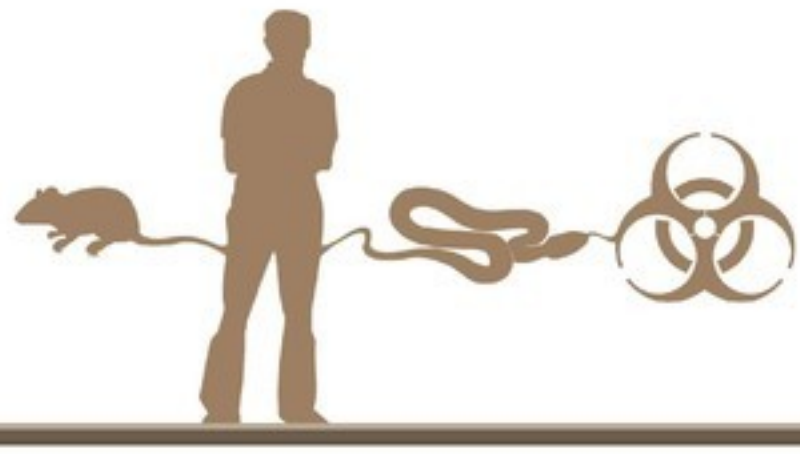
4. Immunology and Vaccines



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

4.01 Upregulation of growth fator receptors in hematopoietic/stem progenitor cells is associated with high acute inflammatory response

Albuquerque LL[1], Katz ISS[1], Suppa AP[1], Rossato C[1], Moreira CN[1], Grund LZ[2], Cabrera WHK[1], De Franco M[1], Starobinas N[1], Jensen JR[1], Lima C[2], Ibañez OCM[1] and Ribeiro OG[1]

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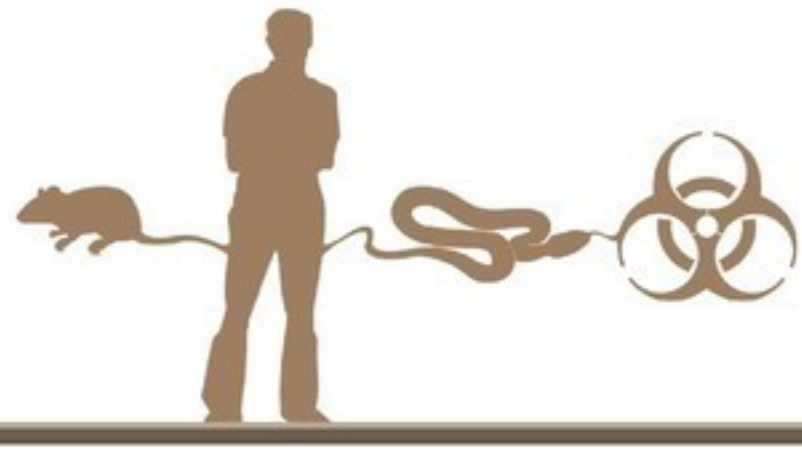
Introduction: As a result of an inflammatory stimulus, the hematopoietic system accelerates the expansion of myeloid progenitors by increasing the production of granulocytes, a phenomenon called emergency granulopoesis. The regulation of myeloid cells expansion and differentiation to mature granulocytes is essential in the control of infections and diseases in which the inflammatory component shown to be important. Mice phenotipically selected for high (AIRmax) or low (AIRmin) acute inflammatory response represent an interesting model for identifying genetic factors and functional mechanisms involved in the production of granulocytes. **Objectives**: To investigate the regulation of myelopoiesis and its association with acute inflammatory response (AIR) in AIRmax and AIRmin mice. **Methods:** The inflammatory exudate and BM cellularity were evaluated by cell count in basal mice and $1^{1/2}$, 3 and 24 h after s.c injection of polyacrylamide beads (Biogel). Mature hematopoietic lineages were depleted by magnetic beads and further analyzed the dynamics of the progenitor populations (Hematopoietic stem cell (HSC), common myeloid progenitor (CMP) granulocyte-macrophage progenitor (GMP) and megakaryocyte - erytrocyte progenitor (MEP)) by flow cytometry and the gene expression was evaluated by quantitative PCR. **Results and Discussion:** Our results showed that only AIRmax undergoes emergency granulopoesis elicited by Biogel. This phenomenon was confirmed by significant increase in total leukocytes, CMP and GMP at 1.5 h after AIR induction. Unlike, any significantly changes at any time point after inflammation was observed in BM from AIRmin mice. The higher differentiation and progenitor expansion to mature granulocytes was also confirmed after 5 days of *in vitro* cultures stimulated with 23 different combinations of hematopoietic growth factors, in which the AIRmax mice showed higher capacity of proliferation. Furthermore, gene expression analysis of GM-CSFRα, G-CSFR and IL-3Rα showed significant differences between AIRmax and AIRmin lines under basal conditions. In AIRmax, the increased expression of GM-CSFR and IL-3R was also confirmed by flow cytometry. After the inflammatory stimulus, we observed an increased mRNA expression of the GCSFR, GMCSFRβ and IL3Rα genes only in AIRmax mice. Taken together the results suggest that there is a correlation between the expression of growth factor receptors, which enables an augmented differentiation, and cell lineage commitment, with the ability to develop an acute inflammatory response.

Supported by: FAPESP and CNPq



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4.02 Identification of B Cells Expressing Anti-Tetanus Toxoid IgG in Human Peripheral Blood by Flow Cytometry

Aliprandini E[1], Kalil J[2,3], Nussenzweig MC[4], Boscardin SB[5], Moro AM[1,3]
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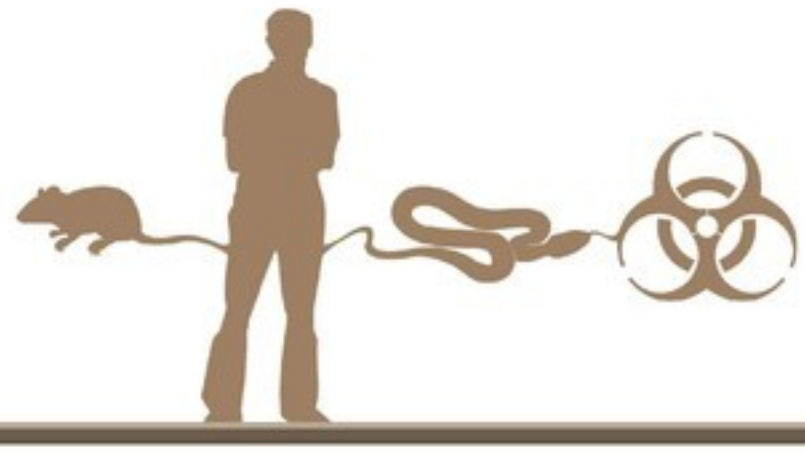
Introduction: The interest in monoclonal antibodies (mAbs) is increasingly observed and many researches are devoted to obtain "fully human" mAbs for therapeutic purposes. Recently, a technique was developed for the production of recombinant human mAbs. It is based on the single cell sorting of peripheral B lymphocytes using a biotinylated protein recognized by their surface IgG receptor. The sorted cell mRNA corresponding to the variable regions of the heavy and light chains can be amplified and used to transfect host cells that will produce the mAbs for screening of the desired properties. The success of this protocol depends on the collection of memory B lymphocytes, which may be present in the blood, or in secondary lymphoid organs. **Objectives:** In this work we tested four potential candidates aiming to determine the presence of B cells that produce anti-tetanus toxoid (TT) antibodies and thus select the more suitable candidate for blood donation for subsequent obtainment of human monoclonal anti-tetanus toxoid IgGs. **Methods:** Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation from blood collected by venipuncture from four donors after signature of informed consent in accordance to the Ethics Commission of the Biomedical Sciences Institute, USP. These donors were selected based on the elevated titers of anti-tetanus antibodies. TT obtained from the Butantan Institute was biotinylated by chemical conjugation. ELISA was performed to evaluate the influence of biotinylation on the binding of anti-TT antibodies to the antigen. **Results and Discussion:** Analysis of the population of anti-tetanus toxoid IgG producing B lymphocytes was performed by flow cytometry after incubation of PBMC with biotinylated tetanus toxoid and staining with anti-CD19 FITC, anti-sIgG APC, and streptavidin PercP-Cy5.5. The results, expressed in number of B lymphocytes expressing IgG anti-tetanus toxoid/10^6 PBMC, were: (1) 16.13; (2) 36.18; (3) 11.15; and (4) 2.82. The control group (no booster immunization after the infant vaccination) showed no detectable cells with this triple staining. Considering the major probability of finding memory B lymphocytes in secondary lymphoid organs, the detection of this population in the blood of the three selected donors in this prospective screening qualifies them as candidates for further development of anti-tetanus human mAbs.

Supported by: CAPES, FAPESP and CNPq



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4.03 Crotoxin from *Crotalus durissus terrificus* is able to down-modulate Th17 response in the acute intestinal inflammation in mice

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Introduction: Crohn's disease is classically regarded as a Th1 mediated inflammatory disorder. Recently, it was verified that the Th17 cells are also involved in inflammatory intestinal response. The crotoxin (CTX) is the main component of the *Crotalus durissus terrificus* rattlesnake venom and has immunosuppressive action. **Objectives:** Here, we investigated the effect of CTX on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. **Methods:** After 18 hours of the TNBS-colitis induction, mice were treated with CTX or PBS and sacrificed 4 days after induction. The colon was separated in parts for cytokines measurement, extraction of mononuclear lamina propria (MLP) cells for flow cytometry and for histological analysis. The cytokines expression was also analyzed in mRNA obtained from mesenteric lymph nodes (MLN) cells. **Results and Discussion:** Clinical and histological scores showed that the CTX-treatment decreased the disease progression. High secretion of IL-6, TNF-α, IFN-γ and IL-17 was verified in cell supernatants from TNBS-mice and the RT-PCR confirmed these data. In contrast, CTX treatment decreased the cytokine secretion in TNBS-induced colitis mice. Furthermore, the CTX administration also induced higher IL-10 and TGF-β secretion when compared with the TNBS-group. The TNBS colitis mice showed higher percentage of $CD4^+ROR\text{-}\gamma t^+$ and $CD4^+T\text{-}bet^+$ cells compared with the CTX treated TNBS-induced colitis group. These results suggest an immunomodulatory role for CTX in Th17/Th1 response observed in acute intestinal inflammation induced by TNBS.

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4.04 Aged [$H_{III}xL_{III}$]F_1 hybrid mice immunoresponse to *M. leprae* Hsp65

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Introduction: Aging process is followed by humoral and cell-mediated immunity changes; alongside these modifications, chronic-degenerative processes, such as autoimmunities, can emerge. Heat shock proteins (Hsp) are involved in autoimmune and inflammatory events and can interfere in immunosenescence course, since induce imbalance of the immunological homeostasis. Our previous data showed *M. leprae* Hsp65 inoculation abbreviates survival of aged genetically selected high responder H_{III} female mice, and not interferes on young female or aged male of this line. **Objectives:** Here, the eventual cell immune alterations after Hsp65 injection and the interference of Hsp65 in aged F_1 hybrid mice obtained from H_{III} x L_{III} reciprocal crosses were evaluated. **Methods:** Two hundred days-old F_1H and F_1L were intraperitonially injected with 2.5µg/animal of *M. leprae* Hsp65 or PBS (control group) (n= 3-6 animals/group). Splenic immune cell phenotypes were analyzed by flow cytometry at day 7 and 14 days after Hsp administration. **Results and Discussion:** The F_1H♀ (at 14 days) and F_1L♀ (at 7 days) mice presented significant increased frequency ($p<0.001$) of $CD3^+CD4^+CD45RA^+$ (10-times), $CD3^+CD4^+CD154^+$ (30-times), $CD3^+CD8^+$ (3-times), $CD3^+CD8^+CD44^+$ (10-times) and $CD11c^+$ (5-times) cells; however, only aged F_1L♀ showed reduced percentage of $CD3^+CD4^+$ and $CD11b^+$ cells ($p<0.001$, 4-fold reduction for both cell types), with amplified frequency of $CD11c^+CD80^+$ ($p<0.001$, 10-fold higher) compared to control group. In contrast, aged F_1H♂ and F_1L♂ mice presented significant reduction of $CD3^+CD4^+$ ($p<0.05$, 1.27-fold) and $CD3^+CD8^+CD44^+$ lymphocytes ($p<0.001$, ~2-fold) and an increase in $B220^+$ ($p<0.001$ in F_1H♂ and $p<0.01$ in F_1L♂, ~2.3-fold) cells percentage 14 days after administration. Besides these quantitative modifications, Hsp65 inoculation did not interfere on F_1 hybrid mice survival. *M. leprae* Hsp65 administration increased the frequency of naïve and activated T CD4 and CD8 lymphocytes and dendritic cells expressing coestimulatory molecules in F_1 female mice, while F_1 male mice it reduced T CD4 and activated T CD8 cell populations and increased B lymphocytes percentage. These results may reflect a sex-effect, since it was observed difference in the immune cells compartment between F_1 female and F_1 male mice.

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4.05 Molecular investigation of *Schistosoma mansoni* Venom Allergen Like protein 6 isoforms

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

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4.06 Role of inflammation in regenerative and non-regenerative types of wound repair

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Introduction: Wound repair is one of the most complex biological processes and can be subdivided into two distinct categories, regenerative and non-regenerative. Homozygous AIRmax and AIRmin sublines for *Slc11a1 S* alleles, produced by genotype-assisted breeding, differ in ability to completely repair ear holes. AIRmax^{SS} mice showed fast ear tissue regeneration while AIRmin^{SS} mice did not show regeneration after ear punch. **Objectives:** Our aim in this work was to evaluate the influence of the early inflammatory response in the determination of those distinct phenotypes. **Methods:** Two-millimeter ear holes were punches in mice of each subline and the inflammatory reaction was characterized. **Results and Discussion:** The local inflammatory response was more intense in AIRmin^{SS} than AIRmax^{SS} mice 24 and 48h after ear punch, which was demonstrated by histomorphometric analysis and MPO levels. Global gene expression analysis demonstrated over-represented distinct biological themes between AIRmax^{SS} and AIRmin^{SS} control mice. Inflammatory response biological category was observed only in AIRmin^{SS}. At 24h after punch, both AIRmax^{SS} and AIRmin^{SS} mice showed significant ($P<0.001$) up-regulated genes related to inflammation. However, angiogenesis, epidermis development and collagen catabolic process were expressed only in AIRmin^{SS}. All down-regulated genes in response to wounding in AIRmax^{SS} were represented to muscle contraction which is known to be involved in tissue repair with scarring. Microarray results were validated by quantitative PCR. Some pro-inflammatory cytokines were measured by multiplex assay and AIRmin^{SS} mice showed amounts significantly elevated compared to AIRmax^{SS}. The higher initial inflammatory response in the inflamed ear tissue combined with elevated RNA expression of genes involved in inflammation could inhibit epimorphic regeneration in AIRmin^{SS} mice. These results suggest that the controlled inflammatory response has a beneficial effect in tissue resolution.

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4.07 Inverse susceptibility to hepatic and lung cancer in mouse lines selected according to the acute inflammatory response

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Introduction: Inflammatory components are an essential part of the tumor microenvironment. It is the expression of various inflammatory mediators and the amount and state of activation of different cell types in the tumor microenvironment that will guide the response for promotion or inhibition of tumor growth. The hepatocellular carcinoma (HCC) is a cancer closely related to sex and inflammation, being three times more prevalent in men than in women and related to infectious diseases like hepatitis. Lung cancer is another type of cancer related to inflammation, mainly to inhalation of toxic substances, such as those present in cigarette smoke. **Objectives:** Our objective was to study the influence of genetic factors relevant to inflammatory response regulation on cancer development by the comparative analysis of carcinogen-induced liver and lung tumors in AIRmax and AIRmin mouse strains, genetically selected for maximum and minimum inflammatory responsiveness. **Methods:** Lung and liver carcinomas were induced by the injection of specific chemical carcinogens, Urethane and Diethylnitrosamine – DEN, respectively, and long term cancer development was observed in the two mouse strains. An analysis of the inflammatory mediators induced by the carcinogens was done hours after the drug injection. Comparative transcriptome analysis was carried out in normal lungs and livers of AIRmax and AIRmin mice. **Results and Discussion:** 38 weeks after DEN treatment, most AIRmax (80%) had multiple and large liver tumors whereas AIRmin mice were more resistant (33.3%) presenting small lesions. According to human HCC, females were more resistant to this carcinoma compared to male from the same strain and none AIRmin female presented tumors. Interestingly, the susceptible lineage of lung carcinoma was AIRmin. 38 weeks after urethane injection, all AIRmin mice were affected and AIRmax incidence was only 27.3% showing an inverse phenotype. Early expression of IL-6 and TNFα mRNA seems to be important in this phenotype, with local (lung and liver) production of these acute phase proteins hours after drug injection. Microarray analysis of normal lung and liver tissue of AIRmax and AIRmin showed differential expression of inflammation-associated genes involved in pathways such as *leukocyte transendothelial migration*, *cell adhesion* and *tight junctions*. These results provide a demonstration of the role of local cells in the control of immunity to tumor development and also show the importance of inflammation during tumor progression and resolution.

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4.08 Characterization of a neutralizing recombinant monoclonal antibody, scFv, against BaP1, A P-I metalloproteinase from *Bothrops asper* snake venom

Castro JMA[1], Ohashi A[1], Caporrino MC[1], Oliveira TS[1], Pereira NA[1], Rucavado A[2], Gutiérrez JM[2], Magalhães GS[1], Fernandes I[1] and Faquim-Mauro E[1]

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Introduction: BaP1 is a P-I class of snake venom metalloproteinase relevant in the local tissue damage associated with envenomations by *Bothrops asper*, a medically important species in Central and parts of South America. We constructed a recombinant single chain fragment variable (scFv) monoclonal antibody against BaP1 (scFvBaP1). It contains VH and VL domains linked by a flexible $(G_4S)_3$ polypeptide. **Objectives:** To express the scFvBaP1 and evaluate its capacity to neutralize important actions of BaP1. **Methods:** ScFvBaP1 was cloned into pMST3 vector in fusion with SUMO protein. Citoplasmic expression of this construction was sucessfully active in C43 (DE3) bacteria. Both scFv and SUMO (control) were analyzed by SDS-PAGE to confirm their purity. The ability of monoclonal antibody (MaBaP1) and the scFv to recognize total venom from *Bothrops asper* and BaP1 was assessed by ELISA. The capacity of scFv to neutralize fibrin degradation induced by BaP1 was evaluated using agarose gel substrate containing fibrin. The ability of scFv to neutralize BaP1-induced hemorrhage in skin mice was estimated by incubating one Minimum Hemorrhagic Dose (35 μg) of BaP1 with scFv (10:1 molar ratio). **Results and Discussion:** Samples of scFv and SUMO presented bands of 38.9 and 13.6 kDa, respectively. ELISA showed that scFv was able to recognize BaP1 as well as whole venom, while SUMO did not. BaP1-induced fibrinolysis was significantly neutralized by scFv, but not SUMO, in a concentration-dependent manner (ratio 20:1 and 10:1 resulted in 73.8% and 46.7% of inhibition of the fibrin degradation, respectively). ScFv, as well as MaBaP1 completely neutralized hemorrhage induced by BaP1. Our data showed that scFv specifically recognized and neutralized biological effects of BaP1 and the whole venom of *B. asper*, while SUMO did not interfere with this ability.

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4.09 The effect of *Slc11a1* gene polymorphism on macrophage activation during pristane-induced arthritis

Corrêa MA[1,2], Canhamero T[1,2], Borrego A[1], Starobinas N[1], Jensen JR[1], Cabrera WH[1], Ribeiro OG[1], Ibañez OC[1], De Franco M[1,2]
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Introduction: Macrophages and their products are involved in chronic autoimmune diseases, such as Rheumatoid Arthritis. Erosion of bone, joint destruction and pain result in severe deformity and disability. Our group observed previously that the presence of *S* allele of *Slc11a1* increased the incidence and severity arthritis in mice selected for high acute inflammatory response (AIRmax). The *Slc11a1* gene is involved in the ion transport at the endosomes in macrophages and neutrophils. **Objectives:** We investigated the effect of *Slc11a1* gene polymorphism in the activation of peritoneal macrophages during pristane-induced arthritis (PIA). **Methods:** AIRmax mice homozygous for *Slc11a1 R* and *S* allele received 0.5 mL i.p. pristane injection and on 2, 7, 14 and 180 days the peritoneal macrophages were isolated. Culture supernatants (48h after pristane injection) were harvested and cytokines levels of several inflammatory (IL1b, IL6, TNFa and MIP-2) were detected by multiplex assay. H_2O_2 and NO detection were also performed. **Results and Discussion:** Pristane treatment decreased significantly the infiltrated cell number in both AIRmax^{RR} and AIRmax^{SS} peritoneal cavity on days 7 and 14 ($p<0,001$), but it was increased on day 180. IL1b and TNFa were produced in high levels in AIRmax^{SS} macrophages culture on 2 and 180 days. On the other hand, IL6 and MIP-2 secretion significantly increased in AIRmax^{SS} only on day 180. Susceptible mice (bearing *Slc11a1 S* allele) macrophages produced high significant amounts of H_2O_2 on 7, 14 and 180 days. In addition, significant NO production ($p<0,001$) was observed in AIRmax^{SS} on 7 and 180 days after LPS stimulation *in vitro*. These results showed that those cytokines were often higher in AIRmax^{SS} than AIRmax^{RR} macrophages after PIA, which suggests that the *Slc11a1* gene modulates macrophage activation during arthritis progression.

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4.10 Protection and memory immune response against *Bordetella parapertussis* induced by a whole cell pertussis vaccine with low levels of endotoxin (Plow)

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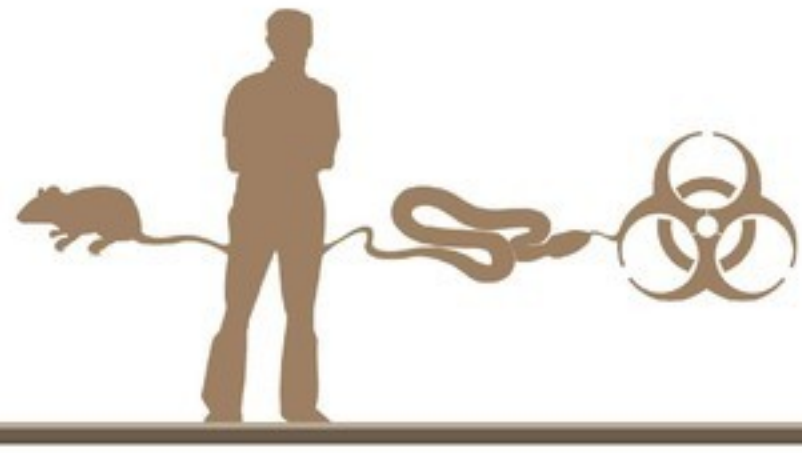
Introduction: Pertussis is a serious transmissible disease of the human respiratory tract, caused by gram-negative bacteria *Bordetella pertussis* and *Bordetella parapertussis*, of universal distribution. The World Health Organization estimates the annual incidence of 50 million cases of pertussis disease, causing 300.000 deaths. Nowadays is being observed a increase in the prevalence of *B. parapertussis*, as an epidemiological consequence of global immunization with pertussis vaccines, most of them although highly effective in reducing the incidence of pertussis infection have little or no efficacy against *B. parapertussis*. The Instituto Butantan is developing a new whole cell pertussis vaccine (Plow), with low-endotoxin content, which proved to be effective, but less reactogenic. **Objectives:** To evaluate, in mice, the impact of the vaccination with Plow in the infection with *B. parapertussis*. **Methods:** Balb/c mice were immunized with Plow, in two independent experiments. The vaccine was administered subcutaneously (a human dose-HD or 1/5 HD), in two doses of 200µl, within 15 days intervals, mixed with aluminum hydroxide (1.2mg/ml). The control groups received just the adjuvant. The animals were challenged intranasally with virulent *B. parapertussis* (10^7 CFU/20µl in PBS) and with virulent *B. pertussis* (10^7 CFU/20µl in PBS) 5 and/or 20 days after the immunization. The upper respiratory tract colonization was evaluated by the number of CFU in nasotracheal wash. The total IgG against Plow or *B. parapertussis* was measured by ELISA, on individual sera before and after challenge. **Results and Discussion:** A HD of Plow led to 91% of reduction in the upper respiratory tract colonization after challenge with *B. parapertussis*, and two doses decreased in 99.6%. When it was used 1/5 of HD, Plow induced a reduction of 95.8% in the colonization with *B. pertussis*, 5 days after challenge and 100% after 20 days, as compared to the residual number of colonies in the control group, with significant increase in antibody titers, due to the booster effect. In the animals immunized with Plow and challenged with *B. parapertussis,* it was observed 52.5% of reduction in the colonization, 5 days after the challenge and 100% over the residual number of colonies in the control group, after 20 days, with significant increase in antibodies titers, possibly due to a booster effect represented by the challenge with *B. parapertussis.* Our data demonstrate that Plow was able to induce an effective protection against *B. pertussis* and against *B. parapertussis* by cross-reaction and also able to induce a memory response, three months after immunization.

Supported by: FAPESP, CNPq, Fundação Butantan



4.11 Study on the correlation between the antigenic content and alum concentration in hepatitis B vaccine

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Introduction: For the hepatitis B vaccine lot release, among several required tests, two are particularly important: alum concentration and antigen content. The first one aims to demonstrate the filling uniformity, and the second indicates the efficacy of the product. Once alum is in an excess when compared to the protein amount in the vaccine, the total adsorption is assumed. Therefore, a fine correlation between these determinations is expected. **Objectives:** The aim of this work is to show if there is any correlation between the results obtained for alum content and antigenic activity of hepatitis B vaccine samples, in order to establish a marker for the filling quality. **Methods:** By using the regular quality control assays, the alum content was determined by aluminum titration and hepatitis B antigen quantified by ELISA (commercial kit). Results for 40 samples were statistically compared. **Results and Discussion:** Data for antigen content and for alum quantitation were compared and did not showed statistically significant correlation. The methods have different focus and very different sensitivity (μg/mL versus mg/mL). That explains the difficulty to have a direct correlation between the results for each technique. So the filling quality marker is supposed to be the analysis of the data ensemble, not only one test.

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05 a 07 de Dezembro 2012, São Paulo, SP

4.12 Stability of the *Bordetella pertussis* monophosphoryl lipid A in emulsion

Macarini FL[1], Kubrusly F [2], Iourtov D[1], Akamatsu MA[1], D'Epiro TTS[1], Cuccovia MI[3], Darini E[1], Sakauchi MA[1], Prado SMA[1], Raw I[2]
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Introduction: The Butantan Institute developed a whole cell pertussis vaccine with lower reactogenicity at affordable prices and we established a method that removes of the LPS from *Bordetella pertussis*. This procedure allows recovery of the LPS as by product which, following acid hydrolysis, leads to the production of *Bordetella*-derived monophosphoryl lipid A (MPLA) a potent adjuvant. **Objectives:** We have evaluated the stability of MPLA emulsion in diferentes concentrations after two years of storage. **Methods:** Butantan emulsion were formulated by mixing a buffered aqueous phase (PBS) with one surfactant, the bacteria derivative immunomodulator MPLA in the following concentration 20, 100 and 400 μg/ml and metabolizable oil for constituting the oil in water (o/w) emulsion. The oil used was squalene at a concentration of 4% and the surfactant was 0.4% polysorbate 80, free of animal derivatives. The ultrahigh shear processor Microfluidics M110-EH Microfluidizer® was the equipment used to homogenize. A previous determined number of passes at 30,000 psi were done to guarantee the emulsifying process. Butantan emulsions quality control program studied the following parameters: sterility, pH, toxicity, pyrogenicity *in vivo* (rabbits) and *in vitro* (LAL) and the emulsion stability by phase separation (visual aspect) and definition of particle size and particle distribution determined by dynamic light scattering (DLS) with the Zetasizer Nano-S equipment after long term storage conditions (temperatures 4-8°C). **Result and Discussion:** The emulsions concentrations of 20, 100 and 400 μg/ml were sterile for bacteria and fungi, atoxic (the individual gain weight was above 60% of the weight gain of the controls) and non-pyrogenic. The pH values were between 7.1-7.3. The visual aspect of all emulsions after 24 months showed that they are all monophasic, whitish and opaque emulsions. The average particle size/ polydispersity index of the emulsion droplets during 1, 6, 18 and 24 month were respectively emulsion+MPLA 20 μg/ml: 127.8/0.122, 133.6/0.096, 126.0/0.096 and 191.7/0.065; emulsion+MPLA 100 μg/ml: 154.1/0.095, 152.8/0.088, 142.4/0,090 and 140.1/0.069; emulsion+MPLA 400 μg/ml: 158.5/0.079, 174.9/0.204, 151.7/0.051, 147.6/0.062. As the average particle size and distribution are indicative of the emulsion stability, the emulsion+MPLA 20 μg/ml were stabile until 18 month but show a particle growth superior to 10% after 24 month. Butantan emulsion+MPLA 100 μg/ml and emulsion+MPLA 400 μg/ml were considered definitely stable as they did not show particle growth superior to 10% during the evaluation period.

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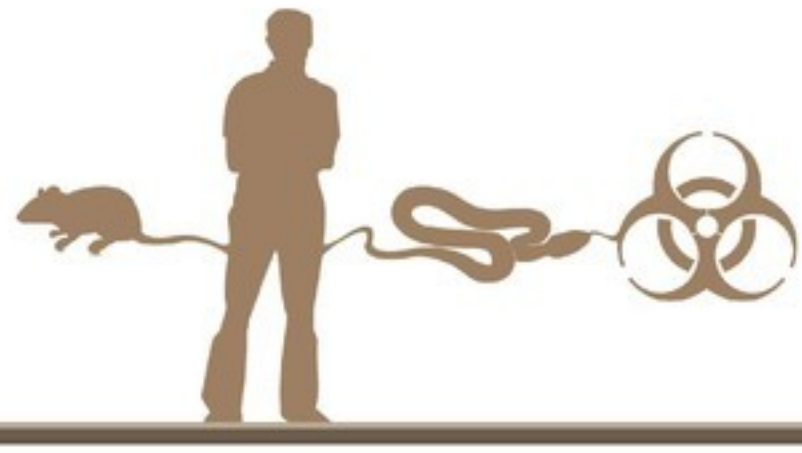

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05 a 07 de Dezembro 2012, São Paulo, SP

4.13 AIRmax and AIRmin lines selected for acute inflammatory response also differ in chronic inflammatory reactivity after a subcutaneous Biogel injection

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Introduction: AIRmax and AIRmin mouse lines differ in terms of acute inflammatory response after Biogel injection. These lines were developed in order to identify genes that affect the acute inflammatory response intensity (AIR) and to understand their cellular and molecular roles. The distinct AIR in these lines is well established, however, differences in late or chronic inflammatory response to Biogel were not described yet. **Objectives:** In the present work we decided to check if the genetic selection that modified the acute inflammatory response in these lines, also affected the development of a chronic inflammatory response to Biogel. **Methods:** AIRmax and AIRmin mice were injected with Biogel-P100 in the subcutaneous dorsal region and 48h and 30 days after, the exudates were recovered for cellular count and cytokines dosage by ELISA method. The local tissue was excised and the mRNA was extracted for microarray and Real Time PCR analysis and histological study. **Results and Discussion:** We found that AIRmax mice had statistically higher cellular influx in the inflammatory exudate than AIRmin mice in both analyzed periods (48h and 30 days) and that after 48 hours of Biogel injection, AIRmax mice showed higher cytokine levels in inflammatory exudate, probably contributing to the cellular influx profile in these lines. The global gene expression analysis in sc tissue showed higher number of up-regulated genes ($P<0.001$) in AIRmax than in AIRmin mice involved with inflammatory response, immune response and signal transduction. Furthermore, these results showed that some of the differentially expressed genes are located in *Irm1* locus region, a previously mapped QTL shown to be involved in AIR regulation. Some acute inflammatory response genes, besides being differentially expressed between the lines 48 hours after stimulus, also showed differences on day 30. Our results indicate that the genetic selection for acute inflammatory response may also have affected the chronic inflammatory response to Biogel. In this way, this work contributes to identify genetic factors controlling not only the acute inflammatory response intensity, but the chronic inflammatory response as well.

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4.14 Investigation of cercariae-schistosomula secreted SmVALs (*Schistosoma mansoni* Venom Allergen-Like Proteins) as potential vaccine candidates

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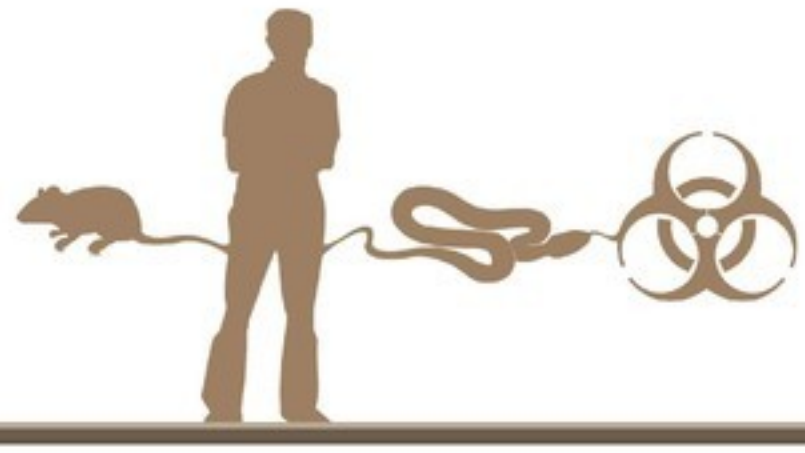
Introduction: The search for vaccine candidates against schistosomiasis using genomic, transcriptomic and proteomic databases led to the identification of a gene family named SmVAL (*Schistosoma mansoni* Venom Allergen-Like Protein) which is part of the superfamily SCP/TAPS (Sperm-coating protein/Tpx-1/Ag5/PR-1/Sc7 domains). SCP/TAPS proteins are structurally related and phylogenetically divided into group 1, containing proteins with features that suggests extracellular localization; and group 2, which contains proteins likely to be involved in intracellular interactions. Recent studies of cercarial/schistosomula transformation suggests that secreted group 1 SmVAL proteins might act as immunomodulators to facilitate the entrance of parasites in the mammalian host. **Objectives:** The objective was first to describe the acetabular glands morphological dynamics of schistosomula cultivated *in vitro* in a time course manner. Next, correlate the emptying of the glands contents with the presence of SmVALs in parasite's secretions. In parallel, we aimed to clone, express and purify group 1 SmVAL1, 4, 10, and 18 to evaluate these recombinant proteins as vaccines candidates in the murine model for schistosomiasis. **Methods:** Western Blot was performed to identify secreted SmVALs using anti-rSmVAL4 that also recognizes SmVAL10 and 18 in a cross-reactive manner. To label the pre and post acetabular glands of parasite stages we used PNA (lectin from peanuts) conjugated with Alexa fluor 647. In parallel, SmVALs cDNAs were cloned in the pAE vector and *E. coli* BL21 DE3 were transformed with these vectors containing SmVAL1, 4, 10 and 18 inserts. The expression of recombinant proteins was induced with IPTG. **Results and Discussion:** Anti-rSmVAL4 was able to recognize SmVALs in the secretions of cercariae, 3 h and 3 day-old schistosomula. In 5 day-old parasite secretions only a small amount of SmVALs could be detected and no protein was detected in 7 day-old schistosomula secretions. Conjugated PNA was able to delimitate the acetabular glands by interacting with glycoprotein in the glands of germballs, cercariae, and 3 h-old schistosomula. In the 3 day-old parasites, there is only residual staining, and no signal in 5 and 7 day-old schistosomula, revealing the complete emptying of the glands. Expression was obtained for all recombinant SmVALs. Here, we show that SmVALs are secreted by the acetabular glands of *in vitro* cultivated schistosomula, reinforcing that these proteins could be potential vaccine candidates. The next step in this study is to purify the recombinant proteins by nickel affinity and use them to immunize mice for challenge assays.

Supported by: FAPESP



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4.15 MYD88 and NF-κB are required for IL-12p40 production during macrophages activation by *Bordetella pertussis* and *B. parapertussis*

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Introduction: Macrophages initiate the innate immune response by recognizing pathogens, phagocytosing them and producing inflammatory mediators. IL-12 is generated especially by dendritic cells and macrophages as a heterodimeric cytokine (70kDa) comprising two disulphide-linked subunits designated p35 and p40. Their production can be differentially regulated by several signaling components through multiple pathways. MyD88 is critical for the signaling from TLRs. This pathway activates NF-κB and the induction of pro inflammatory cytokines. IL-12 is critical for host defense against a variety of pathogens acting directly on development of Th1 response. Whooping cough is a respiratory disease of humans caused by *B. pertussis* and *B. parapertussis*, which express differences in LPS and *B. parapertussis* is a mutant in pertussis toxin gene expression. Studies have demonstrated a dominant role for IFN-γ secreted by Th1 cells in the protection against *B. pertussis*. **Objectives:** This study was conducted to analyze signals pathways responsible for the control of IL-12p40 synthesis, specifically, MyD88 and NF-κB, during the murine bone marrow-derived macrophage (BMDMO) activation by *B. pertussis* and *B. parapertussis*. **Methods:** BMDMO obtained from femur and tibiae of C57BL/6 and MyD88$^{-/-}$ mice were differentiated in complete RPMI medium supplemented with supernatant of L929 cell culture plus 10% of FBS. On the 7th day the adherent cells were pretreated or not with NF-κB inhibitor (BAY 11-7082, 10μM) for 1hr prior stimulation with soluble protein from *B. pertussis* and *B. parapertussis* (30μg/mL). IL-12p40 was quantified by ELISA after 20 hours of incubation. **Results and Discussion:** Our results showed that macrophages from MyD88$^{-/-}$ mice activated with the lysate of *B. pertussis* or *B.parapertussis* significantly reduced the production of IL-12p40 as well as inhibition of NF-κB. Thus, MyD88 and NF-κB are involved in the regulatory pathway of IL-12p40 during the activation of macrophages by soluble protein from *B. pertussis* and *B. parapertussis*. These data suggest that TLRs play role in the host response to both bacterial.

Supported by: FAPESP, CAPES

4.16 Hybrid proteins containing PspA and pneumolysin derivates confer protection against fatal pneumococcal challenge

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Introduction: Pneumococcal surface protein A (PspA) and Pneumolysin derivatives (Pds) are important vaccine candidates, which can confer protection in different models of pneumococcal infection. Furthermore, the combination of these two proteins was able to increase protection against pneumococcal sepsis in mice. **Objectives:** The aim of this study was to produce a hybrid protein PspA-Pds and evaluate the immune response induced. **Methods:** Recombinant hybrid proteins was obtained by gene fragment fusion of pneumolisoids to the C-terminus of clade 1 or clade 2 *pspA* gene fragments, expressed in *E. coli* and purified by affinity chromatography. BALB/c mice were immunized with PspA, Pds, coadministered proteins or the hybrid proteins and the antibody production was evaluated by ELISA. The anti-sera were tested for their ability to bind onto pneumococci bearing family 1 PspAs and promote complement deposition by FACS. Also, the mice protection induced by hybrids protein immunization was tested by fatal pneumococcal challenge. **Results and Discussion:** Mouse immunization with the fusion proteins induced high levels of antibodies against PspA and Pds, able to bind to intact pneumococci expressing a homologous PspA with the same intensity as antibodies to rPspA alone or the co-administered proteins. However, when pneumococcal strains bearing heterologous PspA molecules were used, binding of anti-hybrid antibodies, as well as the levels of C3 deposition on the surface of the bacteria were significantly higher than that of antisera against the co-administered proteins. In agreement with these results, antisera against the hybrid proteins were more effective in promoting the phagocytosis of bacteria bearing heterologous PspAs *in vitro*, leading to a significant reduction in the number of bacteria when compared to co-administered proteins. Finally, mice immunized with fusion proteins were protected against fatal challenge with pneumococcal strains expressing heterologous PspAs. Taken together, the results suggest that PspA-Pd fusion proteins comprise a promising vaccine strategy, able to increase the immune response mediated by cross-reactive antibodies and complement deposition to heterologous strains, and to confer protection against fatal challenge.

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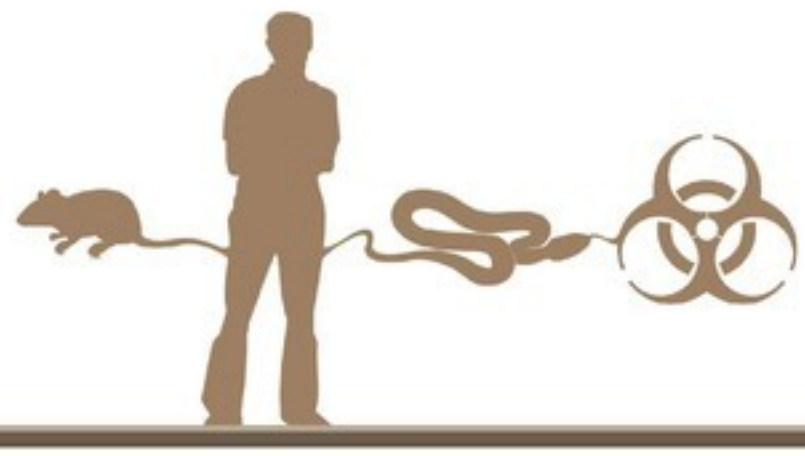

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4.17 Influence of formaldehyde in the determination of the ovalbumin residual content of A/California/7/2009 monovalent influenza vaccines produced by Butantan Institute for the 2011 season

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Introduction: Influenza vaccine is a sterile, aqueous suspensions of strains of influenza virus, type A and B, which have been grown individually in embryonated hen's eggs, inactivated and treated so that the virus particles are disrupted. It contains predominantly haemagglutinin and neuraminidase antigens. As a consequence of the production system, all influenza vaccines produced in embryonated eggs contain varying amounts of residual egg proteins. Ovalbumin is used as a marker, representing the amount of egg-derived proteins present in the vaccine and its content depends on the specific manufacturing process of the vaccine. As egg proteins are considered sensitizing agents, they can be related to adverse effects of influenza vaccines. Considering that influenza vaccines are intended for annual reimmunization, the quality control of influenza vaccines regarding their ovalbumin content is of major importance. **Objectives:** The aim of the present study is to evaluate the influence of formaldehyde in the determination of the ovalbumin residual content of A/California/7/2009 monovalent influenza vaccines produced by Butantan Institute for the 2011 season. **Methods:** The monovalent influenza vaccines used in this study were produced by Butantan Institute from A/California/7/2009 (H1N1) virus strainfor the 2011 season. The monovalents were diluted in 1/7000 and 1/1000 formaldehyde solutions. The content of ovalbumin in the monovalent influenza vaccines was measured by Sandwich ELISA. Calculations are made using linear regression and allow establish an ovalbumin content for the samples in comparison with a standard of purified ovalbumin. **Results and Discussion:** There was no statistically significant difference in the ovalbumin content of the samples diluted in 1/7000 and 1/1000 formaldehyde solutions. These preliminary results demonstrate that formaldehyde did not interfere with the measurement of ovalbumin in monovalent influenza vaccines.

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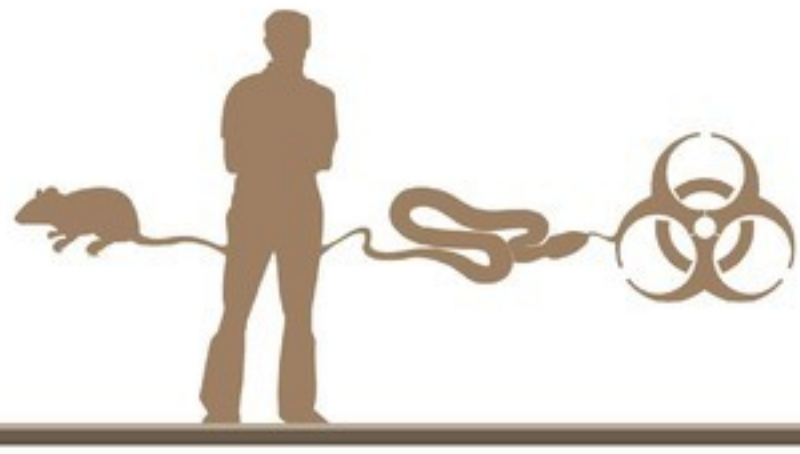

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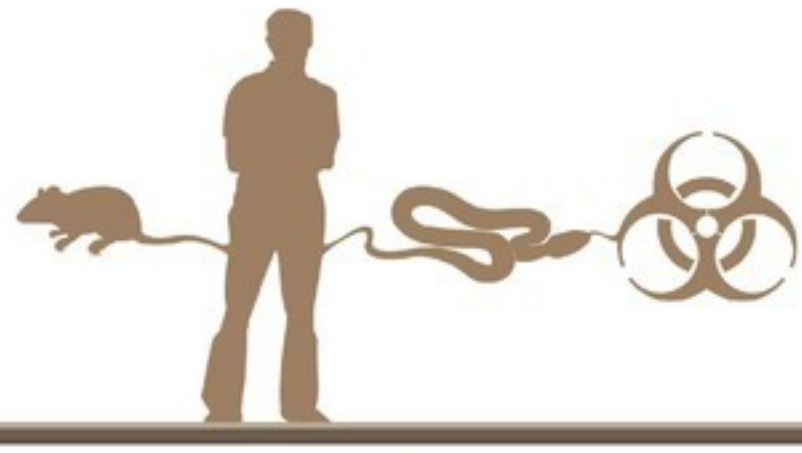
4.18 Myelotoxicity contribute to the phenotypes of susceptibility to Cancer in AIRmin selected mice

Katz ISS[1], Albuquerque LL[1], Suppa A[1], Moreira PC[1], Da Silva GB[2], Carvalho LV[3], Jensen JR[1], Starobinas N[1], Cabrera WHK[1], De Franco M[1], Sant`Anna AO[3], Borelli P[2], Ibañez OM[1], Ribeiro OG[1]

[1]Laboratório de Imunogenética, [3]Laboratório de Imunoquímica, Instituto Butantan, Brasil; [2]Faculdade de Ciências Farmacêuticas, USP, São Paulo, Brasil

Introduction: DMBA is a genotoxic agent that reacts with DNA directly, inducing p53-dependent cytotoxicity resulting in an immunosuppressive state. DMBA metabolism depends on the activation of the aryl hydrocarbon receptor (AhR). Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response presented a complete segregation of *Ahr* alleles endowed with low (Ahr^{d}) or high (Ahr^{b1}) affinity to PAHs, respectively. AIR mice differ in the susceptibility to toxic and carcinogenic effects induced by DMBA. **Objectives:** We investigated the myelotoxic effects of DMBA treatment and its impact on the immune response. **Methods:** AIR mice were treated with DMBA (50mg/kg i.p.) in olive oil. Humoral immune function was evaluated in serum level of specific IgG antibody for HGG adsorbed in SBA-15. Acute inflammatory response was evaluated 1 day after sc injection of Biogel P100. BMC were characterized by flow cytometry and cytospin preparations. Proliferation index and differentiation of BMC was determined by *in vitro* GM-CSF stimulus. The protein and gene expression were determined by Western Blot and Q-PCR analysis, respectively. DNA repair was assessed by Comet assay. **Results and Discussion:** Specific IgG anti-HGG antibody production and the cellular migration to the inflammatory site after sc Biogel injection were suppressed after treatment with DMBA in AIRmin mice. Hypocellularity was observed in BM from AIRmin mice at 1 day post DMBA treatment, mostly in the neutrophil. However there was an increase of blast cells and immature neutrophils at 1 and 50 days after treatment due to a blockade in the cell cycle progression from G1 to S phase in immature cells. These results are consistent with the high levels of p53 and p21 genes and proteins expression. Repair kinetics in BMC demonstrated the early removal of DNA lesion from DMBA-treated AIRmax mice. The *parp-1* gene showed 3 fold increased mRNA expression in DMBA-treated AIRmax mice. Low differentiation and proliferation capacity were observed in DMBA-treated AIRmin mice. Our results indicate that DMBA produced long lasting genotoxicity and cytotoxicity in BMC from AIRmin mice and blocking cycle progression of undifferentiated cells, affecting immune response development. On the other hand, AIRmax mice have a high capacity of DNA damage repair and protection. In conclusion, these mechanisms might contribute to the phenotypes of susceptibility or resistance to DMBA toxicity presented by AIRmin and AIRmax mice, respectively.

Supported by: CNPq and Capes



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4.19 Susceptibility to tumorigenesis is associated with high antibody production

Lavezo AA[1,2], Carvalho LR[1,2], Canhamero T[1,2], Aguilar-Ramirez P[1,2], Borrego A[2], Cabrera WHK[2], Jensen JR[2], De Franco M[2], Ribeiro OG[2], Ibañez OM[2], Starobinas N[2]
[1]Departamento de Imunologia, Instituto de Ciências Biomédicas – USP, Brazil; [2]Laboratório de Imunogenética, Instituto Butantan, Brazil

Introduction: Skin is an organ under intense physical and chemical stress resulting in disorders, including cancer. Applications of the procarcinogen 7,12-dimethyl benzanthracene (DMBA), a polycyclic aromatic hydrocarbon, provide a system for study skin tumorigenesis. Lines of mice genetically selected according to High (H) and Low (L) antibody production can be used to investigate how humoral immunity can affect tumor susceptibility. **Objectives:** The aim of this work was to study the influence of genetic factors relevant to antibody production on tumor development. **Methods:** Skin tumor was induced at the shaved back of mice by epicutaneous application of DMBA (50μg in 0,1mL acetone) for 5 consecutive days, and controls were treated with acetone at the same time. Skin samples had RNA isolated and cytokine gene expression was analyzed by Real Time PCR. **Results and Discussion:** After DMBA application, both strains showed an intense superficial cutaneous inflammation around 15 days, but between 30 – 60 days, L mice healed skin lesions (74%, n= 31) while H mice developed papillomas (76%, n= 33). Analyzing skin and lung, L mice were more resistant, showing few lesions and tumors. Skin tumor multiplicity increased and the incidence around 90 days post treatment was significantly higher ($p < 0,001$) in H mice (76%, n= 30) than in L mice (14%, n= 29). Lung tumors were observed in all H male animals (100%, n= 8). Gene expression of *il-1β, il-6* and *tnf-α* was carried out in skin tissue with 120 and 240 days after DMBA application, and no difference was observed between the strains. Our results suggest that genes related to high antibody production provide susceptibility for chemical tumorigenesis in this model.

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4.20 Analysis of cross-reactivity and effect of anti-PspC antibodies on binding of Factor H (FH) and secretory IgA (sIgA) to pneumococci

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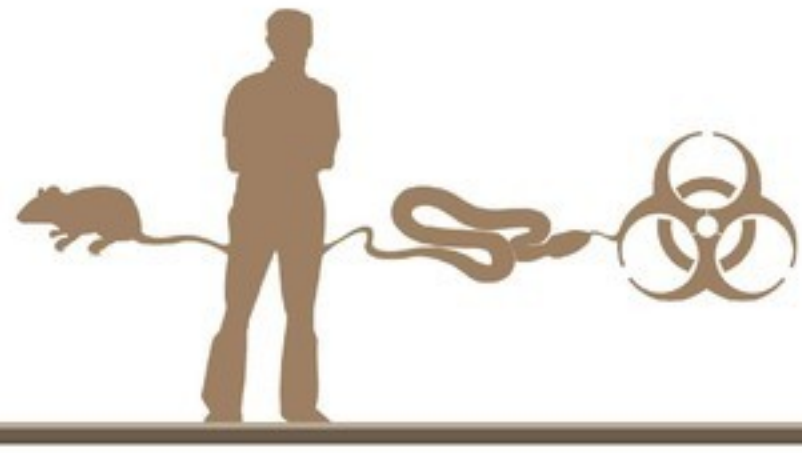
Introduction: *Streptococcus pneumoniae* colonizes the upper respiratory tract of healthy individuals, from where it can be transmitted to the community. Occasionally, bacteria invade sterile niches, causing diseases. Pneumococcal surface protein C (PspC) is a virulence factor that is important during colonization and systemic phases of the diseases. Previous studies have shown that *S. pneumoniae* is able to bind to both human Factor H (FH), an inhibitor of complement alternative pathway, and human secretory IgA (sIgA), via PspC. PspC was classified in 11 groups based in variations of the gene. **Objectives:** The proposal of the present work is to evaluate of cross-reactivity and effect of anti-PspC antibodies on binding of FH and sIgA to pneumococci. **Methods:** BALB/c mice were immunized with three different PspC molecules (PspC3, PspC5 and PspC8) for the production of antibodies that were used for Western blot and FACS analysis. **Results and Discussion:** Immunization with PspC3 induced antibodies that were able to recognize the majority of the pneumococcal isolates as analyzed by Western Blot of whole-cell extract and FACS of intact bacteria. We have also evaluated the interaction of FH and sIgA with pneumococcal extracts and the majority of the isolates tested showed a strong binding to FH and weaker interaction with sIgA through Western Blot. Inhibition of binding assays with whole-cell extract of pneumococci using anti-PspC3 IgG and anti-PspC5 IgG showed a reduction in binding with FH and sIgA. Inhibition in intact bacteria was not observed for the majority of the isolates. The protection against nasopharyngeal colonization was evaluated by nasal immunization of C57BL/6 mice with PspC3. Although a reduction in nasopharyngeal colonization was observed when immunized mice were challenged with the clinical isolate HU23F, the group immunized only with adjuvant also showed lower levels of colonization. It was not possible, therefore, to show the protection mediated by immunization with PspC in this model. Importantly, the interaction of PspC is specific for human FH and sIgA. The various murine models of pneumococcal challenge only check the ability of anti-PspC antibodies to opsonize the bacteria and to mediate subsequent phagocytosis, but do not take into account the blocking of PspC function.

Supported by: FAPESP, Fundação Butantan and CNPq



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4.21 Metabolic and Mathematical Modelling for the simulation of the kinetic behavior of recombinant S2 cells of Drosophila melanogaster based on their metabolic flexibility

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Introduction: The metabolism of the S2 cells (Schneider 2) *Drosophila melanogaster* is not yet fully known. There have been few specific studies on the metabolism of S2 cells, whether recombinant or wild (rS2), for example, transfected to express glycoprotein of rabies virus (RVGP). As the genome of *Drosophila melanogaster* have been mapped, the key enzymes that act on the metabolic processes in general have been identified and are available in the KEGG (Kyoto Encyclopedia of Genes and Genomes). Thus, KEGG presents all possible pathways with the enzymes that can be encoded. **Objectives:** It was proposed a metabolic model based on a set of metabolic pathways and were found characteristic elementary modes of the system through the Metatoolprogram. **Methods:** The mathematical model was defined by addressing these elementary modes. **Results and Discussion:** This process was repeated until a set of metabolic pathways, by mathematical modelling, consistently responded to a set of ten experiments (in various conditions). We came to a basic metabolism for rS2 containing 33 pathways comprising glycolysis, pentose, Krebs cycle and oxidative phosphorylation. Previous data indicate that rS2 is a cell with high metabolic flexibility, which was confirmed by some reactions in the process proposed as reverse breakdown and synthesis of glutamine. The proposed metabolism resulted in 37 elementary modes. Another interesting factor was the use of the production of purines and pyrimidines for the estimation of cell growth. After the modelling performed, the tests were simulated using a Simulator of the Kinetic behavior of rS2 cells (developed in MATLAB), also used for simulation of other experiments with different initial conditions and methods of cultivation, coming to a general adjustment of experimental and simulated values with R^2 in the order of 0.98

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4.22 Adaptation of cells HEK 293T and Huh 7 in differents medium free of fetal bovine serum for the study of gene expression using viral pseudoparticles

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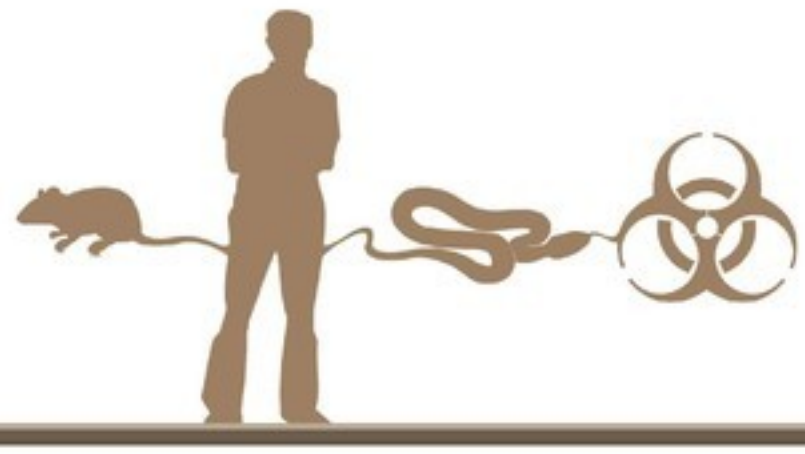
Introduction: The fetal bovine serum (FBS) is a component of higher value added to the medium, which may cause difficulty in the process of recovery and purification of bioproduct. The adaptation of cellular lineages to culture FBSor animal protein free medium can allow optimization of cell growth and expression of heterologous genes, facilitating recovery of recombinant proteins expressed in these cells. These parameters will be evaluated by using an expression system derived from MLV and HCV pseudoparticles (ppHCV) transfected / infected cells grown in serum free medium (SFM). **Objectives:** To improve expression of heterologous proteins using SFM to optimization of transfection of genetic material and subsequently purifying the protein or recombinant virus produced. **Methods:** Adaptation ofHEK 293T and Huh 7.0 cells in four SFM, Pro293TMa, VP-SFM, SFM-Hybridoma, CHO-S-SFM II. **Results and Discussion:** We established protocols to adapt HEK293T and Huh7 to growing cells in SFM. By changing gradually of medium, we obtained two lines adapted to four SFM. The Huh 7.0 cells showed morphology and growth better than HEK293T cells.

Supported by: Fapesp Capes and CNPq



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4.23 Influence of SLC11A1 *R* and *S* alleles on DMBA-Induced skin cancer susceptibility

Perlati-Santos M[1], Cabrera WHK[1], Canhamero TG[1], Borrego A[1], Jensen[1] JR, Ribeiro OG[1], Starobinas N[1], Ibañez OCM[1], De Franco M[1].
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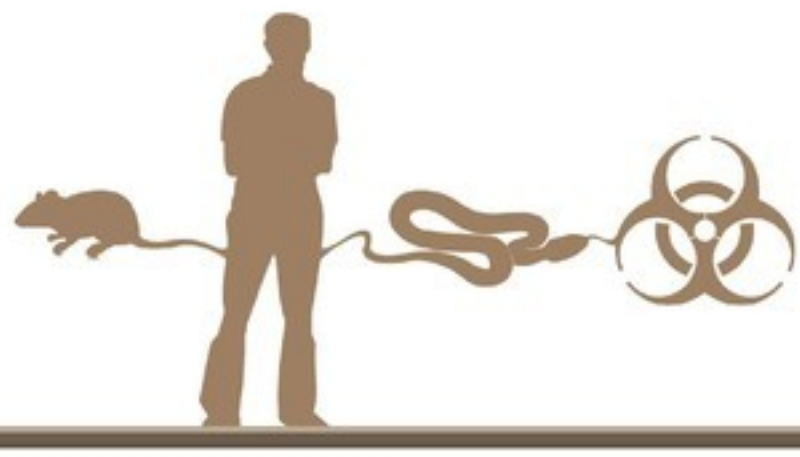
Introduction: Mice selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response differ on susceptibility to carcinogenesis. AIRmin mice are significantly more susceptible to skin carcinogenesis than AIRmax mice due to the genetic background and the polymorphism of aryl hydrocarbon receptor (Ahr) gene. The *Slc11a1* gene (formerly *Nramp1*) polymorphism modulates macrophage activity and the susceptibility to infections and autoimmune diseases. To study the interaction of resistant (R) or susceptible (S) *Slc11a1* alleles with acute inflammatory reaction loci found in AIRmax and AIRmin mice, homozygous sublines for these alleles were produced. These mice were nominated AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS}. **Objective**: The objective of this study was to investigate the skin carcinogenesis induced by DMBA agent in AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS}. **Methodology:** To induce carcinogenesis, 50μg DMBA diluted on 0.1mL acetone were applied epicutaneously to the shaved dorsal skin of mice for five consecutive days. Gene expression analysis of several inflammatory cytokines was made by Real Time PCR. **Results and discussion:** The incidence of skin cancer was 7% in AIRmin^{RR} mice and 13% in AIRmin^{SS} mice, and the incidence of internal organ tumors was similar between these sublines (about 57%). AIRmax^{RR} and AIRmax^{SS} mice did not show skin cancer, but the incidence of internal organs cancer was 100% only in AIRmax^{SS} mice. Expression of several inflammatory cytokines was detected in control and experimental mouse skins 48 hours after the last DMBA application. All control mice had a basal expression of *Il6, Tnfa, Il1b* and *Cxcl2*. In DMBA treated AIRmin^{RR} mice, mRNA levels of these cytokines were increased 9-, 19-, 30-and 215-fold respectively. In AIRmin^{SS}, these mediators were increased 11-, 4-, 6- and 75-fold in relation to their controls. AIRmin^{SS} differ significantly from the other sublines in some cytokines, but AIRmin^{RR} presented the highest number of differentially expressed genes ($P<0.001$) after DMBA application. AIRmax^{SS} showed a high expression of *Ccl2* and *Cxcl2* in the spleen. **Conclusions:** These data showed higher susceptibility to skin carcinogenesis in AIRmin^{RR} than in AIRmin^{SS} mice. The AIRmax^{RR} animals were resistant to treatment. However, AIRmax^{SS} animals showed higher susceptibility, suggesting that the *S* allele in the AIRmax background could influence susceptibility to cancer.

Supported by: Fapesp and CNPq.



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4.24 Role of *Slc11a1* gene in macrophage activation during inflammatory response

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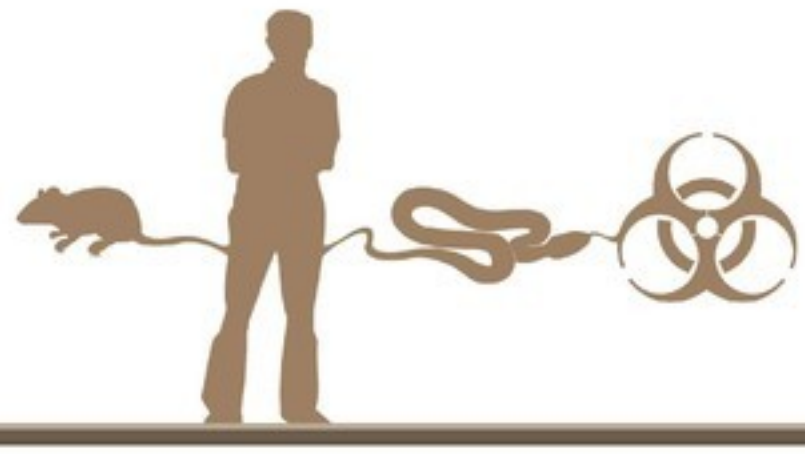
Introduction: The *Slc11a1* gene regulates resistance against *S. enterica* Typhimurium, *L. donovani* and *M. tuberculosis*. AIRmax and AIRmin mouse sublines, homozygous for *Slc11a1* R and S alleles ($AIRmax^{RR}$, $AIRmax^{SS}$, $AIRmin^{RR}$ and $AIRmin^{SS}$) were produced in our Laboratory to study the effect of this gene in the inflammation background. **Objective**: the aim of this work is to evaluate the effect of this gene in peritoneal macrophage (MΦ) activation induced by thioglycollate (TIO) or *M. bovis* BCG infection. **Methods**: Mice were inoculated ip with PBS (control), TIO (96h) or BCG (14 days). The peritoneal cells were collected and placed in culture, the adherent Mϕ stimulated or not with LPS were used in all experiments. Spleens were homogenized and cultured in MB7H10 medium and the bacterial colonies were counted. Production of NO, H_2O_2 and cytokine were determined in culture supernatants. **Results and Discussion**: TIO induced weak cellular activation, with reduced migration, basal synthesis of hydrogen peroxide (H_2O_2), nitric oxide (NO) and cytokines in all sublines tested. After *in vitro* LPS stimulation, $AIRmax^{RR}$ and $AIRmax^{SS}$ macrophages produced higher amounts of NO, IL-1β, IL-12 and TNF-α than those of AIRmin sublines. IL-10 was preferably secreted by AIRmin MΦ, independent of *Slc11a1* alleles. These results indicate that inflammation and MΦ activation induced by TIO were dependent on the genetic background for acute inflammatory response, while *Slc11a1* alleles have little effect on this phenotype. In BCG infection only $AIRmax^{RR}$ mice were capable of controlling bacterial proliferation, which was accompanied with high levels of IL-1β, IL-12, TNF and IL-6 produced by activated macrophages. On the other hand, susceptible $AIRmin^{SS}$ mice produced higher amounts of NO, H_2O_2 and IL-10 suggesting that both inflammatory background and *Slc11a1* alleles interfere on resistance to BCG infection. Thus, we conclude that the high inflammatory response associated to *Slc11a1* R alleles (in $AIRmax^{RR}$ mice) are mechanisms for efficient bacterial killing, through production of cytokines by MΦ which activates the immune response.

Supported by: CAPES, CNPq, FAPESP



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4.25 Recombinant BCG-LTA-K63 induced a Th1 immune response in mice

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Introduction: *Mycobacterium bovis* BCG (Bacille Calmette-Guérin) has been widely used as a live anti-tuberculosis vaccine, administered to over 3 billion individuals with a very good safety record, despite showing highly variable efficacy (0-80%) in different trials. The efficacy of BCG in adults is poor in tropical and subtropical regions. BCG is considered a high potential candidate for the presentation of heterologous antigens in the development of new vaccines, since recombinant BCG (rBCG) based vaccines would carry many of the advantages of BCG. The induction of long lasting cellular and/or humoral immune responses with one dose, which can be administered at birth, could greatly increase vaccine coverage. Adjuvant properties were described for the heat-labile toxin (LT) from *Escherichia coli*. The LT is composed of two subunits, a monomeric enzymatically active A and nontoxic B subunit. **Objectives:** the aim of this work was analyzes the cellular immune response induced in BALB/c mice immunized by rBCG expressing the mutant of nontoxic subunit A (rBCG-LTA). **Methods:** BALB/c mice were immunized subcutaneously with 10^6 cfu of BCG, rBCG-S1PT or rBCG-LTA. After 60 days, the spleen was removed and the cells were restimulated in culture with PDS. After incubation, the cells were collected for intracellular cytokine staining with FITC-, PE-, or PE-Cy7-conjugated monoclonal antibodies (mAbs) against: CD4, CD8a, IFNγ, IL-4, IL-17, TNF-α and IL-2. The cytokine secretion was also evaluated in the supernatant of spleen cell culture. **Results and Discussion:** A proportion of CD4 T cells produced IFN-γ and TNF-α (47.13% and 34.6%, respectively) was significantly higher in spleen cells of mice immunized with rBCG-LTA than in BCG (21.4% and 18.9%), rBCG-S1PT (17% and 15%) or saline controls (13% and 8.4). IFN-γ production was significantly higher in rBCG-LTA cells (1231+/- 119.8 pg/mL) when compared with the other groups; BCG (862.2+/- 258.5 pg/mL), rBCG-S1PT (1084.3+/- 92.2 pg/mL). IL-17, IL-2 showed a similar concentration in immunized and control groups and IL-4 was not detected. We demonstrated that immunization with rBCG-LTA can induce a Th1 response characterized by increased production of IFN-γ and TNF-α and these responses are significantly higher than BCG and rBCG-S1PT.

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4.26 Protection against *Schistosoma mansoni* induced by immunization with tegument nucleotidases associated with a subcurative chemotherapy dose

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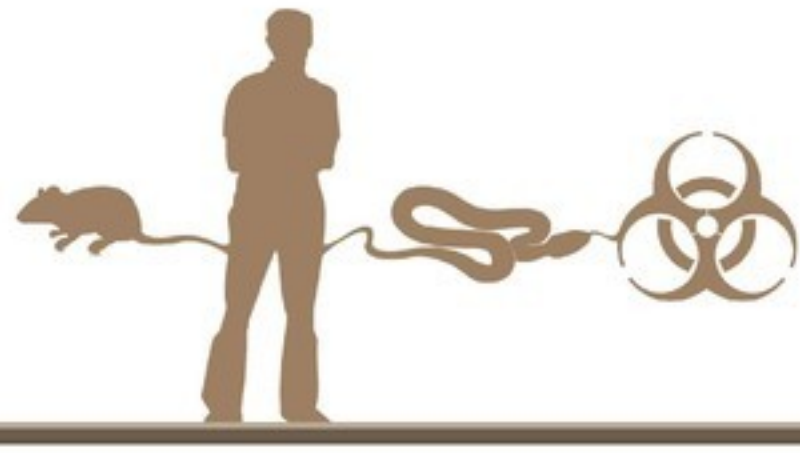
Introduction: Morbidity, transmission and infection control are different targets towards the elimination of schistosomiasis, some of which chemotherapy are not effective. An effective vaccine could help to eradicate this disease. The schistosoma's tegument is the main host-parasite interface and a potential source of antigens: three ectonucleotidases, acting on the metabolism of nucleotides, have been identified by proteomics: an alkaline phosphatase (AP), a phosphodiesterase (PhoD) and an apyrase (APY). These are proteins associated to the plasma membrane with their catalytic site directed to the extracellular area. **Objectives:** We assessed whether mice immunized with these recombinant proteins, associated or not with a subcurative praziquantel (PZQ) treatment, are able to elicit a protective immune response against infection with *S. mansoni.* **Methods:** Mice were immunized subcutaneously with 3 doses in 15-day intervals of the recombinant proteins with Freund's adjuvant. Spleen cells were collected 15 d after the last immunization to assess signaling genes and cytokines expression by real time RT-PCR. For the protection assay, mice were challenged 15 d after the last dose with infective cercariae. In the subcurative PZQ protocol, the animals were treated with a subcurative dose of PZQ (150 mg/Kg) 35 d after the challenge. Blood was collected before and after infection to measure total IgG levels. Worm burden and parasite fecundity were evaluated 45 d post infection in both protocols. **Results and Discussion:** There were no differences in the relative expression levels of Myd88, NF-κB1 and 2, IFN-γ, IL-4, IL-5, IL-10, IL-12p40 or IL-13 between the treated and control groups. The splenocytes of AP-immunized mice expressed ~8 times more TNF-α, while the group immunized with PhoD expressed ~8 times more TGF-β. Both groups expressed more IL-17 when compared to the APY-immunized mice. All animals presented higher levels of IgG and only PhoD-immunized animals reduced their IgG levels after infection. PZQ treatment did not interfere in the IgG levels. Mice immunized only with the recombinant proteins did not present reduction in worm burden or parasite fecundity. However, when animals received a subcurative dose of PZQ, AP-immunized animals reduced their worm burden by ~40%. Immunization with the combined recombinant proteins did not alter their independent immune response profile, and reduced the worm burden to a similar extent as AP-immunized animals. The sub curative chemotherapy treatment may expose the AP protein in such a way as to allow the immune response to effectively attack the parasite.

Supported by: FAPESP



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4.27 Outer Membrane Proteins Cross-Reativity from two *Neisseria* species

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Introduction: *Neisseria spp* is a genus of diplococcus Gram-negative bacteria. Most of species prefer to inhabit distinct human mucosal niches and are categorized as commensals. However, *Neisseria meningitidis* can causes septicemia or meningitis especially in young infants. *Neisseria lactamica* shares surface antigens with *Neisseria meningitidis*. Vaccines to prevent meningococcal disease have been developed from outer membrane vesicles (OMV) from *N. meningitidis* B. It is actually postulated that colonization by *N. lactamica*, contributes to the natural development of immunity against *N. meningitidis*. **Objectives:** The aim of this study is to purify OMV from both species of *Neisseria*; to obtain sera from mice immunized with OMV prepared from *N. lactamica* and OMV from *N. meningitidis*; to analyze the possibility of cross-reactivity. **Methods:** *N. meningitidis* was cultivated in shaker, at 250 rpm, 36°C by 6 hours in modified Catlin medium MC2LAA. *N. lactamica* was cultivated in 5L bioreactors at 36°C, 0.2 bar, overlay air flow rate of 1L/min, 250 to 850 rpm and 10% oxygen saturation for 12 hours in MC2LAA plus yeast extract. OMV were purified by centrifugation followed by ultracentrifugation, yield was determined by Lowry's method. Groups of 10 female swiss mice, 3 weeks old, were immunized with 3 dose contained 2 μg of OMV from *N. lactamica* or *N. meningitidis* in 0.1 mL of 0.85% saline solution by subcutaneous injection. Doses of vaccine were administered on days 0, 14, and 21. On the seventh days after the last immunization retro-orbital bleedings were performed. OMV from *N. meningitidis* were transferred to nitrocellulose membrane after SDS-PAGE. Antibodies were evaluated by western blot. **Results and Discussion:** The sera were reactive with OMVs of each kind and also with one another, although the proteins recognized to be different in every situation. These data indicate cross-reactivity between species.

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4.28 Mast cell (mc) degranulation induced by mt-i, a phospholipase a_2 isolated from *bothrops asper* snake venom. Involviment of kinases and intracellular phospholipases in this effect

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Introduction: Mast cells are central elements of innate immune response. Upon stimulation these cells release a vast array of inflammatory mediators through degranulation process. Several snake venom phospholipases A_2 (PLA_2) have been shown to induce *in vitro* MCs degranulation. However, the mechanisms involved in this effect are still unknown. **Objectives:** This study aimed to evaluate the ability of MT-I, a GIIA-PLA_2 isolated from *Bothrops asper* snake venom to degranulate MCs and the involvement of both kinases (p38MAPK, PI3K and ERK1/2) and intracellular phospholipases (calcium-independent and citosolic PLA2, PLC and PLD) in this effect. **Methods:** RBL-2H3 mast cell lineage was used. Citotoxicity of MT-I (0.7 – 15 μM) on RBL-2H3 cells ($6x10^4$) was evaluated by MTT and LDH assays after selected periods of incubation (30 min - 2 h). MC degranulation was determined by measuring β-hexosaminidase release. Involvement of kinase and intracellular phospholipases in MT-I-induced MCs degranulation was evaluated by pharmacological interferences with specific inhibitors. **Results and Discussion:** MT-I was cytotoxic to MCs at concentrations higher than 8.8 μM. Stimulation of MCs with MT-I (3.5 μM) resulted in increased MC degranulation by 62.6%, 103% and 95% at 0.5, 1 and 2 h, respectively, in comparison with control MCs (3.96 ± 0.53%, 3.72 ± 0.3% and 3.60 ± 0.24% at 0.5, 1 and 2 h, respectively). Pre-treatment of cells with PD98059 or SB202190, inhibitors of ERK1/2 and p38MAPK, respectively, did not affect MT-I-induced MC degranulation. However, pre-treatment with wortmannin, inhibitor of PI3K, significantly reduced MT-I-induced MC degranulation by 53.7% at 30 min as compared with controls. Inhibition of iPLA2 by Bel compound did not modify degranulation induced by MT-I, but inhibition of cPLA2, PLC or PLD by treating cells with compounds Pyr-2 or U-73122 or FIPI, respectively, reduced MT-I-induced MC degranulation (29%, 25.2% and 25.4%, respectively).: MT-I is able to induce a rapid and sustained degranulation of MCs *in vitro*. This effect is dependent on PI3K, iPLA2, PLC and PLD, but not ERK1/2, p38MAPK and iPLA2.

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4.29 SBA-15 Silica: How does this adjuvant works?

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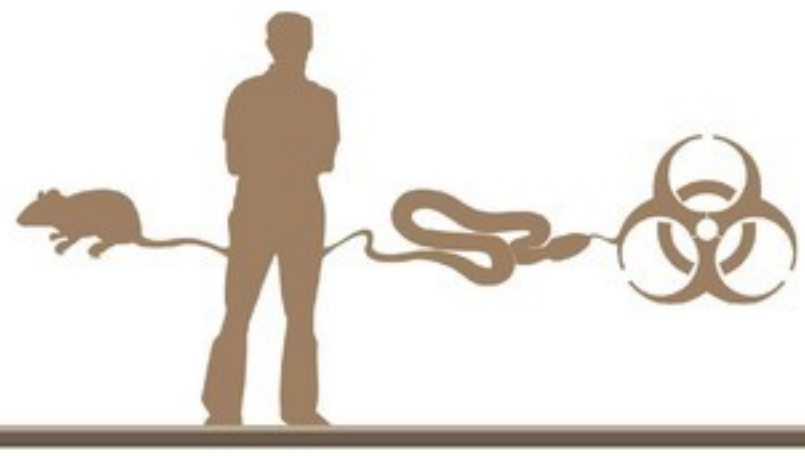
Introduction: Amorphous silicon oxide particles formed of hexagonally ordered nanopores known as SBA-15 are promising adjuvant vectors. **Objectives:** The aim of this study is to explore how silica might act in promoting immune responses. **Methods:** To explore the ability of SBA-15 to activate DC, bone marrow derived DC from C57BL/6 mice were pulsed for 6 hours with different doses (50, 250, 500, 1000 and 5000 μg/mL) of the particles and the expression of MHCII, CD40, CD80 and CD86 and IL-6 levels were assessed by flow cytometry. The capacity of SBA-15 loaded DC to present OVA and OVA peptides to $CD4^+$ and $CD8^+$ T cells was determined by T cell proliferation and γIFN production from co-cultured OT2 and OT1 TcR transgenic cells *in vitro*. T cell responses *in vivo* were analysed by transferring CFSE labelled $CD8^+$ OT1 or $CD4^+$ OT2 T cells to C57BL/6 mice. One day after transfers, the recipients were immunised footpad (s.c.) with 10 μg OVA or 10 μg OVA: 250 μg SBA-15 and the clonal expansion of donor cells was assessed 4 days later in the popliteal lymph node by flow cytometry. OVA-specific CTL activity in SBA-15 immunised mice was monitored by the killing of transferred CFSE labelled OVA peptide loaded spleen cells. To confirm the adjuvant effect *in vivo*, C57BL/6 mice were immunised footpad (s.c.) with 10 μg OVA or 10 μg OVA: 250 μg SBA-15 on 2 occasions 30 days apart and serum IgG specific antibody titers were measured by ELISA. **Results and Discussion:** DC activation experiments showed no upregulation of costimulatory molecules and no effect on IL-6 production after silica treatment. SBA-15 did not alter the ability of DC to induce proliferation and γIFN production by TcR transgenic $CD4^+$ and $CD8^+$ T cells after presentation of ovalbumin (OVA) or OVA peptides *in vitro*.There were no significant differences in the T cell response when SBA-15 was used as adjuvant and SBA-15 also did not allow OVA to induce specific cytotoxic T lymphocyte activity *in vivo*. It was shown a significant increase in specific IgG antibody titers, especially after booster, in SBA-15 immunised mice. These preliminary data confirm that SBA-15 acts as an adjuvant for antibody responses and suggest that its effects may reflect enhanced availability of antigen, rather than direct effects on antigen presenting cells such as DC.

Supported by: CNPq, FAPESP, INCTTOX Program and Cristália Pharmaceutics. This research is under the scope of the Patents WO 07030901, IN248654, ZA2008/02277, KR 1089400 andMX297263



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4.30 Characterization of mice immune response elicited by three novel adhesins of *Leptospira interrogans*

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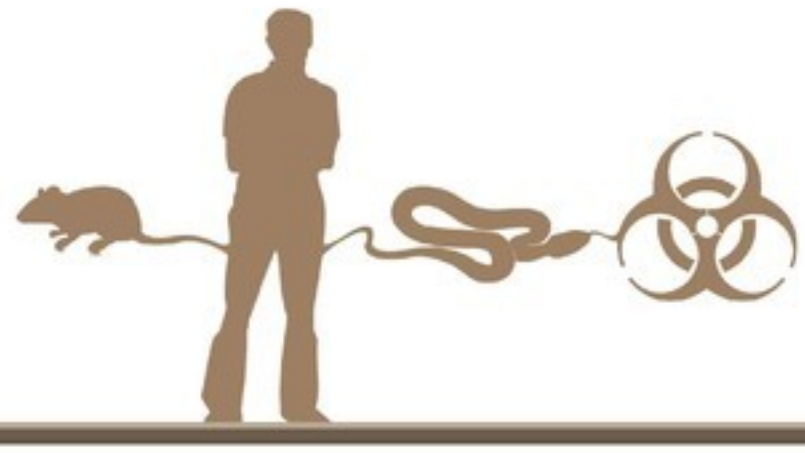
Introduction: Leptospirosis is an emerging infectious disease caused by pathogenic *Leptospira*. The strategy for disease control involves prophylactic measures that have been hampered by the lack of protective conserved antigens. Since the sequencing of leptospiral genomes, putative membrane proteins conserved among *Leptospira* strains have been searched for the development of vaccines. The immune response characterization of antigen candidates is an important step in the investigation of subunits that may confer protection. **Objectives:** Evaluation of the immunogenic activity elicited in mice by predicted membrane proteins of *Leptospira interrogans* expressed in *Escherichia coli.* **Methods:** LIC11087, LIC11228 and LIC11084 genes encode for predicted hypothetical outer membrane proteins identified in the genome of *Leptospira interrogans* sv. Copenhageni and therefore were chosen for our studies. The genes were cloned into pAE expression vector. The recombinant proteins expression was performed in *E. coli* BL21 SI or BL21 DE3 strains. The proteins were purified by metal chelating chromatography. The interaction of the purified proteins with extracellular matrix or serum components was analyzed by ELISA. Mice were immunized with the recombinant proteins, and the immune response was characterized as antibodies titers, lymphocyte proliferation and cytokine measurements. **Results and Discussion:** rLIC11087, rLIC11228 and rLIC11084 were successfully purified. The three proteins were characterized as leptospiral adhesins. All proteins were able to interact with laminin and plasminogen. Additionally, rLIC11087 binds to plasma fibronectin and C4BP, and rLIC11228 interacts with plasma fibronectin. The proteins stimulated mice immune system as titers of antibodies reached 1:400,000 for the antigens rLIC11087 and rLIC11084, and 1:100,000 for rLIC11228. Moreover, statistically significant proliferation of lymphocytes was obtained with rLIC11084 after *in vitro* stimulation of spleen cells from immunized mice. In addition, rLIC11084 was able to elicit the production of IFN-γ cytokines. The results suggest that the proteins rLIC11087, rLIC11228 and rLIC11084 are novel leptospiral adhesins and plasminogen binding receptors that may be involved in pathogenesis. Additionally, rLIC11084 constitutes a potential vaccine candidate, as was capable of inducing humoral and cellular immune responses.

Supported by: FAPESP, CNPq and Fundação Butantan



4.31 *In vitro* activation of the complement system by horse antivenoms

Squaiella-Baptistão CC[1], Marcelino JR[2], Cunha LER[3], Gutiérrez JM[4], Tambourgi DV[1]
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Introduction: Envenomation by poisonous animals is a tropical neglected disease according to the World Health Organization. The only effective treatment is the antivenom therapy. Antivenoms are in the WHO List of Essential Medicines and should be part of any primary health care package where snakebite accidents occur. Intact heterologous antibodies can activate the complement system (C) via Fc, leading to early adverse reactions, which justify the production of F(ab')2 antivenoms. However, studies have indicated that these fragments can also activate C, leading to such reactions. **Objectives:** Herein, we aimed to evaluate the anticomplementary activity of antivenoms (horse antibodies against snakes, spiders, scorpions or caterpillar venoms) produced by Butantan, Vital Brazil and ClodomiroPicado Institutes. These antivenoms have different composition, with the presence or not of Fc portion, and different protein contents. **Methods:** Samples of the antivenoms were incubated with normal human serum and the activation of the three C-pathways was analyzed. Antivenoms were also analyzed by SDS-PAGE and Western Blot, to determine their protein profiles and the possible correlation with C-activation.**Results and Discussion:** Only the classical pathway was activated by some antivenoms from the three Institutes, indicating that anticomplementary activity is not only associated with the presence of the IgG-Fc portion, since F(ab')2 antivenoms also activated the classical pathway. The C-activation/consumption was positively correlated with the amount of heterologous protein only for the antivenom from Vital Brazil Institute. SDS-PAGE showed the presence of several contaminant proteins in most antivenoms, and protein aggregates were detected in those from Clodomiro Picado Institute. Western Blot analysis showed that some antivenoms from Butantan Institute contained some intact IgG heavy chains, indicating that the cleavage of the antibodies for the production of F(ab')2 fragments was not complete. Our results suggest that several factors, such as composition, contaminant proteins and aggregates, are contributing for the C-activation/consumption by the antivenoms.

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4.32 Effect of ischemia and reperfusion on changes in leukocyte-endothelium interactions in microcirculation in mice genetically selected for Acute Inflammatory Response

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Introduction: Ischemia and reperfusion (I/R) is often associated with microvascular dysfunction characterized by dilatation in arterioles, trafficking of leucocytes and protein extravasation in post capillary venules. In early study we evaluated the inflammatory response at 4 hours of reperfusion in mice selected for maximal (AIRmax) or minimal (AIRmin) Acute Inflammatory Response (AIR) obtaining a higher inflammatory infiltrate in I/R AIRmax. **Objectives:** The aim of this study was to evaluate the leucocytes-endothelium alterations in AIRmax and AIRmin mice after I/R. **Methods:** The mice were subjected to superior mesenteric artery ischemia for 45 minutes and 1 or 4 hours of reperfusion. Control groups were sham operated and basal (not manipulated). The local intravital microscopy was measured in mesenteric venules after 1h. At four hours of reperfusion we evaluated the proteomic expression in injured remote organ (lung) by 2D-PAGE and sequencing in electrospray. **Results and Discussion:** We observed in mesenteric intravital microscopy on homeostatic condition (basal) a significant rolling in the AIRmin mice ($p<0.05$) when comparing with AIRmax and it remains in the I/R group at 1h. Unlikely the adherence level in I/R AIRmax was significantly higher ($p<0.001$) than all other groups at 1h of reperfusion. Corroborating with these results the AIRmax and AIRmin lung proteomics expression at 4 h of reperfusion identified qualitative and quantitative differences concerning Profilin-1, Cofilin-1, Tropomyosin beta chain, S100A9 and Peroxiredoxin-6, proteins. These proteins are related to a wide range of functions such as cell adhesion, migration and metabolism regulation. Our results suggest that the exacerbate infiltrate inflammatory observed in AIRmax mice after I/R induction may be regulated by differential protein expression involved in migration and metabolism regulation increasing the leucocyte-endothelium adhesion and migration in these lines.

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4.33 Study of dengue virus replication in Vero cells

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Introduction: Dengue virus is a Flaviviririus that present four serotypes 1, 2, 3 and 4 and can cause an important viral disease in humans. There are approximately 100 million cases and 25,000 deaths annually in the world. Dengue virus cultivated in Vero cell is used to dengue vaccine production at Instituto Butantan. **Objectives:** The objective of this study was to evaluate the dengue virus replication in Vero cell cultures infected with DEN1, DEN2, DEN3 and DEN4. **Methods:** Vero cells maintained in 225cm2 T-flasks with serum-free medium for cell culture (OptiPro- Invitrogem) were infected with DEN1, DEN2, DEN3 and DEN4 dengue virus strains from NIH (National Institutes of Health). The values of MOI (Multiplicity of Infection) used were 0.01 for dengue virus 1, 2 and 4 and of 0.05 for DEN 3. Infected cultures were incubated at 37ºC during 14 days and samples of the supernatants were taken every day to determine the virus titers by PFA test (Plate Forming Assay). The results obtained in this test were expressed in PFU/ml. **Results and Discussion:** The virus titers found in the samples obtained of the cultures infected during 14 days were Log10 2.2 to 7.4, 2.2 to 7.1, 3.0 to 6.8 and 3.8 to 7.1 PFU/ml for DEN1, DEN2, DEN3 and DEN4 respectively. The higher virus titers (viral pick) obtained for DEN1 (7.1 to 7.4 PFU/ml), DEN2 (6.8 to7.1 PFU/ml) and DEN4 (6.6 to 7.1 PFU/ml) were after eight to eleven days of the cellular infection. For DEN3 (6.0 -6.8 PFU/ml) this occurred between ten to fourteen days. The results showed that the virus replication pick of each serotype occurred in different times. These data are very important to determine the optimal days to harvest of dengue suspensions in virus dengue vaccine production.

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4.34 Modulatory effect of ordered mesoporous silica SBA-15 on LPS response of mice genetically selected for high inflammation (AIRmax) bearing *Slc11a1*$^{R/S}$ alleles

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Introduction: Ordered mesoporous silica SBA-15 is an inorganic substance able to interact with atoms, ions and molecules by incorporating them into their nanopores. The adjuvant property of SBA-15 has been studied, evidencing that for various antigens this nanostructure increases the production of antibodies. SBA-15 also reduced the toxicity of diphtheria toxin when used for horse immunization, however, it is unknown if this silica property is also applicable to other toxins. Mice genetically selected for high inflammatory response (AIRmax) bearing *Slc11a1*$^{R/R}$ or *Slc11a1*$^{S/S}$ alleles are susceptible to endotoxic shock induced by LPS treatment. Thus, the AIRmax *Slc11a1*$^{R/S}$ may be considered a good model for the study of the potential modulatory effect of SBA-15 on LPS response and toxicity. **Objectives:** The aim of this work was to evaluate a potential modulatory effect of SBA-15 on LPS toxicity and to determine the adjuvant outcome on the anti-LPS antibody production. **Methods:** Groups of 4-5 AIRmax *Slc11a1*$^{R/S}$ mice were intravenously injected with 25µg, 50µg or 75µg of *Salmonella* Typhimurium LPS, adsorbed or not to SBA-15 at a 1:10 ratio, and mortality was recorded for 72h after injection. Surviving mice were bled by the retro-orbital plexus and the serum IgG and IgM titers were determined by ELISA. **Results and Discussion:** All mice receiving LPS at the three doses survived, while those injected with LPS in SBA-15 presented 50% and 40% death rates at 75µg and 50µg of LPS, respectively. Mice treated with LPS showed specific IgM titers 4 times higher than the controls, irrespectively of the LPS dose. Regarding the specific IgG production, mice receiving LPS only showed titers 5 times higher than controls, while in the LPS-SBA-15 groups the difference was about 7-fold. The IgM responses showed high variance, which were lower in the LPS-SBA-15 groups. Considering the concentrations of LPS, these results suggested that SBA-15 potentiates the effect of LPS in endotoxic shock; conversely, it seems clear an adjuvant effect on the anti-LPS IgG antibody production.

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4.35 Evaluation of Immunodominant and Potencially Protective Epitopes of Pneumococcal Surface Protein A (PspA) Variants by "Spot Synthesis" Technique

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Introduction: PspA (Pneumococcal surface protein A) is a promising candidate antigen for the development of protein vaccines against pneumococcal disease. **Objectives:** This work aims at identifying the linear epitopes recognized by polyclonal serum of mice immunized with PspA variants, notably PspA4 and PspA5, which have higher cross-reactivity with pneumococci expressing different PspAs. Mature PspA is composed of an N-terminal variable region, followed by a proline-rich region (PRR) and a repeat region for attachment to the bacterial surface. **Methods:** Female BALB/c mice were subcutaneously immunized with the recombinant proteins (PspA clades 1 to 5, from the mature N-terminal region till the PRR) adjuvanted with alum and serum antibodies were used to incubate slides (Celluspots - Intavis) containing 15mer peptides covering the complete sequence of all PspA variants. **Results and Discussion:** All sera recognized more peptides within their homologous sequence, having a large number of epitopes recognized at the final third part of the N-terminal region. All sera (except anti-PspA2 serum) recognized similar epitopes at the beginning of the N-terminal portion of all PspAs. The anti-PspA3 serum had the greatest number of recognized peptides, while anti-PspA2 had the lowest number. The peptides recognized by the anti-PspA3 serum were mostly exclusive to PspA3. The anti-PspA1, anti-PspA4 and anti-PspA5 sera could recognize several epitopes in the sequences of the different variants. The initial portion of the N-terminal region has a large number of peptides recognized by all sera on all PspAs, whereas the final third region displays more peptides that are exclusive to each PspA variant and are recognized only by the homologous serum. The sera that had previously been shown to have the lowest cross-reactivity with protein extracts of pneumococci show either a low number of recognized peptides (anti-PspA2) or recognize peptides present exclusively in the homologous sequence (anti-PspA3). Though anti-PspA4 and anti-PspA5 sera could recognize peptides in all PspA variants, the basis for their broad reactivity with different pneumococci has to be further evaluated.

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4.36 Immunomagnetic enrichment of Drosophila S2 cells expressing the rabies virus glycoprotein

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Introduction: A recombinant rabies virus glycoprotein (RVGP) produced in insect cells has been expressed, purified and characterized. Aiming to increase the protein expression, an alternative to approach immunomagnetic cell population enrichment can be used, in order to select the higher RVGP expressing cells. **Objectives:** To separate higher RVGP expressing cells by positive immunomagnetic selection. **Methods:** *Drosophila melanogaster* Schneider 2 cells (S2) were transfected with a plasmid containing the RVGP gene under the control of an inducible promoter and first selected by hygromycin resistance. After cell growth and culture induction, cells (S2MtRVGP-His1) were rinsed with PBS, EDTA and BSA (for blocking receptors and avoid unspecific binding). Cell suspension was then incubated with anti-RVGP mouse monoclonal antibody and further incubated with magnetic microbeads associated with anti-mouse rabbit antibody. Magnetic labeled cells were applied to a column placed in a magnetic field. Cells labeled with microbeads were retained on the column while unlabeled cells passed through. The magnetic field was removed and retained cells were eluted, obtaining a RVGP enriched cell line (S2MtRVGP-His2). **Results and Discussion:** In preliminary assays, the S2MtRVGP-His2 cell line showed 20% higher RVGP expression than S2MtRVGP-His1 (0.57 and 0.47 $\mu g/10^7$cell, respectively). This result is associated with the elimination of unlabeled cells, which did not express RVGP at all, and the elimination of cells that expressed low levels of RVGP, for bearing low RVGP cDNA copy number or by the placement of this cDNA in a *locus* with low expression. The immunomagnetic enrichment system constitutes a very promising approach for increasing the specific production of S2 cell lines.

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