3: Immunology and Vaccines

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

Aires KA[1,3], Bazan SB[1], Campos IB[1], Cianciarullo AM[2], Oliveira MLS[1], Ho PL[1]
[1]Centro de Biotecnologia, [2]Laboratório de Genética and [3]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Albani SMF[1], Takagi M[1], Silva MR[1], Zangirolami TC[2], Cabrera-Crespo J[1]
[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Departamento de Engenharia Química, Universidade Federal de São Carlos, SP, Brasil

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

Supported by: FAPESP and Fundação Butantan.

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

Alves RCB[1], New RRC[3], Mancini DAP[1], Andrade GR[2], Mendonça RMZ[1], Domingos MO[2]
[1]Laboratório de Virologia and [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil;
[3]Proxima Concepts, United Kingdom

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

Supported by: Proxima Concepts.

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

Andrade GR[1], Abreu PAE[1], New RRC[3], Sant'Anna OAB[2], Domingos MO[1]
[1]Laboratório de Bacteriologia and [2]Laboratório de Imunoquímica, Instituto Butantan, SP, Brasil; [3]Proxima Concepts, United Kingdom

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

Supported by: CNPq.

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

Araujo-Montoya BO[1], Farias LP[1], Wilson RA[2], Leite LCC[1]
[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Biology Department, The University of York, United Kingdom

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

Supported by: FAPESP.

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

Astray RM[1], Benmaamar R[2], Wagner R[2], Pereira CA[1]
[1]Laboratório de Imunologia Viral, Instituto Butantan, SP, Brasil; [2]Département Récepteurs et Protéines Membranaires, Université de Strasbourg, France

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

Supported by: FAPESP and CNRS.

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

Atzingen MV[1], Gómez RM[2], Schattner M[3], Pretre G[2], Gonçales AP[4], Morais ZM[4], Vasconcellos SA[4], Nascimento ALTO[1]
[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Instituto de Biotecnologia y Biología Molecular and [3]Instituto de Investigaciones Hematológicas, CONICET, Argentina; [4]Laboratório de Zoonoses Bacterianas, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Baldon EJ[1], Scaramuzzi K[1], Carvalho LV[1], Starobinas N[2], Marengo EB[3], Sant'Anna OA[1]
[1]Laboratório de Imunoquímica and [2]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [3]Laboratório de Imunologia Clínica, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil

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

Supported by: CNPq and FAPESP.

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

Barazzone GC, Tanizaki MM
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan and FAPESP.

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

Borrego A, Peters LC, Vorraro F, Gasparelo TC, Jensen JR, Cabrera WHK, Starobinas N, Ribeiro OG, Ibañez OM, De Franco M
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP and CNPq.

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

Botelho CRM, Fujimura SH, Siqueira JR, Frazatti- Gallina NMF
Seção de Raiva, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Nuñez SP[1], Zapata-Quintanilla LB[1], Carbonare CB[1], Tino De Franco M[1], Palmeira P[2], Carbonare SB[1]

[1]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [2]Laboratório de Investigação Médica - LIM 36, Departamento de Pediatria, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil

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

Supported by FAPESP and CNPq.

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

Carvalho LV[1], Scaramuzzi K[1], Baldon EJ[1], Scivoleto R[3], Pagani E[3], Jacysyn JF[4], Fantini MCA[5], Tambourgi DV[1], Faquim-Mauro EL[2], Sant'Anna OA[1] [1]Laboratório de Imunoquímica and [2]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [3]Laboratório Cristália, SP, Brasil; [4]Instituto de Ciências Biomédicas and [5]Instituto de Física, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP, CNPq and Cristália.

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

Carvalho DT, Brilhante GM, Sbrogio-Almeida, ME, Liberman C, Gonçalves VM, Leite LCC, Dias WO
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

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

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

Silva AS[1], Castiblanco MM[1], Barbosa AS[2], Isaac L[1]
[1]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil; [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP and CNPq.

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

Chaves AAM [1,3], Bazan SB[1], Silva JRSN[1, 3], Cianciarullo AM[2] and Ho PL[1,3]
[1]Centro de Biotecnologia and [2]Laboratório de Genética, Instituto Butantan, SP, Brasil; [3]Instituto de Química, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Da Silva JB[1], Carvalho E[1], Ching ATC[1], Favaro RD[1], Lima SS[1], Moreira VB[2], Mattaraia VGM[2], De Franco M[3], Pereira MM[4], Ho PL[1], Martins EAL[1]
[1]Centro de Biotecnologia, [2]Biotério Central and [3]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [4]Fundação Oswaldo Cruz, RJ, Brasil

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

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

3.18 DLCs as new vaccine candidates against schistosomiasis

Diniz PP[1], Nakajima E[1], Miyasato PA[2], Kawano T[2], Martins EAL[1]
[1]Centro de Biotecnologia and [2]Laboratório de Parasitologia, Instituto Butantan, SP, Brasil

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

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

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

Trezena AG[1], Estima MA[1], Santos AS[1], Rós N[1], Kyoma CH[2], Moraes TTR[2], Utescher CLA[3], Nicolau B[3], Mendes E[3], Yamaguchi IK[3], Gioielli MLR[4], Duboc MA[2], Higashi HG[5], Prado SMA[4]

[1]Seção de Vacinas Anaeróbicas, [2]Laboratório Especial de Garantia de Qualidade, [3]Serviço de Controle de Qualidade, [4]Serviço de Bacteriologia and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Farias LP[1], Rofatto HK[1], Montoya BOA[1], Tararam CA[1], Miyasato PA[1], Levano-Garcia J[2], Kawano T[1], Verjovski-Almeida S[2], Wilson RA[3], Leite LCC[1]
[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Instituto de Química, Universidade de São Paulo, SP, Brasil; [3]Department of Biology, University of York, United Kingdom

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

Supported by: FAPESP.

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

Favoretto BC[1], Silva SR[1], Macedo MS[1], Câmara NOS[1], Jacysyn JF[1], Faquim- Mauro EL[2]
[1]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil; [2]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CNPq and CAPES.

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

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

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

Supported by: FAPESP and CNPq.

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

Fraga, TR[1], Chura-Chambi, RM[2], Morais, ZM[3], Gonçales, AP[3], Vasconcellos, SA[3], Morganti, L[2], Martins, EAL[1]

[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Instituto de Pesquisas Energéticas e Nucleares, SP, Brasil; [3]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP.

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

Gasparelo TC, Peters LC, Borrego A, Carneiro PS, Albuquerque LL, Cabrera WHK, Ribeiro OG, Starobinas N, Ibañez OM, De Franco M
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CAPES and CNPq.

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

Guglielmi PM, Mourão-Fuches RM, Santos ADA, Quintilio W, Moraes JF
Seção de Controle Biológico, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Hashimoto VL and Ho PL
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Hernani M De L, Ferreira PCD, Ferreira DM, Miyaji EN, Ho PL and Oliveira MLS
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Kanno AI, Farias LP, Leite LCC
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP.

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

Katz ISS, Alburqueque LL, Suppa AP, Starobinas N, Cabrera WHC, De Franco M, Ibañez OM, Ribeiro OG
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

Introduction: Polycyclic aromatic hydrocarbons (PAHs), such as DMBA, are genotoxic compounds that react with DNA directly, inducing decrease in bone marrow (BM) cellularity resulting in an immunosuppressive state. This process involves the metabolism of the PAHs that depends on the activation of the aryl hydrocarbon receptor (Ahr). Two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute onflammatory response (AIR) to a non-immunogenic substance (Biogel) showed a complete segregation of the low PAHs affinity *AHR* allele (*AHR* ^{d}) and of the high affinity allele (*AHR* ^{b1}). **Objectives:** Here, we studied the effect of *in vivo* DMBA treatment on the BM of AIRmax and AIRmin mice and on immune response. **Methods:** AIRmax and AIRmin mice were treated with a single i.p. dose of 50 mg/kg DMBA in olive oil. Proliferation index of bone marrow and spleen cells was determined in response to GM-CSF and LPS, respectively, after in vivo treatment with DMBA. Apoptosis levels were observed in BM cells stained with propidium iodide and Annexin V. DNA damage was evaluated by the single-cell gel electrophoresis (Comet) assay, which detects DNA strand breaks. **Results and Discussion:** Hypocellularity was observed in BM from AIRmin ($0.26\pm0.01x10^6$/ml) when compared to controls ($0.51\pm0.02x10^6$/ml) at 24 h post-DMBA treatment, mostly in the myeloid population. On the other hand, AIRmax mice showed normal levels of BM cells (0.54 ± 0.03 $x10^6$/ml). Myeloid cells from DMBA-treated AIRmin mice showed low proliferation capacity after 1 ($0.59x10^6$/ml) or 7 ($0.45x10^6$/ml) days *in vitro* GM-CSF stimulation, whereas AIRmax BM cells displayed normal proliferation (8.57 ± 0.1/ml). Apoptosis levels were observed in BM cells stained with propidium iodide and Annexin V only in AIRmin ($28.7\pm5.1\%$ versus control mice $1.45\pm0.26\%$). A significant increase in tail moment index (TM) was observed in AIRmin mice after DMBA (2.7 ± 0.2). Spleen cells from DMBA treated AIRmin mice showed impaired proliferation after in vitro LPS stimulation. AIRmin mice were more susceptible to the DMBA toxic effects than AIRmax. DMBA-treatment produces DNA strand breaks due to direct hematotoxic effects with significant increase of apoptotic cells in AIRmin mice. These effects on myeloid BM cells reflect on an impaired immune response.

Supported by: CAPES and CNPq.

3.30 Comparative study between two bioreactor methods for rabies virus production

Frazatti-Gallina NM, Lantieri VS, Fang FLW, Pena CAS, Medeiros FM, Takinami VH
Seção de Raiva, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Lara PG[1], Prudente MFS[2], Diniz FM[1], Papotto PH[1], Tambourgi DV[1], Carvalhaes MS[2], Spadafora-Ferreira M[1]

[1]Laboratório de Imunoquímica, Instituto Butantan, SP, Brasil; [2]Departamento de Imunologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, GO, Brasil

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

Supported by: FAPESP and FAPEG.

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

Marçal MS, Rinaldi DP, Frazatti Gallina NM
Seção de Raiva, Instituto Butantan, SP, Brasil

Introduction: Porcine or bovine trypsin derivates are used in cell culture to remove adherent cell from surfaces. However, manufacturers of human vaccines that use cell cultures and serum free- medium need a non-animal trypsin derivates. **Objective:** To evaluate an animal origin-free trypsin (Trype™ Select- Gibco) in Vero cell culture used for rabies vaccine production. **Methods:** Five different procedures to detach the cells were tested: 1- washing the culture with 3.0 ml of trypsin and afterward adding 1.0 ml of trypsin; 2- washing the culture with 4.0 ml PBS and afterward adding 1.0 ml of trypsin; 3- washing the culture with 4.0 ml of 0.1% EDTA and afterward adding 3.0 ml of trypsin; 4- only 4.0 ml of trypsin and 5- use of 4.0 ml of 0.1% EDTA + trypsin. Vero cells ($1.1x10^7$) were grown in 75-cm^2 flasks. The parameters evaluated were: time to detach, cellular integrity and growth. **Results and Discussion:** The time of detachment was the same for all the procedures; the cellular integrity was satisfactory in procedures 1, 2, and 3 and unsatisfactory for 4 and 5. After four days the cell concentrations were 1.4×10^7, 1.5×10^7, 1.4×10^7, 8.4×10^6 and 1.2×10^6 respectively for 1, 2, 3, 4 and 5. The results showed that procedures 1, 2 and 3 are adequate for detaching Vero cells. However, procedure 2 (washing the culture with PBS before the use of the trypsin) showed lower costs when compared to the others.

Supported by: Fundação Butantan.

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

Menezes MA, Aires KA, Ruiz RM, Ozaki CY, Elias WP, Piazza RMF
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP.

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

Menezes MA[1], Rocha LB[1], Koga PCM[1], Fernandes I[2], Nara JM[1], Magalhães CA[1], Elias WP[1], Piazza RMF[1]

[1]Laboratório de Bacteriologia and [2]Laboratório de Immunopatologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP.

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

Monaris D[1], Gonçales AP[2], Morais ZM[2]; Vasconcellos AS[2], Barbosa AS[1], Abreu PAE[1]
[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Laboratório de Zoonoses Bacterianas, Faculdade de Medicina Veterinária, Universidade de São Paulo, SP, Brasil

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

Supported by: CNPq and FAPESP.

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

Mourão-Fuches RM, Guglielmi PM, Quintilio W, Santos ADA, Moraes JF
Seção de Controle Biológico, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Murata VM, Tsuruta LR, Moro AM
Laboratório de Anticorpos Monoclonais, Instituto Butantan, SP, Brasil

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

Supported by: CNPq.

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

Nakajima E[1], Abreu PAE[2], Gonçales AP[3], Morais ZM[3], Souza GO[3], Vasconcellos SA[3], Martins EAL[1]

[1]Centro de Biotecnologia and [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [3]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

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

Supported by: CAPES, FAPESP and Fundação Butantan.

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

Nascimento IP[1], Dias WO[1], Quintilio W[2], Hsu T[4], Jacobs Jr WR[3,4], Leite LCC[1]
[1]Centro de Biotecnologia and [2]Serviço de Controle de Qualidade, Instituto Butantan, SP, Brasil; [3]Department of Microbiology and Immunology and [4]Howard Hughes Medical Institute, Albert Einstein College of Medicine, USA

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Nunes KSR[1,2], Barbosa AS[1], Abreu PAE [1]
[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brasil

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

Supported by: CNPq and FAPESP.

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

Oliveira RN[1], Miyaki C[1], Botosso VF[1], Quintilio W[1], Comone P[1], de Souza JM[1], Adami EA[1], Prado JCM[1], Carneiro SM[2], Higashi HG[1], Raw I[3]
[1]Divisão Bioindustrial, [2]Laboratório de Biologia Celular and [3]Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Oliveira BH[2], Silva, MR[1], Carvalho RJ[1], Braga CJM[3], Massis LM[3], Ferreira LCS[3], Sbrogio-Almeida ME[1], Takagi M[1]

[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Departamento de Engenharia Química, Universidade Federal do Paraná, PR, Brasil; [3]Departamento de Microbiologia, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP and Fundação Butantan.

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

Oliveira DCA[1], Botosso VF[1], Gouvea MN[1], Miyaki C[1], Tenório ECN[1], Faquim-Mauro EL[2], Fernandes I[2]
[1]Seção de Hepatite and [2]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

3.44 Effect of aflatoxin on the immunological system of cut chicken

Otacílio HS[1], Santos BM[3], Lopes DC[2], Souza AVC[2]
[1]Departamento de Medicina Veterinária and [3]Departamento de Zootecnia, Universidade Federal de Viçosa, MG, Brasil; [3]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: CNPq.

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

Paoli RL, Costa FF, Brittes AS, Frazatti-Gallina NM
Laboratório Experimental de Rotavírus, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Peters LC[1], Carneiro PS[2], Gasparelo TC[1], Vorraro F[1], Borrego A[1], Jensen JR[1], Cabrera WHK[1], Starobinas N[1], Ribeiro OG[1], Broman K[3], Rossato C[1], Ibañez OM[1], De Franco M[1]
[1]Laboratório de Imunogenética and [2]Serviço de Bacteriologia, Instituto Butantan, SP, Brasil; [3]Department of Biostatistics, Johns Hopkins University, USA

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

Supported by: FAPESP and CNPq.

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

Portes Jr JA[1], Oba-Shinjo SM[3], Oliveira MLS[2], Uno M[3], Rocha MC[3], Alves MJF[3], Butera D[1], Marie SKN[3], Magalhães GS[1]

[1]Laboratório de Imunopatologia and [2]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [3]Departamento de Neurologia, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP and CAPES.

3.48 Applicability of tetanus antigen conjugated to derivatives of monomethoxypolyethylene glycol

Prado SMA[1], Olivier FBG[1], Simões AA[1], Marcelino JR[2], Higashi HG[3], Guidolin R[3], Abrahão-Neto J[4]

[1]Serviço de Bacteriologia, [2]Serviço de Imunologia and [3]Divisão Bioindustrial, Instituto Butantan, SP, Brasil; [4]Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brasil

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

Supported by: Fundação Butantan.

3.49 Process validation of bulk diphtheria anatoxin

Sakauchi MA[1], Siquini TJ[1], Ferreira AA[1], Akamatsu MA[1], Viegas R[3], Koyama CH[3], Carvalho BP[1], Sakihara VA[1], Suzuki ME[4], Utescher CLA[4], Yamaguchi IK[4], Morais JF[4], Gioielli ML[2], Moraes TTR[3], Duboc MA[3], Higashi HG[5], Prado SMA[2]
[1]Seção de Vacinas Aeróbicas, [2]Serviço de Bacteriologia, [3]Laboratório Especial de Garantia de Qualidade, [4]Serviço de Controle de Qualidade and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Aguilar-Ramirez P, Cabrera WK, Ribeiro OG, Ibañez OM, De Franco M, Starobinas N
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

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

Supported by: CAPES and FAPESP.

3.51 Media hold test for bulk diphtheria anatoxin production

Sakauchi MA[1], Siquini TJ[1], Ferreira AA[1], Akamatsu MA[1], Viegas R[3], Koyama CH[3], Carvalho BP[1], Sakihara VA[1], Utescher CLA[4], Orozco S[4], Gioielli MLR[2], Moraes TTR[3], Duboc MA[3], Higashi HG[5], Prado SMA[2]
[1]Seção de Vacinas Aeróbicas, [2]Serviço de Bacteriologia, [3]Laboratório Especial de Garantia de Qualidade, [4]Serviço de Controle de Qualidade and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Ricardi R[1], Favoretto BC[1], Lima-dos Santos I[3], Okada SS[2], Della-Casa MS[3], Faquim- Mauro EL[3]

[1]Departamento de Imunologia, Instituto de Ciências Biomédicas and Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brasil; [3]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP, CNPq and CAPES.

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

Rodríguez D, Cuña V, Farias LP, Leite LCC
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP and Fundação Butantan.

3.54 Retrospective process validation of bulk tetanus anatoxin production

Trezena AG[1], Estima MA[1], Rós N[1], Santos AS[1], Kyoma CH[2], Moraes TTR[2], Utescher CLA[3], Suzuki ME[3], Farina J[3], Yamaguchi IK[3] Gioielli MLR[4], Morais JF[3], Duboc MA[2], Higashi HG[5], Prado SMA[4]

[1]Seção de Vacinas Anaeróbicas, [2]Laboratório Especial de Garantia da Qualidade, [3]Serviço de Controle de Qualidade, [4]Serviço de Bacteriologia and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Rossato C, Albuquerque LL, Cabrera WHK, Starobinas N, De Franco M, Ribeiro OG, Ibañez OM, Jensen JR
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP and CNPq.

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

Santos FL[1], Dias SC[4], Darini E[1], Furuyama N[1], Alves RF[4], Sakauchi MA[1], Iourtov D[3], Prado SMA[2], Higashi HG[3], Raw I[4], Kubrusly FS[4]
[1]Seção de Vacinas Aeróbicas, [2]Serviço de Bacteriologia, [3]Divisão Bioindustrial and [4]Centro de Biotecnologia, Instituto Butantan, SP, Brasil

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

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

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

Santos MF, Andrade GB, Ozaki CY, Domingos MO
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

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

Supported by: CNPq and FAPESP.

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

Santos SMR[1], Ferreira TL[1], Quintal VS[3], Frazzatti-Gallina NM[2], Carbonare SB[1], Tino De Franco M[1]

[1]Laboratório de Imunogenética and [2]Laboratório Experimental de Rotavírus, Instituto Butantan, SP, Brasil; [3]Banco de Leite, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP and CAPES.

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

Santos SMR, Rizzo R, Paoli RL, Frazatti-Gallina NM
Laboratório Experimental de Rotavírus, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Scaramuzzi K[1], Carvalho LV[1], Ferreira-Jr JMC[1], Faquim-Mauro EL[2], Tino De Franco M[3], Carbonare SB[3], Ribeiro OG[3], Sant´Anna OA[1]
[1]Laboratório de Imunoquímica, [2]Laboratório de Imunopatologia and [3]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

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

Supported by: CNPq, FAPESP and Cristália.

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

Barbosa AS[1], Silva LB[1], Gonçales AP[2], Morais ZM[2], Vasconcellos SA[2], Abreu PAE[1]
[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP, CNPq and Fundação Butantan.

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

Silva MR[1], Albani SMF[1], Pinto JV[1], Cabrera-Crespo J[1], Pradella JGC[2], Zangirolami TC[3], Takagi M[1]

[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Centro de Ciência em Tecnologia do Bioetanol, Brasil; [3]Departamento de Engenharia Química, Universidade Federal de São Carlos, SP, Brasil

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

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

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

Silva SR[1], Jacysyn JF[1], Macedo MS[1], Faquim-Mauro EL[2]
[1]Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil; [2]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil

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

Supported by: CNPq, CAPES and FAPESP.

3.64 Validation of purification process of tetanus anatoxin final bulk

Guilhen FB[1], Simões AS[1], Takahashi ACYAS[1], Evaristo JA[1], Marchi SA[1], Matarazzo RA[1], Utescher CLA[2], Suzuki ME[2], Morais JF[2], Yamaguchi IR[2], Nicolau BA[2], Mendes E[2], Moraes TTR[3], Gioielli MLR[4], Duboc MA[3], Higashi HG[5], Prado SMA[4]
[1]Central de Purificação de Produtos Bacterianos, [2]Serviço de Controle de Qualidade, [3]Laboratório Especial de Garantia de Qualidade, [4]Serviço de Bacteriologia and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

3.65 Validation of purification process of diphtheria anatoxin final bulk

Guilhen FB[1], Simões AS[1], Takahashi ACYAS[1], Evaristo JA[1], Marchi SA[1], Matarazzo RA[1], Utescher CLA[2], Suzuki ME[2], Morais JF[2], Yamaguchi IR[2], Nicolau BA[2], Mendes E[2], Moraes TTR[3], Gioielli MLR[4], Duboc MA[3], Higashi HG[5], Prado SMA[4]
[1]Central de Purificação de Produtos Bacterianos, [2]Serviço de Controle de Qualidade, [3]Laboratório Especial de Garantia de Qualidade, [4]Serviço de Bacteriologia and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

De Franco M[1], Colombo F[2], Galvan A[2], De Cecco L[2], Spada E[3], Milani S[3], Ibanez OM[1], Dragani TA[2]

[1]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [2]Departmenti Experimentale di Oncologia, Istituto Nazionale Tumori, Italy; [3]Istituto di Statistica Medica e Biometria "GA Maccacaro" Università di Milano, Italy

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

Supported by: FAPESP, CNPq, UICC and ARC.

3.67 Validation process of pertussis vaccine final bulk product

Sakauchi MA[1], Furuyama N[1], Dias EDF[1], Viegas R[4], Ferrari L[1], Bezerra MFB[1], Campos MRV[1], Santos FL[1], Darini E[1], Cunha RD[1], Suzuki ME[3], Utescher CLA[3], Yamaguchi IK[3], Morais JF[3], Gioielli MLR[2], Carneiro PS[2], Moraes TTR[4], Duboc MA[4], Higashi HG[5], Prado SMA[2]

[1]Seção de Vacinas Aeróbicas, [2]Serviço de Bacteriologia, [3]Serviço de Controle de Qualidade, [4]Laboratório Especial de Garantia de Qualidade and [5]Divisão Bioindustrial, Instituto Butantan, SP, Brasil

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

Supported by: Fundação Butantan.

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

Ventini DC[1,2], Astray RM[1], Lemos M[1], Greco KN[1], Jorge SAC[1], Tonso A[2], Pereira CA[1,2]
[1]Laboratório de Imunologia Viral, Instituto Butantan, SP, Brasil; [2]Laboratório de Células Animais, Departamento de Engenharia Química, Universidade de São Paulo, SP, Brasil

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

Supported by: FAPESP and TACnet.

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

Vorraro F[1], Galvan A[2], Cabrera WHK[1], Ribeiro OG[1], De Franco M[1], Starobinas N[1], Broman K[3], Dragani T[2] and Ibañez OM[1]

[1]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [2] Department of Experimental Oncology, Istituto Nazionale Tumori, Italy; [3]Department of Biostatistics and Medical Informatics, University of Wisconsin, USA

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

Supported by: FAPESP and CNPq.

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

Vorraro F[1], Galvan A[2], Cabrera WHK[1], Ribeiro OG[1], Pazzaglia S[3], Mancuso M[3], Zolin A[4], Milani S[4], Saran A[3], Dragani T[2] and Ibañez OM[1]

[1]Laboratório de Imunogenética, Instituto Butantan, SP, Brasil; [2] Department of Experimental Oncology and Laboratories, Fondazione IRCCS Istituto Nazionale Tumori, Italy; [3]ENEA Laboratories, Italy; [4]Istituto di Statistica Medica e Biometria "GA Maccacaro", Università di Milano, Italy

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

Supported by: FAPESP and CNPq.

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

Wolf PS[1,3], Lopes APY[1], Ferreira VF[1], Sakauchi MA[2], Quintilio W[3], Cainelli Gebara VCB[1]
[1]Centro de Biotecnologia, [2]Seção de Vacinas Aeróbicas and [3]Controle de Qualidade, Instituto Butantan, SP, Brasil

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

Supported by: FAPESP and Fundação Butantan.