and expressed as a full-length protein in *E. coli.* The recombinant protein rLIC10821 was expressed as an insoluble form, with the expected size of 37 kDa, as evaluated by SDS-PAGE. The recombinant protein was purified by metal chelation chromatography in an ÄKTAPrime plus system with on-column refolding by gradually removing urea (6 to 0 M) and gradient elution of recombinant protein (imidazol concentration of 0-500 mM). After extensive dialysis against phosphate-buffered saline, the protein concentration was estimated to be approximately 10 µg/ml. Evaluation of the immunogenicity in mice is currently underway.

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6.05 Expression of an antiapoptotic protein obtained from *Lonomia obliqua* hemolymph in a baculovirus/SF-9 cell system

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Introduction: Apoptosis plays a central role in many cellular processes, including embryonic differentiation and the development of some diseases such as cancer and Alzheimer's disease. Substances that interfere with the apoptotic process 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 control and understanding of their mechanisms of action are essential for further progress in this field. **Objectives:** The aim of this study was to produce an antiapoptotic protein in a recombinant baculovirus system/insect cells (Invitrogen ™). **Methods:** The protein fraction of hemolymph with antiapoptotic activity was identified and isolated by ion exchange chromatography, gel filtration and Maldi Q-TOF mass spectrometry. To synthesize cDNA, RNA of *Lonomia obliqua* was extracted and used in polymerase chain reactions using reverse transcriptase polymerase (RT-PCR) with primers specific for the antiapoptotic protein (sequence deposited in GenBank database). Restriction sites were inserted in the cDNA for connection to the donor plasmid pFastBac1™. The recombinant plasmid was selected in *Escherichia coli* TOP10 and subsequently used in the transformation of DH10Bac *E. coli*™ (Invitrogen), to obtain the recombinant bacmid. **Results and Discussion:** In this system, the recombinant virus produced was used to infect Sf9 and UFLAG cells. Three passages of recombinant viruses in cells grown in spinner flasks were performed. These cultures were maintained with mixing at 100 rpm with a working volume of 15 ml. We observed an intense granulation in the infected cells after 48 h in Sf9 cells. Few granules were observed in infected UFLAG cells. After 7 days, all infected cells died, while in control, cell viability was over 80% at the same time. Samples of the supernatant of infected cultures were collected daily, concentrated and subjected to SDS-PAGE chromatography. A protein band around 20 kDa was observed. A sample of the infected culture was examined by electron microscopy for visualization of viral particles. We also observed an increase in volume of the cell nucleus in infected cells. Currently, the recombinant proteins obtained in the supernatant are being purified by affinity chromatography and their activity is being tested in cell cultures where apoptosis is induced by chemical agents.

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6.06 Media hold test of hepatitis B fermentation processes

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Introduction: Butantan Institute's recombinant hepatitis B vaccine is composed of physicochemical purified hepatitis B surface antigen (HBsAg). Using recombinant DNA technology, the protein is expressed by the transformed strain of *Hansenula polymorpha* yeast as 22-nm particles. The first part of the production is the cultivation of the yeast in four steps: inocullum I, inocullum II, inocullum III and production cultivation. The facility systems (air, pressure, pure water and steam) are validated and the fermentor used in the production process is qualified (installation qualification, operational qualification and performance qualification). The media hold test is an instrument of process validation that demonstrates product safety, which is the focus of regulatory requirements and official inspections. The media hold test consists of a production process simulation where the product is replaced by the culture medium (tryptic soy broth – TSB), which is exposed to the same risk factors as the product. **Objectives:** The aim of this study was to provide documented evidence of the operational procedures, equipment and operators involved in the fermentation processes of VRHB to assure that they are efficient and able to achieve a reproducible aseptic process. **Methods:** Three consecutive tests simulating production lots were performed. Usual media were substituted by TSB medium as well as the solutions used in the fermentation process, such as anti-foam and methanol. The media were not inoculated with the yeast used in production. Samples of each "cultivated" medium were collected in the beginning and end of each step and sent for analysis by the Butantan Institute's Quality Control Service for sterility and fertility. The criterion of acceptance for the sterility test is the absence of bacterial and fungal growth for 14 days at 20 to 25°C and 30 to 35°C, respectively. For the fertility test, samples were considered fertile if growth of specific microorganism occurs after 72 h at 20 to 25°C. **Results and Discussion:** In every media hold test, the samples showed no microbial growth. Sterility and fertility tests were in accordance with acceptance criteria. Application of the media hold test for the fermentation process of VRHB production showed no deviations.

Supported by: Fundação Butantan

6.07 Evaluation of expression patterns of three proteins from *Schistosoma mansoni*: an insight into the parasite's biology

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Introduction: *Schistosoma mansoni* is a blood-dwelling parasite that infects humans and causes liver and spleen disorders. As a way to know more about the parasite's biology and as a means to reveal potential key molecules important for its life cycle (in the context of a rational vaccine design), we decided to study three putative proteins, namely: carboxypeptidase (SmCPN), sphingomyelinase (SmnSMase) and alkaline phosphatase (SmAP). **Objectives:** The aim of this study was to determine the proteins' expression patterns at different life cycle stages and evaluate their distribution using qRT-PCR, Western Blot and immunolocalization. **Methods:** qRT-PCR amplification was performed in a GeneAmp®PCR System 9600 (Corbett Research). For Western blotting, 20 μg of total protein from the stages were electrophoresed and transferred onto PVDF membranes (Amersham Pharmacia Biotech, England). Blots were incubated with corresponding immunosorbed rat antisera and specific antibody binding was visualized using the ECL Western Blotting Detection System (Amersham Pharmacia Biotech). For immunolocalization, 8-μm cryostat sections of adult worms were adhered to silanized glass slides (DakoCytomation) and incubations were made with corresponding immunosorbed rat antisera. Specific antibody binding was visualized with Alexa Fluor® 488 rabbit anti rat IgG (H+L) (Invitrogen) with a Zeiss LSM 510 Meta Confocal System. **Results and Discussion:** SmCPN showed high transcript levels in cercariae, and lowest levels in adult worms. The protein was expressed in all life cycle stages, secreted to culture media, with a decreased level of protein in adults, and a very strong expression in miracidia. Immunolocalization in adult sections localized it at the tegumental fraction more than in other tissues, raising the hypothesis of its importance in host-parasite interaction. SmnSMase showed high levels of transcripts in eggs and adults, and low levels in 7-day old culture schistosomula. This protein is expressed in all stages except in 7 day-old schistosomula, female and stripped worms. SmAP showed the highest level of transcripts in the cercarial stage; however, protein expression profiles revealed the presence of another band in eggs, with schistosomula having the lowest signal. The huge amounts of mRNA made during cercarial stage would serve for protein synthesis as soon as the parasite enters the skin, which is confirmed by protein levels seen in the schistosomula stage 3-h and 12-h after transformation. Immunolocalization in adult sections showed the protein widespread in all tissues in both male and female worms, with a very strong labeling in vitellary glands in the female. This demonstrates the importance of the protein for egg production in females.

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6.08 Screening of *Streptococcus pneumoniae* serotype 1 strains for capsular polysaccharide production

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Introduction: *Streptococcus pneumoniae* is an important human pathogen in infections of the respiratory tract. There are more than 90 known pneumococcal serotypes, each one identified by a chemically and immunologically distinct capsular polysaccharide (PS). The capsular PS is the main virulence factor of this microorganism and the antigen of the current pneumococcal vaccines. Type 1 is a prevalent serotype in Brazilian children and shows high incidence of pneumococcal invasive diseases. *S. pneumoniae* is a Gram-positive anaerobic bacterium, which is considered nutritionally demanding. The major obstacle to reach high productivities in the cultivation of *S. pneumoniae* is lactic acid formation, but other factors such as the ability of undergoing autolysis can also influence growth and PS production. **Objectives:** This work aimed to select serotype 1 strains for PS production and to establish a method for determining the PS concentration in culture broth samples. **Methods:** Initially, only growth was evaluated, since other works have shown a growth-associated PS production. Nine strains were kindly supplied by Instituto Adolfo Lutz. They were cultivated from frozen stocks directly into 50 mL complex medium containing 3% acid hydrolyzed casein, 2% glucose, 2% yeast extract, minerals and choline. The cultures were statically incubated at 37ºC and 3% CO_2. Three inoculum volumes were tested for each strain: 10, 25 and 50 mL. The biomass was measured by optical density (OD) at 600 nm (OD=1.0 corresponds to approx. 0.35 g/L dry cell weight), and viability was measured by colony forming units per mL (CFU/mL) after plating serial dilutions of the cell suspension in blood agar plates. The m-hydroxydiphenyl method, which detects galacturonic acid, was used to determine the concentration of serotype 1 PS in the supernatant of culture broth samples previously dialyzed against distilled water. **Results and Discussion:** Cell growth was observed for 7 strains. The OD and viability were reproducible for 4 strains. A relationship between maximum biomass and CFU/mL of the frozen stocks was not observed, but the increase of the inoculum volume reduced the lag phase by about 1 h. The strain St595/01 reached the highest biomass (OD = 3.35) and PS concentration (130 mg/L). Kim et al. (1996) reported OD = 0.7 and PS = 60 mg/L in optimized cultivation conditions for production of capsular PS from pneumococcus serotype 1. Compared to the literature, a significant increase in biomass as well as in PS production was obtained by simply screening pneumococcal strains. Further improvement of PS production should be achieved under optimized conditions using the selected strain.

Supported by: CAPES, FAPESP

6.09 Expression of viral capsid protein of bovine papillomavirus in a baculovirus/SF-9 cell system

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Introduction: Bovine papillomavirus (BPV) is a double strand DNA virus that induces lesions (warts) in the epithelium. These lesions can evolve into tumors due to co-factor actions causing massive economic losses. BPV is frequent in several regions of Brazil, but without systematic evaluations of its occurrence. **Objectives:** In order to improve the development of a prophylactic vaccine for BPV-1 targeting L1 protein, this study aimed to build recombinant bacmid containing a gene sequence encoding the viral capsid protein in a baculovirus/Sf-9 cell system. **Methods:** In this work, the Bac-to-Bac® Baculovirus Expression System kit (Invitrogen ™) was used, following the methods described by the manufacturer. **Results and Discussion:** BPV-1 L1 was amplified by PCR using an upstream primer including a SauI restriction site and the downstream primer including an EcoRI site. L1 codon gene sequence of BPV-1 was cloned into pGEM-T (Promega), excised with SauI and KpnI enzymes, and subcloned in pFastBac1 (Invitrogen™) for protein expression in baculovirus. The recombinant plasmid was selected in *Escherichia coli.* The top 10 were subsequently used in the transformation of DH10Bac *E. coli*® (Invitrogen™) to obtain the recombinant bacmid. For confirmation of these results, M13 primers were used for PCR and sequencing. If positive results are obtained for recombination, the bacmid recombinant containing the gene sequence of a protein will be used for expression of this protein in baculovirus/Sf-9 cells system.

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6.10 Antimicrobial secondary metabolites produced by Antarctic bacteria

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Introduction: Infectious diseases frequently cause several deaths around the world and new chemotherapy is a challenge. Microbial secondary metabolites are a rich source of bioactive substances, and microorganisms from extreme environments such as Antarctic are being studied for this purpose. **Objectives:** The aim of this study was to evaluate the potential of Antarctic bacteria biotechnology for producing antimicrobial secondary metabolites. **Methods:** At least 106 bacteria strains isolated from Comandante Ferraz Station in the Antarctic were cultivated for a week in tryptone soy broth at 15-18°C and 150 rpm to produce organic extracts. These were assayed up to 1,000 µg/mL for antifungal activity against *Candida albicans* ATCC 36802/ IOC 3704, *C. albicans* IOC 4525, *Cryptococcus neoformans* ATCC 90112, *C. neoformans* IOC 4528, *Aspergillus fumigatus* IOC 4525 and *Trychophyton rubrum* IOC 4527 by the minimal inhibitory concentration (MIC) assay. Antibacterial activity was also evaluated for the extracts at 400 µg/mL against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 10240 and against *Staphylococcus aureus* ATCC 25923 by inhibition percentage of pathogen growth by measuring the optical density at 595 nm. For taxonomic classification, the strains A8, A12, A16, A17, A24, A25, A27, A31, A45, B1, B5, B22 and D5 were analyzed by the fatty acids methyl ester technique (FAME) and sequencing of the 16S gene rDNA. The fatty acids shown in the bacteria membrane were analyzed by gas chromatography and compared with those of the TSBA6 6.10 library. **Results and Discussion:** For antifungal activity, 48 extracts showed MICs from 125 to 1,000 µg/mL and among them, the extracts A16-HEX, A31-HEX and B1-HEX showed a wide spectrum, acting on four pathogens. The extract A24-HEX was the most promising against *C. albicans* IOC 4525 and *C. neoformans* IOC 4528 with MIC of 200 and 125 µg/mL, respectively. The other 164 extracts were not effective up to 1,000 µg/mL. The extracts B26-AE and C32-AE were effective only against *S. aureus* ATCC 25923 and inhibited 52% and 44% of growth, respectively. However, the extracts C1-HEX, C3-HEX, C6-AE and C10-AE significantly promoted the growth of the *M. luteus* ATCC 10240. Based on the FAME analysis, the strains A45 and B1 were classified as *Bacillus atrophaeus* with similarity indices ≥ 0.7%. The strains A17, A24, A31, B5 and B22 were classified as belonging to more than one taxonomic group with similarity indices ranging from 0.297% to 0.556%. Due to the low similarity indices with the library strains, the Antarctic strains A8, A12, A16, A25 and D5 could not be classified. The sequencing of the 16S gene rDNA is in progress to complement the bacterial taxonomic identification.

Supported by: FAPESP

6.11 Influence of low dissolved oxygen concentration on increasing the expression of recombinant rabies virus glycoprotein (RVGP) by *Drosophila melanogaster* S2 cells cultivated in a bioreactor

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Introduction: *Drosophila melanogaster* Schneider 2 (S2) cells have been used to express heterologous proteins. The recombinant rabies virus glycoprotein (RVGP) is an interesting biotechnological product since it is responsible for the induction of protective immune response against rabies infection and for the virus entry into cells upon virus infection. Some microorganism and all animal cells need aeration during cultivation, and some works describe the influence of dissolved oxygen (DO) concentration on cell growth and protein expression. **Objectives:** The aim of this work was to compare the inducible RVGP expression by S2 cells cultivated in a bioreactor at 10 % and 50 % DO of air saturation. **Methods:** The cells were cultivated in a BioFlo110 bioreactor. The culture conditions were: work volume of 1 L, temperature of 28°C, 10 % or 50 % DO, sparging aeration (air, nitrogen and oxygen – 0.1 L/min), 90 rpm agitation, pitched blade impellers, SF-900 II medium and initial cell seeding of $5x10^5$ cells/mL. The cells were induced at $3\text{-}5x10^6$ cells/mL with 700 µM $CuSO_4$. **Results and Discussion:** Specific RVGP expression was about 30 % higher in assays with 10 % DO as compared to that with 50 % DO (1.9 and 2.0 µg/10^7 cells at 10 % DO, and 1.3 µg/10^7 cells at 50 % DO). The influence of dissolved oxygen on protein expression is not well known. A high oxygen concentration in the culture medium may cause oxidative damage to the protein and/or alter some metabolic pathways, influencing protein expression.

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6.12 Purification of factor VIII – influence of $CaCl_2$ concentration in citrate buffer

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Introduction: Factor VIII (FVIII) is a plasma protein involved in blood coagulation, which is of great biotechnological interest, because it is used in the replacement treatment of hemophilia A. In plasma, FVIII is complexed with von Willebrand factor (vWF). In the last years we studied the purification of plasma proteins using chromatographic methods. Gel filtration of plasma in Sepharose FF columns resulted in one peak of FVIII activity, while the addition of anion-exchange, to concentrate plasma, preceding gel filtration, resulted in 2 peaks of FVIII activity. In the first peak, FVIII can be obtained with a higher purification factor, while in the second peak, FVIII/vWF complexes coelute with lower molecular mass proteins. In order to understand the effect of the salts present in the citrate buffer, we varied the concentration of NaCl and $CaCl_2$. Variation of NaCl concentration has some influence on the size of the FVIII/vWF complexes. **Objectives:** In this study we compared the results observed in: 1) Sepharose 6FF, when the anion-exchange resins Q-Sepharose FF or ANX-Sepharose FF were used in the first step and 2) concentration of $CaCl_2$ was 5 mM and 1 mM in the citrate buffers. **Methods:** Chromatography: direct application of plasma to Q-Sepharose FF followed by Sepharose 6FF. Analytical methods: Bradford for protein content; chromogenic for FVIII biological activity; and SDS-PAGE, to see the protein profile of the collected fractions. **Results and Discussion:** We observed similar results in Sepharose 6FF, when Q-Sepharose FF column was used in the first step purification, for buffers containing 5 mM and 1 mM $CaCl_2$. On the other hand, there was a remarkable improvement in FVIII recovery when the concentration of $CaCl_2$ in the citrate buffer was lowered from 5 mM to 1 mM. In the Q-column, the recovered activity in the first peak was approx. 75%, while in the ANX-column, it was 57% and 84%, using buffers containing 5 mM and 1 mM, respectively. Therefore, the effectiveness of gel filtration depends on the $CaCl_2$ concentration in the citrate buffer used as the mobile phase when the ANX-column was used in the first step purification.

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6.13 Expression and stability of a complementation plasmid in good manufacturing practice production of a recombinant BCG-pertussis vaccine

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Introduction: The live attenuated *Mycobacterium bovis* BCG (Bacille Calmette-Guérin) vaccine has been given to newborns as a safe vaccine to prevent severe and fatal tuberculosis, following WHO recommendations. Moreover, BCG has been investigated as a potential live vehicle for heterologous antigens in the development of new vaccines. We have previously shown a rBCG strain expressing the genetically detoxified S1 subunit of pertussis toxin-9K/129G (S1PT) which induced high protection levels in the mouse against an intracerebral challenge with *B. pertussis*. More recently, we have described the construction of an unmarked BCG lysine auxotroph and its complementation recombinant plasmid without antibiotic resistance marker gene, which contains the lysine and S1PT genes placed under the control of the same promoter (rBCG-pertussis). This complemented auxotrophic rBCG-pertussis strain was also able to induce protection in neonate mice immunized through a similar mechanism, where it is now suitable to be evaluated in humans. **Objectives:** In the present work, we evaluated the expression and stability of the complementation plasmid in rBCG-pertussis during serial passages in Sauton and Sauton-potato media following Good Manufacturing Practices (GMP). **Methods:** One or more clones of complemented auxotrophic rBCG-pertussis were used to inoculate two subsequent cultures of 50 mL of Sauton medium. After fifteen days the bacteria were centrifuged and the pellet expanded in three consecutive passages in pellicle Sauton-potato medium. A different sample of each cultured batch was collected for S1PT expression analyses by immunoblotting. Total protein extracts were prepared and protein concentration in the culture lysates was determined. Approximately 30 mg of protein extracts were separated by gel electrophoresis (SDS-PAGE). The proteins were then electrotransferred onto a nitrocellulose membrane and the presence of S1PT was detected using a mouse polyclonal antiserum raised against detoxified PT. **Results and Discussion:** Western blotting showed that all samples from different passages expressed S1PT at comparable levels. These results indicate that the complementation plasmid in rBCG-pertussis is stable after serial passages in Sauton and Sauton-potato and maintains the S1PT expression levels. Thus, this complementation system is efficient for the production of the rBCG-pertussis vaccine under GMP conditions. The rBCG-S1PT vaccine seed lots and certified vaccine lots will be produced for clinical trials.

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6.14 Multilineage differentiation of canine adipose tissue after cryopreservation

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Introduction: Stem cells are promising tools for tissue regeneration, due to its characteristics of proliferation, differentiation and plasticity. The literature shows that such cells when reintroduced into an organization are able to restore the fabric and function. **Objectives:** Thus, this study aimed to demonstrate that stem cells isolated from canine adipose tissue (cATSCs) have the capacity to develop into osteogenic, chondrogenic and adipogenic cells after cryopreservation. **Methods:** Canine adipose tissue was collected, isolated, cryopreserved and frozen for approximately 1 month. The cells were subsequently thawed and evaluated for their proliferative potential, analysis of expression of pluripotent stem cell markers and capacity for osteogenic, chondrogenic and adipogenic differentiation. **Results and Discussion:** cATSCs can be isolated and expanded *in vitro*. The data showed that after thawing the cells retained their "fibroblast-like" morphology. These cells were characterized by flow cytometry and found to be positive for CD44s mesenchymal marker and immunostaining for Sox-2, Oct3/4 and vimentin. Osteogenic differentiation was demonstrated by the mineralization of extracellular matrix at day 11, which became stronger at day 21 and by positive Von Kossa staining. Differentiated cATSCs also showed positive immunostaining for osteocalcin and bone sialoprotein antibodies. After induction of adipogenic differentiation, the cell morphology changed within 24 h from elongated fibroblastic cells to oval-shaped cells. After 4 days, vacuoles within the cell cytoplasm were observed, and at the day 6 an increased number of these cells, which contained vacuoles, showed positive oil red O staining. Chondrogenic differentiation was observed 21 days after induction by the staining of extracellular cartilage matrix proteoglycans. Our data suggest that the cells isolated from canine adipose tissue can be successfully isolated and expanded *in vitro*. We also demonstrated that cATSCs showed a high proliferative rate and capacity to differentiate into derivates of mesoderm. The canine adipose tissue stem cells can potentially serve as a source of multipotent stem cells in order to develop future cell therapy strategies.

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6.15 Immunization of hamsters using attenuated Salmonella as carrier of recombinant proteins from *Leptospira interrogans* serovar Copenhageni and challenge with different virulent Leptospira

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Introduction: Leptospirosis is one of the most important zoonosis in the world caused by spirochetes of the genus *Leptospira*. In Brazil, in 2009, 3755 cases were registered, with 331 deaths occurring mainly in the rainy season in places with lack of sanitation. Some leptospira antigens were presented as good vaccine candidates, LigA and Lip32. In our studies we observed good results using the antigens LIC10191 and AnkB expressed *in vivo* by attenuated salmonella. Stimulation of both humoral and cellular immune response is expected after immunizations with recombinant attenuated salmonellas, and it is desired against infections with most pathogens. **Objectives:** The goal of our work was to compare the protective potential of the four antigens considered good vaccine candidates against leptospirosis considering the immune response when the antigens are presented as purified proteins or carried by recombinant salmonella. We also proposed to test recombinant salmonella carrying two different antigens. **Methods:** Hybrid plasmids were constructed to simultaneously express the antigens LIC10191 and Lip32 *in vivo*. The genes were cloned in the same pAEsox vector in salmonella. LigA or AnkB were also cloned in pAEsox vectors. The sox system can be activated *in vivo* by oxidative stress and *in vitro* by the bipyridyl paraquat. The recombinant proteins were purified by IMAC. Hamsters were immunized for challenge with virulent leptospira to measure immune protection. **Results and Discussion:** The recombinant proteins LIC10191, AnkB, Lip32 and LigA were successfully purified. Recombinant vaccine strains *S. typhimurium* SL3261 carrying the genes in pAEsox were obtained. As expected, purified proteins induced higher titers of antibodies when compared to the recombinant salmonella presenting the same leptospiral antigens. The recognition of purified proteins by the sera of the animals immunized with the recombinant salmonella confirmed that the antigens were expressed *in vivo.* Although the challenge assay allowed us to observe some degree of protection, further assays should be performed for confirmation, considering even more carefully some critical aspects, such as the age of the animals and the virulence of the bacterial strain.

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6.16 Toxicity evaluation of adjuvant formulations with detoxified *Bordetella pertussis* LPS

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Introduction: The new trends in vaccine development have increased interest in adjuvants in the last two decades. Several substances are being evaluated for possible adjuvant activity and many candidates have been tested clinically, featuring high efficiency but also high toxicity, which prevents them from being introduced into clinical practice. The outer membrane of the Gram-negative bacterial cell wall (lipopolysaccharide or LPS) has sourced the development of LPS derivatives with potential usefulness. Studies have shown that this LPS after heating at 100 °C for several hours, loses significant endotoxic activity. **Objectives:** The aim of this study was to evaluate the toxicity of the formulations prepared with detoxified LPS. **Methods:** LPS extracted from *Bordetella pertussis* was submitted to acid hydrolysis detoxification at 100 ºC. The product formulations were prepared in suspension at a concentration of 10 μg/mL and 100 μg/mL and emulsion water in oil (squalene) in different concentrations (10 μg/mL, 20 μg/mL, 100 μg/mL and 400 μg/mL). They were tested for specific toxicity, safety, and pyrogen *in vivo*. **Results and Discussion:** The safety tests showed no significant variation in weight gain in treated animals compared to the controls, and all survived during the observation period. In the pyrogen test *in vivo*, the highest variation of temperature was product concentration dependent especially in relation to the emulsions. However, all results were within normal. Mice in the specific toxicity test had a weight gain over 60% of weight variation in the control group, showing that all samples were nontoxic. The adjuvant concentration reported in many clinical trials was about 10 μg/mL (5 μg/dose), and the biological tests demonstrated that formulations prepared with detoxified LPS did not show toxicity even at a concentration of 400 μg/mL.

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6.17 Cloning and expression of two leptospiral membrane proteins in *Escherichia coli*

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Introduction: Leptospirosis is a widespread zoonosis caused by pathogenic species of the genus *Leptospira* and was recently classified as an emerging infectious disease. Human infection by leptospires is accidental and results from direct or indirect exposure to urine of infected animals. The genome of *L. interrogans* serovar Copenhageni has been sequenced, and several genes coding for surface 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 two genes encoding hypothetical proteins (LIC11975 and LIC13477) using *E. coli* vectors and BL21 SI (salt induced). **Methods:** The genes were amplified without the signal peptide sequence by PCR from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genomic DNA, as template, and complementary sequence primers. The DNA inserts subcloned in vector pGEM-T were subsequently cloned into the pAE expression vector at XhoI and HindIII restriction cloning sites. The recombinant proteins were expressed with 6 x His-tag at the N-terminus, thus facilitating protein purification by metal-affinity chromatography. **Results and Discussion:** The choice of the coding sequences was mostly based on their cellular localization, and the two selected are predicted to be outer membrane proteins. Both recombinant proteins, rLIC11975 and rLIC13477, were expressed in *E. coli* BL21 SI with the expected size of 36 kDa and 57 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.

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6.18 Purification of capsular polysaccharide produced by *Streptococcus pneumoniae* serotype 6B

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Introduction: *Streptococcus pneumoniae* is an important human pathogen that causes diseases such as pneumonia, bacteremia, and meningitis. The capsular polysaccharide (PS) is considered the main virulence factor and is involved in evasion of the host immune system. There are more than 90 distinct capsular serotypes, but most cases of pneumococcal pneumonia appear to be caused by 23 serotypes. Efforts to develop polysaccharide-protein conjugate vaccines are currently being made due to the limited efficacy of the currently used 23-valent polysaccharide-based vaccines in infants. *Streptococcus pneumoniae* serotype 6B is the second most prevalent in Brazil, and purification of this polysaccharide is the theme of this study. The traditional purification process of polysaccharide serotype 6B is very complex, including many ethanol precipitations (inflammable), organic solvent extraction (phenol, toxic and corrosive) and centrifugation/ultracentrifugation steps. **Objectives:** The contribution of this study was to establish an alternative to the centrifugation with the ethanol precipitation using tangential microfiltration (hollow fiber - 0.22 μm), in order to make the operation more practical and feasible for scaling up. **Methods:** Cells were separated from the cultivation broth by tangential microfiltration and the microfiltrate, that contains the polysaccharide PS6B, was concentrated and diafiltered by tangential ultrafiltration with a 50-kDa cut-off. The concentrate was submitted to hydrolytic enzyme treatment to reduce the protein and nucleic acids; the resulting low molecular weight hydrolyzed impurities were removed by tangential ultrafiltration/diafiltration with a 30-kDa membrane. The concentrated 30-kDa fraction was precipitated with ethanol at 25% final concentration. The soluble fraction with 25% ethanol, containing PS6B, was separated by tangential microfiltration (hollow fiber 0.22 μm). This microfiltrate was submitted to a second stage of precipitation, with ethanol at 50% final concentration. The precipitated PS6B was separated from the 50% ethanol solution by a second tangential microfiltration (hollow fiber 0.22 μm). The insoluble PS6B was dissolved in pure water, and next, the soluble PS was collected in the microfiltrated fraction by another tangential microfiltration (hollow fiber 0.22 μm). The soluble PS6B fraction was concentrated using a 30-kDa tangential ultrafiltration membrane in order to reduce the volume. **Results and Discussion:** The relative purity based on protein as well as nucleic acid increased in each purification step: 25% ethanol precipitation RP_{prt} = 3.4 mg PS/mg prt.; after precipitation with 50% ethanol: RP_{prt} =62.4 mg PS/mg prt and RP_{AN} =830.3 mg PS/mg; and the final purified PS fraction: RP_{prt} =104.3 mg RP_{AN}= 3795.7 mg PS/mg AN and with a final recovery of 81% of PS. In conclusion, the purification process of polysaccharide using tangential microfiltration instead of centrifugation after ethanol precipitation steps was shown to be efficient with a purity of ≥34 mg PS/1 mg protein and PS recovery of 81% which makes this process technically feasible for scaling up.

Supported by: FAPESP

6.19 Influence of pH on DNA and protein adsorption on DEAE-cellulose in intermediate purification step of hepatitis B vaccine

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Introduction: Butantan Institute's hepatitis B vaccine (VRHB) has supplied the Brazilian Health Ministry's Immunization Program since 1998. VRHB is the first vaccine in Latin America using DNA recombinant technology. Virus surface S-antigen (HBsAg) is expressed by the transformed yeast *Hansenula polymorpha*. After the fermentation process and downstream purification steps, the final product is submitted to quality control analysis, including determination of residual DNA. Intermediate product incubation with a cationic adsorbent was adopted for the purpose of removing nucleic acids. Protein and DNA adsorption is known to be directly influenced by several factors, including pH. In order to obtain a higher degree of purity in the final product, such condition could be optimized. **Objectives:** The aims of the study were 1) to optimize selective DNA removal from HBsAg containing intermediate product by pH variation, and 2) to evaluate the effect of pH on the adsorption of other yeast proteins. **Methods:** Suspensions of DEAE-cellulose (DE-52, Whatman) were equilibrated at different pH (4.9 - 8.7). After pH adjustment, samples (HbsAg intermediate product) were incubated with resin for 1 h and centrifuged, and the supernatants were analyzed for proteins (passive hemagglutination, optical density and SDS-PAGE) and DNA content (ethidium bromide staining in agarose gels). **Results and Discussion:** The influence of pH on protein and DNA adsorption to DEAE-cellulose was confirmed. DNA clearance was more effective at higher pH (8.7 - 6.8) and less evident at 5.8 or 4.9. SDS-PAGE showed that lower pH values promoted greater adsorption of nonspecific proteins without substantial loss of the S antigen, which was corroborated by the fact that HBsAg titer showed no significant change at any pH.

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6.20 Rapid determination of DNA content for in-line monitoring purposes

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Introduction: Butantan Institute's hepatitis B vaccine (VRHB) is composed of the virus recombinant surface S-antigen (HBsAg), expressed by the transformed yeast *Hansenula polymorpha*. One impurity targeted for clearance during the downstream process is residual host DNA. DNA is mainly released in the cell disruption step and removed in the following purification steps. The regulatory guidance for our product specifies that DNA content in the final product should be less than 100 pg/dose. The traditional DNA determination assays, such as dot-blot hybridization or PCR based systems are unfeasible for in-line monitoring purposes, due to time-consuming and laborious sample preparation. DNA determination by ethidium bromide staining in agarose gels is a simple and rapid method and has already been described, but comparison of single sample spots with DNA standard curves is only enough for rough estimation. We have optimized this technique, reaching sufficient accuracy to evaluate DNA clearance in intermediate products of purification steps. **Objectives:** The aim of this study was to improve samples and standard curve incubation conditions as well as agarose gel design to evaluate residual DNA in intermediate products compared to dot blot quantification. **Methods:** Samples were pipetted on 0.8% agarose horizontal gels containing 0.5 mg/ml of ethidium bromide. Different alternatives of application (directly on surface, round wells and comb wells) were tested, as well as incubation time (0 - 60 min). Gels were visualized in a UV-transilluminator. *Hansenula polymorpha* DNA was used in standard curves, and the influence of protein absence was minimized with bovine serum albumin (BSA). Three to five dilutions were applied in series for each product sample. Dot blot quantification was done using the Gene Screen Plus kit (Perkin Elmer). **Results and Discussion:** Best results were obtained with comb wells, which must be thin (e.g., 0.75 mm) to maximize wall adsorption and deep enough to accommodate large samples (e.g., 10 µl). Incubation time interferes with the results, as DNA migration goes on - two intervals for analysis are recommended for each test (e.g., 10' and 30'). Patterns of sample adsorption were slightly different between DNA standards and products - BSA was used to partially compensate for this effect. One of the main improvements was accomplished by analysis of dilution series of the tested sample instead of comparing a single spot with the control curve. All these details made this simple technique suitable for evaluating DNA removal in biopharmaceutical protein purification.

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6.21 Gene 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 different viral proteins of different viruses. In this work we used ppHCV-RVGP to study RVGP expression in human cells. **Objectives:** The aim of this work was to establish an expression system using pseudo particles (pp) with E1 and E2 HCV glycoproteins, GAG and POL proteins of MLV (murine leukemia virus) and mRNA of the rabies virus glycoprotein. **Methods:** The pTG-RVGP vector was constructed by digestion of the RVGP DNA fragment extracted from pMtiGPV and by ligation with pTG13077. Three vectors (pTG-RVGP, pGagPol, pE1E2) were co-transfected in HEK 293T cells, producing ppHCV-RVGP. The ppHCV-RVGP produced was used to infect hepatocarcinoma Huh 7.0 cells and the RVGP expressed was measured by ELISA assays. **Results and Discussion:** We obtained the pTG-RVGP vector. This vector has the RVGP gene under control of the cytomegalovirus promoter and the MLV encapsidation signal. We produced pseudoparticles containing RVGP mRNA in HEK 293T cells after co-transfection procedure. Samples from pseudoparticles were taken and used to infect hepatocarcinoma cells (Huh7.0). RVGP produced in infected cell will be measured by ELISA assays. The preliminary results with RVGP expression showed the system efficiency but demonstrated better results. This system will help to analyze its efficiency and can be used to infect animal models to determine immunological responses.

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6.22 Construction of promoter-trap vector systems based on transcriptional fusions with EGFP and LacZ for *Escherichia coli*

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Introduction: The investigation of gene expression control in bacteria is important for understanding several biological phenomena, including adaptation to environmental changes. One of the most common methods for evaluating putative or well-known promoter regions is the gene reporter assay. This work describes the construction of a promoter-trapping vector that allows the identification and characterization of bacterial promoter sequences in a heterologous system. **Objectives:** The aim of this study was the construction of a promoter-trapping system for *E. coli*, using either LacZ or eGFP as reporter. **Methods:** The construction of this system was based on modifications of the *E. coli* expression plasmid pAE, including the excision of the T7 promoter, cloning of the reporter genes, addition of a T7 terminator before the cloning sites and the introduction of the ribosome binding site (RBS) positioned immediately adjacent to the reporter genes. Two reporter systems can be used, the e*gfp* (coding for enhanced green fluorescent protein) and *lacZ* (coding for β-galactosidase) transcription fusions, resulting in vector pTRAPGFP and pTRAPLacZ, respectively. The DNA sequences to be tested can be cloned adjacent to the RBS at either a *Bam*HI or *Apa*I restriction site. **Results and Discussion:** The *lacZ* coding sequence was obtained by amplification from the genomic DNA of *E. coli* BL21 (DE3) which has beta-galactosidase activity; e*gfp* was amplified from eukaryotic vector pEGPF-N1. Once these amplicons were cloned in the multiple cloning site (MCS) of pAE, *E. coli* carrying the resulting expression vectors showed the expected phenotype for functional beta-galactosidase and eGFP. On the other hand, when cloned in the *Nco*I-*Hin*dIII of pTRAP, these phenotypes were no longer observed. PCR with primers flanking the promoter region confirmed that the modified vectors do not show approximately 50 bp, corresponding to the length of the T7 promoter. The sequence of pRSET-derived T7 terminator was amplified using primers which added a *Bam*HI or *Apa*I restriction site downstream of it. This amplicon was cloned at a *Sap*I site immediately upstream of the RBS. These modifications were confirmed by sequencing. Finally, the sizes of the two constructed vectors, pTRAPLacZ and pTRAPGFP, were confirmed by gel electrophoresis of the linearized vectors. These vectors will be tested by the cloning of well- characterized *E. coli* promoters, such as those from *recA* and *katE*. This will prove the functionality of the reporter gene construct by the phenotypes of the transformed bacteria subjected to inducing conditions. Afterward, the capacity to clone unknown functional promoters will be further assessed.

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6.23 Plasmid construction to produce a pseudoparticle virus of hepatitis C

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Introduction: Chronic hepatitis caused by hepatitis C virus (HCV) is a world health problem. The estimate of infected people by HCV is 170 million, almost 3% of the world population. Approximately 70% of the chronically infected patients reach a cirrhosis state. Hepatitis is caused by a *Flaviviridae* virus from *Hepacivirus* and its genome is RNA positive that makes a polyprotein. This form of hepatitis is difficult to cure, and there is no vaccine yet. **Objectives:** Our objectives were to construct plasmids to produce pseudoparticles of HCV carrying a RNA positive to express a non structural protein (NS3) of HCV, using two systems, one with Semliki Forest Virus and another with a particle with HCV structural proteins associated with MLV Gag/Pol. **Methods:** To clone HCV, protease (NS3) was cloned into a plasmid, it was amplified by PCR from a patient serum with the genotype 1b. The product was digested with the enzymes Age I and Hpa I to ligate into a plasmid under control of the cytolomegalovirus promoter. We tested the plasmid construction–by sequencing. We co-transfected this plasmid with others into HEK 293T cells to make the virus particles. **Results and Discussion:** There was good amplification of the plasmid, and sequencing confirmed that it truly was NS3 protease from the 1b HCV genotype. After transfection, we performed RT-PCR, and our data suggest that the cell culture supernatant contained RNA for NS3 protease and possibly pseudovirus particles.

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6.24 Expression and purification of four surface leptospiral proteins in fusion with DnaK in *Escherichia coli*

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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 who can be infected by exposure to urine of chronically infected animals. Currently available veterinarian 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 antigen in the formulation of vaccines, due to its exceptional degree of immunogenicity (humoral and cellular) and its 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 chosen genes were amplified by PCR methodology from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genomic DNA. The DNA inserts were cloned into pAE, an *E. coli* vector, at BamHI and NcoI restriction cloning sites. The open reading frame for DnaK was modified to incorporate an in-frame glycine-proline hinge region, which allows structural conformation of the fused proteins to occur free of steric hindrance. The recombinant proteins were expressed in fusion with DnaK at the C-terminus and a 6xHis-tag at the N-terminus, which facilitates protein purification by metal-affinity chromatography. **Results and Discussion:** Genetically fused proteins were successfully obtained and high expression level of recombinant proteins was achieved using BL21 (SI) *E. coli* strain. Only the rDnaK and rLIC12730 proteins were expressed in a soluble form and purified from the supernatant. The other proteins were expressed in the insoluble form, as inclusion bodies, and were purified after solubilization in 8M urea and purified with on-column refolding. SDS-PAGE analysis showed that each protein was successfully purified as a single major protein band. Our studies show that DnaK, genetically fused with proteins, constitutes a good strategy to "rescue" expression of proteins that are otherwise difficult to express. Characterization of the immune response of these proteins in fusion with DnaK should provide important data that may uncover antigens against leptospirosis.

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6.25 Cultivation strategies for capsular polysaccharide production by *Haemophilus influenzae* b

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Introduction: *Haemophilus influenzae* type b (Hib) is a Gram-negative capsulated coccobacillus that causes meningitides in children less than 2 years old, the elderly and those with immunodeficiency. The capsular polysaccharide type b, a polymer composed of ribosyl-ribitol-phosphate (PRP) has been considered as the main virulence factor, and it is currently used as antigen in the vaccine formulation against Hib. The production of the Hib conjugate vaccine consists of three steps: fermentation, purification and conjugation of purified PRP to a protein. Despite its high efficiency, the conjugate vaccine results in high cost due to the complex purification step and low yield of the conjugated final product. The improvement of cultivation conditions for PRP production may contribute to reducing the specific cost of this vaccine and increase the cost-effectiveness. **Objectives:** Therefore, different strategies of fed batch cultivation were tested to increase PRP production and identify the best economically feasible process. **Methods:** Cultivation strategies were: batch with pulse of glucose (BPG) and fed-batch at constant feed flow (FBCFF), exponential fed-batch (EFB) and fed-batch followed by cell recycle and perfusion (FBCRP). An economical analysis was performed to identify the most economically feasible process. **Results and Discussion:** The results showed that BPG is the simplest process reaching a productivity of 63 mg/L.h and a production cost of US$721.23/g PRP. FBCRP resulted in a productivity of 129 mg /L.h, but it is a very complex process costing US$ 520.06/g PRP, which is relatively high. In the EFB experiments, the cost per gram of PRP was lower than for FBCRP and BPG, at US$ 456.46/g PRP, and productivity was 65 mg/L.h. The most acceptable production cost was achieved with FBCFF, at US$ 425.50/g PRP, with a productivity of 84 mg/L.h. In conclusion, according to the results, the fed-batch at constant feed flow was found to be the most economically feasible process and easier to scale up from an operation point of view.

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6.26 Cloning and expression of two *Leptospira interrogans* genes

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Introduction: Leptospirosis is a neglected infectious disease caused by pathogenic spirochetes of the genus *Leptospira*. The leptospirosis burden has a great impact on public health and the veterinary field. Humans become infected by direct or indirect exposure to the urine of chronically infected animals. Prophylactic intervention against the disease includes vaccines preparation employing killed whole leptospiral cells, but this strategy has many drawbacks, such as low efficacy, the requirement for annual booster immunizations, and failure to confer cross-protective immunity against different serovars. Aiming to identify protein antigens that overcome these problems, outer membrane proteins (OMPs) have been studied regarding their potential to be recognized by the immune system of mammalian hosts and their ability to confer immunoprotection. **Objectives:** We aimed to evaluate the immunogenic activity and conservation among *Leptospira* spp of two predicted surface-coding sequences (CDS): LIC11087 and LIC11121. **Methods:** The CDS of both LIC11087 and LIC11121 were amplified by PCR using specific primers and genomic DNA of *L. interrogans* serovar Copenhageni, as template. The DNA inserts were cloned into the expression pAE vector at XhoI and HindIII restriction cloning sites and used to transform *E. coli* TOP10. Ampicilin-resistant recombinant clones were selected for plasmid DNA isolation and confirmed by restriction analysis and PCR. Positive clones were sequenced in a DNA automated sequencer. Plasmids containing the DNA inserts cloned at the correct reading frame were introduced in BL21 SI, BL21 Star (DE3) pLysS and C43 (DE3) *E. coli* strains for protein expression studies. Heterologous expression was tested using different conditions for protein induction. **Results and Discussion:** The CDS, LIC11087 and LIC11121, were successfully cloned into the *E. coli* expression vector pAE, without the signal peptide sequence tags. The expected recombinant proteins of 30 and 37 kDa, corresponding to LIC11087 and LIC11121, respectively, were observed by 12% SDS-PAGE. Both proteins were expressed in their insoluble form. These proteins will be further purified by metal-chelating chromatography. Future perspectives for this study include the evaluation of the immunogenic activity of both antigens in mice, as well as their degree of conservation among pathogenic *Leptospira* spp.

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6.27 Toxic effects of botulinum type A toxin during long-term repeated use in guinea pigs

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Introduction: Botulinum neurotoxins are increasingly being used to treat a variety of conditions where a functional paralysis of neuromuscular junctions is useful as therapy. For this reason, control testing of biological medicines is morally and legally necessary to establish satisfactory safety requirements. **Objectives:** The aim of this study was to determine the toxicity of botulinum type A toxin, after 14-day chronic test in guinea-pigs, identifying chronic toxic effects during long-term repeated usage of botulinum type A toxin. **Methods:** A total of 46 guinea-pigs (male and female) were divided into four groups according to the dose: small-dose group (0.25 IU/animal), intermediate-dose group (0.50 IU/animal), large-dose group (1.07 IU/animal) and placebo group (vehicle). Control group comprised 6 non-inoculated guinea-pigs. Each animal received daily a single intramuscular injection of toxin or vehicle into the left gastrocnemius muscle for 14 repeated days. Animals were carefully monitored for clinical symptoms of botulism or any behavior abnormalities. The body weight and the amount of food and water consumed were monitored daily. **Results and Discussion:** Control and placebo groups showed body weight gain that was significantly higher than in the other groups, about 32.1% of initial body weight. The small-dose group and intermediate-dose group showed a similar body weight gain of 2%. Large-dose group showed a weight loss of -19.8% of initial body weight. At day 5 of repeated injections, all groups revealed total flaccid paralysis of the inoculated limb. Large-dose group developed some alterations during the following days. At day 9, 100% of those animals developed first symptoms of toxicity, including fuzzy hair, muscle weakness of posterior limbs and a wasp-like narrowed waist. At day 11, 80% of those animals developed cervical muscle weakness, limiting their ability to move freely or to feed or drink adequately. Food and water intake decreased significantly, and weight loss was progressive. Abnormal behavior was observed, where those animals showed no common vocalization and they were very quiet and inactive, accept after being picked up. After stopping chronic inoculations, all toxic effects, including abnormal behavior, completely disappeared, but muscle paralysis remained the same until the end of experiment (15 days after the last inoculation). The information that emerges from this work will be helpful in considering the need for accurate and precise determination of toxicity of these neurotoxins, including safety, efficacy, and toxicity, which are imperative for therapeutic use.

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6.28 Evaluation of *Clostridium botulinum* growth in different culture conditions

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Introduction: *Clostridium botulinum* produces toxins of therapeutic and cosmetic interest. The botulinum toxin type A (BT-A) has pharmaceutical importance and has been used in humans for the treatment of facial wrinkles and a number of muscle hyperactivity syndromes. Therefore, culture media that are in current use for growth and fermentation of bacteria usually comprise one or more animal-derived ingredients, such as cooked meat. Culture conditions of *Clostridium botulinum* are a very important point in toxin production, since the culture medium should be free of animal proteins. **Objectives:** The aim of this study was to compare different culture conditions of *Clostridium botulinum* growth in order to provide preliminary results for the replacement of animal-based products typically employed in such media. **Methods:** *Clostridium botulinum* growth was tested in three different media: cooked meat medium (base: beef heart), thioglycollate fluid medium and Kosaki's medium (base: proteose-peptone). Bacterial growth was monitored by measuring the optical density (O.D.) of the culture. For all media, the first inoculation was tested after 24 h or 48 h. **Results and Discussion:** There was no difference between the growth of *Clostridium botulinum* in cooked meat medium at 24 h or 48 h after first inoculation. In addition, the experiments with thioglycollate and Kosaki media resulted in greater growth at 48 h ($p<0.05$). At 24 h, the cooked meat medium showed better growth than the thioglycollate and Kosaki media ($p<0.001$). However, at 48 h, thioglycollate medium showed better growth than cooked meat and Kosaki media ($p<0.01$). The preliminary results suggest that thioglycollate medium can be used instead of the cooked meat medium, based on 48 h growth after the first inoculation of *Clostridium botulinum*. Thioglycollate medium meets the World Health Organization (WHO) requirements for the use of media preferentially free or substantially free of animal products.

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6.29 Synthesis and evaluation of humoral immune response to a conjugate vaccine between pneumococcal capsular polysaccharide serotype 14 and pneumococcal surface protein A in mice

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Introduction: Vaccines against pneumococcus are constituted by plain capsular polysaccharide (PS) or conjugated to a protein. The advantage of conjugate PS over plain PS vaccine is the thymus-dependent immune response. Since there are about 90 different serotypes, the use of a pneumococcal protein in a conjugate vaccine could broaden its coverage. **Objectives:** For this purpose, the pneumococcal surface protein A (PspA) was used as protein carrier to bind covalently to the capsular polysaccharide. **Methods:** The synthesis of the conjugate PS14-PspA resulted in a high degree of polymerization with gel formation. To avoid polymerization, lysine e-amino groups of PspA were modified by reaction with formaldehyde and sodium cyanoborohydrate. Antibodies against this modified PspA were able to bind at the pneumococcal surface and to induce complement deposition in a similar profile compared to control PspA. The avidity index of the two was also the same. **Results and Discussion:** The conjugate was injected in BALB/c mice and the sera were measured for IgG titer against PS14 and PspA. IgG avidity index and complement deposition was also measured. IgG titer against PS14 in the conjugate vaccine was significantly higher than in control PS14, indicating the conversion of the PS into a T-dependent antigen. The avidity and the complement deposition of anti-PS14 IgG induced by conjugate were also higher. Nevertheless, IgG titer against PspA was higher in plain PspA. Although anti-PspA IgG levels in the conjugate had decreased, the functionality of these antibodies was similar since the avidity index and the profile of complement deposition was also the same for the two.

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6.30 Characterization of surface proteins of *Leptospira interrogans* expressed in *Escherichia coli*

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Introduction: Leptospirosis is a zoonosis of global importance that has been considered a major emerging infectious disease. In Brazil, as in other developing countries, infections occur through contact with water contaminated with rodents' urine, which constitute the main reservoir of the leptospires. Studies have been conducted to identify and characterize relevant antigens for the development of an effective vaccine. **Objectives:** In this work, we present the selection, amplification, cloning, expression and purification of two surface proteins of *L. interrogans* serovar Copenhageni, LIC11834 and LIC12253. **Methods:** Bioinformatics analysis of the sequences encoded by LIC11834 and LIC12253; design of appropriate primers; genomic DNA extraction and RNA extraction (à cDNA) and amplification by PCR to study of conservation of selected genes in *Leptospira* pathogenic and saprophytic serovars; cloning of PCR products in pGEM-T vector; digestion removal of DNA inserts and subcloning in pAE expression vector; sequencing analysis of the cloned inserts; transformation and expression of recombinant proteins in *E. coli* strain BL21 SI; analysis of expression and solubility of the recombinant proteins; purification of the recombinant proteins by metal-affinity chromatography; production of polyclonal antibodies by mouse immunization and immunogenicity tests by ELISA. **Results and Discussion:** After cloning into pAE vector, the proteins were expressed with 6xHis tag at the N-terminus, making them appropriate for metal-affinity chromatography purification. The recombinant proteins rLIC11834 and rLIC12253 were expressed in the soluble and insoluble form, respectively. SDS-PAGE analysis showed that the purification of the proteins was successful, resulting in a major band in each case, with their expected molecular sizes. Polyclonal antibodies raised in mice for both proteins yielded high serum titers, indicating their immunogenic activities. We showed that the gene LIC11834 is present in almost all leptospiral species tested, but transcription is restricted to *L. interrogans*. The gene LIC12253 is found in all species tested, while the transcription is restricted to the pathogenic species. These recombinants will be further characterized in other immunoassays to evaluate their participation in the leptospiral pathogenesis. Moreover, immunoprotection activities in an animal model will determine whether these proteins are suitable for vaccine development.

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6.31 Cloning, expression and purification of two membrane proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is an important global disease of human and veterinary concern caused by a pathogenic *Leptospira*. Humans are accidental hosts who can be infected by exposure to chronically infected animals and their environment. Functional genomics studies complement *in silico* analysis and should help our understanding of bacterial pathogenesis. The genome of *L. interrogans* serovar Copenhageni has been sequenced and *in silico* analysis identified more than 200 predicted outer membrane proteins. **Objectives:** In this work, our aim was the cloning, expression and purification of two genes encoding conserved hypothetical proteins (LIC11469 and LIC11030). **Methods:** The chosen genes were amplified by PCR methodology, without the signal peptide sequence, and cloned into pAE, an *E. coli* vector. The recombinants were expressed as full-length proteins in fusion with 6xHis-tag at the N-terminus, thus facilitating protein purification by metal-affinity chromatography. **Results and Discussion:** The proteins were expressed in BL21 DE3 (SI) or Star (plysS) induced *E. coli* cultures in insoluble form, as inclusion bodies. Recombinant proteins were solubilized in 8 M urea followed by purification on-column refolded. SDS-PAGE analysis showed that each protein was successfully purified as a single major protein band. After extensive dialysis against phosphate-buffered saline, the protein concentration was estimated to be approximately 50 µg/ml for both proteins. Characterization of these proteins from *L. interrogans* should provide important data for the future development of a vaccine against leptospirosis.

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6.32 Purification and solubilization of the recombinant protein Sm-29 from *Schistoma mansoni* using high hydrostatic pressure

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Introduction: Schistosomiasis is an important parasitic disease affecting 207 million individuals in tropical regions around the world, with about 600 million people living in endemic areas. The disease is considered the most deadly neglected tropical disease, killing an estimated 280,000 people each year. Praziquantel is usually used for treatment, but the drug is not efficient against re-infection. The complex life cycle of the parasite makes the development of vaccines difficult. An ideal vaccine should prevent larval penetration or act during worm development before oviposition. The availability of the transcriptome of *Schistosoma mansoni* allowed the investigation of many antigens as vaccine candidates, such as Sm29, a surface protein found in adult worms. Recent studies using mice immunized with rSm29 showed 50% reduction in adult worm burden. **Objectives:** The objective of our study was to clone and express the recombinant Sm29 in *E. coli* and optimize its purification from insoluble fractions of the bacteria culture. **Methods:** Sm-29 was cloned in pAEsox vector and the recombinant protein was expressed *in vitro* by adding paraquat to the bacterial culture. This protein was expressed in the insoluble form and recovered as aggregates in inclusion bodies. High hydrostatic pressure (HHP) was tested for solubilization and refolding of the protein from the insoluble fraction. We performed tests with 29 Kpsi pressure, varying concentrations of guanidine hydrochloride, L-arginine, and different proportions of oxidized and reduced glutathione, checking the influence of these agents in the process. The samples were dialyzed against phosphate-buffered saline and centrifuged before analysis of protein solubilization by SDS-PAGE. Protein was then purified by IMAC. **Results and Discussion:** The recombinant protein Sm29 was cloned in pAEsox vector, and we observed a high level of expression in *E.coli* in the insoluble form as aggregates. Solubilization of the protein from inclusion bodies was tested under HHP in different conditions using chaotropic, reducing and stabilizing agents. HHP was shown to be a good methodology in helping to obtain this protein. The major soluble mass of the protein was obtained using oxidized/reduced glutathione at a 4:1 proportion. The protein obtained after pressurization was eluted from IMAC with buffer containing imidazol, 500 mM and 1 M. The next step is to obtain more amounts of the protein for immunization and challenge assays in mice.

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6.33 Identification of the leptospiral antigens that mediate attachment to extracellular matrix components

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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 project was to study three genes that encode predicted lipoproteins selected from the genome sequences of *L. interrogans* serovar Copenhageni and to evaluate the binding of recombinant proteins to extracellular matrix (ECM) components. **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. The pAE constructions were used to transform the BL21 SI *E. coli* strain for protein expression. Subsequently, the recombinant proteins were purified using affinity chromatography. The secondary structure content of the purified proteins was evaluated by circular dichroism (CD) spectroscopy; antiserum against each protein was obtained by mouse immunization; the cellular localization was performed by liquid-phase immunofluorescence assay (L-IFA) and the capacity of the recombinant proteins to mediate attachment to ECM components was evaluated by ELISA. **Results and Discussion:** Recombinant proteins were expressed and purified as a major band, as assessed by PAGE-SDS. Structural integrity of the recombinant proteins was evaluated by CD spectroscopy that showed a mixture of secondary structure contents. Liquid-phase immunofluorescence method was performed to evaluate whether recombinant proteins are located at the bacterial membrane. The results obtained with L-IFA suggest that three recombinants, rLIC10258, rLIC12880 and rLIC12238, are surface-exposed proteins. The binding capacity of the recombinant proteins with laminin, collagen type I, collagen type IV, cellular fibronectin and plasma fibronectin was performed by ELISA. BSA and fetuin were employed as negative controls. rLIC10258 exhibited a significant level of attachment to laminin and plasma fibronectin ($P < 0.05$), whereas no specific interaction with the ECM components was detected with rLIC12880 and rLIC12238. The results suggest that rLIC10258 may play a role in mediating adhesion of *L. interrogans* to the host and thus in leptospiral pathogenesis.

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6.34 Use of limiting dilution cloning as a tool to select for cell growth in suspension mode and to increase productivity of EPO

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Introduction: Erythropoetin (EPO) is a hormone that regulates the production of erythrocytes in mammals. This hormone can be produced by the cultivation of genetically modified cells, generally adhered to a substrate in medium supplemented with fetal bovine serum. Adherence is well maintained by serum components, and the elimination of serum, required by regulatory agencies, modifies the culture. The use of serum-free medium (SFM) comes together with cell cultivation in suspension mode, more practical from the production standpoint. Recombinant CHO cells producing EPO, used to grow in adherent mode with sera, had been previously adapted to grow in suspension in SFM with consequences, such as lower productivity and cultivation issues. **Objectives:** The aim of the study was to increase the production of EPO while obtaining a clone with suspension growth mode with lower aggregation. **Methods:** CHO cells transfected with human gene coding for EPO were adapted to grow in suspension in a commercial SFM. The scale-up of the cultivation process was hampered by the formation of aggregates as the cell population increased. Different trials were pursued with no better results. The limiting dilution cloning ensued, plating cells at a density of 70 cells/well in 96-well microplates, which were maintained at 37ºC and 5% CO_2. After 15 days, a few clones appeared. Each one was transferred to 24-well plates for further growth and then to 25 cm^2 T-flasks. Some clones were observed to grow in suspension with few or no aggregates. ELISA was used to monitor if EPO was produced. In the next step, growing cells with mixing in shaker flasks, EPO production was compared between clones. Cells were seeded at the same cell density and, after 48 or 72 h, the cultures were sampled, the cell density was determined (trypan blue exclusion) and ELISA was performed. **Results and Discussion:** Out of 126 clones, 19 showed better suspension growth and productivity in comparison with the original clone used to start the limiting dilution protocol. Tests are in progress to assess cryopreservation recovery of the cells and quality of recombinant EPO.

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6.35 Evaluation of porcine pulmonary surfactant protein A (p-SP-A) immunogenicity in a murine model

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Introduction: Alveolar type II cells produce surfactant protein A, SP-A. This protein belongs to a group of soluble humoral pattern recognition receptors, called collectins, which modulate the immune response to microorganisms. Numerous studies about SP-A therapeutic applications are available; however, specific studies about its exogenous immunogenicity were not easily undertaken. SP-A's high molecular mass, oligomeric structures and glycosylation can contribute to the development of immunogenicity, but these properties are important to SP-A activity in vivo and should be maintained for therapeutic applications of this molecule. **Objectives:** This paper investigated porcine surfactant protein A (pSP-A) immunogenicity in murine model. An unwanted immunogenicity may lead to a loss of product efficacy besides severe side effects, sometimes more deleterious to the patient. **Methods:** The mice received pSP-A subcutaneously on days 0 and 7. The animals were observed for 90 days, and blood was collected on days 30, 60 and 90 for assessment of the immunogenic potential of pSP-A. The study followed suitability criteria for detection of anti-drug antibody response. **Results and Discussion:** Some animals showed circulating antibodies above the screening cutoff point, which was calculated based on control mouse serum levels. Those antibody results were considered false positive read-outs by the competitive inhibition assay performed. Besides, no neutralizing antibodies were detected able to prevent the porcine protein's ability to cause lipid aggregation. In the murine model, pSP-A could be considered non-immunogenic. These promising results prompt us to continue studying the potential use of the protein p-SP-A as a drug for the treatment of lung diseases.

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6.36 Transient expression of gyroxin fused to EGFP in mammalian cells

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Introduction: Mammalian cells are highly specialized to perform various post-translational modifications, and thus, they represent a great potential to express complex molecules. The toxin gyroxin, a thrombin-like serine protease, is present in the venom of the *Crotalus durissus terrificus* snake. The mechanism of its neurotoxic activity is unknown. Therefore, the cloning and expression of this molecule would be an important step in understanding its role in envenoming. However, due to its post-translational modifications its recombinant form is difficult to obtain in bacteria. **Objectives:** The aim of the study was to clone and express gyroxin in mammalian cells fused to EGFP and to evaluate its biological activity. **Methods:** Gyroxin cDNA was amplified by PCR and cloned into pcDNA_EGFP vector. This construction was transiently transfected into CHO cells by lipofectamin reagent. The clones were analyzed by confocal microscope, and subsequently, the cells were submitted to G418 drug treatment to isolate the clones. **Results and Discussion:** Due to its specialized post-translational machinery, mammalian cells represent an interesting and not fully explored system to express snake toxins. Gyroxin, a 33-kDa glycoprotein, is one of main serine proteases of *Crotalus durissus terrificus* venom. This toxin induces hemotoxicity in mice and a neurological condition called barrel rotation syndrome. In this work, we cloned and transiently expressed gyroxin in mammalian cells. Stable transfection is being conducted in order to produce and purify this toxin to be used in biological assays.

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6.37 Identification of *Leptospira interrogans* adhesins by shotgun phage display

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Introduction: Leptospirosis is a worldwide zoonotic disease caused by gram-negative spirochete *L. interrogans,* and more than 200 pathogenic serovares have been identified. Transmission to humans occurs through contact with domestic or wild animal reservoirs or an environment contaminated by their urine. The development of vaccines has been pursued as a strategy for the prevention of leptospirosis. At present, developed vaccines are not effective or ideal. Protein antigens, such as adhesins, conserved among pathogenic serovars may contribute to overcome 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. **Methods:** Shotgun Phage Display was used to construct 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). The libraries contain inserts with average sizes of 1500 bp (BBT1 and BBT5) and 345 bp (BBT2 and BBT6). The phage library BBT5 was used for biopanning against fetal calf serum (FCS) and the resulting subtractive library BBT5 $BSA^{-}FCS^{-}$ was biopanned against formaldehyde-fixed A31 and Vero cultures. **Results and Discussion:** After 4 panning cycles of recombinant phage particles on FCS, A31 and Vero cell culture monolayer surface, random clones from each of the 4 cycles were sequenced. It was possible to verify the enrichment of clone FCS19117 and selection of only one clone FCS69107 in fetal calf serum displaying fusion protein. In A31, only two clones were selected in phase with protein PIII, A3180108 and A3143131. In Vero, it was not possible to verify the presence of clones in phase with PIII protein. The characterization and validation of these clones as true adhesins is underway. This approach would allow advances in the understanding of host-pathogen interactions, and possibly identify candidates for a vaccine to prevent leptospirosis.

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6.38 Construction of a recombinant bacmid for expression of an antiviral protein obtained from *Lonomia obliqua* hemolymph in a baculovirus/ SF-9 cell system

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Introduction: The control of viruses, especially those induced by influenza virus is of great interest to the public health area. Several studies have been conducted that show the presence of pharmacologically active substances in the hemolymph. Recently, we have demonstrated the existence of a potent antiviral protein in the hemolymph of *Lonomia obliqua* caterpillar. **Objectives:** This study aimed to build recombinant bacmids containing sequences encoding this antiviral protein in the baculovirus/SF-9 cell 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 primers specific for the antiviral protein, based on the sequence deposited in GenBank database. Restriction sites were inserted in the cDNA for connection to the donor plasmid pFastBac1™ (Invitrogen). The recombinant plasmid was selected in *Escherichia coli* Top 10 and subsequently used in the transformation of DH10Bac *E. coli*, to obtain the recombinant bacmids. The bacmid recombinant containing the sequence of the antiviral protein was subjected to recombination in *E. coli* DH10Bac for construction of recombinant bacmids. **Results and Discussion:** This bacmid, containing the sequence of a protein with antiviral activity will be used for expression of this protein in the baculovirus/SF-9 cell system. At the moment, we are expressing the bacmid recombinant containing the sequence of the protein with antiviral activity in the baculovirus/SF-9 cell system.

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6.39 Comparison of capsular polysaccharide production by *Streptococcus pneumoniae* serotype 14 in complex and chemically defined media

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Introduction: *Streptococcus pneumoniae* is a major cause of mortality in developing countries and is responsible for the death of more than one million people every year. Vaccines are the most efficient method to prevent pneumococcal infections and are based on the protection provided by the capsular polysaccharides (PS). *S. pneumoniae* is a fastidious anaerobic aerotolerant microorganism and pneumococcal metabolism is restricted to substrate level phosphorylation, producing lactate as the main end-product, which limits the microorganism's productivity. The regulation of the capsular polysaccharide synthesis depends on both the activity of specific regulatory genes and the production of sugar precursors by the central metabolism. **Objectives:** This study aimed to evaluate the growth and PS production profile of *S. pneumoniae* serotype 14, the most prevalent serotype in Brazilian children, in complex and chemically defined media. **Methods:** Essays were carried out in flasks to identify vitamins and co-factors which are essential for growth in chemically defined medium (CDM). Batch cultures were carried out in pH-controlled anaerobic bioreactors to compare complex medium with CDM containing 2 to 10 vitamins. CDM was also used in continuous cultivation at 0.5/h dilution rate and increasing glucose concentration (0.5 to 30 g/L). Samples were taken to measure optical density (OD), glucose, lactate and PS in the supernatant and to check for viable cell counting and contamination. **Results and Discussion:** It was observed that the vitamin requirements differed in flasks and in the bioreactor: in flasks only nicotinamide and pantothenate were necessary, while in the bioreactor, nicotinamide, pantothenate, thiamine, biotin, riboflavin and pyridoxal were added to achieve the same biomass as in complete CDM with 10 vitamins. It was concluded from continuous cultivation that glucose was no longer limiting above 15 g/L in CDM. The PS concentration in the supernatant was higher in complex medium (430 mg/L) than in complete CDM (96 mg/L), and surprisingly, the increase in glucose concentration in CDM did not affect the PS concentration in the supernatant of the continuous culture, which remained approximately 50 mg/L throughout the process. In batch cultures with complex medium, the PS concentration in the supernatant augmented after cell death from 68 to 430 mg/L, indicating that PS was released from the cells into the culture broth. On the other hand, cell death was not observed using CDM, and therefore, further investigation is needed to determine whether the cell-bound PS concentration was different in both media and which compounds of CDM protected pneumococcus from lysis.

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7. Cellular Biology and Genetics

7.01 Circulation of influenza A subtype H3 virus in migrating and wild birds from the Atlantic rainforest in Brazil

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Introduction: Avian influenza A vírus belongs to the family Orthomyxoviridae. There are sixteen subtypes of hemagglutinins and nine subtypes of neuraminidase. The wild and migrating birds may be involved in the H16 subtypes of the virus hemagglutinin and its maintenance and transmission in the interspecies cycle in nature. Several low pathogenic forms of influenza A vírus isolated from birds are transformed into high pathogenic forms and have caused outbreaks and epidemics among both humans and poultry. The samples from species were obtained from reserves and experimental field stations located in São Paulo State - Brazil, during the years 1997 and 1998. **Objectives:** This study aimed to subtype the positive samples using RT-PCR and PCR for rapid detection and identification of the pathogenic subtype. **Methods:** RT-PCR (primer 12U) and PCR (specifc primer, H3) were used. **Results and Discussion:** Of the the 12 samples analyzed, seven showed positivity for subtype H_3 (Samples: 6712, 6715, 6744, 6781, 6782, 6784 and 6841). Among these, 5 samples showed positivity for influenza A, but they were not identified as H_3. Sequencing of the hemagglutinin gene is underway, and it would be worthwhile to compare the sequences of pathogenic potential in these positive samples.

7.02 Study of endogenous regulatory mechanisms in acute systemic inflammation after intestinal ischemia and reperfusion in mice

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Introduction: Ischemia induces a hypoxic situation starting a sequence of events that are amplified after reperfusion, leading to severe organ injury and dysfunction by the production of reactive oxygen species, leukocyte infiltrate and protein extravasation. Inflammation has been considered the most important cause of injury in organs subjected to ischemia and reperfusion (I/R). Lung is an important target for systemic inflammatory response associated with intestinal I/R. The mechanisms that trigger inflammation after ischemia have been studied in several experimental models. **Objectives:** The aim of this study was to evaluate the endogenous mechanisms responsible for the regulation of systemic inflammation after intestinal ischemia and reperfusion in two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute inflammatory response to a nonimmunogenic substance. **Methods:** Intestinal ischemia was induced by clamping the mesenteric artery for 45 min, and the following parameters were studied at 0, 1, 4 and 24 h after reperfusion. Lung injury was evaluated by MPO activity in lysates and cellular infiltration in the lung parenchyma. Hypoxia and inflammatory cytokine mRNA expression were evaluated in the lungs by real-time PCR, and the protein content in lung lysates was analyzed by 2D-gel eletrophoresis. **Results and Discussion:** We observed in AIRmax ischemic lungs a progressive neutrophilia starting after ischemia with a peak at 4 h ($2.7 \pm 0.3 \times 10^6$ cells/ml). On the other hand, in AIRmin mice the neutrophil content was similar to that of control groups ($1.2 \pm 0.2 \times 10^6$ cells/ml). Corroborating these results, MPO activity was higher in AIRmax ischemic lungs with absorbance (A_{450nm}) of 0.62 ± 0.038 compared to AIRmin ischemic lungs (0.308 ± 0.04). I/R induced upregulation of *hypoxia inducible factor (Hif-1α)* and of *von Hippel Lindau (Vhl)* genes, and of the inflammatory cytokines *Il-1b* and *Il-6* in AIRmax lungs. Conversely, in AIRmin mice these genes were not modulated by I/R. In line with gene expression, the profile of proteins in the lungs showed quantitative and qualitative differences between the two strains. These interline differences observed after intestinal I/R are in agreement with the selected phenotypes of AIRmax and AIRmin mice. The results demonstrate a positive correlation between the inflammatory ability of these strains and the expression in the lungs of genes involved in hypoxia and inflammation in the intestinal ischemia/reperfusion experimental model.

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7.03 Selective cancer cell toxicity of crotamine – a cationic peptide/toxin from the venom of the South American rattlesnake

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Introduction: Crotamine is a toxic peptide of 42 amino acids found in the venom of South American rattlesnakes. Due to the presence of nine lysine residues and three disulfide bonds, crotamine is a very compact and highly positively charged molecule, which shares structural features with some cationic antimicrobial peptides (CAPs). Recently, we reported that crotamine at a non-toxic concentration for normal cells (human fibroblasts, embryonic stem cells and others), was harmful for CHO-K1 tumor cells. **Objectives:** The aim of this study was to evaluate the cytotoxic effect of crotamine on primary invasion of cutaneous melanoma produced by injection of B16-F10 cells into C57Bl/6J mice. **Methods:** Crotamine at 1.0 mM (5 mg/ml) was shown to be cytotoxic to B16-F10 cells *in vitro*, using the MTT assay and Hoechst 33342 and propidium iodide (PI) staining. Fluorescent-labeled crotamine has an intracellular distribution and co-localization with syndecan-1, as visualized by confocal microscopy in cultured B16-F10. The effect of crotamine on the tumor transplantation was studied in two groups each composed of 35 animals: one, receiving cells only (non-treated group) and the other, cells and 0.2 mM (~1mg/mL) crotamine daily (crotamine-treated group), for 21 days. **Results and Discussion:** Drastic delay of tumor implantation and significant reduction of animal death was observed in the crotamine-treated group. Average weight of tumor in non-treated group was 4.60 gm, while in crotamine-treated only 0.27gm was observed in a few animals. According to Kaplan–Meier curves, the crotamine-treated group showed significant survival (n=28) in comparison with the non-treated group (n=7). Our data indicate that crotamine, a mild toxin, is selectively toxic to cancer cells *in vitro* and in *vivo,* with regard to an aggressive and fast-growing type of cancer, which deserves further investigation as a tumor-killing compound.

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7.04 In utero transplantation of human immature dental pulp in normal dogs

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Introduction: In-utero transplantation of donor cells and tissues in the fetus during gestation is an efficient treatment of several diseases, showing homing in damaged tissues. Immature dental pulp stem cells (IDPSC) were isolated from dental pulp of young patients and well characterized by our group. The fetus is an immunologically privileged environment, which is not susceptible to foreign antigens. **Objectives:** In present study, we aimed at in-utero transplantation of IDPSC into canine fetuses in order to analyze their biodistribution within normal tissues and organs. **Methods:** All experimental procedures were approved by the Ethics Committee and were performed under appropriate anesthesia. Plasmid vector carrying green fluorescent (GFP) gene was introduced into IDPSC. Six million GFP- IDPSC were transplanted following laparotomy and intraperitoneal injection under intra-operative ultrasound control into 5 fetuses at 45 days of gestation. Control animals did not receive the cells. Ultrasound analyses were performed daily. Seven days after cell transplantation, ovarian hysterectomy was performed. Several organs and tissues were collected and fixed or cryopreserved. Ultrafine cryosections were analyzed by confocal microscopy. **Results and Discussion:** The GFP gene was successfully introduced into IDPSC and following selection an expression of protein was observed in 98% of cells. Transplantation procedure was well accepted by the fetuses and the mother. No hemorrhage or intra-abdominal liquid accumulation was observed. The IDPSC, which express GFP protein were detected in several tissues: in thoracic muscle they were localized in blood vessels in tunica externa; in jejunum (gut) they were found in epithelium of the mucosa (cover villi) and in cerebellum they were found in the molecular layer of cerebellar Purkinje cells. Interestingly, IDPSC were detected in placenta, especially in muscle layer (tunica media) of placental artery. The present study showed that in-utero xenotransplantation of hIDPSC was safe. These cells were able to cross the placental barrier and home in on placental vessels. We observed that biodistribution in normal organism is different from that in injured individuals. As it was reported in injured organisms, the cells tend to localize around damaged sites in response to environmental inflammatory factors. In contrast, in normal organism IDPSC traffic throughout all tissues, where they show cell clusters suggesting cell or cells -“founder” event. Our data provide new insight into better understanding of the mechanism of stem cell homing in normal versus damaged tissues.

7.05 On the way to the clinic: successful corneal reconstruction by human immature dental pulp stem cells

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Introduction: Total limbal stem cells deficiency (LSCD) is caused by a variety of conditions, such as genetic disorders, chemical and thermal injury, inflammatory diseases, and others. It often results in persistent corneal epithelial defect or abnormal reepithelialization by conjunctival epithelial cells, leading to a significant loss of visual acuity. Searching for an alternative that could be potentially used in corneal reconstruction, we turned our attention to a population of stem cells isolated by our group, which are called human immature dental pulp stem cells (hIDPSC). These cells were shown to express mesenchymal and embryonic stem cell markers. Recently, we demonstrated that hIDPSC express a set of specific markers of limbal stem cells (LSC), such as integrin β-1, vimentin, p63, ABCG2, connexin 43 and K12, *in vitro*. **Objective:** To provide a new source of limbal epithelial stem cells, we aimed to determine the outcome of hIDPSC transplantation for ocular surface reconstruction in an animal model of LSCD after chemical injury. **Methods:** LSCD was induced by the application of 0.5 M NaOH to the right eye of rabbits for 25 s (mild chemical burn [MCB]) and for 45 s (severe chemical burn [SCB]). After 1 month, a superficial keratectomy was performed to remove the fibrovascular pannus that covered the animals' burned corneas. A tissue-engineered hIDPSC sheet was transplanted onto the corneal bed and then covered with deepithelialized human amniotic membrane (AM). In the respective control groups, the denuded cornea was covered with AM only. After 3 months, a detailed analysis of the rabbit eyes was performed with regard to clinical appearance, histology, electron microscopy, and immunohistochemistry. **Results and Discussion:** Corneal transparency of the rabbit eyes that underwent hIDPSC transplantation was improved throughout the follow-up, while the control corneas developed total conjunctivalization and opacification. Rabbits from the MCB group showed clearer corneas with less neovascularization. The clinical data were confirmed by histologic analysis that showed healthy uniform corneal epithelium, especially in the MCB group. We also showed that in the SCB group reconstruction of the stromal layer was observed, sugesting the next surgery would lead to complete corneal reconstruction. The corneal tissue showed expression of human cornea-specific proteins, therefore corneal tissue formed after transplantation was of hIDPSC origin. In the control corneas, none of these human antigens were detected. Overall, these data showed that transplantation of a tissue-engineered hIDPSC sheet was successful for the reconstruction of corneal epithelium in an animal model of LSCD. Currently, CONEP permission was obtained by our group for transition of this stem cell technology from pre-clinical to clinical applications of hIDPSC in corneal injuries in humans. It is noteworthy that corneal reconstruction using stem cells is also supported by ISSCR among a very small number of therapies which are considered safe.

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7.06 Epistatic relationship between cyclin D1 and Ki-Ras in Y-1 malignant cell line

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Introduction: Arginine vasopressin (AVP) and FGF2 (fibroblast growth factor 2) are two proteins whose pro-survivor and mitogenic effects are well established in several cell lines. However, our group and others have demonstrated that these factors can also act, selectively in malignant cells, inhibiting cell proliferation. Using a ras-driven mouse malignant cell model (Y-1), we showed that this cytotoxic effect of FGF2 depends on high levels of Ras-GTP protein. On the other hand, the cytotoxic effect of AVP depends on the maintenance of high levels of Ras-GTP protein but also involves the inhibition of cyclin D1. We also know that in these cells cyclin D1 does not play its ordinary role in promoting the transition G0 → G1 → S. This function is restricted to cyclin E. These results suggest that cyclin D1 plays a distinct role other than its classical function, in ras-dependent malignant cells. Our hypothesis is that cyclin D1 is a co-oncogene of Ki-ras, ensuring cell survival against the Ras-oncoprotein stress. In addition, we assume that the Ki-ras gene modulates the function of cyclin D1 gene in an epistatic manner. This hypothesis is supported by the phenotype of the sub-lines resistant to FGF2, which are also resistant to AVP. **Objectives:** We aimed to test the hypothesis of epistatic relationship between cyclin D1 and Ki-ras genes through selection of clones resistant to AVP from the parental line Y-1, and analysis of their phenotype concerning the expression of cyclin D1, level of ras-GTP protein and vulnerability to both AVP and FGF2 cytotoxic effects. **Methods:** The parental line Y-1 was submitted to AVP for selection of resistant colonies. Eleven clones survived in culture and were analyzed for sensitivity to AVP and FGF2 by growth curves and clonogenic assays. The expression of cyclin D1 and Ras-GTP were analyzed by PCR and Western blotting. In cytogenetic characterization of the clones, we used conventional Giemsa staining and G, C and Ag-NOR banding. **Results and Discussion:** On the Y-1 cell line, 2 marker chromosomes, HSR-I and HSR-II, carry the amplification of the Ki-ras gene. The cytogenetic study of the clones resistant to FGF2 revealed that they lost the chromosome HSR-I. HSR-II cannot be lost because it is the only one that transcribes rRNA. Three clones resistant to AVP were analyzed after the selection with AVP. They showed resistance to AVP, as demonstrated by growth curves and clonogenic assays. We also analyzed the effect of FGF-2 on these three clones and found that they maintained sensitivity to this factor, which is consistent with our hypothesis. We are now analyzing the expression of cyclin D1, cyclin E1, p27, and Ras protein.

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7.07 Non-hematopoietic progenitor/stem cells derived from yolk sac, liver and bone marrow of canine fetus

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Introduction: Due to the functional role of nonhematopoietic/progenitor stem (nHPS) cells during fetal development in supporting hematopoiesis, these cells co-localize with transient hematopoietic stem cell niches (tHSCN) such as yolk sac (YS), liver (LV) and bone marrow (BM). These nHPS are also responsible for rapid growth and amplification of hematopoietic stem cells and later for formation of adult permanent hematopoietic stem cell niches (pHSCN). These multiple functions of nHPS cells, which localize in different organs, reflect on nHPS cell properties after *in vitro* isolation. It seems that nHPS cells can be isolated only from functional tHSCN during very time-restricted developmental window. Thus, successful isolation of nHPS cells directly depends on correct definition of fetal age. This is the first study aimed at the isolation of nHPS cells in dogs. **Objectives:** We isolated and characterized nHPS cells from canine fetal tissues (YS, LV and BM) **Methods:** The fetuses at different ages were used in order to establish the best fetal age suitable for the isolation of nHPS cells. Crow-rump technique was used for age estimation. The nHPS cells were obtained from the culture of tissue-explants. **Results and Discussion:** All nHPS cells showed fibroblast-like morphology. Ultraestructural analyses showed that populations of BM- and YS-nHPS cells were composed of cells with embryonic- and mesenchymal-like morphology. The LV-nHPS cells, in turn, were of the mesenchymal- and epithelial-like type. CFU-F assay was more efficient in LV-nHPS cells. All nHPS cell expressed vimentin, nestin and CD44 proteins. Expression of cytokeratin-18 was observed in BM- and LV-nHPS cells, while VE-cadherin was expressed only in YS-Nhps cells. The BM-, LV- and YS-nHPS cells expressed pluripotent stem cells markers, such as Oct3/4 and Sox2. Karyotype analysis revealed a normal diploid chromosome set (2n=78) in all cell cultures. The nHPS cells from YS and BM were able to undergo osteogenic and chondrogenic differentiation, while LV-nHPS cells showed osteogenic commitment only. None of nHPS cells were able to differentiate into adipocytes. None of the nHPS cells showed teratoma formation after injection into muscle of nude mice. The efficient isolation of nHPS cells was delineated during fetal development in dogs. We showed that morphologically more homogeneous population of nHPS cells can be isolated from BM, LV and YS between days: 50 -60, 30-45 and 25- 30 of canine pregnancy, respectively. Overall, we isolated heterogeneous populations of BM-, LV- and YS-nHPS that displayed distinct plasticity, when compared to NHPS cells isolated from adult tissue.

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7.08 Isolation of embryonic-like stem cells from fetal brain without feeder layer

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Introduction: Currently, stem cell research has grown, especially regarding the search for new sources of stem cells. Embryonic stem cells are isolated from the inner mass of the blastocyst. These cells are an important model of mammalian development in vitro and are the focus of a great deal of research for their use in cell therapy. Recently, it was demonstrated that pluripotent stem cells can be isolated from the epiblast. Consequently the epiblast will differentiate very early into primordial germ cells (PGC). During embryo development, PGCs migrate into the aorta-gonad-mesonephros region (AGM), which is responsible for waves of hematopoiesis. This region derives hematopoietic stem cells (HSC) and together with the PGC to promote the colonization of the fetal liver, thymus, spleen and bone marrow (BM). These results are new and there is much to be studied on the characteristics and properties of populations of stem cells from embryo and fetal tissues, mainly in fetal brain. **Objectives:** We aimed at the identification and isolation of embryonic-like stem cells from fetal brain. **Methods:** The canine embryo was used in order to isolate embryonic-like cells. The crow-rump technique was used for age estimation. The nHPS cells were obtained from the culture of tissue-explants in cell culture medium composed of DMEM-F12 supplemented with 15% fetal bovine serum, 1% non-essential amnino acids, 1% streptomycin/penicillin and 1% glutamine. **Results and Discussion:** After 48 h, we observed in the fetal brain explants, as expected, the formation of cells with typical morphology of stem cells from the nervous system, such as neurospheres, rosettes and neurons. At the same time other colonies, which displayed embryonic-like stem cell morphology were found in the culture. These colonies remained undifferentiated for 5 days. After splitting, these colonies were then able to grow on both a feeder layer of mouse embryonic fibroblasts and matrigel, while maintaining an undifferentiated state and typical ES cell-like morphology, without LIF. This finding is innovative because it suggests that ES-like pluripotent cells can be isolated at the advanced stages of the development from the fetus. It opens a discussion about maintaining primordial ES cell niches not only in early embryos (blastocysts), but also in some other tissues, such as brain and testis, during fetal and adult development. The function and developmental sense of this phenomenon needs to be clarified.

7.09 Search for therapeutic strategies in cells transformed by human papillomavirus

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Introduction: Cervical carcinoma expresses E6 and E7 oncoproteins from high risk human papillomavirus (HPV). A more significant role for malignant transformation can be assigned to the *E6* and *E7* oncogenes and their respective proteins, which neutralize cellular tumor suppressor function, through degradation of p53 and pRB (retinoblastoma protein), respectively, contributing to tumorgenesis. The E6 oncoprotein encoded by the virus is multifunctional, demonstrating several cellular targets; however, it is not clear yet if all of these activities are related to tumor malignancy. The E7 oncoprotein shows isoforms which act in different ways. **Objectives:** The aim of this study was to evaluate the distribution of viral oncoproteins E6 and E7, mitochondria, transferrin receptor (TfR), transferrin (Tf), ferritin (Fe), clathrin, cytochrome c, porin and F_0F_1-ATPase, in human cells transformed and non-transformed by HPV, to search for strategies against carcinogenesis. **Methods:** HPV-negative cell lines were transfected with pLXSN vectors, containing complete E6 and E7 genes sequence, used as positive controls. Cells were analyzed through immunofluorescence assays, cellular fractionation for mitochondrial isolation in HPV-positive and HPV-negative cells, for ultrastructural immunocytochemistry and Western blotting. **Results and Discussion:** The antigens E6 and E7 oncoproteins were well recognized by the antibodies in HPV-transformed cells and in pLXSN vector transfected cells. TfR were detected in abundance at the plasma membrane of cells, as well as Fe being labeled in the cytoplasm, nucleus and mitochondria and cytochrome c, porin and F_0F_1-ATPase preferentially in the mitochondria. The great amount of iron suggests a participation of this element in the HPV cell transformation, maintaining mitochondrial cytochrome c levels. Co-localizations of E6 in isolated mitochondria were detected in HPV-transfected and HPV-transformed cells, by ultrastructural immunocytochemistry. In conclusion, these findings point to the fact that E6 acts as an anti-apoptogenic factor, and all anti-apoptogenic factors detected are being evaluated as potential prophylactic and therapeutic strategies in the development of vaccines against cervical and anogenital cancers.

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7.10 Erythropoiesis in the spleen of the snake *Bothrops jararaca* (Viperidae, Crotalinae)

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Introduction: The spleen changes over vertebrate evolution both structurally and functionally.In most adult fish and amphibians, the hemopoietic function of the spleen is remarkable, while in Amniota immunological function is predominant, and the bone marrow is most responsible organ for blood supply. Moreover, in mammals and birds, on ontogeny the spleen has an important role in hematopoiesis. In severe cases of anemia, even in mammals, it can produce red blood cells. These functions appear in some groups of reptiles, but most studies focus on morphological or immunological analysis, and few studies have reported on hemopoietic activity. **Objectives:** The aim of this study was to obtain detailed information on the structure of the spleen, mainly regarding the presence of erythropoiesis in healthy adults, adults with induced hemolytic anemia and in early postnatal *Bothrops jararaca* (Bj) snakes. **Methods:** Snakes recently captured from nature during spring/summer were sent to Instituto Butantan and separated into groups: healthy adults (HA, n=7) and adults with induced hemolytic anemia (IHA, n=4) by saponin *s.c.* injection (7.5 mg/ml/kg). Animals were anesthetized with thiopental (30 mg/kg), and the spleen was removed, fixed in Bouin solution and embedded in paraffin or historesin. Blood was drawn to determine pro-erythrocyte counts and some hematological parameters in IHA. **Results and Discussion:** Adult Bj spleen is closely associated with the pancreas. However, it is enclosed by a fibrous capsule of connective tissue, which is rich in collagen and blood vessels, clearly separating it from pancreas. This capsule penetrates the splenic pulp, forming septa, called capsule/septal tissue. The white (WP) and red (RP) pulp are distinct and evident, but there is not a clear demarcation between them. RP is highly vascularizated, with vessels and sinusoids, and with many collagen fibers. Differently, WP is basically composed by lymphoid tissue, mostly lymphocytes, with a few vessels and fibers. These characteristics are also present in other species of snakes. WP and RP ratio is variable, and seasonality seems to be an important factor for it. Spleen can store thrombocytes and granulocytes, and considerable amount of heterophils were seen in snakes' RP spleen, as previously reported in the alligator *Alligator mississippiensis*. About erythropoiesis, there was no activity in HA. However, an increase in immature cells was found, which were similar to erythroblasts in IHA, whose pro-erythrocytes levels reached 7 to 17%. These cells were found in WP, and additional staining and electron microscope analysis should be undertaken to identify them. Spleen from the early postnatal period will also be studied.

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7.11 Genotoxic and cytotoxic effects of 7,12-dimethylbenz(a)anthracene (DMBA) on bone marrow cells from mice genetically selected for inflammatory response

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Introduction: DMBA is a genotoxic agent that reacts directly with DNA, inducing cytotoxicity. Previous studies have reported that DMBA-induced bone marrow (BM) toxicity is p53-dependent in vivo. Two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute inflammatory response (AIR) to a non-immunogenic substance (Biogel) have shown different sensitivity to DMBA toxic effects. Our laboratory reported significantly decreased BM cellularity and decrease in proliferation capacity in AIRmin mice, following DMBA administration. **Objectives:** We investigated the genotoxic and cytotoxic effects of DMBA treatment on the BM of AIR mice. **Methods:** AIRmax and AIRmin mice were treated *in vivo* with a single i.p. dose of 50 mg/kg DMBA in olive oil. BM cells were used in these studies. Flow cytometric analysis was used to determine apoptosis/necrosis levels in cells stained with propidium iodide and annexin V. DNA damage was assessed by the alkaline single-cell gel electrophoresis (comet) assay and expressed as tail moment. Total RNA was extracted to quantify the expression levels of the poly (ADP-ribose) polymerase family member 1 (*parp-1)* involved in DNA repair, by real-time PCR. The p53 protein levels were determined by Western blot analysis. **Results and Discussion:** The kinetics of cell repair was measured at various times after DMBA treatment over a 24-h period. DMBA treatment induced an increase in the tail moment at 2 h in AIRmax (8.0 ± 0.14 versus 0.17 ± 0.09 control mice) and AIRmin (16.2 ± 0.4 versus 0.11 ± 0.05 control mice). The removal of DNA lesion was observed at 4h in AIRmax and at 8h in AIRmin BM cells. Additionally, 24 h after DMBA treatment, the percentage of necrotic cells increased significantly ($p < 0.01$) in AIRmin mice only (25.1 ± 9.2 versus 8.4 ± 4.7 control mice). No significant effects were observed in the percentage of apoptotic cells. The *parp-1* gene showed a 3-fold increase in mRNA expression in AIRmax cells after 12 h of DMBA treatment. Futhermore, p53 protein level was 7.7-fold lower in cells of AIRmax and 6-fold higher in AIRmin mice after 24 h of DMBA treatment, when compared to their controls. Our results demonstrated that DMBA produces long-lasting genotoxicity and cytotoxicity in BM cells in AIRmin mice only. p53 protein is increased in response to the DNA damage induced by DMBA followed by increased necrotic cell numbers in AIRmin mice. On the other hand, AIRmax mice showed a decreased p53 protein level, increased PARP-1 expression and larger capacity for removal of DNA lesions, which suggests that it has an efficient DNA repair mechanism.

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7.12 Lessons from nature: biological versatility of crotamine – a cationic peptide from the venom of the South American rattlesnake

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Introduction: Natural biodiversity of substances isolated from microorganisms, plants, animals or insects can serve as a prototype for designing biopharmaceutical products with a variety of specific properties. Animal venoms were used by humankind to cure diseases since ancient times. Several toxins and their derivates isolated from the venom are currently used in the clinic and diagnosis, while others appear as a basis for the development of new drugs with potential application for incurable human diseases, such as cancer and others. **Objectives:** This work focused on crotamine, a small basic toxic polypeptide found in the venom of the South American rattlesnake *Crotalus durissus terrificus*, which was discovered more than 50 years ago. Despite the time, the exceptional biological versatility of crotamine was demonstrated only in the past six years. **Methods:** The novel and exquisite biological activities of crotamine were accomplished using very low, micromolar, concentration of crotamine peptide and refined instrumention, such as confocal microscopy analysis in combination with culture of selected lines of actively proliferative (replicating) cells. Moreover, a multidisciplinary study based on structural reduction of crotamine proved to be successful in maintaining the natural cell penetrating and nuclear homing abilities. **Results and Discussion:** Here, we have shown (*i*) crotamine's cell penetrating ability, which allows it to pass through cell membranes and to accumulate preferentially in the nucleus; (*ii*) its property of intracellular vesicle tracking and ability to serve as a cell cycle marker, including interaction with chromosomes, nucleoli and centrioles, and (*iii*) its capability of delivering DNA into different mammalian cells *in vitro*. More recently, the antimicrobial action and possible selective anti-tumor activity of crotamine have been also suggested. Recent knowledge about crotamine may lead readers to examine the paradigmatic way of discovering novel and unpredictable properties of "old" toxins. Multidisciplinary studies used for crotamine investigation placed it in a rare category of versatile biomolecules, in which concentration, molecular target preference, structural ancestrality and specificity toward biological membranes play an integral role. The data demonstrated that crotamine is a druggable peptide ready to be employed as imaging agent for detecting dividing cells, intracellular delivery system for hydrophilic biomolecules, and alternative chemotherapeutic compound for aggressive types of cancer. The strategy of discovering crotamine properties can be stepwise dissected and followed for the investigation of undisclosed and unpredictable biological activities of other potentially useful natural toxins and drugs.

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7.13 Long-term culture of adherent mouse embryonic stem cells

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Introduction: Innumerous protocols, using the mouse embryonic stem (ES) cells as a model for in vitro study of neuron functional properties and features, have been developed. Most of these protocols are short-lasting, which therefore does not allow a careful analysis of neuron maturation, aging, and death processes. **Objectives:** We describe here a novel and efficient long-lasting protocol for in vitro ES cell differentiation into neuronal cells. **Methods:** It consists of obtaining embryoid bodies (EBs), followed by induction of neuronal differentiation with retinoic acid of non-adherent EBs (three-dimensional model), which further allows their adherence and formation of adherent neurospheres (AN, bidimensional model). **Results and Discussion:** AN can be maintained for at least twelve weeks in culture under repetitive mechanical splitting providing a constant microenvironment (in vitro niche) for the neuronal progenitor cells avoiding mechanical dissociation of AN. The expression of neuron-specific proteins, such as nestin, sox1, beta III-tubulin, MAP2, NF-M, Tau, NeuN, GABA and 5-HT, were confirmed in these cells maintained for three months with several splittings. Additionally, expression pattern of microtubule-associated proteins, such as Lis1 (lissencephaly) and Ndel1 (nuclear distribution element-like), which were shown to be essential for differentiation and migration of neurons during embryogenesis, was also studied. As expected, both proteins were expressed in undifferentiated ES cells, AN, and non-rosette neurons, although showing different spatial distribution in AN. In contrast to previous studies, using cultured neuronal cells derived from embryonic and adult tissues, only Ndel1 expression was observed in centrosome region of early neuroblasts from AN. Mature neurons, obtained from ES cells in this work, display ion channels and oscillations of membrane electrical potential, typical of electrically excitable cells, which is a characteristic feature of functional CNS neurons. Taken together, our study demonstrated that AN represent a long-term culture of neuronal cells, which can be used to analyze the process of neuronal differentiation dynamics. Thus the protocol described here provides a new experimental model for studying neurological diseases associated with neuronal differentiation during early development; it also represents a novel source of functional cells that can be used as tools for testing the effects of toxins and/or drugs on neuronal cells.

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7.14 Intense acute inflammation affects the number of bone marrow progenitor cells in emergency granulopoiesis in mice

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Introduction: The production of leukocytes in bone marrow (BM) is crucial for innate and adaptative immunity. Developmental commitment to a particular blood-cell lineage occurs by the sequential differentiation of hematopoietic stem cells (HSCs) into multipotential progenitors and terminally differentiated cells under the action of hematopoietic cytokines and transcription factors. Most knowledge comes from the steady-state process of leukocyte production, although recent studies have demonstrated that acute inflammation alters this process in the bone marrow (emergency granulopoiesis), accelerating myelopoiesis over lymphopoiesis. Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response to Biogel P100, represented by the number of infiltrated granulocytes in inflammatory exudates, constitute a suitable model to study normal and emergency granulopoiesis. **Objectives:** The aim of this study was to comparatively evaluate in AIRmax and AIRmin, the effects of acute inflammation on three populations of progenitor cells in BM: hematopoietic stem cell (HSC), common myeloid progenitors (CMP) and granulocyte-macrophage progenitors (GMP), and the differentiation capacity of BM cells into neutrophils. **Methods:** Progenitor BM cell populations were characterized by flow cytometry in normal mice and 24 h after sc injection of Biogel using a combination of antibodies directed to specific surface molecules. Proliferation and neutrophil differentiation were evaluated in 5-day liquid cultures of BM cells stimulated with G-CSF plus IL-3 combined with all-trans retinoic acid (ATRA). **Results and Discussion:** FACS analysis revealed 4% HSCs ($lin^{-}sca\text{-}1^{+}ckit^{+}$ cells) and 11% GMPs ($ckit^{high}Fc\gamma RIII/II^{high}CD34^{high}$ cells) in BM cells from normal mice of both lines. CMPs ($ckit^{high}Fc\gamma RIII/II^{low}CD34^{high}$) were higher in AIRmin (5%) than in AIRmax (2.5%) BM cells. At 24 h after Biogel treatment, the percentage of HSCs, CMPs and GMPs increased 2-fold in AIRmax, reaching 8, 5 and 20%, respectively. In contrast, the percentages were maintained in AIRmin mice. On day 5 of culture, BM cells from AIRmax mice showed higher proliferation in normal ($6.06\pm1.29 \times 10^{5}$ cells/ml) as well as in Biogel-treated mice ($8.83\pm0.02 \times 10^{5}$ cells/ml) compared to AIRmin (0.90 ± 0.20 and $2.38\pm0.19 \times 10^{5}$ cells/ml, respectively). AIRmax BM cultures had 60% mature neutrophils ($CD38^{-}GR1^{+}$ cells) and AIRmin, 35%. Inflammation-induced extrinsic factors, such as cytokines, probably modulate cell-intrinsic factors, which can trigger distinct mechanisms toward self-renewal of HSCs and consequent differentiation into granulocytes in these lines.

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7.15 Testing the potential of adipose tissue-derived stem cells in the treatment of equine endometriosis

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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 within fibrotic foci. Modifications induced by the disease alter the surface of the endometrium which, as a consequence, lead to infertility (impaired embryo implantation). 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. To the best of our knowledge, there is no information about the use of stem cells derived from equine adipose tissue, for the treatment of endometrosis. **Objectives:** The aim of the present work was to test the use of equine heterologous adipose tissue-derived stem cells to reduce the inflammatory process and to remodel periglandular fibrotic tissues affected by endometrosis. **Methods:** Equine heterologous adipose tissue-derived stem cells, previously obtained and characterized by our group, were used. Six estrus synchronized mares suspected of endometrosis (four for treatment and two for control) and three normal mares, also syncronized, 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, as well as anti-CD 10, anti-cytokeratin (CK) 22, estrogen receptor, progesterone receptor and anti-α-actinin antibodies. Equine undifferentiated 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 $2.5x10^7$ stem cells. The two control mares were infused with a placebo. After 7, 15, 25 and 35 days, new uterine biopsies were obtained and remodeling of uterine tissues by stem cells was analyzed following protocol above. **Results and Discussion:** Histological changes were observed in the uterine biopsies of the 4 treatment mares before treatment, which confirmed the presence of fibrotic periglandular tissue in affected animals. Analysis performed 7 days after treatment confirmed the presence of injected cells in the uterine tissues, by red fluorescent signal provided by vital dye, while control animals did not show fluorescence. No signs of immunologic reaction in response to application of cells were recorded, since the presence of T-cells infiltrating at the site of cell graft was not observed. The presence of equine stem cells within uterine tissues was confirmed. Further investigations are needed in order to prove the remodeling of uterine tissue by stem cell differentiation.

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7.16 Differential protein expression in snake venom gland in quiescent and activated stages

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Introduction: The venom gland of viperid snakes has a central lumen where the venom produced by secretory cells is stored. When the venom is lost from the lumen, the secretory cells are activated and new venom is produced. The production of new venom is triggered by the action of noradrenaline on both a- and b-adrenoceptors in the venom gland. We have shown that the protein composition of the venom gland changes during the venom production cycle. Noradrenaline released just after venom extraction regulates the activation of transcription factors and consequently regulates the synthesis of proteins in the venom gland, which is important to activate the venom gland for venom production. **Objectives:** The aim of this study was to further analyze the proteins of venom gland that are involved in venom gland activation by noradrenaline released just after losing venom from the lumen. **Methods:** Venom gland were obtained from female *Bothrops jararaca* snakes in the quiescent stage (N=3) and in activated stage (4 days after venom extraction, N=3). Extracts of these glands were prepared and the proteins were analyzed by two-dimensional gel electrophoresis (2-DE). For the first dimension, isoelectric focusing was done in precast IPG strips (18 cm, pH 3-10) at 20ºC. For the second dimension, the IPG strips were applied to 12.5% SDS-polyacrylamide gels. Gels were run in triplicate, stained with Coomassie Blue G for 4 days and the density of the spots was quantified using ImageMaster 2D Platinum 7. **Results and Discussion:** The 2-DE images of venom gland extracts showed stained spots with PI ranging from 4 to 10 and molecular mass ranging from 200 to 7 kDa. Comparison between venom gland in quiescent stage and in activated stage pointed out that different proteins are expressed. The analysis of the gel showed that 15 spots of protein were expressed in both stages, but only the density of two of them was significantly higher in the extract of venom gland in quiescent stage ($p < 0.05$). Specific spots were found in each stage. The presence of 260 spots was observed only in the extract of venom gland in quiescent stage, whereas 216 spots were observed only in the extract of venom gland in activated stage. The data obtained from 2-DE analysis demonstrates that a great variation in protein expression occurs between venom glands in quiescent and activated stage. These results are in accordance with our previous results using one-dimensional electrophoresis. The identification of these proteins allows us to understand the mechanism of venom gland activation with consequently new insights into the regulation of exocrine glands of mammals.

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7.17 Production of recombinant FGF2 18 kDa and 22.5 kDa in *Escherichia coli* to probe FGF intracellular signaling in mammalian cell lines

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Introduction: FGF2 (fibroblast growth factor 2), the prototype member of the FGF family, has multiple molecular species, sharing a C-teminal sequence of 155 amino acids, which are translated from different initiation sites of the same mRNA. The smaller of the family, FGF2 (18 kDa), is released to the extracellular milieu and binds to specific receptors (FGFR), initiating a complex array of signals with paracrine and autocrine functions. On the other hand, larger isoforms of FGF2 (21, 22, 22.5 and 34 kDa) are intracellular species, which are translated from alternative codons (CUGs) and remain inside the cell interacting with unknown partners to play still undefined intracrine functions. Recently, our group demonstrated that FGF2 (18 kDa) blocked the cell cycle and induced senescence in ras-dependent malignant cells, in spite of triggering mitogenic pathways ERK-MAPK and PI3K. However, FGF2 (22.5 kDa) triggers only the mitogenic pathway in the same cell lines. **Objectives:** Our objective was to produce the recombinant proteins: FGF2 (18 kDa) and FGF2 (22.5 kDa) with and without His or protein A tags. These recombinant proteins are important for later search of intracellular partners of both FGF2 18 or 22.5 kDa, aiming to elucidate FGF2 signaling in mammalian cells. **Methods:** Different *E.coli* strains were utilized (DH-10β, DH-5a and ArticExpress). These strains were transformed with cDNAs of FGF2 (18 and 22.5 kDa), which was subcloned in pET vector system. Selected colonies were grown in LB medium at 37°C and induced by different IPTG concentration (0.1-1.0 mM). Bacterial lysates were submitted to SDS-PAGE and Coomassie staining to visualize the best concentration of IPTG, and purification was done in the FPLC system. **Results and Discussion:** The expression of FGF2 (18 and 22.5 kDa) with protein A tag in *E. coli* DH-10β was successful on a small scale (10 mL) and, presently, biologic assays and larger scale production are underway. FGF2 (18 kDa), without tags, was cloned in pET-3d plasmid and expressed in *E.coli* (BL21) with 0.5 mM IPTG. Cell lysates were fractionated in an Akta FPLC system using a NaCl gradient. The fraction eluted at 1.7 M NaCl is under analysis using an MS/MS system.

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7.18 Effect of protease-activated receptor-2 activating peptide on B1 cell spreading and its modulation by the C-terminus of the calcium-binding protein S100A9

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Introduction: B1 cells, found in the peritoneal cavity of mice, are able to differentiate into phagocytes. They show similar functions compared to macrophages, suggesting their importance as a new mononuclear phagocyte in the inflammatory process. A number of serine proteases display diverse extracellular and intracellular functions during inflammation via protease-activated receptors (PARs). We have shown that PAR-1 and -2 influence the spreading and phagocytosis by adherent peritoneal cells in mice, and that the C-terminal peptide from murine S100A9 (mS100A9p) inhibits the increment induced by PAR-1 activating peptide in these events. To date, however, the participation of PARs and mS100A9p has not been studied in B1 cell function. **Objectives:** In this work, we evaluated the ability of synthetic PAR-1 (PAR_1-AP) or PAR-2 (PAR_2AP) activating peptides to interfere with B1 spreading, and the putative modulatory effect of mS100A9p on this phenomenon. **Methods:** B1 cells obtained from stationary cultures, originated from the peritoneal cavity of Swiss mice, were cultivated and plated on glass coverslips ($2x10^5$ cells/coverslip) in 0.5 mL of R10 medium/well (control) in order to carry out spreading assays. In addition, B1 cells were incubated in R10 medium containing PAR_1AP (20 or 40 mM/well) or PAR_2AP (5, 10, 20 or 40 mM/well). The effect of the reverse PAR_2AP peptide (5 or 10 mM/well) was also evaluated in the presence or not of PAR_2AP. In addition, B1 cells were treated with mS100A9p (1.17 or 2.35 mM/well) during the spreading period (24 h) in the presence or not of PAR_2AP (5 mM/well). Data were generated by evaluating cells by phase contrast microscopy. **Results and Discussion:** The results demonstrated that only PAR_2AP, at all concentrations tested, increased the spreading ability of B1 cells, and that this effect was blocked by its reverse peptide, suggesting the specificity of this receptor on B1 cell spreading. The reverse peptide of PAR_2AP *per se* did not interfere with B1 spreading. However, mS100A9p inhibited not only cell spreading in the control group, but also the increase in spreading induced by PAR_2AP. These findings demonstrate for the first time that B1 cells respond to the PAR-2 agonist, and that this effect is modulated by mS100A9p. The model used here, in conjunction with the data shown, may be used as a tool for providing a better understanding of the involvement of PARs and B1 cells in the pathophysiology of the inflammatory process, as well as for using mS100A9p as a modulatory molecule to control the function of inflammatory cells.

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7.19 Brazilian advances in stem cell technologies: first report about successful and efficient production of induced pluripotent stem cells

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Introduction: Induced pluripotent stem (iPS) cells have emerged as an alternative to human ES cells, which allows us to overcome ethical problems of embryo destruction and to obtain patient matched pluripotent stem cells for future therapies. The marathon of iPS cells started when Yamanaka in 2006 derived these cells by forcing expression of certain genes in fibroblasts, reversing them into the pluripotent state similar to that of embryonic stem (ES) cells. iPS cells can be generated by reprogramming human terminally differentiated cells and precursors by the overexpression of specific genes, such as Oct3/4, Nanog, Sox-2, c-Myc, Klf-4, which normally express in early development. However, fibroblasts show low efficiency and slow reprogramming compared to other somatic cell types. Indeed, more immature somatic cells and cells isolated from younger organisms have shown higher efficiency of reprogramming. Previously, our group isolated a unique population of immature dental pulp stem cells (IDPSC) from deciduous teeth, which we used as a source for iPS cells generation. IDPSC is a very attractive cell type that can be easily isolated from an assessable tissue of young patients. IDPSC show fibroblast-like morphology, retain characteristics of adult multipotent stem cells and express at least one transcription factor, Oct4. **Objectives:** The aim of the present work was to derive induced pluripotent stem cells from human immature dental pulp stem cells. **Methods:** Using four of Yamanaka's factors, we easily derived iPS from IDPSC, which were named IDPS-iPS. Reprogramming of IDPSC occurred under feeder-free conditions, allowing simple pluripotent colony harvest and avoiding future problem of zoonoses. In order to evaluate teratoma formation, one million iPS cells were injected into nude mice. These reprograming cells were characterized by RT-PCR and immunofluorescence using some specific markers. Karyotype analyses were also performed. **Results and Discussion:** Such reprogrammed cells display all characteristics of human ES cells especially with respect to differentiating capacity. Multiple small colonies were already observed five days after injection. IDPS-iPS cells generated teratomas after intramuscular injection into nude mice demonstrating a wide range of differentiated tissues. It was reported that the suppression of p53 gene increased efficiency of reprogramming, suggesting its use as an improvement tool. Although IDPSC strongly express p53, they show high reprogramming efficiency (2.8%). The suppression of p53 is dangerous because iPS cells carrying genetic aberrations, although showing normal iPS cell morphology, can be obtained. Thus, IDPSC-derived iPS cells show an advantageous cell type for future therapies, since they do maintain expression of p53, while control human ES cells do not, suggesting that IDPSC could be safer for application in stem cell therapies.

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7.20 Immature dental pulp stem cells: biotechnological product ready to go through stem cell-based therapy market

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Introduction: Human immature stem cells from dental pulp (IDPSC) are originated from the neural crest and express embryonic, mesenchymal and epithelial stem cell markers in vitro. They appear to be less problematic, as they are not ethically controversial and painful source. In addition, IDPSC are widely multipotent, not tumorigenic, and maintain their ''stemness'' through several serial passages. Because of short population doubling time, these cells can be scaled up to large numbers. Due to these characteristics, they are considered the most promising tool for regenerative medicine and tissue engineering. **Objectives:** Aiming at establishing a cell bank of IDPSC, we compared IDPSC isolated at the very beginning (early) of dental pulp tissue culture and after three months (late). **Methods:** The phenotype and proliferative potential and also differentiation capacity were evaluated, when IDPSC were cultured in different media. Immunofluorescence and RT-PCR analyses were performed using mesenchymal/embryonic and epithelial stem cell markers. IDPSC were submitted to neural/chondrogenic and myogenic differentiation and were confirmed by morphological, immunofluorescence and specific staining. **Results and Discussion:** Our data demonstrate that IDPSC can be easily isolated and successfully expanded *in vitro*, while maintaining their undifferentiated status, preserving their properties even after cryopreservation. Furthermore, dental pulp tissue can be maintained for a long time in culture providing an unlimited source of IDPSC for future therapeutic applications. We also demonstrated that no significant changes in proliferative potential, stem cell marker expression profile and differentiation capacities in both, early and late isolated IDPSC populations, were observed. These data can contribute significantly to the practical application of IDPSC in regenerative medicine. Therefore, this report describes that the hurdles of adult stem cell technologies have been overcome in the case of IDPSC. These stem cells represent a biotechnological product ready to go through the stem cell-based therapy market.

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7.21 HPV genotype distribution in women seen for routine examination in health department, Ouro Preto, MG, Brazil

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Introduction: Human papillomavirus (HPV) is widely distributed in the world and virus persistent infection is recognized as an important cause for the development of cervical cancer. Primary lesions detected by cytological examination can progress or regress spontaneously.The challenge in cervical cancer screening is to detect the risk of progression to cancer. Besides the presence of the virus, the main determinants of clinical progression of cervical cancer include the involvement of high-risk viral types, viral load, integration of viral DNA in the host chromosome and interaction with different co-factors. **Objectives:** We investigated the types of HPV in women of the city of Ouro Preto, MG, relating to HPV cytological alterations and cervical cancer development. **Methods:** Patients received at the City Health Department for routine gynecological were submitted to anamnesis for assessing socio-demographic characteristics, including family, sexual and reproductive history. Cervical samples were collected for cytological examination and molecular analysis (HPV). Detection and typing were performed by polymerase chain reaction with primers MY09/11, RFLP and sequencing. **Results and Discussion:** We evaluated 569 patients, the majority being from the urban area (63.6%), married (63.4%), with only 1 sexual partner (49.6%). The overall HPV prevalence was 23.4% and 56 women (10%) showed cellular changes in the Papanicolaou test: 33 women had cellular atypia of undetermined significance possibly not neoplastic (ASCUS), 8 showed no cellular atypia discarding injury of high degree(ASCH), 1 had non-neoplastic glandular cells (AGC-SOE), 13 had squamous intra-epithelial lesion of low-grade(LSIL), and 1 had squamous intra-epithelial lesion of high-grade(HSIL). A total of 97 women had viral type identified by PCR-RFLP, and 15 had viral type identified by sequencing. Among the patients, 60% had infections with oncogenic HPV high risk, 21% with low risk HPV, and 3% of indeterminate oncogenic risk HPV, while 16% had multiple viral infections. The most common HPV types were HPV 16 (34.6%), HPV 6 (9.8%), HPV 18 (6%), HPV 53 and HPV 61 (5.3%).The data show the presence of different viral types in the city of Ouro Preto in women with or without cytological alteration, and cytological studies indicate that prevalence studies should be conducted prior to vaccination approaches, for the survey and understanding of the determinants of the development and progression of cervical cancer.

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7.22 Analysis of codon 72 polymorphism of *p53* gene in women from Ouro Preto, Minas Gerais

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Introduction: The p53 gene (17p13.1) has 11 exons, where the first is not transcribed. The p53 protein has 393 amino acids, with four segments with distinct functions. The p53 protein is important in carcinogenesis by acting in the preservation of genomic integrity, the transcriptionally active p21 gene, inducing the synthesis of protein p21. p53 activates the gene GADD-45 (growth arrest DNA damage inducible) which acts in correcting DNA lesions. The p53 protein also activates genes involved in the mechanism of apoptosis and suppresses the action of anti-apoptotic genes. The E6 protein of high risk human papillomavirus is able to bind to p53 leading to its rapid degradation. Codon 72 has different alleles, causing the insertion of different amino acids in this position of the protein: arginine (Arg - GCC) and proline (Pro - CCC), generating the genotypes: Arg/Arg, Arg/Pro and Pro/Pro. **Objectives:** The aim of the study was to determine the frequency of polymorphism in codon 72 of the p53 gene in 348 randomly selected women undergoing routine gynecological examination for HPV detection. **Methods:** Analysis was performed in cervical samples obtained from 482 women of Ouro Preto, Minas Gerais. The polymorphism at codon 72 of exon 4 of gene p53 was determined, using the PCR technique, with specific primers for each allele. The PCR products were analyzed in a 2% agarose gel by electrophoresis. **Results and Discussion:** The frequencies of genotypes were: Arg/Arg 41% (197), Arg/Pro 47% (228) and Pro/Pro 12% (57). HPV was found in 13.0% (65) of patients; 9.0% (42) showed relevant alterations in cytological examination. Correlating cytological alteration and the genotypic frequencies, it was possible to determine the following. ASC-US: Arg/Arg 14.0% (6), Arg/Pro 23.0% (10), Pro/Pro 17.0% (7). LSIL: Arg/Arg 12.0% (5), Arg/Pro 10.0% (4), Pro/Pro 7.0% (3). ASC-H: Arg/Arg 10.0% (4), Arg/Pro 5.0% (2) Pro/Pro 2.0%(1). The genotype Arg/Arg was not more frequent in samples verified as ASC-US and LSIL, but was more frequent than samples cytologically showing a more severe diagnosis.

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7.23 Epithelial lineage fate of human immature dental pulp stem cells

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Introduction: Human immature dental pulp stem cells (IDPSC) are of neural crest origin, and they express *in vitro* a set of human embryonic stem (ES) cell, and mesenchymal and neuroepithelial stem cell (MSC and NESC) markers. We already showed that they were able to reconstruct corneal epithelium of the burned eye surface in an animal model, producing a complex tissue composed of five different cell types, suggesting their capacity to respond adequately to environmental signals and to differentiate into the tissue of interest. **Objectives:** In order to extend our knowledge about epithelial lineage fate of IDPSC, we tested the capacity of these cells to populate germinal epithelium. How can this fate of IDPSC be influenced after transplantation into the testis of fertile (normal environment) and infertile (deficient environment) mice? **Methods:** IDPSC from patients of both sexes were used. Different IDPSC transplantation experiments were performed. First, a cell suspension (10^5 cells) was stained with Vybrant CM-DiI. Additionally, LacZ or GFP gene reporters were introduced into IDPSC. In addition, cells were injected into the testis of fertile mice. The mice were killed after three, five and nine days. Second, to destroy endogenous spermatogenesis, the recipients were irradiated and cells were injected directly into seminiferous tubes. Mice were killed three months after injection. Control mice were injected with physiologic solution. Thin whole-testis sections were prepared. Presence of IDPSC in mouse testis was detected by Vybrant CM-DiI and by expression of the enzyme β-galactosidase or GFP by confocal microscopy. In addition, fluorescent *in situ* hybridization (FISH) analysis using a probe for human sex chromosome was carried out. **Results and Discussion:** Our data demonstrated that after IDPSC transplantation into testis of fertile mice, fluorescent signals were observed within cross-sections in several seminiferous tubules (ST). In fertile mice, the process of population and proliferation was quick. Three days after transplantation, hIDPSC were detected in Leyding and Sertoli cell compartments. On the 5th and 9th days, these cells formed fluorescently labelled clusters similar to those defined as germ cell associations within mouse ST. FISH analysis confirmed this finding and one X chromosome signal was detected in adjacent nuclei of cells with human sperm-like morphology. Three months after injection in ST of infertile mice, expression of GFP or β-galactosidase was demonstrated in supporting cells and in the lumen. Control mouse ST (without hIDPSC) did not show any fluorescence or staining. We determined if differentiation of IDPSC can be a result of the fusion between mouse and human cells. Fusion between cells was observed at a low frequency, and it was only between cytoplasm and not nuclei. We used four different approaches in order to provide clear evidence of hIDPSC survival, proliferation and differentiation in mouse testis. In contrast to previous studies, which used human spermatogonial stem cells able to survive only in mouse testis, we demonstrated that IDPSC showed colonization of different compartments of mouse testis. This work also confirms our previous observation that IDPSC show a strong epithelial lineage fate.

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7.24 Immunochemistry analysis of primary cell lines obtained from BPV-related lesions

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Introduction: Bovine papillomavirus (BPV) is a group of DNA oncoviruses from the family *Papillomaviridae*, which affect cattle, causing warts (papillomas and fibropapillomas), and cancer in the alimentary tract and urinary bladder associated with co-factors. BPV is the first of this group reported to infect species other than cattle (horses and donkeys), and it is also considered an excellent model for comparative studies (as comparing to HPV). **Objectives:** We present here a study of morphological characterization of primary cell lines from skin papilloma, esophagus papilloma and bladder mucosa of animals affected by BPV, which evaluated the expression of cell type-specific intermediate filaments and the presence of BPV-1 proteins. **Methods:** To establish cultures, fragments were collected from lesions detected in animals affected by papillomatosis and a sample of skin without visible lesions used as control. The fragments were incubated in DMEM (Cultilab ™), supplemented with 10% fetal bovine serum and maintained at 37 ° C in an atmosphere of 5% CO_2. All the animals were tested for the presence of viral DNA in the lesions and in different passages of the culture by PCR using generic and specific primers. The cultured cells were also labelled with the cell type-specific antibodies anti-vimentin and anti-pan-cytokeratin (Sigma) for cytological characterization and with anti-BPV-1 antibody (BPV-1 H8 Abcam plc, Cambridge). **Results and Discussion:** The animals showing lesions were confirmed as BP-positive for BPV-1, BPV-2, and some for BPV-4. All cell lines were stained vimentin-positive, which is the intermediate filament that characterizes mesenchymal cells. The same cells were positive for pan-cytokeratin, a marker of cells of epithelial origin, and the most evident expression were found in the esophagus papilloma and bladder mucosa cells. The cell lines were also stained by anti-BPV-1, mainly in the perinuclear region. This double labelling feature demonstrated in the cell lines has already been reported in certain types of metastatic cells, characterizing a process called epithelial-mesenchymal transition (EMT): cells show simultaneous cytokeratin and vimentin characteristics. Another point to consider is that cytokeratin genes in cells with malignant growth have been reported with altered expression, which could indicate the expression of this filament as a possible element for diagnosis in cancer. The positive staining for BPV-1 agrees with the PCR results, indicating viral presence in the cell lines, at least the major segment capsid protein is stained by this antibody.

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7.25 Discussing chromosome aberrations related to BPV in peripheral lymphocytes of cattle (*Bos taurus*)

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Introduction: There are 10 bovine papillomavírus (BPV) types that in synergy with carcinogens such as quercetin can trigger cellular changes. Host chromatin alterations induced by viruses, mainly due to the expression of viral oncogenes, per se significantly increase the number of cells with abnormal centrosomes leading to mitotic aneuploidy and other structural and numerical aberrations. **Objectives:** This study aimed to evaluate chromosomal aberrations resulting from this instability in host chromatin of BPV-infected cells. **Methods:** Peripheral blood samples were collected from *Bos taurus* females. Viral detection was performed using PCR, and lymphocyte cultures were established for cytogenetic analysis. A total of 61 animals were analyzed in a "blind-test" in which slides were submitted to conventional staining, C-banding and silver nitrate staining to observe centromere structure and the nucleolar organizer regions (NORs). Twenty-eight (28) animals were verified as non-infected by BPV. Thirty-three (33) animals showed the presence of BPV. The BPV-infected group was divided into two groups: symptomatic (severe cutaneous papillomatosis) and asymptomatic. **Results and Discussion:** The symptomatic group (17 females) exhibited an average of 42.71% aberrant cells/ individual. The asymptomatic group (16 animals) exhibited an average of 40.19%. The analysis included 50 to 100 cells/sample, total of 2203 cells: 918 with one or more chromosomal aberrations. The chromosomal aberration rate of the control group was 4±2. The chromosomal aberrations identified in this study were: centric association (CA), acentric fragment (AF), telomeric association (TA), telomeric association with a single chromatid (TAcr), chromatid breaks (CtB), chromosomal breaks (CmB), gaps; aneuploidy, polyploidy, addition or loss of chromosomal segment (add or del) and early chromatid separation (EcrS). The AT, ATcr, add or del SPcr are not frequently observed in bovine cells infected with BPV. However, "in vitro" HPV-infected human cells have been described showing similar alterations. Gaps were very frequent in this study; however, we did not consider the gaps as an important chromatin lesion, due to eventual subjective analysis. The differences observed compared to the control support the inclusion of these data. The analysis of NOR localization in autosomal distal telomeres, emphasizes that the centric association described here is related to BPV actions on proximal telomeres. Associations of distal telomeres are not stable and thus difficult to detect.

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8. Animal Biology

8.01 Morphological study of the skin and characterization of the cutaneous secretion in the tree frog *Aparasphenodon brunoi*

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Introduction: One of the most important functions of the amphibian skin is the chemical defence against predators and microorganisms through the secretion of toxins. *Aparasphenodon brunoi,* is a casque-headed tree-frog inhabiting the Atlantic forest from São Paulo up to Espirito Santo. This species is frequently found inside axils of bromeliads, using the head as a lid to close the entrance and protect itself from possible predators, in a behavior known as phragmosis. **Objectives:** The aim of this study was to examine the skin morphology of *Aparasphenodon brunoi* and to present a preliminary biochemical and pharmacological characterization of its cutaneous secretion. **Methods:** Fragments of the dorsal, ventral and inguinal skin were fixed in paraformaldehyde and embedded in glycol methacrylate. The whole head was fixed in Bouin and decalcified in EDTA. Histological sections were stained with toluidine blue-fuchsin or HE. The skin secretion *in toto* was analyzed by SDS-PAGE, and the cytotoxic potential was evaluated by the MTT method using B16F10 murine melanoma cells and L929 fibroblast cells. The antimicrobial potential was tested against two bacterial strains, *Escherichia coli* GFP and *Micrococcus luteus.* **Results and Discussion:** The dorsal skin, including the head, shows a considerable number of granular (or venom) glands while the ventral and inguinal skin, richer in mucous glands, is specialized in water absorption, with prominent *verruca hydrophilica*, a well developed vascularization and the absence of a calcified dermal layer (which is present in the dorsal skin). These skin characteristics are in accordance with the phragmotic behavior of the animal, in which the dorsal skin and the head are much more exposed to water loss and to possible attacks of predators. SDS-PAGE revealed the presence in the skin secretion of at least three different proteins varying between 18.4 and 35 kDa. Different levels of cytotoxicity were observed for both cell lines tested, indicating the potential for further pharmacological studies. This toxicity may be related to the large number of granular glands present in the dorsal skin of *A. brunoi* and is probably used as chemical defense against predators and microorganisms in general. For the two bacteria tested, however, the secretion did not show any effect.

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8.02 Sexual dimorphism in the skin of the tree frog *Aplastodiscus leucopygius* (Amphibia, Anura) correlated to courtship behavior

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Introduction: Amphibian skin is mainly involved in respiration, chemical and mechanical protection, water and ion transportation, sensory reception, temperature control and reproduction. Among amphibians, the anurans possess the greater diversity of reproductive modes. In the family Hylidae (the tree frogs), the genus *Aplastodiscus* shows a sophisticated courtship behavior involving male vocalization, mutual tactile stimuli and, possibly, chemical signaling. Using auditive and tactile stimuli, the male gradually guides the female from the vegetation down to a subterranean nest, where oviposition occurs. **Objectives:** The aim of this study was the comparison of the skin glandular distribution and morphology between males and females of *Aplastodiscus leucopygius* to determine: 1) the occurrence of sexual dimorphism and 2) the possible participation of chemical signaling during courtship behavior, through the correlation of gland distribution with the tactile stimulated regions. **Methods:** Skin fragments from the dorsal, ventral, inguinal, dorso-lateral, mentonian and cephalic regions were fixed in Karnovsky and embedded in historesin. The sections were stained with toluidine blue-fuchsin and submitted to PAS (for mucus substances) and bromophenol blue (for proteins). **Results and Discussion:** The skin of both males and females shows a large number of glands of three different types, mucous, mixed and granular (or venom) glands. In the males, a fourth type of gland is present, with peculiar morphological characteristics. The mucous glands are acinar, formed by a monolayer of cells, which are positive mainly for PAS. The mixed glands are also acinar and show two different types of cells, one positive for PAS and the other positive for bromophenol blue. The granular glands are syncytial and are intensely positive for bromophenol blue. The differentiated glands appearing exclusively in the males are also acinar, but the cells, all of the same type, are columnar and full of tiny spherical glands which are strongly positive for bromophenol blue and PAS. The large number of glands present in the skin of *A. leucopygius*, mainly in the dorsum, is uncommon when compared to the skin of other hylids. This is an indication that these animals have a great dependence on skin secretions for their survival. The presence of a differentiated gland exclusive in males, mainly in the mentonian and dorso-lateral regions of the skin, can be associated with the touching behavior during courtship and is probably related to the secretion of compounds for chemical signaling between the male and female.

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8.03 Diet and feeding behavior of turtles and lizards kept in the Anexo da Recepção, Pátio Externo da Casa Vital Brazil, Herpetologia, Instituto Butantan

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Introduction: Reptiles are ectodermic; they depend on external heat to regulate temperature, which is important for their metabolism and behavior. They require favorable conditions for their maintenance. Their feeding is related to energy flow and temperature. With species kept in captivity, suitability of enclosures to ensure efficiency in thermoregulation is recommended. **Objectives:** The aim of this study was to analyze the diet and feeding behavior of different species of turtles and lizards kept in captivity for three years. **Methods:** This work was carried out in the Anexo da Recepção, Pátio Externo da Casa Vital Brazil, Herpetologia, Instituto Butantan, since 2007, with those reptiles brought to the Reception. Every selected species received a record in the admission in order to detail the procedures, mass (in grams), vermifuge, microchip, quarantine, daily observations about the diet and feeding behavior. The *Trachemys* are placed weekly in a swimming pool to eat newborn animals, various arthropods, and fodder when it is available. *Phrynops*, *Hydromedusa* – wild and *Chelidra* and *Apalone* – exotics, are conditioned in plastic boxes with water and substratum and are given feed, neonates and fishes. Land turtles (*Geochelone)* stay free in an open place with a shelter and water available. They feed three times a week with a varied diet. *Iguanas* are kept in a heated yard, and feed on various items and eat *hibiscus* flowers daily, and occasionally eat *tenebrios*. The adult, omnivorous teius (*Tupinambis*) that settle down in the same place where turtles stay, except the young kept in the terrarium, receive diverse food; the offer and the quantity of food vary depending on the size and weight. Food is provided as donations from the employees and the local restaurant. **Results and Discussions**: It is notorious and proved in the different seasons of the year, that these reptiles make use of feeding for energy control and thermoregulation. The species consume the food items according to their biology, especially in the warm seasons because they need additional energy value to maintain metabolism and spend on their activities. In the coldest months, the acceptance of food is lower, just because they are not active. Land turtles when not heated, do not eat and easily become sick. Despite that teius hibernate in the winter; we can observe a dispute of food and territory among them. We have also observed that the animals are adaptable to the different kinds of food, due to a variety of food offered. This work was hampered since the register of entry and records of feeding were lost during the fire at Butantan Institute last May. Despite that, we have compiled the available data for the conclusion of this compendium.

8.04 Seasonal changes of the renal sexual segment in the male reproductive cycle of *Sibynomorphus neuwiedii* (sleep snake)

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Introduction: In neotropical regions, reproductive aspects of snakes are poorly known, especially for species of the family Dipsadidae (non-venomous snakes). The male reproductive cycle of the Squamata (snakes, lizards and amphisbaenians) is inferred by changes in length/ weight of the testes and vas deferens and mainly seasonal changes of the renal sexual segment (RSS) during the reproductive season. The RSS is an androgen-dependent organ present in the distal urinary tubules of some reptiles, such as the Squamata, but absent in turtles and crocodilians. The hypertrophy of these tubules is caused by increased production of sexual granules, which indicates the species' breeding season. This hypertrophy of the renal sexual segment, is also related to sexual behaviors such as combat ritual, court and copulatory guard. **Objectives:** The aim of this work was to determine the influence of the RSS at the time of mating *Sibynomorphus neuwiedii* (sleep snake). **Methods:** The cranial portion of the right kidney of 27 males *Sibynomorphus neuwiedii* was processed for light microscopy by the methods of hystoresin (glycol metacrylate, Leica) and paraffin. The material was sectioned (2-5 mm) and stained with toluidine blue-fuchsin and hematoxylin-eosin. After preparation of the slides seasonal variations in the diameter of the tubules, epithelial height, density of granules and staining intensity of granules of the RSS were recorded. Variations in tubule diameter and epithelial height were tested by analysis of variance (ANOVA). **Results and Discussion:** Structural changes were noted in different seasons, with significant increase in tubular diameter ($F = 20.6$, $n = 270$, $df = 3$, $p = 0.0002$) and epithelial height ($F = 23.9$, $n = 270$, $df = 3$, $p = 0.0001$) of the RSS in the spring-summer. The presence of many eosinophilic granules intensely stained and scattered throughout the cytoplasm of the cell in the spring- summer indicated a higher activity when compared to autumn-winter. The RSS of the *Sibynomorphus neuwiedii* shows a seasonal cycle characterized by a hypertrophy of the distal convoluted tubules in the warmer seasons of the year. The hypertrophy of the RSS in the spring-summer coincided with increased Leydig cell nucleus (mating), spermatogenic activity and presence of sperm in the female, previously described in the species. The data presented in this paper indicate that it is possible to determine the mating season in *Sibynomorphus neuwiedii*, through the study of the RSS.

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8.05 Early learning in prey preference of the lynx spider *Peucetia rubrolineata* (Oxyopidae)

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Introduction: Spiders are known as generalist predators, although many species show preferences for certain types of prey. For some species, it is not clear which mechanisms are involved in the establishment of this kind of behavior. However, studies indicate that prey preferences in spiders of the family Oxyopidae could be related to imprinting processes, which occur early in the development of this animals. **Objectives:** This work focused on the study of prey choice in young spiders *Peucetia rubrolineata*. **Methods:** In the present study, two types of prey were offered to spiderlings of *P. rubrolineata:* newborn crickets (*Gryllus* sp.) and fruit flies (*Drosophila* sp.). The prey preference was tested using a choice test. Three trials were performed and each one with four combined variables: type of prey (crickets or flies), animal's age (five or 15 days), time of exposure to prey (five or ten days) and the amount of prey offered (one or two). **Results and Discussion:** Among the variables used, animal's age (15 days after leaving the egg sac) and time of exposure (ten days) were the most efficient parameters in the establishment of prey preference. When well fed, spiderlings prefer adult flies (*Drosophila* sp.) over crickets (*Gryllus* sp.). However, when spiders are starved, the preference is for the first type of prey offered. In the second case, we observed a primacy effect, an important factor in imprinting determination. The prey preference based in learning is advantageous in case of changes in the original environment of species, colonization of new habitats, as well as exploring new sources of food. This learning process involved in the establishment of food preference can lead to an optimization of feeding behavior, ensuring rapid adaptability to environmental conditions. It happens early in the development of these animals and certainly is important in the survival of these spiders.

Supported by: CAPES

8.06 Evolution of maternal care in spiders of the superfamily Lycosoidea

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Introduction: Spiders show a high diversity of parental care. These behaviors include from the construction of the egg sac to complex behaviors of extended care in social groups. In the superfamily Lycosoidea, females take care actively of the egg sac and spiderlings until dispersion. Categories of parental behavior in these spiders have been used as characters in some phylogenetic reconstruction studies. There is no consensus in the literature about the homology of these characters. Some authors use all parental behavior as states of one character,while others use just some aspects of maternal care divided into several characters. There is also confusion about the definition of the behavior of building a nursery web for spiderling care. Due to the complexity of this type of character, its delimitation should be made carefully and based on specific studies about these behaviors. In Lycosoidea, parental care has a high internal variability, which makes this group interesting for character delimitation. **Objectives:** Thus, this study proposed new characters to study the evolution of maternal care. **Methods:** By gathering literature data and also laboratory observations of maternal care, we propose 4 characters: care with the egg sac, construction of a silken retreat for the egg sac, helping the spiderlings to emerge from egg sac, and care with the spiderlings. We propose the homology based on the female behaviors and the structures built by her. **Results and Discussion:** The reconstruction of evolutionary history of these characters shows to carry the egg sac under the chelicerae, help the spiderlings to emerge from egg sac, make a silken retreat for the egg sac care and to guard the spiderlings until dispersion are plesiomorphic to superfamily Lycosoidea, and all these behaviors are found in *Pisaura mirabilis*. A synapomorphy of *Trechalea* sp. is to carry the spiderlings on the empty egg sacs and in Lycosidae (*Aglaoctenus* sp. and *Lycosa erythrognatha*) is to carry young on the female abdomen. Parental behavior has been important to phylogenetic studies of the Lycosoidea group. The characters proposed are conservative and there are low rates of homoplasy, so they can be suitable to the study of evolution and phylogenetic analysis.

Supported by: CAPES

8.07 *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wolbachia* sp. (Alphaproteobacteria, Rickettsiales): influence of infection on reproduction and morphological phenotype of the mosquito

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Introduction: *Wolbachia* is an intracellular alpha-proteobacteria endosymbiont capable of infecting various arthropods and nematodes. They are transmitted maternally through the cytoplasm of the egg. These bacteria can alter reproduction of their hosts, inducing feminization of males, parthenogenesis, male killing, cytoplasmic incompatibility and changes in reproductive fitness. The effects of infection may be advantageous or disadvantageous, depending on the host and the strain of the bacterium. One of their hosts is *Culex quinquefasciatus*, a nocturnal mosquito, cosmopolitan, urban, very common in Sao Paulo city and competent vector of filaria and arboviruses. Despite the medical importance of this mosquito and the biological relevance of *Wolbachia*, little is known about the association between these two *taxa*. **Objectives:** In this study we evaluated whether this endosymbiont influences the reproductive fitness and wing form of the mosquito. **Methods:** A colony of *Culex quinquefasciatus* naturally infected by *Wolbachia* was started with mosquitoes collected on the banks of the Pinheiros River in Sao Paulo city. Some mosquitoes were treated with antibiotic, tetracycline hydrochloride, in two consecutive generations in order to eliminate the bacteria. After obtaining a colony of uninfected mosquitoes, comparisons were made between reproductive fitness of infected and uninfected mosquitoes. These two groups of mosquitoes were also compared for the wing geometry to assess whether the phenotype of wing shape changes would suffer after elimination of bacteria. **Results and Discussion:** We found that infected mosquitoes laid fewer eggs and the viability of eggs was lower than that of mosquitoes uninfected. Furthermore, data from wing morphometry showed that the presence of bacteria does not modify wing shape of the following generation. The presence of bacteria does not interfere in the determination of wing shape of *Culex quinquefasciatus*, although it appears to influence the reproductive fitness of mosquitoes. How this bacterial endosymbiont causes such disturbance in this mosquito is still being investigated.

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8.08 A curious case of duplication in spermathecae of *Acanthoscurria suina* Pocock, 1903 (Araneae, Mygalomorphae, Theraphosidae)

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Introduction: Some anomalies are described in the Theraphosidae, as in *Eurypelma tripepii* Dresco, 1985 based on a single male which shows a tubercle on both chelicerae, character considered by Bertani as an anomaly and transferred to *Nhandu tripepii.* Other examples are the occurrence of siamese twinning or conjoining in 14 specimens of *Poecilotheria regalis* Pocock, 1899 found in a single egg sac, and a probable case in the specimen described by Schmidt in 1997 as *Nhandu tripartitus*, since he based his description on only one female holotype which shows three receptacles on the spermathecae. **Objectives:** The aim of this work was to present a case of duplication in spermathecae of *Acanthoscurria suina* Pocock, 1903. **Methods:** The specimens studied are deposited in Instituto Butantan, São Paulo (curator: I. Knysak), Museu de Ciências Naturais (E. H. Buckup) and British Museum of Natural History (Janet Beccaloni). Female epigynum was dissected and cleared in 85% lactic acid at 100ºC for observation of internal structures. The multifocal photos were taken using a Leica MZ 16A stereomicroscope with a Leica DFC 500 digital camera attached. **Results and Discussion:** The spermathecae of *A. suina* normally shows two lobes involved in a common base. Studying the material from Museu de Ciências Naturais, we found a curious duplication of the spermathecae. This fact notes the importance of having a large number of exemplars analyzed, since just one specimen with one or more different characters can be an anomaly and not a new species.

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8.09 Twinning, triplets and bicephaly in a clutch of the green snake, *Philodryas olfersii*

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Introduction: There are numerous reports of twinning and bicephaly in oviparous reptiles. Despite this, such phenomenon appears to be rare and most reports deal with isolated cases in captivity. However, reports of recurrence of twinning within a single clutch are virtually unknown. **Objectives:** Herein, we document the occurrence of twinning, triplets and bicephaly in a single clutch of the green snake, *Philodryas olfersii*. **Methods:** A clutch of 13 eggs was laid by a wild-caught female of *P. olfersii*. Eggs were measured, weighed and incubated in moistened vermiculite with temperature ranging from 22-28 °C. After the incubation period, 10 out of 13 eggs hatched and babies were measured, weighed and sexed. The three unhatched eggs were fixed and then dissected. Extra-embryonic membranes were excised and submitted to standard histological procedures. **Results and Discussion:** Dissection of the three eggs revealed that all contained dead, late-term embryos, each with varying amounts of residual yolk remaining. However, egg no. 1 contained two dead late-term dead embryos (twins) and egg no. 2 contained three dead late-term embryos (triplets). Both twins and triplets were connected to the same yolk sac through their own umbilical cords. Amnion and chorioallantoic membranes were observed in histological analysis. Moreover, twins and triplets were smaller than their normal siblings. All triplets showed cephalic abnormalities and one had the anal slit fully closed. Twins of egg no. 2 were seemingly biting each other inside the egg. Egg no. 3 contained just one dead late-term embryo showing bicephaly almost complete. At the dorsal region, two heads fused close to the eye position deforming the majority of supra-labials scales. At the ventral region, several abnormalities were observed, which makes it difficult to distinguish the separation of the two heads. Although twinning in oviparous snakes was already reported in the literature, the occurrence of more than one twinning within a single clutch is virtually unknown. Twinning in oviparous snakes is not an effect of incubation conditions because early embryonic segmentation (when twins are formed) occurs inside maternal uterus, and at oviposition embryos are well developed. The smaller sizes of twins and triplets in relation to their siblings are clearly explained by the partition of the same yolk during embryogenesis. Despite our twins, triplets and bicephalic dying before hatching, this is not a phenomenon incompatible with life. There are several reports of successful hatching of twins and bicephalic reptiles. The cause of hatching failure may be related to the several abnormalities present in the embryos.

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8.10 Skin morphology and cutaneous secretions support a case of müllerian mimicry involving the dart-poison frog *Ameerega picta* and the frog *Leptodactylus lineatus*

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Introduction: Cutaneous secretion of toxins plays a central role in amphibian defense against predation. Amphibian skin presents high densities of granular (or poison) glands, whose products and morphology vary among species. Highly toxic secretions are often associated with conspicuous color patterns, which are recognized and avoided by predators. This is the case of many dendrobatid frogs, such as *Ameerega picta*. Its color pattern is very similar to that of the presumably non-toxic *Leptodactylus lineatus*, assumed to attain protection through mimicry. **Objectives:** The aim of this work was the morphological and biochemical study of the skin of these two species, in order to assess the hypothesis of mimicry. **Methods:** Dorsal skin fragments were removed, fixed in paraformaldehyde and embedded in paraffin and historesin. For a histological study, paraffin sections were stained with hematoxylin-eosin, and historesin sections were stained with toluidine blue-fuchsin. For histochemical description, we used bromophenol blue for proteins, PAS for polysaccharides and alcian blue pH 2.5 for acid polysaccharides. Skin secretions were manually extracted in aqueous solution, lyophilized and submitted to SDS-PAGE and RP-HPLC monitored at 214, 280 and 339 nm. **Results and Discussion:** *A. picta* possess elliptical granular glands filled with non-protein granules rich in carbohydrates. *L. lineatus* shows spherical granular glands possessing protein granules. Accordingly, SDS-PAGE indicates great amounts of proteins in the poison of *L. lineatus* but not in that of *A. picta*. Chromatographic profiles further suggest marked differences in poison composition between these species. Moreover, both differ greatly in profiles of gland distribution: in *L. lineatus*, poison glands are organized in aggregates whose position coincides with colored elements of the dorsum, suggesting that poison disposition is announced to predators through skin colors. In contrast, *A. picta* shows glands distributed homogeneously and in lower densities than in *L. lineatus*. This simpler profile in *A. picta* suggests that its investment in chemical defense is rather qualitative than quantitative, in agreement with the high toxicity attributed to dendrobatids. In conclusion, our data suggest that both species studied are toxic, in contrast with assumptions that *L. lineatus* is non-toxic. Hence, *A. picta* and *L. lineatus* seem to transmit common warning signals to predators, probably benefiting from their resemblance. Rare among vertebrates, this class of mimicry is referred to as müllerian mimicry.

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8.11 Predatory behavior of *Chironius bicarinatus* (SERPENTS: Colubridae) WIED, 1820 in the exposition of the Museu Biológico of the Instituto Butantan

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Introduction: *Chironius bicarinatus* lives in the southern plateaus of Brazil reaching the northwest of Uruguay. It has as characteristic two keels in the vertebral scales, of green-bluish color, with black scales on the back and ventral side, with a black line appearing in zigzag on the subcaudal scales on the inferior side of the tail. It is non-venomous snake and it has average size reaching up to 1.80 m in length, and shows diurnal activity. Of arboreal habit, it inhabits bushes, orchards and gardens, where it feeds on amphibians, small lizards and birds, and it often goes down to the ground, and when threatened it quickly returns to the foliage of the trees. **Objectives:** The aim of this study was to observe the predatory behavior of the snake *C. bicarinatus* against prey species *Lithobates catesbeiana* offered in captivity. **Methods:** For a young specimen, female, *C. bicarinatus*, found in the Granja Viana - São Paulo, kept in a terrarium with environment with and not reflecting pool in favorable terms were offered frogs (n = 84) of the species *L. catesbeiana* adults and tadpoles, fortnightly. The specimen was fed during the period of 10 months, with 44% of adult frogs and 56% of the tadpoles. **Results and Discussion:** From a total supply of specimens of frogs of the species that were ingested, 33 of 35 adult frogs and 42 of the 49 tadpoles offered, of which there were three regurgitations of tadpoles and four tadpoles and two adult frogs offers not consumed. It was observed that the interest of the specimen, the food offered, was due to the movement that the tadpoles and frogs held in the water, where it was found a game based on the movement of prey. Its posture was predatory when more than half of the lower body attached to the branches of the enclosure, the other half semi-inflected facing the water surface, where the pace of issuance of the tongue (dart) was 10-20 times/min. After the movement of tadpoles or frogs, the specimen strikes a boat for the most accurate in moving prey. In the subsequent capture of the tadpole, the specimen retracts the posterior portion of the body, performing a movement of spring toward the branches of the enclosure space. After ingestion of the tadpole, mostly by the tail or the head of the adult frog, the specimen rubbed its mouth on the trunk or leaves the room to clean the viscosity present in its mouthparts. Although frogs are one of the main food items of some Colubrids snakes, as in the case of *C. bicarinatus,* more detailed records about the interspecific relationship are scarce in the literature and are an extremely useful study in *ex-situ* and *in-situ* to elucidate the behavior of this species.

Supported by: Fundação Butantan

8.12 Diet and feeding frequency of a fossorial snake (*Phalotris lativittatus*)

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Introduction: *Phalotris lativittatus* is a rare, fossorial and medium-sized snake distributed endemically in Cerrado vegetation areas of São Paulo State. Data on feeding habits are unknown, but like congenerics, its diet probably consists of other elongated fossorial vertebrates. **Objectives:** This work aimed to characterize the diet and feeding frequency of the snake *P. lativittatus*. **Methods:** We dissected 51 preserved specimens of *P. lativittatus* to examine the gastrointestinal contents. Specimens were categorized as 'fed' or 'not fed' and stomach content was collected only when prey identification was possible. In parallel, newborn (both live and dead) dipsadid snakes were offered as food to some captive *P. lativittatus* hatchlings to verify the acceptance and ingestion of this item. **Results and Discussion:** We found that 96.1% (n = 49) of the analyzed specimens showed gastrointestinal content. Taken separately, it was observed that 96.4% of the females, 92.8% of the males and 100% of the hatchling/young had gastrointestinal content. It is a strikingly very high and uncommon frequency of fed individuals rarely observed in snakes. To date, the diet of *P. lativittatus* was unknown and it was hypothesized to be constituted of amphisbenids and other elongated fossorial vertebrates. Our observations suggest that *P. lativittatus* diet may be specialized in amphisbenids. Although we were able to identify stomach contents only in three specimens, amphisbenids were observed in all cases. In two females, we observed only skin pieces of *Amphisbaena* sp. and in one hatchling we found an intact prey, identified as *Amphisbaena roberti*. In addition, in all captive feeding trials individuals always refused to feed on dipsadid snakes. Feeding on *Amphisbaena* sp. also suggests that predation events occur underground, because amphisbenids show marked fossorial habits. In conclusion, amphisbenids as food item is also present in other elapomorphini snakes known to date indicating a conservative feeding habit diet in the group. Additional data both in *P. lativittatus* and other elapomorphini are necessary to test this assumption.

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8.13 Comparative reproductive biology of Amphisbaenidae (Squamata, Amphisbaenia) from Brazil

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Introduction: Amphisbaenia are a group of fossorial Squamata reptiles, whose most representative family is the Amphisbaenidae. There are currently a limited number of studies available on their reproductive biology. The reproductive cycle was studied only in eight species, and the histology of genital ducts was studied in three species. The family Amphisbaenidae is represented by 65 species in Brazil. **Objectives:** This study aimed to characterize the reproductive cycle, morphology and anatomy of the genital tract of eleven species of the Brazilian genus Amphisbaena. We also aimed to compare the data obtained from different species and to discuss the results comparing it with morphological and molecular phylogenetic trees. **Methods:** The analyses were performed using museum specimens. The reproductive cycles of males were analyzed from the variation of testis volume and vas deferens diameter during the year. Histological analysis of the testis and vas deferens furnish data on spermatogenic cycle and sperm storage. The reproductive cycles of females were analyzed considering the variation in follicle length during the year, the observation of primary or secondary vitellogenesis and the occurrence of eggs. The oviducts were analyzed by light microscopy, for anatomical description and observation of morphological changes during the reproductive cycle. All data were compared among the eleven species, regarding the different ranges and biomes occupied by each one. **Results and Discussion:** All reproductive cycles observed until now are seasonal, with a short period of spermatogenesis and the occurrence of sperm storage by males in the vas deferens. Females' follicles usually show a major yolk deposition and size increase during the two or three months before ovulating. One to eleven eggs are laid by the female, which varies according to the species. In most species, the eggs are arranged in a line in the female's abdominal cavity. The exception is *Amphisbaena alba*, in which eggs can be arranged side by side.

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8.14 Morphology and natural history of rattlesnake populations (*Crotalus durissus*, Viperidae) from Brazil

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Introduction: Rattlesnakes (*Crotalus*) originated in North America and subsequently spread throughout Central and South America. The species show a wide variation in body size and color patterns. In addition, there is a similar variation in reproductive patterns across the genus with litters of 5 to 8 neonates in smaller species to litters of 15 to 40 neonates in larger species. These snakes generally eat vertebrates with smaller species feeding on lizards and larger species feeding on lizards when they are young, switching to small mammals and birds, when adults. In Brazil, *C. durissus* occurs throughout the country, except in the states of Acre and Espírito Santo. Some populations show considerable variation in morphology, reproduction and diet composition even with neighbor populations differing drastically from each other. **Objectives:** Our aim was to study the relationship between *C. durissus* populations of Brazil, characterizing their morphology (morphometry, pholidosis and pattern coloration), reproduction and diet, to determine the relative influence of environmental parameters such as altitude, temperature and precipitation on these morphological and ecological patterns, such as the importance in the taxonomic diagnosis of the group. **Methods:** A total of 620 specimens of *C. durissus* housed in the Instituto Butantan (IB) from Southeast and Central-West regions from Brazil were examined. Several measures, counts of scales and pattern coloration of the body and the head were recorded for each individual. Fecundity (clutch size), sexual maturity, testicular activity (volume of the testicle), vas deferens diameter and uterine muscular contraction were analyzed. Histological analysis was performed to detect sperm storage structures in males and females. Snake stomachs were dissected to examine the percentage of occurrence of each prey category. **Results and Discussion:** Significant differences were not detected in body size ($F = 3.25$; $p = 0.07$). There were significant differences in the shape variables (MANOVA; Wilks' Lambda = 0.86; $p < 0.001$) and pholidosis variables (MANOVA; Wilks' Lambda = 0.72; $p < 0.000$). Female reproductive cycle for Southeast and Central-West regions was seasonal. The reproductive cycle of male for the same regions was annual. Ontogenetic variation in diet was not detected, which was mainly composed of rodents.

8.15 Geographical ecology of the rattlesnake *Crotalus durissus* (Squamata, Viperidae) in the Brazilian Cerrado

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Introduction: Geographical variation is intimately associated with the ecological characteristics of a species; however, few species of snakes have been adequately studied to determine the existence of geographical variation in morphology, diet and the reproductive cycle. In general, environmental parameters of latitude, altitude and temperature possibly reflect a general pattern of geographic differentiation in these morphological and ecological patterns in some species of rattlesnakes. Two species of rattlesnakes are restricted to South America, *Crotalus simus* and *C. durissus*. There has been little research conducted on *C. durissus* in Brazil involving geographical variation. **Objectives:** We investigated patterns of geographical variation in morphology (morphometry and pholidosis), reproduction, and diet of the rattlesnake *C. durissus* in the Brazilian Cerrado, and the relative influence of environmental conditions on these patterns. **Methods:** A total of 507 specimens were examined, 228 males and 279 females from the Brazilian Cerrado. Several measures and counts of scales of the body and the head were recorded for each individual. The sex of individuals was determined by direct examination of the gonads. In females, the reproductive condition was determined by the presence of vitellogenic follicles and embryos in the oviducts, and in males the reproductive condition was evaluated by the presence of enlarged testes and convoluted epididymides. Snake stomachs were dissected and their contents analyzed. Prey items were identified to the lowest taxonomic level possible. **Results and Discussion:** *Crotalus durissus* shows a marked sexual dimorphism in body shape, with males having bodies, tails and paravertebral stripes relatively longer than females, while females having bodies relatively wider. Clutch size averaged 11 embryos and was significantly correlated with snout-vent length. The female reproductive cycle occurred in the transition at the end of the dry season and the rain season, with the beginning coinciding with the greater activity of small mammals and male reproductive cycle occurred throughout the year. Ontogenetic variation in diet was not detected, which was mainly composed of rodents, a habit related to an ancestral pattern in the group. Association between climatic structure and ecological parameters from rattlesnakes was not detected, probably because of the predictability of the climate in the Cerrado.

8.16 Reproductive strategies in the snake genus Bothrops: gain and loss of genetic diversity

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Introduction: Viperid snakes show an ancestral sexual reproductive pattern, in which delayed fertilization is ensured by uterine muscular twisting (UMT) sperm storage. Female parthenogenesis is rare and poorly recorded, mainly in South American taxa. **Objectives:** The aim was to discuss gain and loss of genetic diversity in snakes in nature. **Methods:** We discuss gain and loss of genetic diversity in snakes in nature based on the observation of five cases of *Bothrops* litters from 3 females in captivity kept isolated long-term from males one from birth and other two for 7 and 9 years, respectively, since arrival. **Results and Discussion:** Five birth cases, one in *B. leucurus* and 4 in two different adult female *B. moojeni* in Laboratório de Herpetologia, were recorded. The female *B. leucurus* arrived in 2001 and laid 31 attretic follicles (af) and 2 stillborn females in 2010. The offspring of the first case of *B. moojeni* (mother born in captivity/January 2002) were observed on three occasions (2005, 2006, 2010). In April 2005, the snake laid 6 af and 1 neonate male which died some hours later. In March 2006, the same *B. moojeni* mother, laid 10 af and 1 stillborn male. The third time, this female laid 23 (af) and 1 teratogenic male. Second case *B. moojeni* offspring female arrived in 2003 and laid 14 (af) and 1 female (sexed by dissection) which survived 18 days. The literature points out 2 previous cases of possible parthenogenesis in *Bothrops* (*insularis* and *moojeni*). In all of them, there were doubts about total isolation from males which could characterize offsprings as a result from sperm storage, differently from the first case *B. moojeni* described here. Facultative automictic parthenogenesis (FAP) in the sexual determination system (ZZ, ZW) has explained the production of normal diploid males. However, here we had 4 males (only one teratogenic) and one female fully developed. Assuming parthenogenesis or alternative sexual mode of reproduction, besides FAP (which originates males) other meiotic events could produce females. Parthenogenetic snakes have been reported to survive longer in captivity. In the wild, despite very rare, we believe parthenogenesis would drastically reduce genetic variability, though vital for species survival, mainly when colonizing isolated places. Different genetic mechanisms could produce males and females and could represent restoration in opportunities of mating that combined with mutations would result in an increase in genetic diversity at least during the period of total isolation. Multiple copulation and sperm storage would contribute to accelerating gain in diversity in periods when contact with other specimens occurred. We carried out population studies through molecular analysis to evaluate polymorphism in natural populations as well as to understand the role of asexual reproduction in the natural history of the *Bothrops atrox* species complex.

Supported by: CNPq

8.17 Predatory behavior of *Oxyrhopus guibei* (Squamata, Dipsadidae) Hoge & Romano 1977 in captivity

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Introduction: *Oxyrhopus guibei is* an opisthoglyphous dipsas, which occurs in most parts of southern, southeastern and central-western Brazil, and also in Paraguay, Bolivia and Argentina. This species is well adapted to the human environment and it feeds on lizards and small rodents. The choice of prey subjugation tactics, which can be constriction, poison or swallow while still alive, varies according to the risk of retaliation the prey shows. **Objectives:** The objective of the present study was to improve the knowledge of feeding behavior of this species while in captivity by offering different prey. **Methods:** Three types of prey were offered: *Mus musculus* (mice), *Rattus norvegicus* (rat) and *Meriones unguiculatus* (gerbils). During the observations, we recorded the time spent eating (stopwatch seconds), the tatics of subjugation of prey (alive down, poisoning and/or constriction), the body site at which prey ingestion began (anterior, posterior or lateral region) and the amount of prey consumed. **Results and Discussion:** Thirty-seven predatory behaviors were observed. The type of the prey, as well as its size, affects the snake's predatory behavior. The amount of prey offered sequentially did not interfere with the choice of subjugation tactics used. Regarding small preys (0-5 g), the tactics of subjugation were swallowing while still alive, while for bigger preys (over 10 g), the tactics varied between constriction and poisoning/constriction. *O. guibei* has a Duvernoy's gland, which is responsible for toxin production and secretions in opisthoglyphous snakes. However, the poison of this snake has low toxicity, and apparently is used to complement constriction. We observed a tendency to start swallowing the prey from the anterior region, which is possibly related to reduction of the ingestion time and the energy invested. The ingestion time for different prey with the same mass illustrated no significant differences in their means, either between mice of different masses. However, the statistical analysis showed significant differences in ingestion time in three comparisons: between mice from 0 to 5 g and from 20 to 25 g; between mice from 0 to 5 g and rat from 20 to 30 g; and between mice from 0 and 5 g and gerbil from 20 to 30 g, probably due to the snake's greater energy spent in swallowing larger prey.

8.18 Psalistopoides Mello-Leitão, a new synonymy of the genus Rachias Simon (Araneae: Nemesiidae)

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Introduction: The genus *Psalistopoides* was proposed by Mello Leitão in 1934 to include *P. fulvimanus*, described from Alto da Serra de Paranapicaba, Santo André, São Paulo, Brazil, based on a single male specimen. The last modification that occured in the genus was proposed by Lucas and Indicatti in 2006, which considered *Psalistopoides* as a valid genus, redescribed the type-species and described a new species from Ribeirão do Pinhal, Paraná, Brazil. The diagnosis they provided was that the species of the genus *Psalistopoides* differ from *Neostothis* Vellard and *Prorachias* Mello-Leitão by the presence of keels close to the embolus; from *Pselligmus* Simon by the presence of tarsal scopulae on legs III and IV; from *Stenoterommata* Holmberg by the absence of a megaspine on tibia I and the aspect of the palpal bulb; from *Rachias* Simon by the absence of spines on all tarsi and from *Pycnothele* Chamberlin by the longer embolus. **Objectives:** The aim of this study was to synonymize the genus *Psalistopoides* with *Rachias* Simon and describe the females of *P. fulvaminus* and *P. emanueli* Lucas & Indicatti for the first time. **Methods:** The examined material was deposited in the Arachnida collection of the Instituto Butantan. The female spermathecae were dissected and submerged in clove oil to study internal structures. The illustrations and morphological observations were made using a Leica MZ12.5 stereomicroscope with a camera lucida. **Results and Discussion:** The study of *Rachias dispar* Simon, type-species of *Rachias*, that has the apical area of the embolus dilated, and of other species of the same genus that do not have spines on tarsi, and of the unknown females of the two species of *Psalistopoides*, led us to conclude that *Psalistopoides* is not a valid genus and that it should be synonymized with *Rachias*. This is because the diagnosis provided by Lucas and Indicatti does not differentiate *Psalistopoides* from *Rachias*, due to it having the same features. The females of the species that belonged to *Psalistopoides* are described for the first time. The female of *Rachias fulvimanus* (Mello-Leitão) comb. nov. differs from those of the other species of the genus by the basal dome of spermathecae being very high and narrow, and *R. emanueli* (Lucas & Indicatti) comb. nov. differs from those of the remaining species by the very reduced basal dome and by the thicker duct. In addition, new records on distribution range of both species are presented.

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8. 19 Chilopod biodiversity from Belterra (Pará, Brazil)

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Introduction: Studies about centipede biodiversity was begun during the project "Butantan Amazonia" in Belterra and Santarem cities. Chilopods are represented by superclass Myriapoda and includes Pauropoda, Symphyla and Diplopoda. They have a long and segmented body, a pair of antenna and a pair of poison claws. There are poor studies about centipedes in Brazil and there is no specific research. **Objectives:** The aim of this work was to study centipede biodiversity from the Amazon Forest in Belterra and Santarem cities and specify the species involved in human accidents. **Methods:** Collections were made during December 2009 to February 2010 in the field and urban area in Belterra and Santarem cities. The collected animals were kept alive and sent to Butantan Institute where the animals were identified. **Results and Discussion:** Until now, four genera from family Scolopendridae have been identified, one from Newportidae and one of the order Geophilomorpha. Data from Belterra and Santarem show a high centipede diversity and these samples are similar to those of other studies previously conducted in the Amazon Forest.

8.20 Lifestyle and reproduction of captive Brazilian insular snakes

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Introduction: Insular habitats are important for evolutionary studies; in addition, the geographical reclusion favors endemic species. Two islands off the coast of SP are known to be important players in the Brazilian ecological scene: Queimada Grande Island, where we find *Bothropoides insularis* and Alcatrazes Island where *Bothropoides alcatraz* is found. Due to the ever increasing habitat destruction, both venomous species are threatened with extinction. The reproduction, lifestyle and venom production of a new third species *Bothropoides jararaca aff.*, which is still in the description phase, is being studied. This new species inhabits Moela Island in the southern coast of SP. Due to the absence of mammals in these three islands these species show different feeding habits. **Objectives:** Our aim was to study the reproduction and behavior of these snakes in order to develop methods to help in *"ex situ"* conservation projects and venom extraction for research. **Methods:** *B. alcatraz*, *B. insularis* and *B. jararaca aff.* kept in captivity were used. **Results and Discussion:** One male and five females of *B. alcatraz* have been kept for about 10 years in captivity with zero mortality rates. In captivity, these snakes spend most of the time wrapped around tree branches even feeding from this location. In the wild, *B. alcatraz* feeds on small lizards, centipedes and small amphibians. In captivity they have accepted rats as food besides the usual diet described. So far, three forms of mating have been observed; however, no female has completed conception. One female and one male of *B. insularis*, born in the Herpetology are now 12 and 10 years old, respectively. In the wild, the young feed on amphibians, lizards and centipedes, and the adults mainly on migratory birds. In captivity they also feed on rats, gerbils and hamsters and show preference for newly hatched chicks. Two matings have been observed, but the female did not complete conception either. From a mating registered in 2008, four snakes were born in March 2009, and in February 2010 another six were produced. All have been developing remarkably and accepting the food offered. Of the new species, *B. jararaca aff.*, 5 specimens are kept (two males and three females). Four snakes were born from a female already pregnant when collected. A mating in captivity was also observed and the behavior studied and described. While the species is still being described, preliminary results indicate that they also feed on small lizards, small amphibians and centipedes. In captivity they have already begun to accept rats as food. The success of the reproduction in captivity of this species is very important for their conservation, furthering the studies related to the behavior, courtship and reproduction. Researchers are seeking new raw materials in order to conduct pharmacological studies and the search for venom is ever increasing. Endemic species, threatened by extinction that live inside protected areas, may not be removed, therefore the success of these endeavors depend on the successful reproduction of these species in captivity.

8.21 Regeneration of the parotoid macroglands in the toad *Rhinella icterica* after mechanical compression

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Introduction: Toads have a pair of parotoid macroglands that secrete venom used in passive defense against predators. These macroglands are composed of juxtaposed alveoli filled with syncytial glands, which are connected to the exterior by ducts. When the parotoids are bitten, the venom is expelled on the predator's oral mucosa in the form of jets, causing several pharmacological actions. The empty secretory syncytia collapse in the interior of the alveoli. **Objectives:** Using morphological methods, we aimed at the study of the parotoid regeneration after manual mechanical compression, simulating a predator's bite. **Methods:** Parotoids of male toads *Rhinella icterica*, were dissected after being compressed at successive times, from 2 hours to 105 days. Animals with normal non-compressed parotoids were used as control. Two positive control animals were also examined: one sacrificed 330 days after parotoid compression and another collected from nature with a defective parotoid. The samples were fixed in Bouin and prepared for histology using paraffin. The sections were stained with HE, Mallory trichrome and picrosirius. **Results and Discussion:** The results showed that after compression, a considerable number of alveoli remained intact. The alveoli which were effectively affected were observed with their syncytia totally collapsed and with an extensive hemorrhagic area and inflammatory infiltrate around them. From the 5th to the 105th day, the syncytia gradually showed signs of recovery, showing conspicuous nuclei and amounts of secretion inside them. The hemorrhagic areas gradually diminished. In the last stages, together with the syncytia almost completely regenerated, there were a few which were only partially recovered and others which seemed to be still in the first phases of regeneration. The presence, in the first stages, of hemorrhage and inflammatory infiltrate around the syncytia, including a great number of macrophages, indicates that the glandular tissue, as well as the connective tissue around it, suffers injuries when compressed. In the last stages of observation, many alveoli were well regenerated. Regeneration, however, seems to occur in different grades, from complete to restricted to a few areas of the syncytia. The alveoli which suffered the worst injuries did not seem to recover their functional state. On the other hand, the fact that not all the alveoli are effectively compressed can be crucial for toad survival, since they do not lose all their venom stock in the case of a bite, remaining protected for new attacks.

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8.22 Tick karyotype test: methodology improvements and technical training

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Introduction: There are some tick species that are still not well studied. One parameter that may be taken into consideration for the better characterization of those species is the karyotype. **Objectives:** The aim of this study was to adapt cytogenetic techniques to be used as a complementary study in the characterization of different tick species **Methods:** Based on a published peer-reviewed methodology, an established cell culture strain from the tick *Ixodes scapularis* (ID8) was used. The cells were incubated in 0.01 µg/ml colchicine for 18 h. The detached cells were centrifuged, incubated in 0.075 M KCl for 30 min, fixed with 3:1 methanol/ acetic acid solution and stained with Giemsa, 10% or 2%. The slides considered suitable for analysis (based on the visual subjective size of the cell population, integrity and percentage of cells in division) were screened. **Results and Discussion:** A good number of metaphases were obtained, but some modifications must be introduced to obtain a better morphology of the chromosomes. Staining with 2% Giemsa worked better than the 10% concentration since it allowed a clearer distinction between chromosomes from the same cell. Future tests are needed to improve our karyotype test technique including KCl time/concentration and adjustment of chromosome dispersion on the slide for better visual conditions for chromosome counting and morphological analysis in order to confirm the identity of cell culture lines.

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8.23 The forgetful spider

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Introduction: Memory is considered by many scientists as the most fascinating product of biological evolution. Use of previous experience helps animals to cope adaptively with ever changing environmental conditions, in habitat selection, mate selection, etc. Animals such as web spiders which hoard food as part their predatory behavior depend on memory to retrieve hoarded items and maximize intake by keeping memory representation of hoarding sites. The Orbiculariae are a group of spiders, the synapormophy of which is to build orb webs. Orbwebs, in the course of evolutionary time, may undergo structural changes and give origin to sheet webs and cobwebs. When spiders capture more than one item of prey, and one prey is taken off from the web, spiders exhibit search behavior which includes locomotion on the web, plucking of web lines, etc. in attempt to recover the stolen prey. *Zosis geniculata*, although from this group of spiders, does not show, under normal conditions, prey searching. **Objectives:** We examined in our study the behavior of *Z. geniculata* when captured prey is suppressed. **Methods:** In condition 1, a first prey (p1) was offered and afterward it was carried to the hub by the spider, a second prey (p2) was offered, and p1 was taken off from the web while Zosis was capturing p2. In condition 2, a single prey was offered and this prey (p1) was taken off when the spider was attracted to the periphery of web. **Results and Discussion:** Zosis did not search for p1 under condition 1, but did search under condition 2. Results show that memory of p1capture is affected by the capture of p2. This may be due to the time spent by *Zosis* wrapping p2. *Zosis* belongs to the unique spider family which has no poison glands and thus needs to spend enough time wrapping its prey. According to another interpretation, the lack of searching of p1 in condition 1 is due to working memory constraints in *Zosis*. Other derived spiders and one ancestral (Desidae) of the Orbiculariae group studied do search for the first prey even when a second is offered, so it seems probable that this behavioral feature has been lost in the Uloboridae. It is possible that there is an adaptive advantage in forgetting p1, in Zosis, but this is still not clear and should be addressed in further research.

Supported by: CNPq

8.24 *Badumna longinqua* (Araneae: Desidae): description of the predatory behavior and plasticity of the web

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Introduction: *Badumna longinqua* is an introduced spider species in Uruguay. It appeared in the 1960s, probably due to the arrival of *Eucaliptus* sp. Both spider and tree came from Australia. Spiders from this genus have been reported to build irregular ground sheet webs. **Objectives:** The aim of this work was to study two populations of *B. longinqua* in Montevideo (Uruguay) and to describe the predatory behavior and the web. **Methods:** We observed two populations of *B. longinqua* (n=60) at the campus of the IIBCE and in the park of the city zoo**. Results and Discussion:** These spiders build two types of irregular sheet web: a two-dimensional and three-dimensional. The aerial web uses leaves from trees as support, and its retreat is inside a group of leaves knit together. The trunk web can be either two- or three-dimensional, with the retreat always inside cavities of the cortex. The first sheet built by the spider is two-dimensional, but later she adds new capture areas and the structure eventually becomes three-dimensional. The differences in the webs can be the result of incipient speciation, of adaptive plasticity or of competition for web sites with large differences in the overall fitness. Predatory behavior has unique features in such spiders. They capture the prey by biting: they approach it, seize it in their chelicerae and bring it directly to the retreat to be wrapped. The wrapping is different from the usual pattern in which alternate movements of legs IV throw silk onto a prey suspended on the web. At the retreat, she releases the prey and starts to rotate over the prey, eventually fixing threads over its legs. While rotating, keeping the prey below her cephalothorax, the spider repeatedly dabs the spinnerets over the substrate and the prey, so as to immobilize it. There are variations in this wrapping technique; sometimes the spider fixes a few threads over the prey and sometimes they do not wrap it at all, and start the ingestion right away. Some scientists describe a similar kind of wrapping in the Theraphosidae, Tengellidae and Agelenidae. These spiders wrap the prey by moving their entire body and spinnerets, fixing threads over the dorsal portion of the prey. *Badumna* also wraps the prey by moving the body, but there are slight differences as to where the spider fixes the threads. Following the proposal of these researchers, the wrapping by moving the body is the ancestral condition in araneomorphae, and this behavior is a homology in all these groups, now including the Desidae.

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8.25 Ultrasonographic evaluation of pregnancy in the snake *Boa constrictor constrictor* (red-tail boa constrictor)

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Introduction: The red-tail boa constrictor (*Boa constrictor constrictor*) is a snake species found in the Neotropical region. They are viviparous and kill prey by constricting them with the robust body which may reach about 4 m in length (Gomes *et al* 1989). Since the physical examination is limited in reptiles, the semiological process of the internal organs is complicated. The ultrasonography is the easiest method to evaluate the internal organs, particularly those of the reproductive system. **Objectives:** The objective of this study was to describe the stages of embryonic development in the uterus of *Boa constrictor constrictor* through ultrasound. **Methods:** The *Boa constrictor constrictor* (MIB Nº 3640) under study is kept in the Biological Museum of Butantan Institute with one male and one female of the same species. It is 2.5 m in total length with a weight of 10.5 kg. Between December 2009 and March 2010, three ultrasound examinations were performed: (1) with portable ultrasound Sukuba 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). **Results and Discussion:** In the spring, the female had an increase in abdominal size. In the first (Dec. 12, 2009) and the second (Dec. 15, 2010) ultrasonographies, 28 eggs were observed in uterine chambers. Sonographic images were hyperechogenic with hypoechogenic peripheral region, with no visualization of cardiac activities such as flickering motion. In the last ultrasonography (Mar. 26, 2010), many eggs were observed together, making it impossible to count individually. On Mar. 29, 2010, 25 offspring were born together with three atresic eggs. The offspring were born in early autumn of 2010. Therefore, we suggest that increased abdominal size was due to fertilization (ovulation) that occurred only in early spring (September). The female may have stored sperm in the reproductive tract during the winter for the fertilization and the pregnancy beginning in the spring with a higher temperature. This fact is corroborated by the high percentage of offspring and few atresic eggs in early autumn. Ultrasound is thus considered the easiest method to diagnose and demonstrate the gestational process.

8.26 Body plasticity and chemical communication: morphology of the pheromone glands among the gymnophthalmid lizards

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Introduction: Lizards make great use of pheromones for chemical communication with co-specifics and with the environment. These substances are, in general, secreted by epidermal glands located in femoral, cloacal and/or ventral regions. The small lizards of the family Gymnophthalmidae are distributed in different types of habitats, and some groups have as common feature progressive levels of appendicular reduction or even the total loss of limbs, associated with grades of adaptation to the fossorial environment. This phenomenon seems also to be correlated with the arrangement and functioning of the pheromone glands. **Objectives:** The aim was to understand the role and morphology of these glands in chemical communication through the study of the pheromone glands in five psamophylic species of the tribe Gymnophthalmini, living in sandy environment, and with different levels of limb reduction. **Methods:** The glands of *Calyptommatus leiolepis*, *Nothobachia ablephara*, *Procellosaurinus tetradactylus*, *Psilophthalmus paeminosus* and, *Vanzosaura rubricauda* were removed, fixed in Karnovsky solution and embedded in historesin. The sections were stained with toluidine blue-fuchsin for the general study of glandular morphology. **Results and Discussion:** The glands are present only in males. In *P. tetradactylus*, *P. paeminosus* and *V. rubricauda* they are located in the femoral region, arranged in a row below the dermis, forming a glandular cord. In *C. leiolepis* and *N. ablephara*, which have a significant limb reduction, they are arranged side by side in the pre-cloacal region. In all species, the glands show an arborescent shape and are divided into lobules 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 eventually desquamate from the secretory epithelium, becoming part of the solid secretion plug, which permanently obstructs the duct. This proccess is typically holocrine and must generate an intense cellular turnover within the gland: at the same time that a dead cell desquamates, another germinative cell takes its place. The secretion is deposited on the substrate through a pore opening in the center of a scale. The general morphology of the glands seems to agree with the psammic environment in which these animals live. The brittle nature of the secretion plug must contribute to the rapidly pheromonal dispersion as the animal moves. Moreover, the primary role of chemical communication among these animals seems to be related to sex, since glands seem to be absent in the females.

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8.27 Ultrasound monitoring of male and female gonads in some species of venomous snakes kept in captivity at the Laboratório de Herpetologia - Instituto Butantan

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Introduction: The use of ultrasound examination in small animals as a diagnostic tool has increased in the last two decades, mainly because it is a non-ionizing examination, painless (does not require sedation of the animal) and noninvasive, and allows visualization of the internal architecture of organs. Although the use of this examination is being currently used on wild and exotic animals, few studies have been published. Studies on snakes are more restricted and are based mainly on the study of non-venomous snakes. Ultrasound examination can be extremely important in reproductive studies of snakes, indicating gonadal changes that occur in different seasons. **Objectives**: The aim of this study was to characterize the ultrasonographic image of the testes and ovaries and monitor changes occurring in these organs in four species of venomous snakes kept in captivity, throughout the seasons. **Methods:** From March to June/2010, six females and five males of the species *B.jararaca*, *B.fonsecai, B.atrox* and *Crotalus durissus terrificus* were examined monthly and had their gonads analyzed and measured with the aid of a portable ultrasound machine using a 6 – 13 MHz tranducer. **Results and Discussion:** The testes and ovaries of the snakes are located in the final third of the body, a few inches below the gallbladder. The gallbladder has an anechoic content (easily located) and is a good reference point to locate the gonads. The testes are less echogenic than the adjacent tissues, have a homogeneous echotexture and an ellipsoidal shape. The testicular tunic is thin and hyperechoic. The ovarian parenchyma has an echogenicity similar to that of the surrounding tissues and also has a homogeneous echotexture. The ovarian follicles may have different echogenicity, depending on their stage: in phases I and II (<0.6 and 0.6 to 1.1 cm, respectively) the follicles are usually anechoic and in phases III and IV (1.2 to 2.0 cm and >2.1 cm, respectively) they are more echogenic, but hypoechoic in relation to the ovarian parenchyma. In March and April, the females of *B. atrox* and *B. jararaca* showed follicles in phases I and II, while in the months of May and June follicles in phases II and III were the most encountered. Females of *B. fonsecai* and *Crotalus durissus terrificus* showed follicles in phases I, II and III in March and April, and in the months of May and June follicles in stage IV were already observed. During the study period, no differences in testicle size was seen.

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8.28 Management of rattlesnakes in captivity: wood or plastic cages?

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Introduction: The Laboratório de Herpetologia at Instituto Butantan currently maintains about a hundred venomous snakes of the genus *Crotalus durissus* to yield poison for anti-venom production and research in animal biology. This species inhabits the open areas of central Brazil, the arid and semi-arid region of the Northeast, and the fields and open areas in the South, Southeast and North of Brazil. Before the reform of the Laboratory in 1993, all venomous snakes were kept in wood cages. From that year on these cages were replaced by plastic ones aimed at the improvement of the sanitary handling of the animals. Some months after the change the rattlesnakes began to have health problems such as pneumonia and attack by fungal dermatitis. *Aspergillus* sp, *Cladosporium* sp, *Fusarium* sp, *Trychophyton* sp, *Trichosporon* sp, *Geotrichium sp* and *Penicillium* sp were the fungi most commonly found. Most of these fungi are saprophytic but, when associated with the decrease in the host immune system, can become pathogenic. Six months after the change, some deaths occurred and all the rattlesnakes were again placed in the wood cages which, because of their porosity and wire mesh slide, provide a greater ventilation and lower humidity, thereby preventing the emergence of infections by fungi. However, the maintenance of rattlesnakes in wood cages hampers the appropriate health management of the snakes, since the porosity of the wood contributes to the development of microorganisms and their disinfection is not satisfactory. **Objectives**: This experiment aimed to keep the snakes in plastic cages, improving their sanitary handling by increasing the number of ventilation openings. **Methods**: The room where the rattlesnakes are kept has 20 m^2 with six shelves. The average room temperature is 25 ºC and the humidity is about 60%. In this experiment, started on February 2, 2010, 18 animals were divided into three groups of six animals each: a control group in wood cages, a experimental group in plastic cages (with an average of 50 holes of 3 mm diameter) and a third experimental group, also in plastic cages (with an average of 150 holes of 6 mm). All the animals of the experiment were kept on the same side of the room. All the snakes of the experiment received the same care as the others of the room. **Results and Discussion**: After five months of the experiment, fungi or other diseases in the snakes were not observed, even in the six specimens kept in common plastic cages, contrary to what occurred in 1993. This result is probably related to the higher frequency in the cleaning of the cages and its disinfection with sodium hypochlorite (4%). We can conclude that if appropriate conditions are provided, rattlesnakes can be kept in plastic cages without harm to their health.

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8.29 Aspects of the reproductive biology of *Crotalus durissus* Linnaeus, 1758 (Serpentes, Viperidae)

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Introduction: Brazil has only one species of rattlesnake, *Crotalus durissus*, and its accident is of great importance due to the high mortality its causes in humans. Reproduction is considered the main component of the life history of organisms; however, most of the work on *Crotalus* reproductive biology is done with North American species and works in Brazil are scarce, which is indispensable in future studies on the species. The litter size is one of the parameters of the reproductive biology, having the advantage of being compared between different populations and species. **Objectives:** The study aimed to determine whether there is any relationship between the litter and offspring size with maternal body length, compare the weight and measurements of young males and females and examine the reproductive effort of pregnant females, contributing to the knowledge of the reproductive biology of South American rattlesnakes. **Methods:** In this study 25 litters of *C. durissus* were used, with a total of 206 offspring. The 25 pregnant females were donated to the Instituto Butantan and had their biometric data and mass taken. At birth, the offspring were measured, sexed and their health conditions verified. Statistical analysis was performed using Fisher's exact test, t test and regression analysis and ANOVA. The level of significance was $\alpha = 0.05$ for all tests. **Results and Discussion:** The offspring's birth occurred mainly in January (n=11) and February (n=10). The average number of offspring per litter was 10.4 (2-28). Several authors suggest that litter size (number of offspring) in snakes is closely related to the body size of the females. The offspring were born with an average snout-vent length (SVL) of 31.23 inches (18-37 cm) and average weight of 24.15 grams (7 to 37.3 g). The ratio between males and females was 1:1. There was no significant difference in SVL ($t = 0.19$, $p = 0.84$) and weight ($t = 0.09$ and $p = 0.96$) for males and females, although males showed a greater relative length of tail ($t = 14.34$ $p <0.0001$). Relationships were found between female SVL and litter size, and between maternal SVL and average size of their offspring. The relative reproductive effort (RRE) shows that the energy investment for reproduction in females is large (RRE= 42%). These snakes are born larger than other neotropical viperids and, in this way, they can feed upon larger prey in the early stages of life, which can promote a rapid growth and avoid predators quicker.

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8.30 Geographic distribution of the Neotropical pitvipers *Bothrops atrox* group (Linnaeus, 1758) (Viperidae: Crotalinae): A survey in the museum collection Alphonse Richard Hoge and troubleshooting systematics and conservation

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Introduction: The *Bothrops atrox* group is a very diverse snake taxon regarding morphological characters, macro- and microgeographic habitat occupation, sexual and ontogenetic variations, but low levels of mtDNA phylogeographic distinction. Depending on the author, *B. atrox* species complex includes the lineages of *B. atrox* (Amazonian distribution), *B. moojeni* (Central Brazil andsurroundings), *B. marajoensis* (described for Ilha de Marajó) and Atlantic forest populations assigned as *Bothrops leucurus* (presently including *B. pradoi*). However, species boundaries are unknown. **Objectives:** The aim of this study was to define their boundaries in geographic distribution (through DIVA-GIS), searching for possible sympatric regions of occurrence and discuss phylogeography of *B. atrox* species group. **Methods:** We surveyed the registry books of the Museum Collection Alphonse Richard Hoge - IBU and scattered literature. **Results and Discussion:** Preliminary data pointed out approximately 54 localities for the presence of *B. leucurus*, 179 of *B. atrox*, 150 of *B. moojeni*, and 5 of *B. marajoensis*. Maps show sympatry areas between *B. atrox* and *B. moojeni* (northern Tocantins) where putative hybrids have been observed and collected. Three records of *B. marajoensis* on the continent were found, namely Monte Alegre – PA, Pinheiro – MA and Macapá – AP. *B. leucurus* including *B. pradoi* shows a wider geographic distribution along the Atlantic forest which should be taken into consideration when discussing its conservation status. We carried out population studies combined with molecular analysis to evaluate polymorphism as well as to check the status of those putative hybrids of the *Bothrops atrox* species complex.

Supported by: CAPES

8.31 Occurrence of *Microsporidia* in larvae of black flies (*Diptera: Simulidae*) in São Paulo State

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Introduction: Biological control of insect vectors has high efficiency because it is a more specific and has less environmental impact. Among the entomopathogenic agents, microsporidia may contribute to the management programs for the control of black flies, but little is known about its occurrence and efficiency in Brazil. The family Simuliidae includes vector species of pathogens, and biological control of this group is a challenge for public health. **Objectives:** The objective of this study was to investigate the occurrence and identify larvae infected by Microsporidia. **Methods:** Larvae were collected fortnightly during a period of four months (April-July 2010) in the municipality of Caraguatatuba, São Paulo. Larvae that showed symptoms of infection by microsporidian were dissected in distilled water, and fresh smears were made and stained with 10% Giemsa according to the method of Undeen and Vavra (1997). **Results and Discussion:** Of a total of 1213 larvae, 1210 were identified as normal and 3 larvae showed symptoms of infection by microsporidians, which were identified by morphological analysis as *Polydispyrenia sp.*

9. History, Education and Science Dissemination

9.01 The art of scientific illustration in Butantan

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Introduction: Since the beginning of Butantan Institute, people such as August Esteves, Carlos Hoehne and Teresa Sarli, had produced engravings used to illustrate scientific articles, didactic materials and complete works, which are examples of scientific publications at that moment. The scientific illustration followed the disciplines' changes, serving still today the researchers as a visual and didactic resource. Therefore, in 2009 in the *National Week of Sciences and Technology: Sciences in Brazil,* the Historical Museum organized, for the purpose of exhibiting the graphics collection of Butantan, the exposition the Art of Scientific Illustration. **Objectives:** The exposition intended to show the work of the illustrators of Butantan in its historical and social context, featuring the art of the representation and of the scientific communication. It adopted different insertions throughout time, going beyond the esthetic and technical aspects, and showing the peculiarities of each tracer. **Methods:** We chose two types of illustration that had been historically significant: zoological and botanical. After raising the authors and the production of the same ones, we related the tradition of inserting them in the practical one of the graphical register on the national scene since the XVIII century, identifying styles, intentions and traditions that had characterized the illustration as a resource for the register and the dissemination of the research of public character. For this, we created an exposition with strong visual appeal, stimulating the visitors to participate in the research that had resulted in engravings of plants, animals and human beings, by means of panels that had presented the subject in different languages. **Results and Discussion:** The exposition remained in the Museum during the period of October 2009 until January 2010, receiving 18,471 visitors where 3,527 were set appointments. The white public participating in the educational action was defined as being the childhood public with ages between 6 and 12 years. The educational action was carried out for 1405 and divided into three moments: 1) presentation of the subject and chronology, 2) inclusion of the public in the workshop, " also a Scientific Tracer" and 3) use of a line of the time where each pupil inserted its illustration, recognizing it in the already identified secular traditions. Argument: The exposition was a chance to show the importance of the tracer as identification and spreading of national the environmental and social elements, beyond characterizing the Butantan of this tradition inside. However, the great motivation of the exposition was to divulge part of this significant quantity and so little known.

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9.02 Architectural heritage inventory

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Introduction: With the opening of discussions in the History Museum about the architectural heritage of Butantan Institute, a study of the construction's history was conducted through examination of the Annual Reports. During the research, we noted a scarcity of data about the constructions. At the same time, we observed in the Institute's iconography an important wellspring to research of some architectural aspects and the occupation's and space's uses. Therefore, the team prepared two instruments for research: an inventory of buildings and a database for iconographic documents, which together, may complement and extend the discussion about patrimonial preservation. **Objectives:** The aim was to extend our knowledge of the buildings that belong to Butantan; to get information about the architectural sections, construction techniques, buildings' history and buildings' changes, to encourage the discussion and policy making for preservation and educational activities using the data obtained. This work was carried out in partnership with the Engineering' Department and the Institute Documentation Center. To determine if the iconography will be used as a research source, an identification form was created to catalog and integrate the collection's database. **Methods:** In order to identify development notes, for the buildings as well as the iconographic material, literature support relative to aspects of cataloging, evaluation and preservation were raised and discussed. For the filing and analysis of records, an extensive bibliography that approaches relevant questions was planned. Parallel to readings and preparation of identifying notes, actual topographies of buildings were prepared to be part of the database. **Results and Discussion:** As to the research work and discussion about to the fields that must be present for the knowledge of the iconography collection and buildings, were elaborated two identifies notes and cataloging. The database has been discussed in partnership with the Butantan Foundation computer sector. For the partial results, we realized some photographic essays of the state of the buildings. The essays will be attached to the buildings inventory. The purpose of preparing this inventory was to enhance in the community the importance of identity and the institutional heritage preservation, which consist of the buildings, old documents and museum collections, and also scientific and intellectual. Through the heritage's observation and documentation, it is possible to think in political and control strategies, security and preservation of property, avoiding loss, dispersions and destruction.

9.03 Implementation of an innovative service: exchange of serum for snakes by the Instituto Butantan (1901-1919)

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Introduction: The service of exchange of snakes for antidote serum was created, introduced and implemented by Vital Brazil, the first director of the Instituto Butantan. In order to continue with the research on snake poisoning and the production of antidote serum, medical doctors required a large number of snakes to extract poison, the raw material for the production of serum. The system of exchange was implemented to face the difficulty of buying or obtaining live snakes in good condition. **Objectives:** This study aimed at the analysis of the implementation of the exchange system at the Instituto Butantan. The service was rendered by the Institute from its foundation until the mid-1990s. It was significantly important for enlarging the snake collection, the development of the study of snake poisoning, the production increase of antidote against snake bites, as well as for the information to the population, which contributed with diminishing mortality due to snake bites. **Methods:** This work was grounded on a systematic research of official documents from the Institute, archived by the Laboratory of History of Science (Laboratório de História da Ciência) as annual reports, statements and mail, as well as the bibliography on the history of health during the first years of the Brazilian Republic. **Results and Discussion:** To debate the implementation of the exchange service, means to discuss the institutionalization of the Instituto Butantan itself, due to the fact that the exchange of serum for snakes is vital for its production: to analyze the difficulties of legitimization and spreading a treatment unknown up to then, which was based on bacteriology –a science just starting at the time; to ponder on an issue of the institutional history– i.e., the institutional mission (production, research, health education); to reconsider the health policies during the first decades of the twentieth century. As a director of the Institute, Vital Brazil organized a service and implemented a project of health education for the population, thus allowing the snake poisoning to become a matter of public health.

9.04 Studies undertaken by the Microbiology Museum concerning the viability of a science laboratory at the Instituto Butantan base in the Amazon

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Introduction: During its first eight years of existence, the Microbiology Museum has established itself as an important center for developing educational activities and disseminating scientific information, with the aim of stimulating the curiosity of the general public about science - especially Microbiology. The administration of the Instituto Butantan took advantage of these experiences and invited Museum team to develop strategies for establishing a science laboratory for public school students in Belterra, a municipality of Santarém, Pará. **Objective:** The aim of this study was to analyze the practicality of establishing a science laboratory for grammar and high school students at the Instituto Butantan base in the Amazon, taking advantage of the abilities and skills of its professionals in the areas of science, the physical possibilities of the locale, and the interest of the municipal administration. **Methods:** The present study was based on interviews with a total of 15 professionals - 5 health workers, 1 laboratory technician, 1 general education coordinator, 1 school director, and 7 science and biology teachers in public schools in the municipality of Belterra. The physical conditions of some public schools' infrastructure were also analyzed. It was found that only 12 teachers in Belterra had studied natural sciences and, of these, 2 had graduated in Biology. Science classes in many schools are given by teachers from other areas. Only one of the 64 public schools has a science laboratory (with a microscope that is not being used). Only one of the teachers interviewed had some notion of the principles of microscopy. **Results and Discussion:** An analysis of the information gathered in Belterra indicates that it would be possible to set up a science laboratory there if certain factors were taken into account. The existing laboratory at one of the schools was only recently built and is of adequate size and physical structure to install a duly equipped science laboratory for the Amazonian Base of the Instituto Butantan and can attend to the needs of students from other schools. The Municipal Department of Education already regularly provides transportation for students to and from other schools. There is an obvious need to prepare the local teachers to use the laboratory and their disposition to do so became evident. However, financing for setting up the laboratory and purchasing materials for class activities are important limiting factors that must be considered. This preliminary survey points to the need for continuing discussions and cooperation between Belterra and the Instituto Butantan, which can result in a work plan contemplating the requirements for establishing and running the proposed science laboratory.

Supported by: Projeto Butantan Amazonia, Secretaria da Saúde de São Paulo

9.05 Education and training activities developed by the "Divisão de Desenvolvimento Científico do Instituto Butantan"

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Introduction: The Instituto Butantan executes several activities focusing on the Sciences, among them the development of scientific projects, new drug discovery, innovation and staff formation can be pointed out as important tasks. The education and students' formation are also important issues included in the attributions of the "Divisão de Desenvolvimento Científico (DDC)." To reach the students' training, four different programs have been established in the Instituto Butantan: PIBIC, PAP, Post-Graduation and NEUAP. **Objectives:** The aim of this communication is to describe the four programs developed by DDC focusing the students' formation and explain how each program contributes to the scientific activities of the Instituto Butantan. **Methods:** Among the programs developed, "**PIBIC** - Programa Institucional de Bolsas de Iniciação Científica" focuses on the graduation students. Its main objective is to awake the scientific vocation and to stimulate potential talents among university graduates. Another program developed here is the "**PAP** - Programa de Aprimoramento Profissional." which aims to complement the training of recent graduates, by training them under direct supervision of specialized professionals in the diverse areas that integrate the program. A really important program created in the Instituto Butantan is the **Post-Graduation Program,** whose main approach is a multidiscipline formation, focusing on poisons and toxins (effects on biological systems, structural aspects, etc), envenomation, therapeutics and bioprospecting. Finally, the youngest program of the DDC is the **NEUAP** "Núcleo de Extensão Universitária e Aperfeiçoamento Profissional" which enrolls several scientific researches of our community who offer courses in different areas to graduate students and graduated people. **Results and Discussion:** All of these programs were created to improve the theoretical and practical knowledge of the different students who are interested in the Scientific Projects of Instituto Butantan, by promoting their involvement with the researchers who have a high scientific level and also by offering them a fellowship support supplied by different agencies.

9.06 Evaluation of the journal Cadernos de História da Ciência of Butantan Institute as a vehicle for divulging the history of science and health

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Introduction: Created in 2005, the journal *Cadernos de História da Ciência* publishes original papers, interviews, reviews and analyses of sources in the area of the History of Science and Health. Since 2009, as a result of the indexing process, the editorial board found it necessary to define more clearly the public to which it is aimed, as well as to improve its identification with other research groups in the area, making it a recognized communication vehicle. **Objectives:** The aim here was to discuss, by means of a survey of the CNPq Research Groups (area of History of Science and Health) and *Scielo* evaluation for indexation, the scope of the *Cadernos de História da Ciência*, as well as to pose a critical analyses of its constitution as an information vehicle of the area, taking into account the institutional relationship of the authors and the discussed themes. **Methods:** The Journal indexation process, started by the *Scielo* evaluation, has offered us the first insight with respect to the influence the Journal upon the professionals of the area, identifying some points that deserve to be reevaluated about the institutional origin of the authors, members and associated researchers. Second, we surveyed the CNPq research groups, mapping their origin, institutions and internal leaderships, relating the themes discussed by those groups and the papers published by the *Cadernos de História da Ciência*. **Results and Discussion:** The revision of the items reported by Scielo/Bireme suggests that by its definition as an information vehicle of the area of History of Science and Health the Journal should have more representation from other parts of the country, as up to this moment, more than 80% of papers published come from the Southeast region, mainly from São Paulo and Rio de Janeiro. On the other hand, of 36 research groups, 26 are from the Southeast region (1 from Minas Gerais, 11 from Rio de Janeiro and 14 from São Paulo) and only 10 are from other regions: 1 from Northeast, 5 from North and 4 from the South Thus, in our opinion, up to this moment, even though we are restricted to the region that produces most of the knowledge in the area, we do not exchange themes outside the interests of the research in health institutes, having little interchange with the universities such as the groups from UFRJ, USP and UNICAMP.

9.07 Hospital care and health policies in the State of São Paulo (1800-1950)

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Introduction: Hospitals play an essential role among the health policies of the state. This has, however, been a rather recent matter of consideration. In the nineteenth century, the hospital was almost unknown for most of the population. Illnesses received home care and hospitalization was recommended only in some cases among the low-income population. In the mid-nineteenth century, changes occurred as to the importance of the hospitals and their role in curing. The discussion, however, lay on the responsibility of the organization of hospital care: if it had to be in the hands of philanthropy, the state, the employers, the ill people themselves through organizations of civil societies or different working groups. At the end of the 1930s, there was an increasing investment by the state in the building and maintenance of hospitals. **Objectives:** This work is part of a broader project on the relationship between public and private health policies in the State of São Paulo, focusing on hospital care. **Methods:** The analysis is grounded on a systematic research of the state legislation for health and on the messages and reports of the presidents of the province and the governors of the State of São Paulo during the mentioned period –a period which witnessed important changes in the political, social, economical, demographic and scientific scenarios in the State of São Paulo and Brazil. **Results and Discussion:** What were the roles played by the state as for hospital care in São Paulo from the beginning of the nineteenth century and the decade of 1950? What were the proposals, projects and measures implemented by the government of São Paulo regarding hospital care? These are some of the issues which guide this work, which aims at the analysis of the official policies and at the comprehension of the changes during the 150 years covered in this study.

9.08 Learning in science museums: young visitors at the Microbiology Museum - Phase II

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Introduction: Very few events in Brazilian museums are specifically planned for children even though these young visitors represent a significant percentage of the population that passes through the Microbiology Museum of the Instituto Butantan every year. As such, studies about this youthful public will be important for planning educational programs and an exposition for the Museum specifically directed to them. This research project is being developed in two phases: the first phase has already been completed, and was designed to examine the degrees of knowledge that children already have about microorganisms; the second phase has been initiated and its preliminary results have been analyzed. **Objectives:** The objectives of the second phase was to evaluate what types of equipment have the greatest potential for stimulating children to learn more about microorganisms, which are most adequate for the expositions and what are the best materials and techniques for teaching about magnifying images. **Methods:** This research was carried out through interviews with young children (4-6 years) who were visiting the Microbiology Museum and from the *Centro de Convivência Infantil* (CCI) do Instituto Butantan. These young people were invited to manipulate several materials such as hand-held or fixed magnifying lenses, a toy called "Eye Clops" (which magnifies objects and transfers their image to a TV screen), boards with drawings and pictures of microorganisms, as well as three-dimensional models of microorganisms (made from resins or fabrics). The children's responses were recorded or filmed with video cameras and subsequently transcribed, notes were taken, and the drawings made by the children were collected for evaluation. **Results and Discussion:** The use of hand-held lenses was found to be more interesting as they can be readily manipulated and their magnifying effect is immediately appreciated. The "Eye Clops" apparatus did not contribute greatly to conveying the idea of amplifying images. All kinds of images was judged possible to use in games and animated films planned for future expositions as well as 3D models that were also found to be attention-holding. The data presented here brings various important elements to the planning and development of museum exhibits to teach young children more about microorganisms. As such, we believe that this work can contribute to strengthening research into learning experiences in museums and also enrich educational practices and aid in disseminating scientific knowledge in these cultural settings.

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9.09 100 years of the book "A defesa contra o Ofidismo" and the building of Public Health in Brazil

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Introduction: In 2011, "A defesa contra o Ofidismo" celebrates 100 years of it first edition. The researchers of Laboratório de História da Ciência of Instituto Butantan had worked with this important text, in the way of emphasizing the importance of the scientist Vital Brazil for building the Brazilian Public Health. **Objectives:** The work aimed at bringing out the importance of the book through the viewpoint of the scientist, his insertion at the Instituto Butantan and in the health service organization in Sao Paulo. **Methods:** In this historical analysis, different sources were used, biographic aspects of the author and his inclusion in the scientific scenario in his time. In addition, the analyses focused on the social, economic and political conditions of the creation of public health institutions and their performance in these periods. **Results and Discussion:** Vital Brazil began his works when infectious diseases and their combat were the great concern of the public health policies, and great health campaigns were developed in a vertical way. In this period Vital Brazil had implemented a movement of health education to the population to fight against mortality of snake bite. This mobilization had proved to be nationwide, reinforcing a trend of precursor form of public policy implemented, especially since the 1920s.

9.10 New educational and scientific dissemination activities by the Microbiology Museum in 2009/2010

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Introduction: The mission of the Microbiology Museum is to serve especially young people stimulating their scientific curiosity and lend support to science education in schools. The museum also seeks to promote a greater understanding of science by the general public. As all fields of science are advancing at a very rapid pace, the museum continually seeks to update its presentations and develop new activities that complement existing exhibits. **Objectives:** The aim of this work was to reformulate the activity modules currently used in the high school science laboratory, to develop a module directed towards grammar school students, to offer night students of Biology the opportunity to visit the science museum, and to better inform the public about A H1N1 flu. **Methods:** The five original modules directed toward high school students were reformulated into just three modules and in one workshop only with the concept of DNA, which is administered independent of the other modules. A workshop was created specifically directed toward *Ensino Fundamental II* students. A night visit sponsored by the staff of the museum received 30 Biology students from the University of São Paulo (USP) enrolled in a course dealing with Methodologies of Teaching Biological Sciences. The students attended a special monitoring with emphasis on educational activities offered by the Museum. The exposition concerning the A H1N1 flu included panels with printed material, booklets made for different age groups, an interactive game, and an educational video about the importance of the vaccine and its production process developed by the Instituto Butantan in an interview with Prof. Isaias Raw. **Results and Discussion:** There was a significant increase in the number of modules offered in the first semester of 2010 as compared with the same period in 2009 (80 versus 55), which indicates that the reduction in the number of modules motivated the students from both public and private schools to participate in these activities as they could complete all of the modules with fewer visits to the Museum. The evening visits to the museum gave the USP students the opportunity to experience for themselves non-formal educational strategies that can help young people become more aware of the role of science in the modern world. Approximately 68,000 people (including 20,400 students) visited the temporary exposition "*The H1N1 virus in the gun-sights of Brazilian science*" over a period of 7 months - reaffirming the interest of the general population in scientific exhibits. These encouraging statistics reinforce the mission of the Microbiology Museum, which is to bring scientific information and education to the general public and stimulate critical reflections on the role of informed citizenship in modern society.

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9.11 Belterra's Center of Memory: a space for the rescue of local memory

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Introduction: By the end of 2009, the group *Research on Patrimony and Memory of Belterra*, responsible for creating and organizing the historical heritage and the activities of patrimonial education in the municipality of Belterra, recognized that it was a duty to make heritage accessible and inserting it in the Education Secretariat. Doing that, the municipality becomes the main agent responsible for the creation of a policy for the local heritage. The Instituto Butantan became a partner and collaborated, by the project Butantan at Amazonia and INCTTOX, to define these policies. **Objectives:** The aim of this work was to support the creation of the Belterra's Center of Memory in order to develop research related to guarding and conserving documents containing the history of Belterra and to provide a space for the public to have access to its own heritage, performing its main duty of promoting the community's participation in the process of official recognition as a historical city, and the inclusion in the federal Plan for Acceleration of Development–Iphan PAC Cidades Históricas. **Methods:** A multidisciplinary group made a survey of the heritage collected by the institutions of Belterra and Santarém, the public and the cultural places of the municipality. Besides being used as sources of the research performed by Butantan, all the material had to be submitted to a conservation and cataloguing process to be made publicly accessible. It is also responsibility of the municipality and the Butantan to qualify multiple agents through courses and trainings on Patrimonial Education, involving documents' management and workshops to rescue the memory and to value the inhabitants' histories of life, seen as a cultural heritage of Belterra. The Butantan and the Education Secretariat developed these workshops, including these issues in education programs of the public schools. **Results and Discussion:** In May 2010 the Belterra's Center for Memory was inaugurated, settled in a historical building, originally used as the physicians' house and more recently as the Health Secretariat. The restoration of the building was performed by the organization AmaBrasil, maintaining the same structure as the original, from the 1940's. Since the beginning the City Hall, it has been responsible for the administration of the Center, with the technical support of the Education Secretariat. Nowadays, the Center is consolidated in the scholastic calendar and on daily lives of inhabitants. Our initiative to open a discussion about history, heritage and culture led us to congregate people, ideas and documents. The challenge for the future is to maintain initiative, gaining new partners and financial support.

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9.12 Evaluation of the activities of the Historical Museum in 5 years of management

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Introduction: The Historical Museum of the Butantan Institute has undergone some reformularizations, generating the necessity of one practical of systematic and clear evaluation, becoming more compromised with the use of instruments or processes that evaluate the degree of reaching its action. **Objectives:** This work aimed at correcting the absence of this practice, a strategic planning was elaborated to present the results and the evaluation of the innovation proposals during the five years of this management. For this, general elements had been considered that evaluated the involvement of the internal team and the exploitation of the visiting public, serving as an element of analysis for the future managements. **Methods:** We used analyses cross related to the involvement of educators of the Museum and the public, by means of the following elements: elaboration, execution and scientific production subsequent to the application of the activity (internal evaluation) and number of visitors and impressions registered in the notebook of visitation and for the email of the Museum (external evaluation). **Results and Discussion:** During 5 years, the Museum developed activities where the public participated in different ways, between expositions of short duration, workshops and special guided tour. The coefficient of participation in the elaboration and application of the activity did not always follow the same degree of interest in the scientific production of the group, resulting in cases in which the initial research took advantage of dissemination and the later production, mainly in the workshops and special guided tour. The opposite occurred in the expositions of short duration that had also motivated employees and educators in the article elaboration, abstracts, presentations and internal records of the actions promoted. When crossing these elements with the participation of the public, we corroborated the perception that the expositions, for the dynamics, involvement and knowledge acquired from the work team, more had been used to the advantage of its pedagogical aspects and of leisure. We determined, however, that the involvement of the public does not only depend on the treated subject, but on the dissemination (contact by telephone, folders, posters, email list, etc). It is therefore one practise little used, but basic for the Museum: to consider the spreading as integrant of the elaboration of the initial proposal, and not only as the completion of the actions. It is clear that there is a need to articulate the internal projects of research with the promoted actions, in ways to stimulate scientific production. In this direction, the team dedicated the different forms of registering that join internal and external impressions to the action, as a blog of the Historical Museum, which shows the stages of the activities and the results obtained by means of the notations of the proper participating pupils.

10. Others

10.01 Process validation methodology applied in immunobiological production at Butantan Institute

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Introduction: Validation of the production process is a requirement of Good Manufacturing Practice (GMP) and consists in documented evidence that the immunobiological production process is effective and reproducible. The validation process starting with equipment qualification (Installation Qualification - IQ, Operation Qualification - OQ and Performance Qualification - PQ) such as the bioreactor, followed by Media Hold Test that simulate the production process with the bacteriological culture medium. The last step in process validation is performed with the product. **Objectives:** The aim of this study was to describe a methodology applied in order to validate the immunobiological production process according to the national requirements (ANVISA) and World Health Organization (WHO). **Methods:** The measuring instruments and control process of the bioreactor were calibrated. All specifications of the equipment parts were checked in IQ. OQ was performed by functional tests, such alarms and critical point test while simulating the production procedure. PQ consisted of three cycles of the sterilization process with the empty equipment, and the temperature was monitored by sensor and biological indicator geometrically distributed inside the bioreactor. Three batches of the Media Hold Test were performed using tryptic soy broth (TSB) and the samples collected during the test were evaluated by sterility and bioburden tests. The environment was monitored by viable and non-viable particles. Process validation was carried out with three consecutive batches of immunobiological product, and the quality control tests were performed during the production and in the final product. **Results and Discussion:** IQ demonstrated that the equipment was in accordance with the project and user requirements. The results of functional tests demonstrated that the equipment was certified by OQ. In PQ, all sterilization cycles showed a temperature at 121 ± 1°C and pressure at 1.2 ± 0.1 bar, and the biological indicator proved the efficiency of the sterilization process. The result of sterility test in the Media Hold Test showed no microbial growth after 14 days at 37 °C and the bioburden test showed that the amount of microbial particles acquired during the process was in accordance with the requirements for the immunobiological production. The quality control tests performed during process validation demonstrated that the product met the criteria established by ANVISA and WHO requirements.

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10.02 Comparative studies between monogamous and polygamous mating in the Swiss mouse strain

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Introduction: The Central Animal House of Butantan Institute sets high demands for laboratory animals, requiring quality standards and providing animal well-being. These factors create a challenging scenario to administer, since the Swiss mice reproductive lifetime decreases over time, the interval between birthing increases and proliferation decreases, leading to low productivity. **Objectives:** The objective of the present study was to perform a comparative study of two mating types: monogamy (1:1) and polygamy (2:1) in order to verify and define the mating types that better match with demand and to maintain quality standards, providing animal well-being. **Methods:** Swiss mice were housed in polypropylene boxes and kept in rooms with flow set, protected by sanitary barriers (autoclave, air filtration system, differential pressure), with ambient temperature of 22±2°C, light cycle of 12 L:12 D and free access to water and feed. The animals were divided into two types of mating systems: monogamous (n = 25 females : 25 males) and polygamous (50 females : 25 males). Animals were mated to 60 days old and kept together until the 7th birth. After this period, the groups were analyzed as to birthing intervals, number of births per female, and number of weaned as well as pre-weaning mortality. **Results and Discussion:** In both mating types analyzed, intervals between births was reduced in 2nd to 4th parturition, but after this period, the intervals gradually increased. The interval average between births from 1:1 mating was 27.41 days. However, in the polygamous system, it was 25 days. Moreover, it was observed that the average weaned between two mating systems was similar, around 7.19 and 7.3 animals weaned per female respectively for monogamous and polygamous groups. The mortality rate of pre-weaning in both groups was low (0.20 and 0.27% of monogamous and polygamous groups, respectively). The highest yield from monogamous mating occurred on 3rd parturition with 8 born per female. In the 2:1 system, the highest production occurred at the 4th birthing, reaching 8.5 animals per female. There was no significance for the traits analyzed between mating systems, suggesting a need for more studies with larger numbers of animals with the aim of total elucidation of these results. However, we can affirm that the polygamous system (2:1) appears to be more suitable for intensive animal production with high demands, as it requires less space and material, which reduces labor hours and the skilled workforce needed, allowing the optimization of production.

10.03 Snake welfare: "Curious" food items offered to feed snakes coming to Butantan Institute

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Introduction: The Butantan Institute in its history has stood out in the knowledge of snakes. The institution has received snakes from all over Brazil since 1903, when Vital Brazil set established his effective work on environmental education. Thanks exclusively to the cooperation of suppliers of venomous animals, we could develop this work. The snakes sent to Butantan Institute came in different types of boxes, some suitable for this transportation and others with less safety, such as pet bottles, cardboard boxes and a number of other kinds of containers. To accommodate the snake in the container used for transportation, some supplies were added, in addition to moistened cotton (as recommended), a little soil as substrate, newspaper, rags and even small branches and leaves, presumably to better accommodate the animal besides some food items. All snakes received were destined for different research areas of the Institute and used for several purposes: for antivenom production and also for research, systematic studies, such as general biology and physiology, public exhibition and environmental education. Besides, some specimens were included and registered in the Herpetological Collection and others used for feeding ophiophagous snakes. **Objectives:** As the diet of snakes is poorly known we recorded here different food items offered to the snakes by the suppliers. **Methods:** Only items clearly placed to feed the snake which could not have come together with the substrate or vegetation placed in the container were considered during a one-year investigation period. **Results and Discussion:** We recorded food items such as slugs, ball-armadillos, ants, spiders, beetles and other arthropods. In most cases, prey offered could be ingested by the snake, concerning size relationship, but items did not belong to the usual known diet list. We also recorded food for human feeding purposes (rice, beans, hotdog, lettuce, cauliflower, bread, cookies and a coconut sweet piece). Such information emphasizes the supplier's concern regarding the snake's welfare despite the fear of being bitten. Moreover, it corroborates previous environmental educational studies carried out in the Laboratory of Herpetology, Butantan Institute.

10.04 Searching for the control of nuclear DNA replication in trypanosomes

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Introduction: Chromosomal replication begins with the assembly of the prereplication complex (pre-RC) at replication origins. In eukaryotes, the pre-RC is composed of the ORC complex containing six proteins, Orc1-Orc6, two proteins called Cdc6 and Cdt1, and the minichromosome mainenance (MCM) complex, which is composed of Mcm2 to Mcm7 proteins and shows helicase activity, essential for DNA replication. Once pre-RC is organized in the chromatin, origins become licensed to replicate. Since ORC, Cdc6, and Cdt1 are required for loading MCM onto DNA, but are not required for the continued MCM-DNA interaction, the downregulation of their expression and/or activity at the end of G1 represents, in eukaryotes, an effective way to block DNA replication. Trypanosomes do not contain an ORC complex, Cdc6 or Cdt1. Instead, they contain a protein homologous to Orc1 and Cdc6, called Orc1/Cdc6 which is a component of pre-RC. Orc1/Cdc6, however, does not seem to be involved in DNA replication control, since it is bound to DNA during the entire cell cycle. **Objectives:** Therefore, we wondered if Mcm proteins could be involved in this control in trypanosomes. **Methods:** In this work, we searched for sequences in trypanosome databases, and we found 10 genes for *T. cruzi* and 8 genes for *T. brucei* annotated as Mcms. By alignment analysis, we identified the likely Mcm7 gene. *T. cruzi* Mcm7 was then cloned and expressed by a prokaryote system. The recombinant protein rTcMcm7 was used to immunize mice. **Results and Discussion:** The antibody obtained was able to recognize the *T. cruzi* recombinant Mcm7 protein as well as the putative *T. brucei* recombinant Mcm7, expressed by Sf9 cells using the bacullovirus system. The anti-rTcMcm7 serum will be now used in Western blotting and immunofluorescence assays in order to analyze the expression and localization of Mcm7 during the cell cycle of trypanosomes.

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10.05 Comparative evaluation of antioxidant activity of raw and processed pequi fruit (*Caryocar brasiliense* Camb.)

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Introduction: The pequi is a characteristic tropical fruit that is cultivated in the Central-West and Northest regions of Brazil. This fruit is very popular in Brazil and normally is consumed with a rice and chicken meal. The pequi fruit is normally sold in the raw or processed forms. The phenolic compounds present in many foods, including fruits, vegetables and spices, exhibit antioxidant properties. **Objectives:** The objective of this study was to evaluate the thermal processing effect on pequi's phenolic composition, its antioxidant activity "in vitro" and in cellular metabolism in MDCK cultivated cells. **Methods:** The phenolic compounds were evaluated by the Folin-Ciocateau method. The antioxidant activity was measured by β-carotene/linoleic acid and DPPH• methods. Cell viability was measured by MDCK cell cultivation at 37 °C in L15 medium in six 24- well plates. The phenolic compounds in aqueous extract of raw and processed pequi fruit were 57.36 mg/100 g and 45.18 mg/100 g, respectively. **Results and Discussion:** The antioxidant activity measured by β-carotene/linoleic acid was 90.70% and 85.01% in raw and processed pequi fruit and in the DPPH• the half maximal effective concentration EC50 in μg/mL was 289.9 and 240.6, respectively. The viability of MDCK cells was 68.6% and 64.9% for raw and processed pequi extracts. Therefore, both induced higher cell viability levels than observed in the control. Thermal processing induces a minor decrease in the content of phenolic compound and antioxidant activity, as well as in cell viability in response to aqueous pequi extract, which was not a significant loss with regard to the importance of these phytochemical compounds in pequi.

Supported by: CNPq

10.06 New technique for undoing slides and recovery of type specimens and material of mites (Acari) in deteriorated Hoyer's medium

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Introduction: The Acari Collection of the Instituto Butantan (IBSP) includes a large slide collection. This collection has many types of mites that are deteriorated. Since 1996, we have tried to recover the types, dismounting slides and remounting them based on a traditional technique using Hoyer's medium. However, most of the types were impregnated with old gum arabic, and the remounted slides were not satisfactory so far. **Objectives:** The aim of this study was to recover important material of types and other specimens in mite collections that are in deteriorated Hoyer´s medium. **Methods:** A) Undoing slides: After copying the data label, the slides were placed in a Petri dish with distilled water to 55ºC in an oven to remove the sealant. Usually, the slide could be separated from coverslip after around 24 h. If the coverslip still stayed attached to slide, the process was repeated. When the coverslip was free, it was carefully removed with a micropin to avoid damaging the specimen. The specimen was removed with a small paint brush and placed into a cavity slide. Sometimes the specimen still stayed fixed in old and dirty glue from Hoyer's medium, and in this case, it was placed in a Petri dish with distilled water to 80ºC until the specimen appeared clean. For each specimen, a clean cavity slide and paint brush were used, to avoid contaminating the next mite. B) Remounting slides: A drop of Hoyer's medium was placed on a new clean slide. The mite specimen was placed in the ventral position over it, and the slide was heated at 55ºC for 10 min for fixation. Hoyer's medium was replenished, and specimen covered with a coverslip; the slide was then placed at 55ºC for a week or more. The excess around the coverslip was removed with a blade and the slide returned to the oven at 55ºC until completely dry. The slide was labeled. Finally, the coverslip was sealed with Glyptal sealant. Material recovered was photographed and prepared in CorelDraw. **Results and Discussion:** The techniques used to date were not efficient in the undoing of mite slides, because they failed to take into account the differences in their external structures. In most cases, the specimens were damaged, not allowing the identification of basic characters for their taxonomy. The same did not occur with this new technique that uses only heated distilled water. It was excellent in separating the coverslip from the slide and in diluting the Hoyer's medium completely without damaging the specimen. Another reason to remove the sealant with water is that other chemical products are toxic, and can penetrate and deteriorate the specimen. With this new technique of undoing slides, the types and other material of mites of the IBSP Acari Collection have been successfully recovered.

10.07 Cellular absorption and antioxidant activity of free phenolic acids from pomegranate (*Punica granatum* L.)

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Introduction: Oxidation reactions are of major significance in both human physiology and food preservation. Oxidative stress in humans has been associated with several diseases, and food rancidity with oxidative spoilage. The phenolic acids present in many fruits and vegetables possess antioxidant activities. **Objectives:** The present study aimed to evaluate the antioxidant activity of free phenolic acids obtained from pomegranate pulp and its transport across MDCK cultured cells, focusing on protection from oxidation. **Methods:** The antioxidant activity was measured by β-carotene/linoleic acids and DPPH• methods. The phenolic acids were determined by high performance liquid chromatography (HPLC), based on their spectral characteristic and retention time, comparing with standards. The transwell plate technique with MDCK cells cultivated at 37°C in L15 medium was used to measure the transport of 70 μg/0.15μL of pomegranate free phenolic acids (FPA) using gallic acid as control. **Results and Discussion:** The antioxidant activity of FPA measured by β-carotene/linoleic acids was 68.18% and by DPPH• was half maximal effective concentration, EC50 =1.04 μg/mL. The principal phenolic acids transported by MDCK cells after 40 min were caffeic, catechinic, feluric, protocatechinic, sinapic and fumaric at 25% of the aforementioned initial level and against 35% of gallic acid as control. Thus, phenolic acids from pomegranate were absorbed by MDCK cells, which can confer oxidative protection in them. The free phenolic acids from pequi fruit have antioxidant activity and are absorbed by these cells. These properties could be useful in regular diets and cosmetics industry.

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10.08 An investigation of the host range of human influenza viruses

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Introduction: Studies on the host range of influenza viruses have been of great importance to determine the role of animals, once unlikely links, in the virus transmission chain. **Objectives:** This study aimed to investigate the circulation of the influenza virus in cats in Brazil. **Methods:** Domestic cats, seen at the clinic of the Faculty of Veterinary Medicine at the University of São Paulo, were grouped according to gender and age (young and adult). Serum samples were collected and, prior to titration, were examined for antibodies to influenza A and B viruses by the hemagglutination inhibition (HI) test using the corresponding antigens from the circulating viruses in Brazil. **Results and Discussion:** In cats between 6 and 20 years old, 20 % responded with high antibody titers ($\geq$640 HIU/μL) against human influenza type A (H1N1). Lower percentages of the animals in the same age group, 11% and 8%, showed the same high titers in response to human influenza types A (H3N2) and B, respectively. In the gender group, 17 % of males and 8% of females showed a poor antibody response against the influenza A (H1N1) virus (titers of $\leq$20 HIU/μL). Protective titers of $\geq$40 HIU/μL against human influenza viruses suggest viral infection transmitted to the domestic cats by humans. In conclusion, our results show that domestic cats, like other mammals, may play a role in interspecies transmission and spread of the influenza virus.

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10.09 Chronic treatment with BPP-5a and BPP-10c attenuates the hypertension and cardiac hypertrophy in spontaneously hypertensive rat (SHR)

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Introduction: Some BPPs isolated from *B. jararaca* venom have an antihypertensive effect independent of ACE inhibition. A recent study proposed that the activation of AsS enzyme is a mechanism for the antihypertensive effect caused by BPP-10c, which raises an NO-dependent effect. **Objectives:** The aim of this study was to evaluate the effect of chronic treatment with BPPs 5a and 10c on cardiovascular parameters and cardiac hypertrophy in SHR. **Methods:** Experiments were conducted in male SHRs (±300 g). Blood pressure measurements were performed by a non-invasive tail cuff method. Rats were anesthetized (tribromoethanol, 250 mg/kg, i.p.) for subcutaneous insertion of mini-osmotic pumps (Model 2002, ALZET) containing BPP 5a, 10c (n=5-8) or vehicle (0.9% NaCl) (n=7). The doses administered of each BPP were 71 and 710 nmol/kg/day. Cardiovascular parameters were determined at 0, 1, 4, 7, 10 and 14 days. At the end, blood samples (6 mL) were collected and the heart was removed. The left ventricle (LV) was weighed and mass index (LVMI) was calculated by the ratio LV/body weight (mg/g). L-Arginine (L-Arg) and NO plasma levels were measured by HPLC and NO analyzer, respectively. Statistics: Student's *t* test or one-way ANOVA; $p<0.05$. **Results and Discussion:** Compared to vehicle ($p<0.05$), both BPPs reduced blood pressure throughout treatment. At a dose of 71 nmol/kg/day, BPP-5a and BPP-10c reduced mean arterial pressure (MAP) (-24±5 and -26±2 mmHg, respectively). At 710 nmol/kg/day, reductions in MAP by BPP-5a and BPP-10c were -17±3 and -26±3 mmHg, respectively. There were no differences in the range of antihypertensive effect between peptides and doses. In addition, slight bradycardic effect (-20±7 bpm) was found only in animals treated with BPP-10c (71 nmol/kg/day). LVMI was smallest after BPP-5a and 10c treatments (71 nmo/kg/day) compared to control (2.53±0.05 and 2.57±0.03 *vs.* 2.76±0.11 mg/g; $p<0.05$). Based on LVMI results, LV histological investigation is required. Preliminary results with 71 nmol/kg/day showed that BPP-5a increases L-Arg levels (2.92±0.63 nM, $p<0.05$), while BPP-10c decreases L-Arg levels (0.38±0.06 nM, $p<0.05$) compared to control (0.8±0.03 nM). Despite L-arg differences, no changes in NO levels were observed after either treatment, compared to control. Chronic treatment with BPPs at both doses caused a long-lasting antihypertensive effect, such as reduced cardiac hypertrophy in SHR. Results concerning plasma levels indicate mechanistic differences between BPPs 5a and 10c. However, further studies are needed to elucidate the mechanism(s) involved. Nevertheless, these peptides may be considered potential pharmacological tools to treat cardiovascular diseases.

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10.10 Evaluation of the in vitro activity of piplartine against schistosomula and adult flukes of *Schistosoma mansoni*

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Introduction: Schistosomiasis is a neglected tropical disease, considered a severe public health problem worldwide. Praziquantel is the only effective drug currently used against all schistosome species, and as a consequence, parasite resistance remains a major challenge. Thus, the search for antiparasitic compounds from natural sources, mainly from plants, has been encouraged. **Objectives:** The aim of the present study was to determine the effect of piplartine, an amide isolated from *Piper tuberculatum* (Piperaceae), against schistosomula and adult flukes of *Schistosoma mansoni*. **Methods:** Schistosomula and adult (male and female coupled) were each incubated in vitro using piplartine over a wide concentration range (1–200 µg/ml). The efficacy of piplartine was examined regarding: a) schistosome survival; b) reproductive fitness of adult worms; c) motor activity; and d) alterations in the tegument of *S. mansoni* as determined by means of laser scanning microscopy. **Results and Discussion:** Piplartine significantly reduced worm motor activity and caused death in schistosomes of all larval- and adult-stages within 24 h at 25 and 5 µg/ml, respectively. At the highest sub-lethal concentration for adult worms (2 µg/ml), an inhibition of 75% in egg laying was observed despite the parasites remaining coupled. In addition, piplartine at 5 to 200 µg/ml induced morphological changes in the tegument and suckers in adult worms. These findings revealed that piplartine is an effective compound against larval and adult stages of *S. mansoni in vitro*, and that this natural amide is promising for further development as an antischistosomal drug.

Supported by: FAPESP

10.11 In vitro activity of dermaseptin, cationic antimicrobial peptide, against *Schistosoma mansoni*

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Introduction: Schistosomiasis is a neglected tropical disease that remains of considerable public health significance worldwide. Since the mainstay of schistosomiasis control is chemotherapy with a single drug, praziquantel, drug resistance is a concern. Dermaseptins (DSs) are cationic antimicrobial peptides found in the skin secretion from frogs of the genus *Phyllomedusa*. Besides the fact that DSs are active against a large spectrum of microorganisms, they have been considered promising candidates for new anti-infective drugs. As an advantage, it has been demonstrated that this compound does not induce significant cytolysis against mammalian blood cells. **Objectives:** The aim of the present study was to determine the effect of dermaseptin 01 (DS 01), on *Schistosoma mansoni* adult worms. **Methods:** In this study, the viability of 49-day-old adult worm pairs of *S. mansoni* was assessed *in vitro* with incubation in RPMI 1640 medium with different concentrations of DS 01. Worm motor activity, egg output (oviposition), tegumental alterations, and survival of parasites were monitored on a daily basis for 5 days using a confocal microscope and a stereomicroscope. **Results and Discussion:** Dermaseptin 01 at 100 µg/ml reduced worm motor activity and caused death of all worms within 48 h in RPMI 1640 medium. At the highest sub-lethal concentration of antimicrobial peptide (75 µg/ml), a 100% reduction in egg output of paired female worms was observed. Additionally, dermaseptin 01 induced morphological alterations in the tegument of *S. mansoni*, and a quantitative analysis carried out by confocal microscopy revealed extensive destruction of the tubercles in a dose-dependent manner in the range of 50-200 µg/ml. As stated previously, the dermaseptin family of peptides may have potential use as therapeutic drugs, as they are not toxic to animals or plants. Consistent with the data reported above where the schistosomicidal activity of dermaseptin 01 was demonstrated, combined with its effect on reproductive fitness and tegumental alteration in adult worms, it is clear that these peptides should be considered important candidates as antihelminthic agents.

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10.12 Cardiovascular effects of synthetic analogues of BPP-10c: in vivo and in vitro assays

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Introduction: BPPs from *B. jararaca* venom were used for the development of captopril, a powerful ACE inhibitor. Recently, *in vivo s*tudies showed that BPP-10c reduced blood pressure of SHRs, without affecting ACE activity. Also, BPP-10c boosts argininosuccinate synthetase (AsS) activity, increasing plasma L-arginine levels. **Objectives:** The aim of this study was to develop stable and active synthetic molecules with similar cardiovascular effects found for the natural molecule, BPP-10c. **Methods:** In vivo assays: *1) Cardiovascular parameters:* 24 h after femoral catheterization, SHRs and Wistar rats (WT) (±300 g) received i.v. injections of: BPP-10c, analogues A and B (71 nmol/kg); or vehicle (0.9% NaCl) (n=5-6 each). Cardiovascular parameters were monitored for 6 h. At the end, blood samples were collected for plasma levels. *2) Bradykinin potentiation (BK):* After measuring standard hypotensive responses evoked by BK (0.5 and 1.0 µg) in anesthetized WT, i.v. bolus injections of BPP-10c, analogues A and B (60 nmol/lg) or vehicle (0.9% NaCl) were given (n=4-5 each). Afterward, injections of BK (0.5µg) were repeated every 5 min. In vitro assays: *1) AsS activity:* kinetic assays using colorimetry were performed with BPP-10c and analogues. It was based on inorganic phosphate produced from pyrophosphate, by cleaving with pyrophosphatase. *2) Quantification of NO:* HEK-293 cells were treated with specific inhibitor of AsS and/or stimulated with 1, 20 and 30 µM BPP-10c and analogues. NO values in cell medium were determined by NO analyzer and compared to a nitrate standard. *3) Plasma levels*: L-arginine levels were measured by HPLC. Statistics: Student's *t* test or one-way ANOVA; $p<0.05$. **Results and Discussion:** Compared to vehicle (-9±4 mmHg), analogues A and B reduced mean arterial pressure (MAP) of SHR (Δ: -26±3 and -26±2 mmHg, respectively; $p<0.05$) and did not change MAP of WT, which means an antihypertensive but not hypotensive effect. However, MAP changes caused by both analogues were smaller than those for BPP-10c (-53±6 mmHg). Analogue B was more effective in potentiating BK (306 %) than either analogue A (212%) or BPP-10c (232%). Unlike what was found for BPP-10c, AsS activity was not changed by any analogue. L-arginine levels found after treatment with analogues were lower in WT than in SHR (analogue A: 0.42±0.04 and 0.61±0.05 nM; analogue B: 0.46±0.07 and 0.86±0.14 nM, respectively). Only analogue B increased NO production (A: 8.44±1.9; B: 26.2±1.6 vs. vehicle: 8.9±0.7 nmol/10^6 cells). Analogue A seems to act independently of NO-related mechanisms. Further studies using different approaches are needed to develop active and stable molecules useful in treating hypertension.

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10.13 Snake diversity in upper Tietê municipalities

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Introduction: São Paulo state, Brazil, has ca. 80% of the Atlantic Rainforest remains, but this number is decreasing due to the anthropic influence on this biome. It is believed that the Atlantic Rainforest has ca. 130 reptile species, but many may have been already lost. Originally, this biome was largely distributed on the coast of Brazil, having about 1.2 million square km. Currently, only 7% of it still exists (2% in conservation units). The study area: Salesópolis, São Luis do Paraitinga, Biritiba Mirim and Mogi das Cruzes are within the Atlantic rainforest domain, where there are many river springs, including the Tietê river spring (Salesópolis). The climate is humid (rainfall is between 2,100 - 2,400 mm a year and the relative humidity, around 70%). **Objectives:** The aim of this study was to compile a list of snake species of the region and to characterize their species richness and abundance. **Methods:** The data presented here are records of the collection "Alphonse Richard Hoge" – Instituto Butantan, collected from 1989 to 2009. From this data, we estimated the family and regional richness and relative abundance. **Results and Discussion:** A total of 1,146 specimens were collected in the study area during the period considered. The most abundant family was Dipsadidae (67%), followed by Colubridae (14%), Viperidae (11%), Elapidae (0.5%) and Boidae (0.3%). The commonest species found were *Bothropoides jararaca* (27% of the records), *Caudisona durissa* (13%), *Oxyrhopus guibei* and *Sibynomorphus neuwiedi* (9% and 7%, respectively). These last ones are non-poisonous and easily mistaken for poisonous ones by the public, which is the reason why they may have been brought to Instituto Butantan. Mogi das Cruzes showed the largest abundance (31 species) and followed the pattern described above. Biritiba Mirim had 21 species, with *B. jararaca* as the most abundant, but, unlike the other municipalities, only one *C. durissa* was found. The whole region has undergone an intense vegetation suppression (mainly for coffee plantations and ranching). *C. durissa* is known to be an alien species in the Atlantic rainforest. It is, probably, present there due to the impact caused in the last decades. Salesópolis and São Luis do Paraitinga recorded 20 species each. The most abundant for Salesópolis were *B. jararaca, Oxyrhopus clathratus* and *O. guibei*. São Luis do Paraitinga had a larger record for *C. durrisa* (90 specimens) than for *B. jararaca* (75 specimens). The third and fourth scores were for *Xenodon neuwiedi* and *X. merremii.* The results here may have some bias, as most of the snakes were collected by the local population, many times, because they are similar to poisonous snakes. Species not fit in this case were rare on the records. Mogi das Cruzes is the most urban of them; this increases the chance of human encounters.

10.14 Kinetic parameters calculated from application in MATLAB

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Introduction: The treatment experimental data is very important in a biotechnological process, and for that there is a need for a suitable fast tool that supplies reliable results. **Objectives:** This work is an application developed in MATLAB, including all the results and analysis involved in biotechnological processes. **Methods:** Starting from the fundamental kinetic variables of such bioprocess as cell concentration, and metabolite and product concentrations, the program developed in MATLAB calculates several parameters, such as growth rate, and several conversion factors, and it allows for smoothing of experimental data, using a spline algorithm. The program imports data from Microsoft Excel. Conversion factors are certain starting from the kinetic correlation of variables during the exponential phase. **Results and Discussion:** The use of that application results in less time spent for this treatment and also in a standardization of results. This application shows better results in its analysis, besides presenting the information in a clearer way to be understood by anybody. Besides, the results are standardized and obtained in a shorter time, using a single program.

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10.15 Genetic and morphological variability of *Aedes aegypti* populations from metropolitan area of São Paulo based on microsatellite loci and wing morphometrics

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Introduction: *Aedes aegypti* (Diptera: Culicidae) is a mosquito species of medical interest, where it is vector of etiological agents of diseases such as dengue and yellow fever. Its geographical distribution is tropical and subtropical, and its habits are synanthropic and anthropophilic. In the absence of a vaccine to combat dengue virus, the alternative is to control the vector. **Objectives:** Population studies are important to develop control strategies, and with the lack of population studies of this mosquito in the State of Sao Paulo, our purpose was to characterize *Ae. aegypti* populations combining microsatellite molecular markers and phenotypic wing study. **Methods:** Samples of *Ae. aegypti* were collected at four different locations in the state of São Paulo (SP): Butantã (BUT), Guarulhos (GUA), Osasco (OSA) and Suzano (SUZ), all located in the metropolitan area of Sao Paulo city. Locations were separated by a minimal distance of 13.5 km and a maximal distance of 50 km. For the genetic analysis five microsatellite *loci* were used, and for geometric morphometric analysis, wings were analyzed regarding 18 landmarks. **Results and Discussion:** The number of alleles observed for each *locus* differed between populations: six for 38/38 *locus*, five for T3A7 and 34/72 *loci*, and four for AED19 and C2A8 *loci*. A high frequency of heterozygotes was observed at *loci* 34/72 and C2A8, surpassing the rate of homozygotes, whereas the opposite was observed at *loci* AED19 and 38/38. For the *locus* T3A7, homozygotes and heterozygotes occurred at equivalent frequencies. The population of SUZ showed a 90-bp allele at *locus* 38/38 which was not present in other populations. Multivariate analysis of wing shape showed small interpopulation differences between BUT, GUA and OSA. Mahalanobis Distance Analysis revealed that the population of the BUT is morphologically closer to GUA than to OSA, for both sexes. Such morphological distances were not correlated to geographical distances between the same locations. The five *loci* examined were polymorphic. A deficit of heterozygotes was observed at 38/38 and AED19 *loci*, a pattern that may have been caused by the presence of null alleles. The microsatellite *loci* revealed possible diagnostic alleles for GUA, OSA and SUZ. Morphometric analysis showed morphological variability in wings, which, however, does not indicate population structure. The two population markers used here apparently have different degrees of resolution for microevolutionary events, and microsatellite DNA was slightly more sensitive in revealing population structure.

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10.16 Prolyl oligopeptidase and aminopeptidases in adipocytes

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Introduction: Adipose tissue has important endocrine functions whose disruption is related to the high incidence of pathological states, such as obesity**.** Among the molecular signals and regulators of lipogenesis and lipolysis in adipose tissue are several peptides susceptible to hydrolysis by exo- and endopeptidases. **Objectives:** The aim of this study was to detect prolyl oligopeptidase (POP) and the diversity of representative aminopeptidase activities, i.e., acid (APA), basic (APB), puromycin-insensitive (APM) and puromycin-sensitive neutral (PSA) and dipeptidyl peptidase IV (DPPIV) in adipocytes. **Methods:** Adipocytes were isolated from eight healthy male Wistar rats by the method of Rodbell. Briefly, the retroperitoneal fat was collected and incubated (30 rpm, 37°C for 1 h) in appropriate buffer with type 2 collagenase (1.25 mg/mL) and then centrifuged (200 rpm, 25°C for 1 min). The supernatant, which contains the suspension of adipocytes, was aspirated, suspended in 20 mM Tris-HCl buffer (pH 7.4, 20ºC) (1.67 mL/g of initial fat), sonicated (20 s at 20% of amplitude) and centrifuged (1,500 rpm, 4°C for 10 min). The lower layer formed was aspirated, homogenized in the same buffer with 0.1% Triton X-100 (800 rpm for 3 min) and centrifuged again (1,500 rpm, 4°C for 10 min). After this last centrifugation, the lower layer formed was collected and subsequently submitted to fluorometric measurements of peptidase activities using synthetic naphthylamide substrates. **Results and Discussion:** Peptidase activities were expressed as picomoles of hydrolyzed substrate/min/mg protein and presented as mean±s.e.m. (n=8) as the following results: APA (14±6), APB (4331±480), APM (137±22), DPPIV (83±18), PSA (156±34) and POP (25±6). This is the first report describing the existence of peptidase activities belonging to the M1 (APA, APB, APM and PSA) family in addition to insulin-regulated aminopeptidase (EC 3.4.11.3., LAP/IRAP, VP165, gp160), and to the S9 (DPPIV and POP) family in mammalian adipocytes. The interaction of these novel adipocyte enzyme activities with insulin, angiotensins, bradykinin, oxytocin and vasopressin is now under investigation in our laboratory in order to elucidate new mechanisms of adipocytes acting in energy balance.

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10.17 APM/CD13 and FOS are altered in the hypothalamus of obese and fasting rats

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Introduction: Peptides such as substance P, somatostatin, angiotensin III, vasopressin, kallidin, dynorphin, leu- and met-enkephalin and endorphin exert significant effects on the nutritional status and regulation of energy balance by the central nervous system and are susceptible to hydrolysis by exopeptidases. **Objectives:** Protein (Western blotting) and gene (PCR) expressions, catalytic activity of puromycin-insensitive membrane-bound neutral aminopeptidase (APM/CD13) and *in situ* regional distribution of CD13 immunoreactivity (ir) and FOS(ir) were evaluated in the hypothalamus of rats in order to explore the association of APM/CD13 and cellular activity with monosodium glutamate obesity (MSG) and/or fasting (FD). **Methods:** Induction of monosodium glutamate obesity (MSG) and/or food deprivation was carried out in rats. Western blotting and RT-PCR were used for measurements of protein and gene expression (RT-PCR) of hypothalamic CD13. The distribution of CD13 was immunohistochemically studied by the ABC technique and catalytic activity of APM/CD13 by fluorometry. **Results and Discussion:** Variations in protein and gene expressions of CD13 in relation to controls coincided in the hypothalamus of MSG and MSG-FD (decreased 2- to 17-fold). Compared to controls, the reduction of hypothalamic CD13 content may reflect a negative balance with its regional distribution in the supraoptic, paraventricular, periventricular and arcuate nuclei. CD13(ir) increased in the supraoptic nucleus in MSG (2.5-fold) and decreased in the paraventricular nucleus (2-fold) together with FOS(ir) (1.5-fold) in FD. In MSG-FD, FOS(ir) decreased (7-fold) in the paraventricular nucleus, while CD13(ir) decreased in the periventricular (5.6-fold) and the arcuate (3.7-fold) nuclei. All these changes of CD13 were not related to catalytic activity of APM. The data suggest that CD13 in these hypothalamic areas may function in the regulation of energy metabolism but not by means of APM enzyme activity.

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10.18 Role of HSP70 in the survival of *Biomphalaria glabrata*: evidence based on heat shock

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Introduction: HSP70 (heat shock protein) is a member of an important family of cell proteins involved in the regulation of protein homeostasis. Due to evidence that exposure to a wide variety of stressors, including elevated temperatures, ultraviolet (UV) radiation, heavy metals, and xenobiotics, can induce the heat-shock response, many studies are trying to establish the family of heat shock proteins as biomarkers of environmental stress. **Objectives:** The aim of this work was to investigate the role of HSP70 in protecting organisms from external injury after induction by a sublethal stimulus and a subsequent challenge with a lethal harm. **Methods:** We followed the HSP70 expression in the digestive gland of freshwater snails by Western blotting. Heat shock was chosen as the injurious stimulus for a group of 70 snails *Biomphalaria glabrata*; they were pre-exposed to sublethal temperatures of 33 and 36 °C to induce HSP70, and then exposed to a lethal temperature of 42 °C. Control group was not exposed to sublethal temperatures. The 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:** The animals pre-exposed to sublethal temperatures survived longer to the lethal temperature than the control ones. Western blotting showed that the induction of HSP70 in the digestive gland of pre-induced snails was clearly higher than in the controls, which strongly suggests that HSP70 played a protective role against the lethal effects of heat. *Biomphalaria glabrata* has been used as experimental model by our group. The results found here reinforce the proposal of HSPs as biomarkers of environmental damage, providing further evidence to establish *Biomphalaria glabrata* as a bioindicator in ecotoxicological studies.

10.19 Serologic incidence of Chagas disease in patients treated at Sanatorinhos Hospital in 2006 and evaluation of two different methods for diagnosis

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Introduction: Chagas disease was named in honor of its discoverer, the Brazilian physician Dr. Carlos Chagas. The disease has a ubiquitous geographical distribution on the American continents, and it is characterized by a generalized infection, with endemic nature and chronic development, by the flagellate protozoan *Trypanosoma cruzi.* Humans are intermediate hosts, and infection occurs via mucosa exposed to contaminated feces of triatomine insects. **Objectives:** The aim of this study was to evaluate the incidence of Chagas disease in serum samples of suspected patients sent to the diagnosis laboratory of Sanatorinhos Carapicuiba Hospital (2006). **Methods:** Serum samples were taken from 993 patients from five different municipalities of metropolitan São Paulo. The samples were analyzed for the presence of anti-*T. cruzi* IgG by commercial Pathozume Chagas ELISA kit (OMEGA) and by indirect immunofluorescence (IFI) with commercial Imuno Com kit (WAMA). **Results and Discussion:** The incidence of positive or indeterminate results in samples was 14.1% (140 patients of 993). Except for samples that came from Itapevi that showed just 5.26% positive results, samples of other municipalities showed equivalent rates of incidence of chagas disease: Vargem Grande (17.24%), Carapicuiba (15.35%), Osasco (13.46%) and Cotia (12.19%). Among the 140 patients with positive results to Chagas disease, we found a higher prevalence in adults from 32 to 87 years (70.71%). Regarding sex, most positive samples were from women (57.14%). When these results were analyzed together with data from medical handbooks of positive patients, we found that physicians adopted similar procedures for Chagas diagnosis in the period. Most suspected patients (65%) received medical attention due to heart disease at the moment of blood collection. Just 25% of patients were hospitalized, but around 50% of patients were not yet diagnosed for Chagas disease. In general, ELISA and IFI are requested for the exact diagnosis of the disease. In our analysis, the two methods showed similar results in 95% of samples (133 patients). Results were divergent or inconclusive in only seven patients (5%).

11. PIBIC Program

11.01 Maternal exposure of Wistar rats to the venom of the *Tityus bahiensis* during lactation and its effects on the offspring.

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Introduction: Previous studies with the venom of the scorpion *Tityus bahiensis* demonstrated that it causes deleterious effects in the offspring when injected into rats during pregnancy. There are no studies in the literature demonstrating if there are some effects on the offspring when the venom is inoculated during lactation. **Objectives:** The aim of this work was to evaluate the effects of the injection of *T. bahiensis* venom during lactation on the offspring. **Methods:** The dose of venom was 2.5 mg/kg (sc). Pregnant females were separated into control groups injected with saline on the 10th (10C) or 16th postnatal days (16C) and into experimental groups injected with venom on the 10th (10E) or 16th postnatal day (16E). Pups were evaluated for their behavioral development. The parameters measured were: forced swimming, box activity and enriched environment. **Results and Discussion:** The following results were obtained for animals injected on the 10th day and assessed for forced swimming: latency to stop swimming (females: C 3.4±2.9, E 17.4±14.6; males: C 8.3±8.3, E 17.7±22.5) and immobility (females: C 107.8±53.9, E 64.1±12.6; males: C 99.0±47.2, E 69.9±26.4) and For the injection on the 16th day the results were: latency (females: C 13.1±11.6, E 13.7±11.6; males: C 11.9±7.8, E 21.5±8.7) and immobility (females: C 64.9±46.5, E 68.6±57.6, males: C 71.9± 9.6, E 46.1±35.1). The animals injected on the 10th day assessed for box activity gave the following results: total activity (females: C 453.6±61.0, E 586.7±93.3; males: C 232.5±123.7, E 470.0±229.1) and ambulation (females: C 322.3±79.3, E 440.0±81.0; males: C 145.0±59.4, E 328.5±183.1). For the injection on the 16th day the results were: total activity (females: C 554.6±245.5, E 594.3±147.1; males: C 474.5±90.4, E 482.7±30.9) and ambulation (females: C 421.6±189.2, E 442.6±118.9; males: C 330.0±70.2, E 333.0±41.4). The animals injected on the 10th day assessed for enriched environment showed the following results: total activity (females: C 719.0±342.7, E 720.2±28.1; males: C 408.5±123.7, E 556.0±25.4), ambulation (females: C 536.6±315.3, E 549.2±20.7; males: C 309.0±124.4, E 403.0±12.7), and exploratory activity (females: C 130.2±21.4, E 162.6±60.8; males: C 213.00±5.5, E 191.0±25.4). For the injection on the 16th day the results were: total activity (females: C 686.7±116.6, E 671.7±210.9; males: C 579.5±180.0, E 408.5±279.1), ambulation (females: C 524.7±103.6, E 440.2±228.5; males:C 400.2±157.8, E 201.7±170.5), and exploratory activity (females:C 172.8±35.4, E 170.6±32.3; males:C 206.0±24.7, E 165.2±65.6). There were no significant changes in all parameters studied. Thus, no deleterious effect was observed when the venom was injected during lactation, in contrast to the findings obtained when venom inoculation occurs during pregnancy.

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11.02 Antimicrobial susceptibility pattern of enterotoxigenic *Escherichia coli* (ETEC) and enteroaggregative *Escherichia coli* (EAEC) strains.

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Introduction: *Escherichia coli* are facultative anaerobes in the normal intestinal flora; however, pathogenic strains of these bacteria are an important cause of infectious diarrhea. The diarrheagenic *E. coli* pathotypes include *E. coli* enterotoxigenic (ETEC) and *E. coli* enteroaggregative (EAEC). The emergence and spread of antimicrobial resistance in *E. coli* have been well documented as a serious public health problem worldwide, making it necessary to perform tests to assess drug sensitivity. For the treatment of some bacterial infections, antibiotics that are used such as the β-lactams can be degraded by the extended-spectrum beta-lactamases (ESBLs). **Objectives:** This study was carried out to determine the antimicrobial susceptibility patterns of ETEC and EAEC strains isolated from infantile diarrhea cases in Salvador, Bahia and presumptively to detect ESBL production. **Methods:** The ETEC and EAEC strains (20 strains each) were tested for susceptibility with the Kirby-Bauer disc diffusion method, utilizing Muller-Hinton agar and the following antibiotics: amoxicillin + clavulanic acid (AMC), nalidixic acid (NAL), ampicillin (AMP), cephalothin (CFL), ciprofloxacin (CIP), chloramphenicol (CLO), streptomycin (EST), gentamicin (GEN), sulfamethoxazole + trimethoprim (SUT) and tetracycline (TET). The following antibiotics were used for the presumptive detection of ESBL: cefotaxime (CTX), aztreonam (ATM), ceftazidime (CAZ) and ceftriaxone (CRO). **Results and Discussion:** The ETEC strains showed 100% sensitivity to NAL, AMC, CIP and CLO; resistance to SUT (45%), AMP (40%), TET (25%), EST (20%) and GEN (5%); and intermediate pattern to EST (15%), CFL (10%) and AMP (5%). The EAEC strains displayed 100% sensitivity to NAL, CIP and GEN; resistance to AMP and SUT (55% each), TET (40%), EST (15%), CLO and AMC (10% each), and CFL (5%); and intermediate pattern to EST (25%) and CFL (5%). We found an elevated percentage of strains (45% of ETEC and 50% of EAEC strains) that showed multiple drug resistance, mainly to AMP, EST, SUT and TET. The antibiotics used for detection of ESBL did not detect any strain producing the enzyme. Research on the antimicrobial susceptibility profile of ETEC and EAEC strains has been shown to be lacking. Although *E. coli* strains usually show sensitivity to a broad spectrum of antibiotics, exposure to these substances tends to select resistant organisms. Plasmids encoding β-lactamases in *E. coli* strains are the main form of resistance against β-lactam antibiotics, and its detection is very important, especially in the pediatric area. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control among the main etiological agents of diarrhea.

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11.03 Effects of trypsin/chymotrypsin inhibitors from *Nephilengys cruentata* on the development of the dengue fever vector *Aedes aegypti*

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Introduction: *Aedes aegypti* is the urban vector of dengue viruses worldwide causing significant morbidity and mortality. *Ae. aegypti* is a mosquito that exploits peridomestic water containers as its larval habitats, and human reservoir hosts that are preferred for blood feeding. Efficient alternative control strategies are still required. Spiders are carnivorous arthropods and insects are their major source of prey. Predators ingest a large quantity of peptidases from their prey which have to be controlled. There are some suggestions in the literature of the presence of peptidase inhibitors in the digestive system of some spider species. Our group has shown that the spider *Nephilengys cruentata* contains trypsin/chymotrypsin inhibitors (TCI) in their digestive juice and their hepatopancreas. These inhibitors are very efficient in inhibiting digestive trypsins and chymotrypsins from insects. **Objectives:** The aims of this study were to isolate TCI from *N. cruentata* hepatopancreas, to determine the dissociation constant of inhibitor/chymotrypsin complex, and to observe *in vivo* effects of these inhibitors on *Ae. aegypti* larval development. **Methods:** Hepatopancreas from adult females from *Nephilengys cruentata* were isolated by dissection and were homogenized in 0.1 M acetate buffer, pH 3.5, and centrifuged at 13,000 rpm for 30 min. The soluble portion was then boiled for 5 min. Samples were centrifuged again and the soluble portion was used as inhibitor sample to be applied onto a Superdex G75 column (gel filtration). Fractions able to inhibit insect digestive trypsins were pooled and submitted to anion-exchange chromatography (ResourceQ column). Inhibitory fractions were pooled, lyophilized and applied to a 15% polyacrylamide gel stained with Comassie Blue R. Different concentrations of isolated inhibitor were tested against bovine chymotrypsin, resulting in the determination of the dissociation constant of the inhibitor/chymotrypsin complex. Enriched inhibitory samples were added to the diet of *Ae. aegypti* larvae in order to test the effect of the presence of these inhibitors on larval development. **Results and Discussion:** We observed that *N. cruentata* has at least two important TCIs which are very efficient against insect digestive trypsins and chymotrypsins. We isolated the major TCI form from *N. cruentata* hepatopancreas which is thermal and pH stable and has a molecular mass of 13 kDa. This inhibitor showed a K_D of 67 nM against bovine chymotrypsin. These inhibitors in *Ae. aegypti* larval diet caused a delay in larval development indicating that these molecules may be good candidates in dengue vector control.

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11.04 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*. These spiders have large numbers of urticating hairs on their abdomen that can be ejected from their body when they are disturbed. To our knowledge, there are no reports of allergic type reactions occuring following contact with other groups of spiders. Moreover, several workers of 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 develop methods 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:** Initially there will be a detailed study of the work environment to identify all tasks that involve exposure to spiders from the genus *Loxosceles*. Workers will be assessed for venom allergy using questionnaires and immunological tests. The presence of venom sensitization will be determined through quantification of specific IgE (ELISA). Allergens will be studied using the Western blots and inhibition assays. **Results and Discussion:** Based on the study of the work environment, we developed a questionnaire containing questions regarding personal history of allergy, spider stings, and contact (oral or ocular) with spider venom, as well as work history (length of employment and specific work tasks) and work-related symptoms. We observed that the job of arachnologists entails specific tasks, including spider cage cleaning, spider feeding, spider venom extraction, the handling of spider venom, spider room cleaning and the handling of spiders for identification. Estimates of the exposure times to spiders or spider venom will be derived from the following questions: ''How many days per week do you perform [the specific task]?''; and ''For how many years have you been performing [the specific task]?'' The frequency of exposure to each specific task will be reported as a continuous variable, in days per year. To assess the influence of each task at different frequency levels, an exposure time index will be calculated by multiplying the years of exposure by the frequency of exposure to each specific task. Our next step is to apply the questionnaires, collect blood samples and perform laboratory tests.

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11.05 ***Nephilengys cruentata*** **hemolymph as source of metallopeptidase inhibitors**

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Introduction: Notwithstanding the fact that peptidases are present in all biological processes and correspond to 2% of all genes, these enzymes must be strictly controlled. During evolution, two strategies were selected to diminish proteolytic activity: the synthesis of peptidases as zymogens and specific inhibitors. The discovery of new inhibitors is important to control undesired peptidases as observed in diseases, such as cancer and rheumatism. *N. cruentata* showed two classes of proteolytic enzymes involved in protein digestion: a cathepsin L-like enzyme and a metallopeptidase: an astacin-like enzyme (NcAst) already isolated by our group. There is some evidence in the literature of the presence of metallopeptidase inhibitors in the hemolymph of crustaceans and spiders. The isolated astacin from *N.cruentata* was efficiently inhibited by *N.cruentata* hemolymph samples. However, in order to isolate this inhibitor we needed to establish sensitive assays to NcAst. **Objectives:** The aim of this study was to establish a sensitive method to determine NcAst, and to isolate a NcAst inhibitor from *N.cruentata* inhibitor. **Methods:** *N.cruentata* adult females were collected, immobilized and dissected. Hemolymph was recovered with the help of a micropipette and added to a 10 mM sodium cacodylate and calcium chloride solution. Samples were homogenized with a pellet pestle and submitted to gel filtration on a Superdex G75 column. Hepatopancreas homogenate samples and isolated astacin were used as astacin activity source, and the synthesized Abz-GPKRAPWV-K(Dn)-OH (ASub) was used as substrate. Inhibitory activity was pooled and samples were applied to a 7.5% polyacrylamide gel and submitted to electrophoresis. Gels were silver stained. The kinetic parameters of the isolated astacin to the new substrate were determined using different substrate concentrations. **Results and Discussion:** Previously isolated NcAst was characterized using ASub as substrate. The purification steps used in astacin isolation were repeated and followed with casein-FITC (the former substrate) and with the new one. There was no alteration of astacin isolation indicating that this substrate is a specific substrate to this enzyme. The isolated astacin showed a Km of 45 μM ± 5.5 μM for ASub. The presence of hemolymph sample in astacin activity assay indicated an inhibition of approximately 70%. Hemolymph samples were applied to a SuperdexG75 column. The estimated molecular mass of this inhibitor in gel filtration was 140 kDa. SDS-PAGE of the inhibitory pooled fractions indicated an enriched protein band of 70 kDa suggesting that, under native conditions, this inhibitor dimerizes. This inhibitor will be tested with astacin from *Loxosceles gaucha* venom and with the recombinant astacin from *Astacus astacus*.

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

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Introduction: 5-Aminolevulinic acid (ALA) is a heme precursor accumulated both in inborn and acquired hepatic porphyria, such as acute intermittent porphyria (AIP), tyrosinosis and lead overload. Increased hepatocellular carcinoma (HCC) incidence in patients with AIP has been reported by several authors and has been hypothesized to be related to ALA and its derivatives. *In vitro*, ALA undergoes enolization and subsequent metal-catalyzed aerobic oxidation yielding reactive oxygen species, which can cause oxidative damage to DNA and proteins, which could be involved in the initiation and promotion of cancer. We demonstrated that ALA is able to cause single strand breaks in plasmid and calf thymus DNA *in vitro,* and to increase the steady state level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) in liver DNA of ALA-treated rats. Another mechanism that could be involved in the triggering of cancer is the alkylating property of the final oxidation product of ALA, 4,5-dioxovaleric acid (DOVA). We established that DOVA is an efficient alkylating agent of the guanine moieties in the nucleoside and isolated DNA. Diastereoisomeric adducts were produced through the formation of a Schiff's base involving the N^2-amino group of 2'-deoxyguanosine and the ketone function of DOVA. ALA and DOVA were shown to be mutagenic in Salmonella/microsome mutagenicity assay and Chromotest. **Objectives:** The main objective of this work was to determine the mutation spectra promoted by ALA in a bacterial system, contributing to the elucidation of the mechanism involved in DNA damage promoted by ALA. **Methods:** Competent *Escherichia coli* DH10b strain was transformed with plasmid pAC189, which contains the *supF* gene. The plasmid DNA was extracted and treated with different concentrations of ALA. MBL50 strain was then transformed with ALA-treated plasmid DNA and plated in selective medium containing X-gal, IPTG or *L*-ara. Survival rate of transformed bacterial colonies was calculated. Mutants were selected and *supF* gene was sequenced to obtain the mutation spectra. **Results and Discussion:** We observed an ALA dose-dependent decrease in colony survival rate and an increased mutation rate. The results showed deletions of bases, transitions and transversions. Further analysis is under investigation to obtain more mutants and determine the wide mutation spectra and possible hot spots. These results showed the possible mutagenic events involved in the mechanism of DNA damage induced by ALA and its derivatives, which could act as endogenous weapons, and are consistent with the hypothesis that these compounds could be associated with deleterious processes involved in the development of HCC in symptomatic AIP patients.

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11.07 Search for new human peptidase inhibitors present in the low molecular weight fraction from the venom of *Tityus serrulatus*

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Introduction: Scorpions are chelicerates that had a great evolutionary success and are therefore widely distributed throughout the world. Because of this, it is of medical importance as the major cause of human animal poisoning. In Brazil, the main species is *Tityus serrulatus*, the yellow scorpion, and, until now, little information about both venom's components and their mechanism of action are available. **Objectives:** The aim of this work was to investigate the action of the peptide fractions present in *T. serrulatus* venom (TsV) on human peptidase activities, such as elastase, thrombin and neurolysin. **Methods:** In the first step, TsV (2 mg/mL) was fractionated by a gel-filtration chromatography using a Superose 12 column, which resulted in 11 new fractions. These fractions (F0-F10) were studied with the cited proteases through the use of FRET substrates in a spectrofluorimeter. The fractions that showed inhibitory or activator activities (F4, F6 and F10) were subjected to HPLC purification steps and the peaks were collected manually for later proteolytic assays **Results and Discussion:** It was observed that certain fractions obtained from the first purification step were able to reduce the activity of proteases, such as the F4's inhibitory action on thrombin (40%) and neurolysin (75%), or even increase proteolysis, as with the effect of F10 on elastase (220%). At this moment, we focused on the analysis involving neurolysin, considering its importance for CNS and good inhibitory potential by TsV fractions. F4 was selected and submitted to the HPLC-RP purification, obtaining 14 new peaks, which were tested with neurolysin. Of this total, 3 fractions showed a good inhibitory potential and the analyses of primary sequences of these peptides revealed some sequences already described in literature and other new peptides. The methodology used here led us to uncover a new fragment from the known peptide named hypotensin I (AEIDFSGIPEDIIKQIKET) and the PAPE fragment (AEPAAPAAAAEPEP). In addition, the results showed new interesting molecules similar to sequences in anemone venom proteins, which could suggest a positive Darwinian selection in the evolution of scorpion venom. The observation of neurolysin activity inhibition and the presence of hypotensins are possibly related to the strong pressure drop observed in the post-envenomation symptoms and it may help to further studies of the venom of *T. serrulatus* and its mechanism of action.

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11.08 Taxonomic characterization of *Oxyrhopus guibei* (Serpentes, Dipsadidae, Xenodontinae)

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Introduction: Snakes of the genus *Oxyrhopus* belong to the tribe Pseudoboini, subfamily Xenodontinae. The tribe Pseudoboini comprises nine genera, whose representatives are found from southern Mexico to Argentina. The genus *Oxyrhopus* is currently composed of 14 species distributed over almost all Latin America regions. Snakes of this genus are opistoglyphous and oviparous and have elliptical pupils, strong sexual dimorphism, a pair of apical pits on dorsal scales, single cloacal plate, and divided subcaldals. Its species show predominantly nocturnal activity and their diet is composed basically of lizards and rodents. *Oxyrhopus guibei* is a common species that occurs in western to southeastern Brazil, including areas from the Brazilian state of Paraná, Paraguay, Bolivia, and Argentina. *Oxyrhopus trigeminus guibei* was described based on 13 specimens. The holotype is an adult female from Londrina, Paraná. To date, it is possible that the type series has been lost during the recent fire accident that destroyed most of the Herpetological Collection of the Institute Butantan. The authors that described *O. trigeminus guibei* differentiated it from *O. t. trigeminus* on the basis of the number of subcaudals 59-91 (vs. 53-81 in *O. t. trigeminus*), black snout (vs. snout scattered with white dots), and triads overlapping the belly (vs. belly immaculate or with black cross lines). It was later elevated to specific rank based on hemipenial morphology, color pattern, and pholidosis characters. Currently, the species is still insufficiently diagnosed due to overlapping nature of meristic and color pattern characters. **Objectives:** The main aim of this study was to provide a robust diagnosis for *Oxyrhopus guibei* with respect to other congeners, accurately delimiting their area of distribution. Another objective was to identify the areas where it occurs parapatric or sympatric with *O. trigeminus*. **Methods:** Terminology and nomenclature employed in the study followed the traditional use in snake systematics. We examined specimens of the Instituto Butantan, but additional samples from other institutions should be analyzed in order to finish the study. We took data from external morphology (meristic, morphometric, and color pattern characters) along most of the range of distribution of the species. **Results and Discussion:** To date, we have analyzed a hundred specimens of *Oxyrhopus guibei*, as well as additional specimens of *O. trigeminus* and *O. melanogenys* for comparative purposes. *Oxyrhopus guibei* is distinguished from the other species of the genus by the following combination of characters: 19/19/17 dorsal scale rows; ventral scales 197-217 ($\bar{x}$=207) for females and 186-199 ($\bar{x}$=192) for males; subcaudals 59-82 ($\bar{x}$=73) for females and 67-91 ($\bar{x}$=78) for males; eight supralabials; ten infralabials; 2+3 temporals; single preocular; two postoculars; divided nasal; black snout; and triads overlapping the belly.

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11.09 Isolation of prothrombin from *Bothrops jararaca* plasma: preliminary results

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Introduction: Prothrombin is the most abundant of the vitamin K-dependent blood clotting proteins, circulating at plasma concentrations between 1 and 2 μM. This factor is a plasma zymogen that is converted to thrombin – an essential enzyme that converts fibrinogen to fibrin, the main structural component of the clot. **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B. jararaca*) prothrombin and to compare it with prothrombin of humans and other animals. **Methods:** Prothrombin was partially purified from snake plasma through HiTrap DEAE Fast Flow chromatography, followed by affinity cromatography on HiTrap Cu^{2+}Chelating HP and HiTrap Blue HP columns. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Purification steps were analyzed by SDS-PAGE. Amidolytic thrombin activity was measured using chromogenic substrate (S-2238) after prothrombin activation by *Oxyuranus scutellatus scutellatus* venom. **Results and Discussion:** Our results indicate the presence of prothrombin after HiTrap DEAE Fast Flow and HiTrap Cu^{2+}Chelating HP chromatographies. However, after these purification steps, the protein was not pure and the activity was low. The perspectives for this work are to improve the purification process in order to get higher purity protein and to compare it biologically and biochemically to prothrombin of other animals.

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11.10 Effect of crotoxin on secretory activity of peritoneal macrophages co-cultivated with tumor cells. Involvement of formyl peptide receptors

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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, was not determined yet. 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 activity (nitric oxide-NO) of macrophages co-cultivated with LLC WRC 256 tumor cells (Ethical Committee For Animal Research of Butantan Institute, No. 631/09) was evaluated. **Methods:** In *in vitro* assays, the effect of CTX on nitric oxide-NO production by macrophages co-cultivated with LLC WRC 256 tumor cells was investigated. Macrophages were obtained from peritoneal cavity and cells ($2x10^5$) were incubated with CTX (0.3 μg/mL) for 2 h at 37°C. After this time, the macrophages were co-cultivated in the presence of LLC WRC 256 tumor cells ($2x10^4$), previously plated in 96-well culture plates. After 48 h, at 37°C, in a humidified atmosphere of 5% CO_2 in air, the effect of CTX on the production nitric oxide-NO was evaluated. After this period, cell proliferation was measured by MTT assay. The involvement of formyl peptide receptors with the stimulatory effect of CTX on the production nitric oxide-NO by macrophages was evaluated using Boc2, a selective antagonist of formyl peptide receptors. **Results and Discussion:** The results showed that macrophages previously incubated in the presence of CTX and co-cultivated with tumor cells generated a greater quantity of NO (35%) than control cells. Tumor cells co-cultivated with macrophages pre-incubated with CTX showed reduction (25%) of proliferation. Boc-2 reversed the stimulatory effect of CTX on secretory activity of macrophages and the inhibitory effect of these macrophages on tumor cell proliferation. Taken together, the results indicate that CTX modifies the secretory activity of M2 cells, which may contribute to the inhibitory action of the toxin on tumor growth, and activation of formyl peptide receptors seems to play a major role in this effect. These data reinforce the actions of CTX on defence mechanisms and open new perspectives for the development of a new substance with therapeutic properties.

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11.11 Isolation of toxins with high affinity for heparin from *Bothrops cotiara* venom

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Introduction: Heparin is a highly-sulfated glycosaminoglycan released from mast cells and is involved in anti-coagulant and anti-inflammatory processes. We had previously shown that *Bothrops* venoms contain some toxins with high affinity for heparin *in vitro*. **Objectives:** The aim of this study was to isolate toxins with high affinity for heparin from *B. cotiara* venom. **Methods:** *B. cotiara* venom was chromatographed on a heparin-Sepharose column previously equilibrated with 0.1 M ammonium acetate, and proteins were eluted with increasing ammonium acetate concentration and analyzed by SDS-PAGE. The fractions with high affinity for heparin were submitted to reversed-phase HPLC, using a Shim-Pack CLC-C_8 column (250 mm×4.6 mm, particle size 5 µm, Shimadzu). Alternatively, *B. cotiara* venom was chromatographed (FPLC system) on a gel filtration column (Superdex 75 10/300 GL; GE Healthcare). Fractions containing proteins of ~ 30 kDa were further submitted to cation-exchange chromatography (MonoS HR 5/5; GE Healthcare). Protein fractions were analyzed by SDS-PAGE. Protein identification was performed by *in gel* trypsin digestion followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis on a ion trap mass spectrometer (LTQ-XL, Thermo Scientific). Mass spectra of peptides were submitted to database search (MASCOT 2.2.04, Matrix Science) restricted to Serpentes taxonomy. The isolated serine proteinase with affinity for heparin was submitted to the following assays: platelet-aggregating, hemorrhagic, fibrinogenolytic, coagulant, and amidolytic activities. Moreover, the enzyme was tested for its ability to activate coagulation factors II and X. **Results and Discussion:** Visual inspection of the gels indicated few proteins with high affinity for heparin in *B. cotiara* venom (eluted with 2.0 M ammonium acetate from the heparin-Sepharose column), and these did not show gelatinolytic and hemorrhagic activities. Moreover, immunostaining with specific antibodies showed the presence of metalloproteinases and serine proteinases among the proteins with high affinity for heparin. The gel-filtration chromatography of the crude venom followed by cation-exchange chromatography resulted in the isolation of a novel serine proteinase of ~30 kDa with high affinity for heparin. The enzyme showed amidolytic activity on peptide p-nitroanilide substrates, and it is devoid of hemorrhagic, coagulant, platelet-aggregating and fibrinogenolytic activities. Interestingly, the enzyme was able to directly activate coagulation factor II (prothrombin) but not factor X. This is an unusual activity for a snake venom serine proteinase, and we are currently investigating the activity of the enzyme on plasma recalcification time.

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11.12 Neurobehavioral effects of dantrolene in rats

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Introduction: In previous studies in our laboratory, dantrolene, a drug that inhibits the release of intracellular calcium, was used to reverse the effects of a scorpion toxin. In this experiment dantrolene significantly reduced the hippocampal damage caused by the toxin, but when used individually, lesions were observed in the hippocampus of the animals. **Objectives:** Based on this study, we aimed to investigate whether the administration of dantrolene could cause neurobehavioral changes in rats. **Methods:** Forty male Wistar rats (240-260g) were divided into four groups: control group (C) treated with saline (0.9%), experimental group treated with 5.0 mg/kg of dantrolene (D5), experimental group treated with 10.0 mg/kg of dantrolene (D10), and experimental group treated with 15.0 mg/kg of dantrolene (D15). Tests included: box activity, enriched environment, forced swimming and social interaction. **Results and Discussion:** In animals observed in the box activity, there was a reduction in locomotion with the dose of 10.0 mg/kg and 15.0 mg/kg (C: 594.8±61.02; D5: 456.2±47.34; D10: 268.0±46.69*; D15: 288.6±47.55*) and proportionately in general activity (C: 428.8±33.93; D5: 376.9±39.26; D10: 173.0±18.75*; D15: 195.0±38.16*). In the enriched environment, it was observed that locomotion (C: 482.2±53.61; D5: 226.6±29.69*; D10: 191.0±34.38*; D15: 234.2±49.33*) and general activity (C: 638.6±56.61; D5: 341.6±43.07*; D10: 288.0±44.38*; D15: 362.4±75.94*) were also reduced for all the doses, despite that the time (in seconds) spent in the exploratory activity did not change (C: 149.4±21.97; D5: 129.6±45.84; D10: 41.40±17.23; D15: 112.2±26.60). There was no change in the forced swimming, evaluated as latency to stop swimming (C: 6.600±1.030; D5: 8.400±1.288; D10: 6.200±2.107; D15: 8.000±4.171), and time of immobility (C: 163.6±51.07; D5: 223.6±55.47; D10:78.40±23.80; D15: 41.20±11.65). The dose of 5.0 mg/kg caused a reduction in the time spent in social interaction of the animals (C: 128.4±3.745; D5: 43.60±10.46*; D10: 140.6±4.754; D15: 127.2±8.696). We can conclude that the three doses of dantrolene cause behavioral changes in animals, which can be associated with lesions previously observed.

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

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Introduction: In Brazil, there are about 20,000 accidents with snakes annually, mostly with the *Bothrops* genus (about 90% of all accidents). *Bothrops 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 evaluate the interference of biological variables which caused snakebites in São Paulo State. **Methods:** *Bothrops jararaca* specimens that have caused accidents from 1995 to 2010 are preserved at "Coleção Vital Brazil" at Instituto Butantan. All these snakes were dissected and examined. Data related to seasonal activity, mating season, sexual maturity and diet were collected, analyzed statistically and discussed. **Results and Discussion:** Snake stomachs were dissected to check whether or not they had stomach contents. These data revealed that *Bothrops jararaca* male juveniles fed mainly in spring, whereas female juveniles fed mainly in 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 accidents occurred during the day, between 6 am and 6 pm. However, many accidents occurred during the night period, between 7 pm and midnight. Adult snakes caused more accidents during the day period. Our data show that 71% of the accidents were caused by juveniles, whereas 29% were caused by adults. Adult females caused more accidents than adult males. Males caused more accidents during the fall, whereas females caused more accidents during the summer. The juveniles (male and female) caused more accidents during the spring and summer. Analyzing the female reproductive status of the snakes that caused accidents, reproductive females were found during the summer and pregnant females were found starting at the end of spring. The juveniles were born in the fall. Accident seasonal patterns are different between *B.jararaca* adults and juveniles. However, juveniles caused more accidents than adults. Preliminary analysis shows that juveniles and adult females cause more accidents than juveniles and adult males. 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).

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11.14 Purification and characterization of antimicrobial peptides present in the venom of *Nephilengys cruentata*

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Introduction: The first description of antimicrobial activity in spiders was published in 1989, with one species in China. Since then, many studies involving antimicrobial peptides were performed. More than 1200 antimicrobial peptides have been identified in all living species. In recent years, a large number of these molecules were isolated from insects. Examples of antimicrobial peptides from spiders may be cited: two peptides (lycotoxins I and II) of the venom of the spider *Lycosa carolinensis*, a family of peptides (cupienins) of the spider *Cupiennius salei* and one peptide (gomesin) of hemocytes of *Acanthoscurria gomesiana*. Currently, there is a big problem with respect to the emergence of bacterial strains resistant to conventional treatment with antibiotics. The main reason is due to overuse and inappropriate use for human beings. Increased migration of the population contributes to the spread of these resistant organisms emerging in the world, so there is much interest on the pharmacological application of antimicrobial peptides (AMPs) in the treatment of infections. **Objectives:** The aim of this study was to separate and characterize bioactive molecules with antimicrobial function, previously obtained from the venom of the spider *Nephilengys cruentata* (Araneomorphae, Nephilidae). **Methods:** To obtain the poison, a low-voltage electrical stimulator was used, where the poison was harvested with a pipette and subjected to centrifugation at 14,000x 3 min. The material obtained was concentrated in a vacuum centrifuge (Savant Instruments, Inc.). After centrifugation the material was dissolved in 0.05% trifluoroacetic acid (TFA) and applied in two disposable SEP-PAK C18 columns connected in series, in order to pre-purify antimicrobial peptides. There were three stages of successive elution using different concentrations of acetonitrile (5%, 40% and 80%) in acidified water. The resulting fractions were concentrated in a vacuum centrifuge, resuspended in acidified water and subjected to liquid chromatography (HPLC). The organisms used for testing the presence of antimicrobial activity were the Gram-positive *Micrococcus luteus* A270, Gram-negative *Escherichia coli* SBS363 and yeast *Candida albicans* SBS363 MDM8. **Results and Discussion:** Two fractions eluted at 40% obtained by high performance liquid chromatography showed antimicrobial activity against bacteria *E. coli* and *M. luteus*. When subjected to mass spectrometry ESI-MS type, it was not possible to obtain the masses of the two samples, since they were not pure. A second purification of two samples was then carried out by high performance liquid chromatography. One sample had six fractions, but their antimicrobial activity was lost, probably due to the low concentration of molecules, and the second sample also showed several fractions, where two of them continued to show antimicrobial activity.

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11.15 Studies on the growth of *Neisseria lactamica*

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Introduction: *Neisseria lactamica* and *Neisseria meningitidis* are Gram-negative, diplococcal bacteria which occur in the nasopharynx of humans. *Neisseria meningitidis*, can cause septicemia or meningococcal disease, especially in young infants. Bacterial meningitis remains a serious threat to global health, reaching 500,000 cases a year around the world, with at least 50,000 deaths and at least the equivalent of cases with neurological damage. Serogroup B polysaccharide vaccines fail to induce bactericidal antibodies. An alternative approach to producing a vaccine for serogroup B is the development of an outer membrane vesicle, OMV, vaccine based on the commensal bacteria *Neisseria lactamica,* a closely related species of *N. meningitidis*. During bacterial growth, OMV are constantly being discharged from the surface of the cell. Immunological and epidemiological evidence suggests that carriage of *N. lactamica* contributes to natural immunity against *Neisseria meningitidis*. **Objectives:** The aim of this work was to study the growth kinetics of *Neisseria lactamica* in shaker culture in different growth media and to analyze and compare the yield of OMV and the electrophoretic pattern of major proteins. **Methods:** *N. lactamica* was cultivated on a shaker, at 200 rpm, 36°C for 8-17 h. The culture media tested were: defined culture media MC2LAA and MC with or without the addition of ultrafiltrate yeast extract or enzymatic digested soybean. Biomass was measured by reading optical density at a wavelength of 540 nm, and the yield of OMV was determined by Lowry's method. **Results and Discussion:** Growth was approximately OD_{540} 0.5, 1.7, 2.2 and OMV yield was 15 mg/L; 31 mg/L, 54 mg/L in defined culture media MC2LAA, defined medium with enzymatically digested soybean and defined medium with ultrafiltrate yeast extract, respectively. The electrophoretic pattern was similar in all media. Our results suggest that limiting growth factor of *N. lactamica* is not only due to carbon or nitrogen sources but may be related to the limitation of vitamins present in the yeast extract.

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11.16 Description of the male of *Acanthoscurria rondoniae* Mello-Leitão 1923 and the female of *Acanthoscurria insubtilis* Simon 1892, and new distribution records (Araneae: Mygalomorphae, Theraphosidae)

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Introduction: The genus *Acanthoscurria* Ausserer is represented by 40 species, but several are only known by the holotype male or female. Simon described in 1892 *Acanthoscurria insubtilis* based on a male from San Mateo, Bolívia. The species *Acanthoscurria rondoniae* Mello Leitão, 1923 was described based on a female from Mato Grosso, Brazil. **Objectives:** The aim of this study was to describe the female of *A. insubtilis* and the male of *A. rondoniae.* **Methods:** The material examined is deposited in the collection of the Instituto Butantan, São Paulo, Brazil. Female epigynum was dissected and cleared in lactic acid for observation of internal structures. The drawings were made on a Leica MZ 12.5, with a camera lucida. **Results and Discussion:** Females of *A. insubtilis* resemble *A. theraphosoides* (Dol. in Ausserer, 1871) and *A. rondoniae* by the morphology of the seminal receptacles, with a smaller base involving the seminal receptacles, but can be distinguished by the bulged seminal receptacle without basal constriction and distant one from the other. The male of *Acanthoscurria rondoniae* is close to *A. paulensis* and *A. chacoana* due to the appearance of the embolus with two prolateral keels, one superior and other inferior and presence of a blunt tubercle on the palpal tibia, but can be distinguished by the less developed keels with a well-marked anterior projection of the inferior. The study contributed to the better knowledge of both species, until known only by the holotypes. The distribution range of *A. insubtilis* is enhanced for Brazil: states of Rondônia, Acre, Mato Grosso and Mato Grosso do Sul, and *A. rondoniae* for states of Tocantins, Mato Grosso, Rondônia, Goiás, Minas Gerais and Mato Grosso do Sul (Brazil).

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

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Introduction: Actinic cheilitis is the initial and incipient stage of squamous cell carcinoma, resulting from excessive and long-term exposure to solar ultraviolet radiation. Malignant tumors are characterized by unrestrained cell growth, invasion in adjacent tissue and their ability to metastasize. Laminin-5 is a heterotrimer containing α3, β3, γ2 chains, a playing important role in development of squamous cell carcinoma and its invasive properties. One of the functions of laminin-5 in epidermal cells is its ability to interact with two major epithelial integrin receptors, α3β1 and α6β4. The expression of laminin receptors, in particular α6β4 integrin, has also been shown to have an important role in squamous cell carcinoma progression. The integrin α6β4 is concentrated in hemidesmosomes during the migration of keratinocytes. The development of carcinomas is associated with disassembly of hemidesmosomes. The domain III γ2 chain of laminin 5 interacts with EGFR to induce phosphorylation of tyrosine in the cytoplasmic domain of integrin β4, leading to disassembly of hemidesmosomes and stimulating cell migration. **Objectives:** The aim of this study was to analyze and evaluate through immunohistochemical techniques the expression and distribution of laminin-5 and integrins β1, β4 and α3 in actinic cheilitis and in lip squamous cell carcinomas. **Methods:** Paraffin blocks of actinic cheilitis, superficially invasive squamous cell carcinoma and invasive squamous cell carcinoma, from Hospital das Clínicas da Faculdade de Medicina da USP, were sectioned. Immunohistochemical reactions to laminin-5 gamma-2 chain, β1, β4 and α3 were carried out, and the slides were examined by light microscopy. **Results and Discussion:** The majority of cases of actinic cheilitis and superficially invasive squamous cell carcinoma showed lack of expression of β1, β4 and α3 integrins in basal and parabasal layers of epithelium. In areas of dysplastic epithelium, loss of expression was also observed in cells of granular and spinous layers. Slides of invasive squamous cell carcinomas showed loss of β1, β4 and α3 immunoexpression in peripheral layers of tumor islands and strands. Cytoplasmic staining for laminin-5 gamma 2 chain was absent in actiic cheilitis cases. All the cases of superficially invasive carcinoma and invasive carcinoma showed laminin 5 gamma 2 chain positivity located in the extracellular matrix and in the peripheral cells of tumor invasive front, but expression was not homogeneous. No cancerous tissues close to invasive areas showed cytoplasmic expression in the epithelial basal layer.

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11.18 Separation and identification of components present in egg wax of *Rhipicephalus sanguineus* and *Amblyomma cajennense* (Acari: Ixodidae)

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Introduction: Most species of ixodid ticks lay between 2,000 to 5,000 eggs, which are viable in the natural environment because of a substance surrounding the eggs which is capable of protecting them from desiccation and microbial attacks, mainly because of its lipid components. **Objectives:** The objective of this study was to evaluate the lipid composition of the wax that surround the eggs of *A. cajennense* and *R. sanguineus* species. **Methods:** Eggs were obtained from female ticks fed on New Zealand rabbits. The females were kept at 27ºC ± 1ºC and 90% ± 5% relative humidity to complete the oviposition period. The egg surface material was extracted based on two previously described protocols from the literature. The crude extracts were analyzed by two different chromatographic methods, TLC (thin layer chromatography) and HPLC (high performance liquid chromatography). **Results and Discussion:** TLC allowed a visual determination of qualitative lipid content of the egg wax, suggesting the presence of cholesterol esters, free fatty acids, cholesterol, monoglycerides and diglycerides for both species. Triglycerides seem to be present in the crude wax of *R. sanguineus*. The identification of these compounds may help to develop a substance to be used for therapeutic purposes, since there is evidence of that the wax shows antimicrobial resistance.

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11.19 Evaluation of the accidents caused by spiders, scorpions and lepidopteran larvae (caterpillars) in patients seen at Vital Brazil Hospital, Butantan Institute

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Introduction: In Brazil, in 2006, 37,632 accidents by scorpions were reported, 2,658 by *Phoneutria* and 1,215 caused by larvae of Lepidoptera. The most frequent and key characteristic of these three accidents is local pain, which usually is acute and intense and with variable frequency of other phenomenons of local inflammation. **Objectives:** The objective of this study was to compare the epidemiological, clinical and therapeutic characteristics of accidents caused by spiders, scorpions and caterpillars admitted to the Hospital Vital Brazil. **Methods:** This is a prospective observational study, which collected information about the animals that caused the accident (sex, sexual maturity, identification of genus and species), the patient and also the circumstances in which the accident occurred and the characteristics of the painful phenomenon (intensity, irradiation , temporality, frequency, rhythm, factors of improvement and worsening, duration and the kind of pain sensation), the treatment applied and outcome. **Results and Discussion:** The period of data collection began in November. By June 2010, 108 records were filled and will be submitted later. The inclusion criteria are: patients bitten by scorpions and spiders and contact with caterpillars in less than 24 h, without previous treatment and only mild and moderate cases. In this summary, 61 records were analyzed, 32 of which brought the animal and conisdered the first inclusion criteria. Twenty-one (65%) were men, and 5 accidents occurred in rural and 27 in urban areas. Eighteen spiders were brought (15 specimens of *Phoneutria nigriventer*, a genus of *Corinna*, a copy of *Cterus ornatus* and one genus *Lycosa sp*), 13 scorpions (9 *Tityus serrulatus*, 3 *Tityus bahiensis* and 1 *Tityus costatus*) and 3 caterpillars (1 *Megalopygidae* , 1 *Automeris* and 1 without identification). There was an accident caused by *Centruroides sp*, which was not included because it received analgesia prior to Hospital Vital Brazil admission. With respect to the intensity of pain on a 0-10 scale, there was an average intensity of 7 in accidents caused by spiders, 6.5 by scorpions and 6.0 by caterpillars. Pain intensity in 12 cases (37%) was classified as being of intensity less than 5, in 19 cases (59%) above 5, and in 1 case (3%) the intensity of pain was not recorded. Of the patients whose pain was classified as less than 5, 6 were initially treated with hot water, 7 initially received oral analgesic, and 2 local anesthetic, and in two accidents there was no record of treatment. Of the patients whose pain was greater than 5, 15 were first submitted to hot water, 9 received oral analgesic and 11 were treated with local anesthetic. Some patients received at the time of admission, more than one treatment. There are important epidemiological and clinical differences between these accidents that after the conclusion of this study will allow us to distinguish their characteristics and choose better treatments.

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11.20 Antifungal secondary metabolites produced by endophytic fungi

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Introduction: Fungal infections affect millions of people worldwide and their pathogens have been developing resistance to the antifungal chemotherapy available. Therefore, the development of new antifungal agents is urgently needed, and secondary metabolites produced by microorganisms, such as endophytic fungi, can be a huge source of new pharmacological agents. **Objectives:** The aim of the present study was to evaluate the potential of 66 endophytic fungi isolated from coffee tree on producing secondary metabolites with antifungal activity. **Methods:** Antagonism assay was used to evaluate 66 endophytic fungi strains against *Trychophyton rubrum* IOC 4527, *Candida albicans* ATCC 36802/ IOC 3704, *Cryptococcus neoformans* ATCC 90112 and *Aspergillus fumigatus* IOC 4526. The inhibition halo of the pathogen growth was measured in millimeters (mm) and the strains that inhibited the growth of at least 2 pathogens were selected to produce crude extracts. Thus, 15 strains were inoculated into potato dextrose broth and incubated at 28ºC and 150 rpm for 7 days, and the crude extracts were obtained through the supernatant extraction with hexane (HEX) and ethyl acetate (AE) consecutively. The minimal inhibitory concentration (MIC) was determined for each organic extract against the same above pathogens in the range of 8 to 1,000 μg/mL. The crude extract BG9-IId3 was purified by HPLC in 2 steps using CN and PFP column as stationary phase, and acetonitrile as mobile phase. MIC was determined for each fraction obtained against *C. neoformans* ATCC 90112 and *C. albicans* ATCC 36802. **Results and Discussion:** Fifteen fungal strains out of 66 inhibited the growth of at least two pathogens by the antagonism assay with an inhibition halo larger than 5 mm, and organic extracts were produced by these strains. Among 29 extracts, 16 showed MIC $\geq$ 1,000 μg/mL for all the pathogens, and 11 extracts showed a MIC lower than 1,000 μg/mL against at least one pathogen. The extracts BII-01 HEX, BG1-IIIf HEX and BG9-IId3 HEX were the most effective with MICs lower than 500 μg/mL against *C. albicans* ATCC 36802/ IOC 3704, *C. neoformans* ATCC 90112 and *T. rubrum* IOC 4527. The extract BG9-IId3 HEX showed MICs of 162.5 μg/mL against *C. albicans* and *C. neoformans,* and its purification by HPLC yielded the fractions F1 and F2 in the first step. Fraction F2 with a MIC of 62.5 μg/mL against *C. albicans* was re-purified, yielding 3 isolated compounds named F2a, F2b and F2c, which are being characterized by physical and chemical methods (^{1}H NMR, ^{13}C NMR, and mass spectrometry). These results show that endophytic fungi isolated from the coffee tree are promising sources of bioactive antifungal secondary metabolites that can also be hits for new antimicrobial compounds.

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11.21 Inhibitory effect of rattlesnake (*Crotalus durissus terrificus*) venom on the formation of multinucleated giant cells in an experimental model of chronic inflammation

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Introduction: The venom of the *Crotalus durissus terrificus* (*Cdt*V) alters some functions of macrophages, cells that in chronic inflammatory processes are fused to form multinucleated giant cells (MGC). This process depends on the participation of actin filaments (F-actin) and signaling proteins, such as phosphotyrosine (PTy). Previous studies showed that *Cdt*V changes the patterns of F-actin expression and PTy in macrophages subjected to an acute inflammatory stimulus and, when applied prior to a chronic inflammatory stimulus, this venom reduces the formation of multinucleated giant cells. Yet, it is known that, after an ion exchange chromatography of *Cdt*V, 3 different fractions are obtained: PI, PII (corresponding to the crotoxin- CTX) and PIII. **Objectives:** Our objective was to evaluate qualitatively and quantitatively the effect of the *Cdt*V on the rearrangement of F-actin and PTy in mice subjected to a chronic inflammatory stimulus and to assess the fraction of the venom responsible for the inhibitory effect on the formation of MGC. **Methods:** Glass coverslips were implanted s.c in mice pretreated with *Cdt*V, fractions PI, PII (CTX) or PIII or saline. After 7 days, cover slips were removed and stained with H & E and counting of CGM in different groups was done. Preparations for immunohistochemical identification of F-actin and PTy were made in cover slips removed 4, 7, 14 and 21 days after implantation and analyzed in a confocal microscope, where fluorescence intensity was evaluated with the aid of Image-J software. **Results and Discussion:** The immunostainings for F-actin were significantly inhibited in cover slips removed 4, 7, 14 and 21 days after implantation, when compared to control groups. Regarding PTy, differences were not observed in any of the times studied. The inhibition of F-actin expression in the implants of 4 and 7 days observed in this study were positively correlated with the inhibition of the giant cells formation observed previously in cover slips implanted in the same period and stained with hematoxylin/eosin. Related to the fraction responsible for such inhibition, results indicate CTX as the inhibitor since after the total count of MGC, it was observed that the number of fused MGC in Cdt pre-treated groups was similar to the PII pre-treated ones, and in groups treated with PI or PIII, the results were similar to the control group treated with saline. The compiled data show a significant inhibitory action of *Cdt*V on the progression of the chronic inflammatory response.

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11.22 Characterization of enteropathogenic *Escherichia coli* (EPEC) outer-membrane proteins (OMPs) by two-dimensional electrophoresis and mass spectrometry

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Introduction: *E. coli* is a versatile pathogen in animals and humans. Enteropathogenic E. coli (EPEC) has been identified as the main causative agent of acute diarrhea in populations of developing countries. Diarrhea is still one of the most significant causes of global child mortality. **Objectives:** The goal of this work was to characterize and identify the outer-membrane proteins (OMPs) separated by two-dimensional electrophoresis (2-DE) of extracts derived from one strain of EPEC (strain 9100-83, serotype O125:H6). **Methods:** 2-DE was performed by a two-step protocol: the first dimension by focusing on 13-cm pH 4-7 strips (IPGphor III, GE Healthcare) and the second dimension by SDS-PAGE using 15% SDS-polyacrylamide gels (SE 600 Ruby, GE Healthcare). Characterization of the proteins was performed by in-gel trypsin digestion followed by mass spectrometric analysis (ETTAN MALDI-TOF/PRO – Amersham Biosciences and ESI QTOF Ultima – Waters). The resulting spectra were searched against non-redundant protein database (NCBInr) using MASCOT v2.0 engine (Matrix Science, www.matrixscience.com). **Results and Discussion:** Twenty-two spots were identified with high scores allowing the characterization of eleven distinct proteins. All proteins have membrane localization, a fact that indicates the efficiency of the extraction method. Five proteins were OMPs or porins (OMP A, OMP X, outer membrane channel – specific tolerance to colicin E1, outer membrane Tol C and maltoporin). Two transporters were found (long-chain fatty acid and ferrichrome outer membrane transporters). Moreover, one enzyme (glutamate decarboxylase alpha), a receptor (vitamin B12), an elongation factor (EFTu) and a protection protein (DNA protection during starvation) were detected. OMP A was one of the most abundant gel components. The biological function of OMP X is unknown, although it has been suggested that it binds foreign proteins on the *E. coli* cell surface, possibly as part of a cellular defense mechanism, and that this binding affinity is used to achieve cell adhesion and invasion. These preliminary data indicate that the majority of proteins identified have important roles in membrane permeability and at least two of them, OMP A and OMP X, are involved in the adhesion of the pathogen to host cells.

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11.23 Cytochemical characterization of blood cells of the snakes *Oxyrhopus guibei* and *Xenodon neuwiedii*

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Introduction: Circulating blood cells of reptiles can be classified as erythrocytes, thrombocytes and leukocytes. Four types of leukocytes were identified in the blood of snakes: lymphocytes, azurophils or monocytes, heterophils and basophils. General morphologic characteristics of erythrocytes and thrombocytes from the Reptilia blood are similar, showing little variation among groups while the characteristics of leukocytes, mainly the granulocytes are limited and inconsistent. Furthermore, considerable controversy regarding heterogeneous nomenclature of leukocytes remains. Even the presence of eosinophils in the Squamata remains controversial since criteria have not been well defined to distinguish eosinophils and heterophils in snakes. Thus, cytochemical staining is used to evaluate morphological characteristics of blood cells. **Objectives:** The aim of this study was to provide a morphologic description of blood cells of *Oxyrhopus guibei* and *Xenodon neuwiedii* snakes using cytochemical staining. **Methods:** Three *Oxyrhopus guibei* and four *Xenodon neuwiedii* snakes were anesthetized with thiopental sodium and the blood was withdrawn from the abdominal artery. Blood smears without anticoagulant were prepared immediately after blood collection. Enriched leukocytes were also prepared from at least 2 ml of whole peripheral blood, fixed in 2% glutaraldehyde and 4% paraformaldehyde in Tyrode buffer and embedded in historesin. The cytochemical reactions (benzidine peroxidase, sudan black B(SBB), periodic acid Schiff(PAS) and toluidine blue) were carried out both in blood smears and historesin sections. **Results and Discussion:** Most lymphocytes found were small and round but they often had irregular cell outlines. Lymphocytes did not stain with any of the cytochemical stains used. Azurophils are round or amoeboid cells with the eccentrically placed nuclei. The cytoplasm of azurophils was strongly positive with peroxidase, SBB and moderately stained or negative with PAS. Heterophils were the largest peripheral blood cells with a round to oval with eccentric nucleus. The cytoplasm is filled with numerous eosinophilic granules which were strongly positive for peroxidase and SBB while for PAS the reaction was weak or negative. Basophils are round to oval cells and contained a nucleus that was masked with numerous basophilic granules in the cytoplasm which strongly stained with toluidin blue and PAS. The positive reaction of the granules was better demonstrated in the histological section than in blood smears. Thrombocytes in general were oval or round-shaped cells; the nucleus was centrally located with hyaline cytoplasm which was strongly positive for PAS. There was no evidence of the presence of eosinophils in the blood of these two species.

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11.24 *Ornithodoros mimon* (Acari: Argasidae): third generation under laboratory conditions

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Introduction: The biological cycle of the argasid tick comprises embryonic eggs, larvae, nymphs (many instars), and adults. Adults feed many times, in general, before mating and ovipositions. *Carios mimon* is a tick common on bats, described from Bolivia and Uruguay. It was recently found in Argentina and Brazil where it is very aggressive to human and domestic animals. **Objectives:** Our aim was to study the 3rd generation of *C. mimon* in the laboratory, by observing pre-feeding and feeding periods as well as pre-molting and molting of all stages and instars and number of gonotrophic cycles per females. In addition, pre-oviposition and oviposition periods as well as the eclosion period of larvae of the 4th generation were also observed. **Methods:** The colony of *C. mimon* started from ticks collected in a household in Araraquara municipality, São Paulo State. Ticks were allowed to feed on rabbits in the laboratory of Parasitology of the Instituto Butantan, following the Protocol on Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation. Engorged specimens were left in an incubator at 27°C ±1 and 90% relative humidity, in order to obtain molts to the adult stage or larvae from female ovipositions. Larvae of the 3rd generation (N=78) were placed in a cotton chamber fixed on the dorsum of rabbits. Nymphs from each instar (N=40) and adults (9 females, 9 males) fed directly on animals. All data about the biology of the 3rd generation of this species were monitored. **Results and Discussion:** After feeding, the larvae of the 3rd generation showed different results from those of prior generations. Periods of larval feeding and pre-molts to nymphs of first instar (N1) were shorter (3 to 7 days) than those observed in previous generations (5 to 8 days). Pre-feeding and feeding periods of these N1 were longer (15-50 min, respectively). Although most of N1 from previous generations molted to 2nd instar (N2) after feeding, some of them molted without feeding. However, most of the nymphs N1 from the 3rd generation needed a meal before molting to N2 and they molted 9 days after. Those N1 that did not feed were in the same instar. The N2 fixed and fed quickly. Most of N2 that fed between 30 to 35 min molted to males, while the majority of N2 that spent 45 to 50 min molted to N3 (N=37). Of the N2 that molted to adults, 4 males and 2 females emerged after 9 days. The remainder of the N2 molted to N3. After 11 to 18 days, the engorged N3 molted to adults, and most of them molted to females. After 8-10 days each mated female laid 80-100 eggs in only one gonotrophic cycle. Larvae of the 4th generation (N=280) hatched after 10 days. From the larvae of the third generation to larvae of the 4th generation, the life cycle of *C. mimon* lasted 80 to 120 days.

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11.25 Characterization of antimicrobial molecules found in the venom of the spider *Acanthoscurria gomesiana*

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Introduction: There has been indiscriminate antibiotic use among the public in recent years, causing the selection of resistant microorganisms, making it difficult to treat illnesses. Research has attracted interest in the possibility of new drugs, natural or synthetic, that are efficient against these resistant strains. Peptides have been found to be molecules of innate defense in invertebrates, through the cleavage of its proteins. In the venom of the spider *Acanthoscurria gomesian,* three toxins (gometoxins 1, 2 and 3) with antimicrobial activity had been identified. **Objectives:** This study aimed to identify and characterize new molecules with antimicrobial activity present in the venom of the spider *A. gomesiana*. **Methods:** The purification of the crude venom of the spider *A. gomesiana* was performed by reversed-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). The molecular weights were analyzed by mass spectrometry MALDI – TOF. Fractions with more than one mass were repurified by reverse phase liquid chromatography, using a Jupiter C18 analytical column. Two new fractions that showed antimicrobial activity were analyzed by mass spectrometry. **Results and Discussion:** Two new molecules with antimicrobial activity were isolated, named gometoxin 4 and 5. From the analysis in the mass spectrometer, the molecular weight was obtained for gometoxin 4, and the result was 878.3Da, which showed antimicrobial activity against *M. luteus*. No molecular weight was determined for gometoxin 5, which has antimicrobial activity against *C. albicans*. In future studies, the molecular weight of gometoxin 5 will be determined, and the two substances, gometoxins 4 and 5, will be sequenced.

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11.26 Purification of factor X and protein C from *Bothrops jararaca* snake plasma

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Introduction: The clotting factors II, VII, IX and X, as well as the inhibitors protein C and protein S, belong to the group of vitamin K-dependent proteins. This group of proteins plays a key role in blood coagulation. Coagulation factor X (FX) plays an important role in the regulation of blood coagulation by converting prothrombin into thrombin. Human FX has a molecular mass of about 62 kDa and consists of two polypeptide chains, light (17.5 KDa) and heavy (45 kDa) chains. The protein C (PC) inhibits coagulation by selective inactivation of the active forms of factor V and factor VIII. Human PC has a molecular mass of about 62 kDa and consists of two polypeptide chains, light (21 kDa) and heavy (41 KDa) chains. **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B.jararaca*) FX and PC **Methods:** The purification process consisted of tandem steps on different chromatography columns. Briefly, plasma was applied on HiTrap DEAE FF column. The vitamin K-dependent protein fractions were applied on HiTrap Cu^{2+}Chelating HP, followed by Q-HiTrap FF, and finally on HiTrap Heparin HP. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Protein activity was measured using specific chromogenic substrate. The fractions were analyzed by SDS-PAGE (10%). **Results and Discussion:** FX and PC were partially purified by this developed process, showing that additional purification steps must be included. The perspectives for this work are to improve the purification process in order to get higher purity proteins and to compare them biologically and biochemically with FX and PC of other animals.

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11.27 Convulsant effect of intravenous administration of *Tityus serrulatus* scorpion whole venom in 21-day-old rats: electroencephalographic, behavioral and histopathologic aspects

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Introduction: Clinical data have shown that scorpion venom can induce convulsion, mainly in children. Also, late epilepsy has been described in some of these patients. We have shown that i.v. injection of whole venom of scorpion causes convulsions in adult and newborn rats. In view of these facts, there were some questions we wanted to investigate: Is it possible that convulsion caused by systemic injection of whole venom of scorpion induces late epilepsy in rats? Could treatment with anticonvulsivants just few hours after the beginning of convulsions prevent late epilepsy? **Objectives:** The aim of this study was to investigate the acute and long-term convulsivant effects of i.v. administration of *Tityus serrulatus* scorpion venom in male and female rats aged 21 days. An electroencephalographic, behavioral and histopathological study was performed. **Methods:** Surgery to implant electrodes in the hippocampus area for electroencephalographic analysis was done. After two days, the whole venom of *Tityus serrulatus* scorpion in a 0.2 mg/kg dose was administered (i.v.). Behavior and electroencephalographic activity of rats were observed for six hours uninterruptedly. Seven or 90 days after venom administration, rats were anesthetized with carbon dioxide to do a perfusion, and the brain was processed to histological analysis. The cells of CA1, CA3, hillus and dentate gyrus of hippocampal formation were counted in an area of 100 um^2 and a search for mossy fiber sprouting was performed. **Results and Discussion:** During the acute period of observation (0-24h after venom injection), electroencephalographic recordings were characterized by isolated spikes and epileptic discharges in rats that had received 0.2 mg/kg of crude venom. The behavioral modifications were characterized by paralysis, "wet-dog shakes," intense salivation, convulsion, and respiratory and locomotion difficulties. Long-term effects (1-90 days after venom injection). However, late epilepsy was not observed in this study. Histopathologic analyses of brains of these rats performed 90 days after venom injection did not show mossy fiber sprouting, a phenomenon that is present in epileptic patients. These results showed that the systemic venom injection in rats was able to induce central effects such as convulsion and epileptiform activity only on the day of venom administration. However, spontaneous and recurrent seizures were not observed in a period of 90 days after venom injection. This experimental study was not able to induce late epilepsy as observed in some patients who present with severe neurologic symptoms of scorpion envenomation.

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11.28 Primary culture of B type synoviocytes and effects of a metalloproteinase isolated from *Bothrops* asper venom on these cells

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Introduction: Snake venom metalloproteinases show homology with matrix metalloproteinases (MMPs), which are increased in inflamed articular joints during arthritis. Recently, we demonstrated that the metalloproteinase BaP1 was able to induce inflammatory events in rat articular joints, including the release of PGE_2 and TNF-α, which are the major mediators of pain in inflamed joints. However, the cell sources of these mediators were not identified. During inflammatory processes in joints, the synovial fibroblasts (B-type) are central cells for production and release of inflammatory mediators. **Objectives:** The aim of this study was to establish a primary culture of B-type synoviocytes and to evaluate the action of BaP1 on these cells, focusing on: 1) cell viability, 2) release of PGE_2 and 3) the protein expression of cyclooxygenase-1 and-2 (COX-1 and -2). **Methods:** B-type synoviocytes were isolated from synovial membranes of male Wistar rats (CEUIAB 576/09) and cultivated in culture flasks with complete RPMI medium at 37°C and 5% CO_2. Presence of the membrane protein Thy-1 was evaluated by immunocytochemistry as a marker of B type synoviocytes. To standardize the number of cells to form a monolayer on microplates, $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ were seeded into 6-, 12- and 96-well microplates and incubated with RPMI for 24 or 48 h, and observed the formation and confluence of the monolayers under light microscopy. The synoviocytes were then incubated with BaP1 (6.25, 12.5 and 25 μg/mL) or RPMI (control) for 30 min, 1, 3 or 6 h, followed by evaluation of cell viability by LDH activity and MTT assay, PGE_2 concentration by enzyme immunoassay and protein expression of COX-1 and -2 by Western blotting. **Results and Discussion:** Cell concentrations suitable for experimental assays were $1x10^4$, $1x10^5$ and $1x10^6$ in 96-, 12- and 6-well microplates, respectively. The protein Thy-1 was present in 100% of cells in culture obtained from the fourth passage, indicating the homogeneity of B-type synoviocytes. BaP1 was non toxic to isolated synoviocytes and induced the release of PGE_2 from these cells after 3 and 6 h incubation. In addition, BaP1 induced protein expression of COX-2 at 30 min and 3 h, but did not affect COX-1 expresssion. BaP1 is able to directly stimulate B-type synoviocytes to produce and release PGE_2. Upregulation of COX-2 protein expression may be the primary mechanism for production of these mediators induced by BaP1. Moreover, the B-type synoviocyte is a target for BaP1 and a relevant cell source for production of inflammatory mediators during joint inflammation induced by this metalloproteinase.

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11.29 Identification of the endoparasites affecting colubrids offered to coral snakes (*Micrurus* sp)

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Introduction: In Brazil, there are 321 known species of snakes belonging to nine families and 75 genera, of which 70 are venomous. Among the venomous snakes, the coral snakes were always the most difficult to keep in captivity. Due to the drastic decrease in the receipt of these animals at Instituto Butantan, it has become increasingly necessary to be self-sufficient in the maintenance and breeding of these snakes in captivity for the production of anti-elapidic serum and immunobiologic research. In captivity, colubrids of various genera are offered as prey items for the coral snakes, especially: *Liophis* sp, *Oxyrhopus* sp, *Phylodrias* sp, *Sybinomorphus* sp and *Tomodon* sp. When free-ranging snakes are offered to ophiophagous ones, there is the risk of infecting the latter with endoparasites, as most snakes from nature can harbor a wide variety of parasites. Therefore, it is necessary to establish an effective prophylactic management of the colubrids offered to coral snakes in captivity in order to avoid parasitic infection that, among other consequences, can lead to the death of the animals. **Objectives:** The aim of this study was to identify the endoparasites that affect colubrids commonly offered as prey items for the coral snakes, for the effective prophylactic management of the prey before being offered to coral snakes. **Methods:** During twelve months (August 2009 – July 2010), 50 adult colubrids from São Paulo State: 10 *Liophis* sp, 10 *Oxyrhopus* sp, 10 *Phylodrias* sp, 10 *Sybinomorphus* sp and 10 *Tomodon* sp were euthanized and necropsied. Organs and tissues were analyzed and all the parasites were collected and properly fixed. Fragments of organs were fixed in 10% formalin for histopathological analysis and fecal samples were taken for coprologic examinations. The feces collected were subjected to the Willis method and centrifugation technique for the detection of eggs and/or larvae of parasites. The eggs and larvae of helminths were classified according to their class and, when possible, according to their order; the counting of eggs and/or larvae was made subjectively. The adult parasites were classified according to morphological analysis, identification keys and measurements performed with Image-Pro Express program. **Results and Discussion:** This study showed that 36% of the animals necropsied were parasitized, whereas 84% of the parasites encountered were nematodes, 12% trematodes and 4% cestodes. Most of the parasites were found in the respiratory tract and belonged to the genus *Rhabdias* sp. In the coprologic examinations 58% of the animals necropsied were positive for at least one developmental phase of the helminth. These data demonstrate the importance of identifying these endoparasites for the establishment of appropriate and specific anti-parasitic methods to prevent parasitic infection in coral snakes.

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11.30 Incidence of trematodes in the oral cavity of *Bothropoides jararaca* (Viperidae, Ophidia) of São Paulo State

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Introduction: The species *Bothropoides jararaca* can be infected by a variety of endoparasites, including trematodes. There are 44 species of trematodes affecting Brazilian snakes, belonging to four orders, 12 families and 23 genera. Trematodes are found in several organs of the snakes and affect mainly the digestive system. Clinical signs in most cases are not typical, and disease only occurs when the animal has a large parasite load. Parasitic infections are one of the most important causes of death in snakes. **Objectives:** The aim of this study was to dentify the species of trematodes found in the oral cavity of *Bothropoides jararaca* in the state of Sao Paulo. **Methods:** During the period of May 2005 to May 2010, all the *Bothropoides jararaca* from various cities of São Paulo, donated to the Instituto Butantan (IB), were examined for trematodes. Within five years, Instituto Butantan received 3717 jararacas that had their biometric data recorded (snout-vent length (SVL), total length (TL) and mass), the sex determined by the presence or absence of hemipenis and the oral cavity examined for the presence of trematodes. Whenever present, the trematodes were placed in distilled water for several hours to expel the eggs and afterward were gently compressed between two slides and immersed in a solution of alcohol, formaldehyde and acetic acid (AFA - 93 parts 70% alcohol + 5 parts 10% formaldehyde + 2 parts glacial acetic acid) for a few minutes and preserved in 10% formalin. The parasites were stained with carmine and their main structures were morphologically studied and measured using a light microscope equipped with a camera and attached to a computer with Image-ProExpress program. The genus and species of trematodes were obtained with the aid of identification keys. **Results and Discussion:** From all the jararacas donated to Instituto Butantan in the period studied, 62 (1.6%) showed trematodes in their oral cavity. Males had a higher incidence than females (1.9% and 1.5%, respectively). Young specimens (less than 40 cm SVL) were not affected by this parasite, only the adults and subadults. The incidence of infected animals is higher in summer (1.9%) and lower in winter (1.2%), while in autumn and spring the rate is 1.6%.

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11.31 On the genus Pycnothele Chamberlin: description of the female of *Pycnothele singularis* Mello-Leitão (Araneae: Mygalomorphae, Nemesiidae)

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Introduction: The genus *Pycnothele* Chamberlin was described based on the type species, *P. perdita* Chamberlin from Mendes, Rio de Janeiro, Brazil. Currently, the genus comprises five species: *P. perdita*, *P. singularis* (Mello-Leitão) and *P. piracicabensis* (Piza) from Brazil; *P. auronitens* (Keyserling) from Brazil and Uruguay and *P. modesta* (Schiapelli & Gerschman) from Argentina and Uruguay. Of these five, only *P. singularis* does not have a female description. The species and the genus *Androthelopsis* Mello-Leitão, in which it was originally described, underwent some taxonomic modifications during the last decades. These are shown below. *Androthelopsis singularis* was described based on a male specimen from Serrana, São Paulo, Brazil. In 1973, Lucas & Bucherl reviewed the holotype of *A. singularis* and transferred the species from the family Barychelidae and included it in the Pycnothelidae. Perez-Milles & Capocasale in 1988 synonymized *Androthelopsis* with the genus *Pycnothele* Chamberlin, resulting in a new combination, *Pycnothele singularis*. **Objectives:** The aim of this study was to continue the project "On the genus *Pycnothele*" initiated by the scholar Victor Passanha in 2006, and to describe the female of the species *P. singularis*. **Methods:** The examined material was deposited in the Arachnida collection of the Instituto Butantan. The female spermathecae were dissected and submerged in clove oil to study internal structures. The illustrations and morphological observations were made using a Leica MZ12.5 stereomicroscope with a camera lucida. **Results and Discussion:** During the study of part of the material of the Arachnida collection from Instituto Butantan, it was possible to find the female of *P. singularis*, which was unknown until the present moment. The female of *P. singularis* resembles that of *P. auronitens*, due to the less developed supraspermathecal chamber, and differs by the less twisted ducts. This discovery allowed is to enhance the distribution range of the species to Vargem Grande do Sul, São Paulo and also to complete the review of the described species of the genus *Pycnothele*.

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

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Introduction: Neprilysin, also known as neutral endopeptidase, is a membrane protein that activates or inactivates oligopeptides such as natriuretic hormones (BNP, ANP, CNP), endothelins, and bradykinin. This enzyme is also related to tumor progression and its metastatic capacity. Moreover, recent studies have shown the involvement of neprilysin in the physiopathology of Alzheimer's disease by its activity in the clearance of brain amyloid substance. **Objectives:** The aim of this study was to obtain recombinant neprilysin using a bacterial expression system and to perform site-directed mutagenensis. **Methods:** The NEP cDNA sequence was amplified by PCR from the vector pCR4-TOPO (Invitrogen) using oligonucleotide primers containing recognition sequences for the restriction enzymes *Sal* I and *Not* I, and subcloned into the vector pGEX4T-2, which had been previously digested with *Sal* I and *Not* I. Subsequently, the plasmid was used to transform *E. coli* XL1-Blue, and the clones that contained the insert were submitted to DNA sequencing on both strands to ensure that the coding sequence was correct. The cDNA cloned in the pGEX-4T2 vector, which allows the expression of soluble recombinant proteins in fusion with glutathione S-transferase (GST), was transformed into *E. coli* BL21DE3. Protein expression was induced with 1 mM isopropyl thio-b-D-galactopyranoside for 24 h. Cells were collected by centrifugation at 3500 rpm, 4C for 15 min and suspended in lysis buffer (50 mM Tris HCl pH7,5, 2 mM $MgCl_2$, 0/25 mg/mL lysozyme and 25 U/uL benzonase nuclease). After cell lysis by sonication, the cell lysate was centrifuged and the pellet containing inclusion bodies was submitted to a solubilization/refolding protocol. Purification was finally performed by affinity chromatography on a glutathione-Sepharose column. The eluate containing GST-NEP was digested with thrombin followed by filtration using a Centricon 50 membrane. The expression of NEP was analyzed by SDS-PAGE, Western-blotting, and proteolytic assays using peptide fluorogenic substrates. **Results and Discussion:** Despite its insoluble form, NEP was successfully obtained in fusion with GST in *E. coli* BL21DE3. The SDS-PAGE analysis and Western-blot using an anti-NEP antibody confirmed the expression of NEP. The yield of recNEP was estimated at 2.3 mg/L culture medium. However, no enzyme activity was detected in the purified NEP sample, indicating that the protein was solubilized from inclusion bodies but not properly folded to express its catalytic activity. We are currently working on an improved protocol to obtain the fully active wild-type recombinant enzyme as a basis for our mutagenesis studies.

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11.33 Mygalomorph spiders in the Serra do Japi, State of São Paulo, Brazil: species richness and composition at three different altitudes

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Introduction: Fifteen mygalomorph families are known from Brazil; eight of them are found in the state of São Paulo. Little is known of their taxonomy, biology and ecology. Habits are rarely recorded and nothing is known about the influence of altitude on the distribution of mygalomorphs. **Objectives:** The aim of this study was to make an inventory of mygalomorph spider species of the Serra do Japi and to compare the taxa collected at 3 different altitudes in order to determine differences in the richness and composition among the sampled areas. **Methods:** The collections were made once in each season during one year. Two areas were sampled at 880 m, 1000 m and 1200 m, totaling 6 areas. Three collecting methods were used: 50 pitfall traps in each area; diurnal hand collecting limited by time of one hour and nocturnal direct hand collecting in transects of 60 m2. **Results and Discussion:** After four excursions, a total of 294 mygalomorph spiders were obtained. Four families and 12 species belonging to 9 genera were recorded: Nemesiidae (4 genera, 6 species), Idiopidae (1 genus, 2 species), Dipluridae (1 genus, 1 species) and Theraphosidae (3 genera, 3 species). Considering only the adults, 9 species were found at the lower site, 7 species at the intermediate site and 9 species at the higher site. The frequences are respectively: Nemesiidae - *Rachias* sp.1, 3.2%; *Prorachias* sp., 3.9%; *Stenoterommata* sp.1, 26.9%; *Stenoterommata* sp.2, 7.8%; Gen. sp.1, 14.4%; Gen. sp.2, 5.9%; Idiopidae - *Idiops* sp.1, 17.7%; *Idiops* sp.2, 13.8%; Dipluridae - *Diplura* sp.1, 1.9%; Theraphosidae - *Magulla obesa*, 1.3%; *Homeoma montanum*, 1.3%; *A. gomesiana*, 1.3%. Specimens of *Stenoterommata* sp.1, Gen. sp.1 and *Idiops* sp.1, were found at all altitudes; *Stenoterommata* sp.2 at 880 m to 1000 m; *Prorachias* sp., *Homoeomma montanum* and *Idiops* sp.2, from 1000 m to 1200 m; Gen. sp.2, *Magulla obesa* and *Acanthoscurria gomesiana*, were found only at 880 m. *Rachias* sp. and *Diplura* sp. were found at 880 m and 1200 m, and probably can also be found at 1000 m. All species are being illustrated, and a key to the mygalomorph genera and species of Serra do Japi is being prepared.

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11.34 Establishment of a method for evaluation of molluscicidal activity against *Achatina fulica* (Stylommatophora: Achatinidae)

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Introduction: The giant African snail, *Achatina fulica*, originating from east Africa, can also be found in several countries, including Brazil, where it was introduced for food as an alternative to traditional "escargot" of the *Helix* genus. This snail has a high population growth rate that has drawn the attention of authorities, researchers and local people. This species has been considered a competitor with native species leading some of them to be at greater risk of extinction; it destroys crops, representing an agricultural pest, and assumes a role as an intermediate host of certain human parasites. Among the methods of *A. fulica*'s control, there is the use of chemicals, such as metaldehyde and carbamates. However, these agents showed high toxicity, affecting the environment and requiring a high cost of production and commercialization. Thus, plant extracts have been studied as an alternative for *A.fulica* control. **Objectives:** The aim of this study was to evaluate the molluscicidal effect of a crude extract of *P. gaudichaudianum* (Piperaceae family) on *Achatina fulica*. **Methods:** Thirteen young animals (3 to 5 cm) were used, which were injected with a dose of 200 mg/kg of crude extract of *P. gaudichaudianum* leaf. Besides that, diethyl ether was used to anesthetize the snails before the treatment. Positive control was done using a dose of 30 mg/kg of niclosamide (Baylucide WP70 ®). The negative control was 3%DMSO. **Results and Discussion:** After 24 h, 100% mortality was observed in those treated with niclosamide and 40% for those treated with the crude extract, and there was no mortality in those treated with 3% DMSO. The new method using extract injection was effective, because the amount of extract into the animal and the exact dose to which it was exposed are known. Unlike the ingestion method, mortality and potential molluscicide were observed in *A.fulica* injected with crude extract of *P. gaudichaudianum* leaf. Previously, methods (ingestion) using 1000 ppm intake showed no effect and the increase in concentration resulted in an increase of DMSO, inhibiting snails' appetite. Therefore, an applicable methodology was established for the evaluation of plant extracts, and the crude extract of *P. gaudichaudianum* showed potential molluscicide effect, which should be evaluated in further studies.

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11.35 Polymorphism of gyroxin in *Crotalus durissus* venom by Western blot analysis

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Introduction: Snake venom variability is related to exogenous and endogenous factors occurring at several levels such as interspecies, geographic and ontogenetic variation, resulting in differences in venom composition. Gyroxin is a toxin from rattlesnake venom, with a molecular weight of 33 kDa. It is a serine protease that has fibrinogenolytic activity and when inoculated intravenously causes an effect known as barrel rotation syndrome. The batroxobin is a serine-protease from *Bothrops atrox* venom. It is a single toxin whose genomic sequence consists of five exons and four introns. As homologous toxins, gyroxin and batroxobin show similarities between the gene structures. Data from our laboratory indicate the absence of exon 4 of the gyroxin gene in some rattlesnake genomes. **Objectives:** Our aim was to analyze the polymorphism of gyroxin in *Crotalus durissus* venoms. **Methods:** Forty venoms from São Paulo, Paraná and Goiás were fractionated by denaturing 12% SDS-PAGE and transferred to nitrocellulose membrane. Gyroxin was detected using anti-MSP1 and MSP2 (against serine proteases from *Bothrops moojeni*) as primary antibodies and anti-rabbit IgG conjugated with peroxidase as secondary one, followed by colorimetric development with 4-chloro-1-naphthol. **Results and Discussion:** Western blotting revealed a single band of gyroxin with apparent MW of 32.5 kDa. We also observed a second band with a size close to 35.5 kDa in some samples. Nine of 16 samples from São Paulo and seven out of nine from Paraná showed the larger band, while it was present in only two of 12 samples from Goiás. We did not observe any relationship between the low intensity of the gyroxin band and the absence of exon 4. All samples of rattlesnake venoms tested displayed the band corresponding to gyroxin. We identified a polymorphism of the 35.5 kDa serine protease. Despite that the majority of the samples from Goiás do not show the 35.5kDa band, we cannot establish a geographic variation in relation to this band without an increase in sample size.

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11.36 Evaluation of toxicity of two cyanobacterial strains in mice

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Introduction: Cyanobacteria are known to produce hepatotoxins, dermatotoxins, cytotoxins and neurotoxins. When cyanobacteria die in water reservoirs, such toxins can be released, causing poisoning and even deaths to humans and animals. Since 2006, our group has been investigating the cyanobacteria strains from the Algae Bank of the Botanical Institute through acute toxicity experiments in mice. The symptoms induced in mice are observed, and when the toxic effects are not related to the typical cyanotoxins poisoning, we perform further histophatological analysis of the organs. **Objectives:** The aim of this study was to characterize the toxicity of two cyanobacterial strains, SPC 1044 (*Geitlerinema amphibium*) and SPC 1049 (*Phormidium sp.*), in mice (i.p.), observing the effects on the animals after injection, and performing postmortem examinations (including histopathological analysis). **Methods:** Extract preparation: the cultured cyanobacteria cells were filtered through an AP-20 filter and freeze-dried. The resulting material was then extracted with 0.1M acetic acid (4x) or MeOH/H_2O 75:25(v/v) (5x) with ultrasonication (4 x 10 sec., 50 W) and centrifuged. 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 (19-21g). The symptoms of the mice were observed up to 8 days after administration. After euthanasia, necropsy was performed and tissue samples were taken from the liver, kidneys and lungs, fixed and used for histopathological analysis. **Results and Discussion:** Neither cyanobacterial extract caused death of the mice. However, the animals showed some symptoms of intoxication (SPC 1044, methanolic: dyspnea; SPC 1049, methanolic: paralysis, abdominal pain and contractions). After necropsy, the mice injected with SPC 1044 (methanolic) showed altered color of intestine and pancreas, and darkened testicles. Histological analysis showed lung abnormalities, such as augmented inter-alveolar walls. The liver showed a great quantity of cells with pyknotic nuclei which may indicate necrosis or apoptosis. The mice injected with SPC 1049 (methanolic) displayed, after necropsy, dark stains in the bile vesicle. The histopathological analysis of the organs is still ongoing. These results indicate two new toxic cyanobacterial strains. Although the extracts did not cause death in any mice, the animals showed signs of poisoning unlike those of typical cyanotoxins.

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11.37 Comparison between male and female pups of Wistar rats during their reflexological and behavioral development

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Introduction: Previous studies with *Tityus serrulatus* scorpion venom showed deleterious effects on offspring when injected into rats during pregnancy. There are no studies in the literature showing if there are effects on the offspring, when the venom is inoculated during lactation and no comparison was made between males and females regarding their behavioral and reflexological development. **Objectives:** Our aim was to study and to compare the behavioral and reflexological effects between males and females in offspring of rats in the postnatal period and adulthood after maternal administration of saline on day 10 of lactation. **Methods:** Lactating females were injected with saline on the 10th postnatal day. Their offspring were assessed for their reflex development. The parameters observed were: palmar grasp, surface righting and negative geotaxis. The same pups were evaluated for their behavioral development in adulthood (2 months) and the tests utilized were: activity box (where locomotion and total activity were determined), forced swim (where the time was spent until the animal stopped swimming and the time of immobility were determined in training and test sessions), enriched environment (where the total motor activity and the time of exploration were determined) and social interaction (where the time spent in interaction between animals were determined). **Results and Discussion:** Comparisons were made between males and females, but there was no significant difference between them. These results indicate that both, young males and females could be used for future comparisons with animals treated with venom because there is no difference between them.

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11.38 Dual effect of *Crotalus durissus terrificus* venom on neutrophil functions

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Introduction: Previous works showed that *Crotalus durissus terrificus* snake venom (CdtV) modulates macrophage function, inhibiting the spreading and phagocytic activity but increasing the microbicidal activity and the oxidative burst of these cells. In addition, crotoxin (CTX), the main component of the venom, was reported to inhibit this phagocytic activity. Recently, CdtV was shown to inhibit phagocytosis by neutrophils, via CR3 receptors. Moreover, CTX was characterized as the CdtV component responsible for the inhibitory effect on phagocytosis by neutrophils. However, phagocytosis and microbicidal activity of neutrophils obtained from rats treated with CdtV and CTX have not yet been investigated. **Objectives:** The aim of this study was to investigate phagocytosis activity, via CR3 receptors, and microbicidal activity by neutrophils obtained by carrageenan-induced peritonitis from rats treated with CdtV or CTX. **Methods:** In an *in vivo* study, male Wistar rats were treated with CTX (18 μg/300μl/rat, s.c.) or saline (control) (CEUAIB 734/10) administered subcutaneously to rats at different time periods: 2 h, or 1, 4 or 14 days before or 1 h after inoculation with carrageenan (cg, 4.5 mg/kg). Neutrophils were obtained 4 h after the intraperitoneal administration of cg. *In vitro* assay. Neutrophils were obtained 4 h after the intraperitoneal administration of cg and incubated with CdtV (0.125, 0.25, 0.5, 1.0 and 2.0 μg/mL) or CTX (0.02, 0.04, 0.08, 0.16 and 0.32 μg/mL) for 1 h at 37°C. Phagocytosis and microbicidal activities of neutrophils were evaluated after in vivo or in vitro treatment with CdtV or CTX. **Results and Discussion:** *In vivo*, the injection of a single dose of CTX reduced the percentage of phagocytosis by peritoneal neutrophils at all times of treatment: 2 h: 24%, 1 day: 31%, 4 days: 25%, 14 days: 18% and 1 h after cg: 35%. These data confirm the results obtained previously *in vitro* which indicate CTX as the CdtV component responsible for phagocytosis inhibition. Moreover, the results show that the treatment with CTX induces a long-lasting inhibitory effect on phagocytosis by neutrophils. However, CdtV and CTX *in vitro* and *in vivo* did not alter the microbicidal activity of neutrophils, unlike macrophages. Thus, the results demonstrate a dual effect of CdtV on neutrophils, since it inhibits phagocytosis but did not modify microbicidal activity. 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.

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11.39 Survey of Linyphiidae (Arachnida, Araneae) of litter in fourteen areas of the Atlantic Forest

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Introduction: The order Araneae is the second largest among arachnids, with 40,462 described species and distributed among 109 families. This number probably represents only a portion of the actual number, since recent estimates suggest the existence of more than 70,000 species worldwide. Of these families, about 70% have representatives that live exclusively in leaf litter. The family with the highest incidence in leaf litter is Linyphiidae, with about 4000 described species in 585 genera. However, the soil spiders, despite their high diversity in the neotropics, are still poorly studied. It is estimated that over 50% of material deposited in the South American collections consists of undescribed species. This project seeks to use the material collected and deposited in the collection of Instituto Butantan, through the thematic project BIOTA-FAPESP program, which also allows the ecological investigation of the soil araneofauna. **Objectives:** This study aimed to learn more about the spiders of the Linyphiidae, to survey and compare the diversity of areas, and to assess the composition of arachnids and to deposit the material in the collection of the Institute Butantan. **Methods:** The material was collected with pitfall traps and then sorted according to morphospecies and deposited. We used the collector's curve to examine the quality of sampling, correspondence analysis where it was possible to identify the characteristics of arachnids of sites, and their similarities and differences, and finally to perform comparisons with the literature that used pitfall traps as sampling methodology. **Results and Discussion:** Spiders were identified in 2132, divided into 62 morphospecies of which 49 the species could not be determined since we found in the literature, suggesting a more comprehensive taxonomy of this family because of the amount of richness found and not yet known. The area was the richest in Caraça, with 19 morphospecies and the largest number of copies in the samples. The species mostly occurring in the areas was *Meioneta* sp. 1, found in eight areas, followed by the *Vesicapalpus simplex* occurring in seven areas, allowing the conclusion that these species are generalists. The collectors' curves showed an increasing pattern, suggesting that most of the areas sampled could have been better, except in areas of Maquiné and Órgãos, which plateaued. In CA, we observed a pattern north and south; only the area is nonstardard because of generalist species and similarities with the area and Copasa Órgãos. In comparison with studies using pitfall traps, it was observed that the interior forests have an abundance of Linyphiidae in comparison with coastal forests, as in comparisons between the areas sampled in Brazil. However, we note that studies of soil spiders in the country are still preliminary and that collections of spiders in the soil should be conducted more frequently, so we can better know the litter spiders.

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11.40 Diversity of Oonopidae (Arachnida, Araneae) of litter in fifteen areas of the Atlantic Forest

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Introduction: Spiders are the dominant invertebrate predators in most terrestrial habitats. It is the second order in richness among the Arachnida, behind only the Acari, with 41,253 described species, distributed among 109 families. The family Oonopidae contains 543 described species, distributed among 74 genera. Due to their small size (1-3 mm), they are abundant in any area in the Neotropical region. **Objectives:** The objectives of this study were to evaluate the structure and estimate the diversity of soil oonopids in fifteen areas of the Atlantic Forest, and compare the data with information from other samples reported in the literature. **Methods:** The material was collected during the project BIOTA/FAPESP conducted between 2001-2003, with pitfall and Winkler traps in 15 areas of the Atlantic Forest. For analysis, we used the the collector's curve, correspondence analysis (CA) and simple linear regression. **Results and Discussion:** The total number of individuals collected in 15 areas sampled was 1326, of which 882 were adults and divided into 33 morphospecies. Of these, only three were determined to the species, suggesting that most species are still undescribed. Returned 801 specimens distributed in 31 species in the pitfall traps, 20 of which were unique to that method. Winkler obtained 81 specimens, belonging to 14 species, of which three were captured exclusively by this method. The layout of the areas revealed by the CA showed a pattern of north/south fauna, although there are some exceptions to this pattern, which presents some generalist species. Works that sampled the fauna of the canopy record a richness and diversity in general lower than that observed in the litter. The collector's curves plateaued, in general, with only those areas that had a large sampling effort, such as Caucaia Caparaó Caraça and Órgãos. Pitfall traps were more effective than Winkler, obtaining a larger number of species and individuals, while Winkler contributed some unique species. In reviewing the literature, we found that the richness of the areas sampled was similar to or greater than that recorded in the references to Atlantic Forest areas, but was less than that observed in Amazon areas. However, one must take into account that there is a wide variation in collection methods employed, sampling effort and sampling period in these surveys. The family's richness tends to grow toward the equator, which is consistent with the latitudinal gradient of species richness, a known ecological pattern. In the collections made by the project BIOTA, many potentially new species were obtained, which will broaden the diversity of species in this family.

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11.41 Antibacterial activity of fractions from the marine sponge *Amphimedon viridis*

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Introduction: The emergence of bacterial strains with multidrug resistance, besides the indiscriminate use of antibiotics, has created an urgent need for the development of new antimicrobial compounds and new strategies to treat bacterial infections. The potential contribution of marine organisms to the discovery of new bioactive molecules, mainly with antibacterial activity is very promising. The substances isolated from the sponge of genus *Amphimedon* have shown antimicrobial activity against Gram-positive and Gram-negative bacteria. **Objectives:** The aim of this study was to evaluate the antibacterial activity of the fractions obtained from the marine sponge *Amphimedon viridis*. **Methods:** The sponge *A. viridis* (Niphatidae, Haplosderida) was collected in the urban coast of Maceió, Alagoas, Brazil. The aqueous extract was desalted and the concentrated aqueous layer was submitted to chromatography on Sephadex G100 column with deionized water as mobile phase. The last fraction (LC) showed antimicrobial activity and was re-chromatographed. All fractions were submitted to microdilution antimicrobial susceptibility testing against ATCC strains of *Escherichia coli* and *Staphylococcus aureus*. The fractions were analyzed in 96-well plates, using Poor broth and bacteria at 10^4 CFU/well. The microtiter plates were incubated at 37°C for 18 h, and culture turbidity was then measured in an ELISA reader at 595 nm to assess bacterial growth. The fractions with small quantities and similar content were combined. **Results and Discussion:** The LC fraction showed a minimal inhibitory concentration of 12.5 µg/mL against *S. aureus* and 25 µg/mL against *E. coli*. The antimicrobial activity against both *S. aureus* and *E. coli* was detected in the fractions obtained from the LC fraction: 1, J (these two fractions showed the highest antimicrobial activity and also hemolytic activity), 29, H, L, N, T, U, V and AL (21.9% to 46.6% - *S. aureus* and 14.7 to 29.7 – *E. coli*). Some fractions inhibited mainly the growth of 37% to 48.6% of *S. aureus* growth: 7, 13, 15, 16, 19, 20, E, P, R and S; while others inhibited mainly *E. coli* (27.7% to 39.1%): 10, 11, M and Z. Alkylpyridine polymers called halitoxin show toxic and cytotoxic activities and have been identified previously in sponges of the same genus. These results confirm the potential antimicrobial activity of fractions obtained from *Amphimedon viridis* extract, which may contain halitoxin or other active substances in its composition.

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11.42 Characterization of glycine release from striatal tissue in superfusion: understanding Parkinson's disease

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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 largely studied, many striatal circuits are still poorly understood. Previous investigations have shown that glycine interferes with 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 report describes 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 gased with carbogen. Tissue was cut into prisms using a McIlwain tissue chopper, suspended and pre-incubated at 37°C for 5 min. An aliquot of 20 µL of 3H-glycine was added to the incubation that lasted 20 min. Tissue was then filtered and washed twice with ice-cold KRB, transferred to a beaker and suspended for distribution into 18 parallel superfusion chambers (Brandel SF2500 – USA). A stabilization superfusion lasted 45 min and then, eighteen 3 min aliquots of effluent were taken from each chamber for radiometric quantitation. Drugs were included in the superfusion medium according to the experimental protocol. Results are expressed as fractional release, i.e., the percent of radioactivity released in a given moment of the perfusion. **Results and Discussion:** Glycine was released at a steady baseline of 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 stimulated release included nicotine (cholinergic), muscimol (GABAa agonist), NMDA (glutamate agonist), glutamate itself and tetrodotoxin. A complete characterization of glycine release in this tissue including second messenger signaling and neurotransmitter interactions should add to the proposal of new therapeutic strategies to fight Parkinson disease.

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11.43 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 then were submitted to the pharmacological treatments for 5 h. The cells were stimulated with norepinephrine (1 μM) associated 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 with 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 could not be observed when the TNF- α inhibitor was used. The evidence obtained supports 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.

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11.44 Study of the action of melatonin on the injury induced by toxins from the venom of *Micrurus lemniscatus* in cultured rat hippocampal neurons

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Introduction: In elapid venom of *Micrurus lemniscatus,* there are neurotoxins present with presynaptic and postsynaptic actions. Studies show that different neurotoxins in this venom are powerful pharmacological tools for studying neurotoxic processes and discovering novel neuroprotective agents. Melatonin, secreted by the pineal gland, has antioxidant function and studies show that melatonin enhances GABA receptors, hyperpolarizing the cells, and then decreases neuronal excitability, which may contribute to the neuroprotective effects of melatonin. **Objectives:** The aim of this study was to investigate the possible neuroprotective action of melatonin on the neurotoxic effects of toxins Mlx-11 and Mlx-12 isolated from the venom of the snake *Micrurus lemniscatus* in cultured primary rat hippocampal neurons. **Methods:** The neuronal culture was prepared from hippocampus taken from fetuses of 18-19 days. The tissue was dissociated with trypsin and with a Pasteur pipette. The cells in culture were placed in microplates pretreated with poly-L-lysine. These are incubated in 5% CO2. After 6-7 days in culture, hippocampal cells were exposed to 6 or 24 h to toxins Mlx-11 and Mlx-12 at concentrations of 10, 100 or 1000 ng/mL and to KCl (250 mM) as a positive control. To test the neuroprotection of melatonin, this was incubated at concentrations of 10^{-7} M and 10^{-9} M, about 30 min before the toxins. The experiment was carried out using the MTT colorimetric assay, which measures cell viability. **Results and Discussion:** The group submitted to Mlx-11 for 6 h showed statistically reduced cell viability when compared to the control group just in the group treated with 1000 ng/mL. When incubated for 24 h, the group with Mlx-11 at a concentration of 10 ng/ml was the only one that showed no statistically significant difference in the control group. In both periods, there were no statistically significant differences between the groups that received only the toxin and those who received it with melatonin. In the groups incubated with Mlx-12 for 6 h, there were no statistically significant differences between the groups treated with toxins and control. By incubating Mlx-12 for 24 h at different concentrations, all showed differences compared to the control. At a concentration of 10 ng/mL, there were no statistically significant differences between the groups that received only the toxin and those who received it with melatonin. For groups that received Mlx-12 at concentrations of 100 ng/ml to 1000 ng/ml, there were significant differences between the groups incubated with melatonin. The results show that different concentrations of Mlx-11 and Mlx-12, used in this study, significantly reduced the viability of hippocampal neurons maintained in culture. Melatonin showed a neuroprotective role against cell death caused by the toxin Mlx-12 at concentrations of 100 and 1000 ng/ml, when cells were exposed for 24 h. Melatonin at a concentration of 10^{-9} M was shown to have a higher profile than with the neuroprotective concentration 10^{-7} M.

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11.45 DNA damage in hemocytes of *Biomphalaria glabrata* (Say 1818) measured by the comet assay after treatment with different classes of genotoxins

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Introduction: The comet assay or single cell gel electrophoresis (SCGE) assay is a simple, sensitive and rapid technique for the detection of DNA damage in individual cells. This technique has been the major tool in the evaluation of genotoxic effects in genetic toxicology. In this work, two chemicals from different classes of mutagens (ethyl methanesulfonate - EMS and cyclophosphamide - CP) and hydrogen peroxide - HP were tested to evaluate the expression of DNA damage in hemocytes of *B. glabrata* in the SCGE assay. **Objective:** The aim of this study was to evaluate genotoxic activity of direct and indirect genotoxins in hemocytes of *B. glabrata* using the SCGE assay. **Methods:** Snails were exposed for 3 days to different concentrations of EMS (1, 10 and 50 mg/L) and 7 days for CP (10, 100 and 500 mg/L) with the solutions renewed every 48 h. Isolated hemocytes were exposed to hydrogen peroxide (10 30 and 50 µM) for 5 min. To perform the comet assay, about 100 µL of hemolymph containing hemocytes of each animal were collected by pedal stimulus, and then added to 500 µL of LMP agarose 0.5% (w/v), mixed, and placed on two microscope slides pre-coated with NMP agarose 1.5% (w/v). Slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 20% DMSO, pH 10.0), kept at 4 °C and protected from the light for 2 h. They were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 200 mM EDTA, pH>13) for 20 min for DNA unwinding. Electrophoresis (20 min at 300 mA and 23 V (0.74 V/cm) was performed in the same buffer. After electrophoresis, the slides were neutralized in 400 mM Tris (pH 7.5) and fixed for 10 min in ethanol. Prior to the examination, the slides were stained with 20 µg/ml ethidium bromide and 100 cells per slide (200 per each animal) were analyzed using a Zeiss Axioplan epifluorescence microscope. The extent of the DNA damage was determined by visual anaysis. **Results and Discussion:** DNA damage was measured as percent number of comets (classes 1, 2 and 3) and normal cells (class 0). There was no increase in DNA migration with any of the CP concentrations. The lack of genotoxic effect in the comet assay with CP was observed by other authors and was attributed to the induction of inter-strand cross-linking. The direct genotoxins EMS and HP induced a significant increase in DNA migration. The trypan blue exclusion test showed no cytotoxicity for all agents.

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11.46 Secretion of a toxin by atypical enteropathogenic *Escherichia coli* depends on the culture medium used

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Introduction: EPEC is associated with diarrhea in many developing countries. It is subdivided into typical (tEPEC) and atypical (aEPEC). aEPEC differs from tEPEC in that it does not possess the EAF plasmid and in that it shows a greater heterogeneity of virulence factors, which explains the rise in the prevalence of this group. It is known that the expression of toxins is an important factor in diseases caused by different pathogens. Still, the expression of toxins by aEPEC remains very poorly studied. Our group has been searching for toxins described in other *E. coli* categories in aEPEC. These studies led to the identification of aEPEC isolates which express the toxin Pet (plasmid encoded toxin), classically known as enteroaggregative *E. coli*. On the other hand, although there are aEPEC isolates positive for the ***sat*** gene, which encodes the toxin Sat (secreted autotransporter toxin) and described in diffusely adhering *E. coli* (DAEC) and uropathogenic *E. coli* (UPEC) strains, the expression of this important toxin is still not clear. **Objectives:** The aim of this work was to establish better culture conditions for aEPEC in order to improve studies on the expression of the toxin Sat in *sat*+ isolates. **Methods:** Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or supernatant of the culture from isolates 589 (O5:H2) *sat/pic/east*; 1887 (O111:H38) *sat/hly*; 2294 (O9:H33) *east/Sat*, DAEC (C1845 and 114) and C600 (non pathogenic) grown in DMEM or DMEM + 1% tryptone. The cell parameters used in the study of toxin expression were: morphology, detachment and cell viability, evaluated by staining with Giemsa, crystal violet/Giemsa or trypan blue, respectively. **Results and Discussion:** Our results show that isolates positive for the *sat* gene were unable to produce alterations in cells grown in DMEM. On the other hand, when 1% tryptone was added, vacuolization, cell detachment and a significant reduction in cell viability were observed. These effects were more intense than those we described previously when concentrated supernatant from bacterial cultures, containing only substances over 50 kDa, were used. Comparative cytotoxicity assays also performed with aEPEC isolates pet + showed that the cytotoxic effects, observed only after 24 h of incubation with the supernatant from bacterial cultures grown in DMEM, were observed after 5 h when 1% tryptone was added to the DMEM medium. These findings are important not only for the detection of Sat in aEPEC, but also because they open the possibility of discovering new toxins with the use of tryptone. This will help improve the understanding of aEPEC pathogenesis, which is known to show highly heterogeneous virulence factors and which is, up to this time, practically unknown.

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12. PAP Program

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Supported by: PAP/SES

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

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

Supported by: PAP/SES

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

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

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

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

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12.15 Plasma FVIII and protein C purification by IMAC

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

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

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

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

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

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

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

Supported by: PAP/SES

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

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

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12.20 Assessment of maternofetal transfer of anti-rotavirus serotype G9

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

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

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

Supported by: PAP/SES, INCTTOX Program - CNPq, FAPESP

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

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

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12.23 4th Introductory course to the "Programa de Aprimoramento Profissional (PAP) da Secretaria de Estado da Saúde no Instituto Butantan"

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Introduction: The "Programa de Aprimoramento Profissional (PAP)" was created in 1979 aimed at complementing the training of new university graduates involved in the health area. In the Instituto Butantan (IBu) this program lasts 2 years and consists of 40 hours/week of activities under direct guidance and supervision of specialized professionals. Since 2007, before the beginning of their laboratory activities, the students have to attend a course which is organized by a commission composed of researchers from different laboratories of the Institute. In this course, pertinent themes are covered by scientific researchers, specialists and also 2nd year PAP-students. In addition, the course focuses on the integration of the grant-holders to the different areas of the Institute (Production, Development, Research and Museums). At the end of the course, the students must answer an anonymous questionnaire which gives them the opportunity to express their point of view about this activity. **Objectives:** The aim of this work was to describe and evaluate the planning, organization, and application of the fourth course offered in 2010. **Methods:** The activities lasted 2 weeks and were divided into hands-on and theoretical classes distributed in an 8 hours/day workload. In this period, all of the Divisions of the IBu were presented, and the Museums and the Collections were visited. Theoretical classes concerning several topics including routine equipment operation, first aid and laboratory security, animal care and ethics, and the preparation of solutions were presented. At the end of the course a representive was elected by their colleagues, and a questionnaire was answered by the grant-holders in order to find out their opinion about the course. **Results and Discussion:** In 2010, 46 new graduates with different trainings (35 biologists, 4 veterinarian scientists, 3 pharmacists, 2 biomedical scientists and 2 nurses) were received by the program, and among them 75% did not belong to IBu. The questionnaire was answered by 44 students and the average of the course grade was 8.1. The program was considered totally satisfactory to all of the grant-holders, as well as the content and workload. The helpfulness and attentiveness of teachers and coordination staff were totally satisfactory to them. The main subjects of interest were laboratory techniques (64%), biosafety guidelines (61%), biotherium (61%), bioethics (61%), sterilization (59%), routine equipment operation (57%), pharmacologic assays (52%), museums (50%) and reagents and solutions preparation (50%) Moreover, organization chart (46%), SUS (34%) and São Joaquim Farm (38%) were pointed out as areas of medium interest. The feedback received at the end of the course can play an important role in the organization and improvement of the course in future years. In general the grant-holders evaluated the course positively. Although satisfaction does not assure the learning process, certainly it stimulates the performance of the grant-holders in their laboratory activities.

Supported by: PAP/SES

12.24 Comparing BPV-1 and BPV-2 E6 genes

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

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12.25 PCR identification of atypical enteropathogenic *Escherichia coli*

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

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

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Introduction: There is an increasing interest in the pharmacological application of antimicrobial peptides (AMPs) to treat infections due to the growing problem of pathogenic organisms resistant to conventional antibiotics. AMPs are widely distributed in nature and represent an ancient host defense mechanism present in organisms across the evolutionary spectrum. Over 1200 AMPs have been reported so far in the literature, of which a third have been isolated from arthropods, including arachnids. The AMPs of the first type are mainly detected in the hemolymph of various arthropods such as sacrotoxin in the housefly, cecropins in *Lepidoptera* and spinegirin in termites. The AMPs of the second type were found in the venom glands of bees, wasps, ants, scorpions and spiders such as *Lycosa carolinensis*, *Cupienius salei* and *Oxyopes kitabensis.* **Objectives:** The aim of this study was to characterize gometoxin-3, antimicrobial peptides of the venom of the spider *Acanthoscurria gomesiana*. This work could be useful in the scope of future pharmaceutical use. **Methods:** The venom was obtained from venom glands of *A. gomesiana* spider, which were macerated with water, centrifuged and the soluble part was dried by vacuum centrifugation and reconstituted with acidified water (TFA - trifluoracetic acid 0.05%). The soluble part was separated by HPLC reversed-phase chromatography on a semi preparative or analytical Jupiter C18 column. Elution was performed with different linear gradients of ACN/TFA 0.05%. The presence of antibacterial activity was determined by a liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli* (*SBS363)*, Gram-positive bacteria *Micrococcus luteus* (*A270)* and yeast *Candida albicans* (MDM8). Molecular weight and purity of the molecules were analyzed by mass spectrometry (MALDI- TOF). **Results and Discussion:** We recently isolated three molecules with antimicrobial activity in the venom of *A. gomesiana*: Gometoxin-1, 2 and 3. Gometoxin-3 with anti-*M. luteus* activity and molecular weight of 1658.9 Da was reduced, alkylated and cleaved by trypsin and subjected to mass spectrometry (MALDI-TOF-MS). After obtaining the masses of the fragments, they were analyzed in the databases PROTEIN BLAST and MASCOT, where similarity was found with a fragment of a neurotoxic molecule, called huwentoxin, Chinese spider *Ornithoctonus huwena* (family Theraphosidae), as well as the spider *Cupiennius salei* (family Ctenidae), and the scorpion *Tityus discrepans* (subfamily Tityinae). Analysis of gometoxin-3, showed that this peptide is linear without disulfide bounds. The complete characterization and sequencing of gometoxin-3 are in progress.

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12.27 Noradrenergic regulation of RAS aminopeptidases in the rat pineal gland

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

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

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

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

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

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12.30 Comparative skin morphology in toads from Atlantic forest and Caatinga

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

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

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

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

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

Supported by: PAP/SES

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

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

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

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

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

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

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