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OBESITY MODEL INDUCED BY MONOSODIUM GLUTAMATE IN FEMALE BALB/c MICE

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Introduction: Obesity has reached epidemic proportions worldwide. Animal models are crucial for studies on the pathogenesis and therapy of this complex metabolic disorder. Monosodium glutamate (MSG) neonatal administration in mice provides a model of obesity through ablating cells in the arcuate nucleus of the hypothalamus and destroying circumventricular neurons, reducing cellular survival, altering the production of orexigenic and anorexigenic molecules, leading to neurochemical, endocrine and metabolic abnormalities, resulting in obesity. The strain BALB/c is widely used to study experimental allergy models however is known to be resistant to high-fat diet-induced obesity. Standardize the obesity model induced by MSG in the BALB/c mice strain was the aim of this study.
Methods and Results: To induce obesity in BALB/c mice, newborns received, for five consecutive days, subcutaneous injections of 4mg/g body weight of MSG. Control group received injections of saline solution 0.9% (mg/g body weight). For estimation of body mass, mice were weighed and measured weekly between weeks 5 to 12 of age. The lengths of the mice were measured from nose to anus using calipers; the weights were recorded and Lee index was calculated by dividing the cube root of body weight (g), by the nose-to-anus length (cm). For analyze the body fat the histology of adipose tissue in the 12th week was evaluated. The perigonadal adipose tissues were dissected and fixed in buffered 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.
Results: Obese BALB/c female mice did not show a significant increase in their body weight, but exhibited shorter body length when compared to lean control. However an increase in fat weight was observed with an excessive accumulation of gonadal fat at 12 weeks age (700%). The calculated Lee index was increased in obese group (339.29) in comparison to control group (317.47). The histological analysis of perigonadal adipose tissue of obese mice showed increased adipocytes.
Conclusions: Our results indicate that early administration of MSG induced important increases of fat to body weight ratio in female BALB/c mice being a good model to study obesity associated to other diseases.

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M. leprae Hsp65 K409A pep CHANGES CYTOKINE PATTERN AND THE MICROENVIRONMENT IN LUNGS OF [NZBxNZW]F1 LUPUS MICE

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Introduction and aim: The role of Hsp60, a highly conserved family of intracellular proteins, in chronic inflammatory processes such as autoimmune diseases is well documented. We have previously shown that wild type [WT] M. leprae Hsp65, and its related peptide leader pep increased disease severity in the induced organ-specific Autoimmune Uveitis and in the spontaneous Systemic Lupus Erythematosus [SLE] mice models. In contrast, the point-mutated K409A Hsp65, and its derived peptide K409A pep were able to delay the disease progression. Although, the mechanisms underlying lupus modulation by Hsp molecules are unknown, it is suggested that early death of the WT mice group can be related to the aggravation of vasculitis. To address this hypothesis we have analyzed the leader pep and the K409A pep inflammatory response in the lungs, liver and kidney of [NZBxNZW]F1 lupus **mice Methods:** Female mice at 45 days-old were intraperitoneally inoculated with leader pep or K409A pep peptides (n=8-9/group). At 55 days-old, lung was removed for leukocyte phenotype, cytokine production; lung, liver and kidney were collected for histopathological evaluation. **Results:** The K409A pep group exhibited a lower frequency of CD3+ cells when compared to the control group ($p<0.05$). However, no differences were found in CD4+, CD8+, CD19+, PanNK+ and CD3+PanNK+ cell frequencies, and in the expression of activation markers CD62L and CD44 among the groups. No differences in the levels of IL-10, IFN-□, IL-4, TNF-□, IL-5, IL-6, IL-12p70 and IL-17A in the lungs were found. Nevertheless, mutant peptide showed a lower IL-4/IL-10 ($p<0.05$), IL-4/IL-12p70 ($p<0.05$) and IL-4/IL-5 ($p<0.01$) ratio than leader pep group. Also, K409A pep group exhibited lower ratios of both IL-17A/IL-5 ($p<0.05$) and TNF-□/IL-5 ($p<0.05$) compared to controls. Leader pep group presented increased infiltrating polymorphonuclear cells in the lung compared to the other groups (leader pep: n=4/8; K409A pep: n=2/9; control: n=0/8). Initial analyses revealed no differences in kidney and liver tissue. **Conclusion:** Altogether, our findings indicate that K409A pep leads to a microenvironment richer in regulatory and

TH1 cytokines than leader pep, which could explain the antagonistic effects of the two peptides. Also, the observed differences between K409A pep group and Control group regarding cytokine ratios and lymphocytes frequencies reaffirm the K409A pep ability of mitigating lupus progression.

Financial support: CNPq and FAPESP.

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PREVENTION OF EARLY ALLERGEN SENSITIZATION BY MATERNAL IMMUNIZATION IN MICE: OFFSPRING CYTOKINES MODULATION.

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Introduction and Objectives: Previously we have been observed that pre-conceptional maternal immunization to ovalbumin (OVA) is able to prevent the development of type I hypersensitivity response in BALB/c mice, mainly due to the transference of maternal Ab to offspring and up-regulation of FcγRIIb inhibitory receptors on offspring B cells. We sought to verify the effect of maternal immunization on offspring FcγRIIb expression and cytokines production in C57BL/6 mice.
Methods and Results: C57BL/6 female mice were immunized with OVA in alum, and mated with normal males. Offspring from immune or control mothers were immunized with OVA at 3 days old (d-o) and boosted at 13 d.o. Offspring serum were evaluated at 20 d.o. and splenic cells phenotype and intracellular cytokine production at 3 d.o. and 20 d.o. Maternal OVA-immunization was able to up-regulate the expression of FcγRIIb inhibitory receptors on offspring B cells at 3 d-o when compared to control group. This effect was maintained at 20 d-o after offspring immunization. Serum analysis revealed that maternal immunization could down regulate offspring IL-6 levels at 20 d-o compared to offspring from non-immune mothers. Newborns from immune or non-immune mothers show similar percentage of TCD4+ cells producing IL-10, IL-17, IFN-γ or IL-4. However, at the same period, offspring from immune-mothers show augmented IL-10+ and IL-17+ B cell levels when compared to control group, with no change with the IFN-γ+ and IL-4+ B cells frequency. After 20 d-o immunized offspring of immune mothers showed higher levels of IL-17+ and IFN-γ+ TCD4+ cells than offspring from nom-immune mothers, no effect was observed with IL-4+ and IL-10+ TCD4+ cells. Moreover, after immunization at 20 d-o, increased percentage of IL-17+ and IFN-γ+ B cells was detected in offspring from immune mothers.
Conclusion: We confirmed our previously observations on BALB/c mice that maternal OVA immunization could up-regulate the expression of FcγRIIb on offspring B cells, corroborating this evidence, lower IL-6 serum levels were detected in offspring from immune mothers indicating diminished B cells proliferation. The augmented IL-10+ B cells frequency in immune mother's

newborns suggest an involvement of B regulatory cells. The role of IL-17 on offspring from immune mothers must be investigated. Together these results indicate that maternal effect could be mediated by offspring regulatory cytokines/cells production.

Financial support: FAPESP/LIM-56/HCFMUSP.

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AGED [$H_{III}xL_{III}$]F_1 HYBRID MICE IMMUNORESPONSE TO *M. leprae* Hsp65

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Introduction and aim: 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. 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 and Results:** 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. 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. **Conclusions:** *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.

Financial Support: FAPESP, CNPq, INCTTOX Program.

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TITLE: STUDIES ON FOXP3+ REGULATORY T CELLS IN EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

Authors: SYNARA NÔ SEARA CORDEIRO; ALESSANDRO MARINS-DOS-SANTOS; RAFAELLA FERREIRA REIS; BIANCA PERDIGÃO OLIVIERI; ANDRÉA HENRIQUES-PONS; VINÍCIUS COTTA-DE-ALMEIDA.

Institution: Oswaldo Cruz Institute (IOC)/Fiocruz

Introduction: Several studies have demonstrated that FoxP3$^+$ regulatory T (Treg) cells are important controllers of immunity to infections. Patients with the indeterminate form of Chagas disease were shown to bear high numbers of circulating FoxP3+ Treg cells compared to patients that progressed to the cardiac form. However, antibody depletion studies in mice showed controversial results regarding a potential role of Treg cells in experimental *Trypanosoma cruzi* infection. Here, we searched for specific subgroups of Treg cells following the acute course of experimental T. cruzi infection. **Methods and Results**: We used an experimental model of acute infection with the *T.cruzi* Y strain (parasite load 10^4) in C57BL/6 inbred (females 6-8 weeks). The animals were divided into three groups: N = Non-infected (control), I = Infected, Infected IBZ = treated with Bz (Benznidazole). Thus we evaluated the phenotypic profile of nTregs in spleen and thymus by flow cytometric analysis. Our data show a significant decrease in the frequency of CD4$^+$FoxP3$^+$ cells in the spleen of infected animals, as compared to noninfected controls. These results prompted us to investigate the relative contribution of thymic-generated Treg (nTreg) cells compared to those induced in the periphery (iTreg). Thus, we analyzed the intracellular expression of the transcription factor Helios, which are regarded as a marker for the nTreg cells. Our data revealed that the frequency of both nTreg and iTreg cells are markedly decreased in infected animals. Moreover, analysis of absolute numbers show that Treg cells expand at lower level relative to the major expansion seen with the total CD4$^+$ subset. Analyses of thymus of infected mice showed a slight decrease in Treg cells, although not as important as the decrease seen for other thymocyte subpopulations. In addition, we also demonstrated that treatment of infected mice with the trypanocidal drug benznidazole does not preclude the lower levels of splenic Treg cells. **Conclusion**: In the spleen, we found that the Tregs cells are not the target cell depletion in animals infected when analyzing numbers cells but there is an important decrease in relative number, being the natural Treg cells mainly responsible for this decrease. In the thymus, these cells maintained their absolute number. Bz treatment did not cause an improvement in the relative

profile of Treg cells in the spleen, but the absolute level of natural Tregs increased compared to group N.

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THE ROLE OF HOST IMMUNITY ON THE BIOLOGICAL FITNESS OF *Aedes aegypti* MOSQUITO VECTOR

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Introduction: Many hematophagous insects are vector of several pathogens that cause human diseases such as malaria, dengue, Chagas disease and leishmaniasis. Due to lack of an effective vaccine for many of these diseases, the search of new methods to control these insect vectors are desirable. After repeated exposures to salivary antigens of hematophagous insects, the immune system of the vertebrate host is able to mount a strong response to these salivary components, resulting in some cases in decreasing infectivity of the pathogens transmitted. However it is not known the effect of this immune response on the biological fitness of the mosquito *Aedes aegypti*. In this work we evaluated whether the host immune response affect the mosquito choice of target blood meal, as well as the fitness parameters of this vector.
Methods and Results: In order to generate hosts with different "immunological history", BALB/c female mice were sensitized by *Ae. aegypti* bites to mimic natural exposure. Other groups of mice were immunized with the mosquito salivary gland extract (SGE), either in presence of alum or Freund's adjuvants. Serum antibody levels were evaluated by ELISA and presented a Th2 response in natural exposure to mosquito bites (specific IgG2a: 27,3 µg/mL; specific IgG1: 70,8 µg/mL; total IgE: 10,9 µg/mL) and SGE/alum-immunized mice (specific IgG2a: 11,7 µg/mL; specific IgG1: 38,49 µg/mL; total IgE: 8,4 µg/mL). On the other hand, SGE/Freund-immunized mice induced a mixed response (specific IgG2a: 164,5 µg/mL; specific IgG1: 174 µg/mL; total IgE: 14,3 µg/mL). No change in fertility was noted for those mosquitoes fed in mice immunized or sensitized. However, the insects that took their blood meal in sensitized mice showed reduction on fecundity (45%) when compared to control groups. Preliminary data indicates that control mice (non-exposed/non-immunized) are less attractive to mosquitoes when comparing to immunized/sensitized mice (6 and ~20 mosquitoes, respectively), but fewer mosquitoes fed in groups immunized/sensitized (~80%) compared to control group (100%).
Conclusion: No significant changes in fitness parameters of *Ae. aegypti* were observed when mosquitoes were blood-fed in hosts with different types of immune response against salivary antigens. However, the preliminary results

indicate that mice immunized or sensitized are more attractive to mosquitoes than control mice.

Financial Support: FAPESP and CNPq

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PRESENCE OF ACTIVE VITAMIN D DURING IMMUNIZATION WITH PROTEOGLYCAN TRIGGERS SPECIFIC TOLERANCE IN BALB/C MICE

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Introduction: Vitamin D has an important immunomodulatory effect in the innate and adaptive immune system. Active vitamin D (VitD3) promotes antimicrobial responses to pathogens in macrophages and suppresses inflammatory responses. In addition, VitD3 inhibits the maturation of dendritic cells (DCs) (suppressing their capacity to present antigens to T cells) and induces DCs with a tolerogenic phenotype. *In vitro* studies indicate that VitD3 inhibits T cell proliferation and IL-2 and INF-g synthesis. The aim of this study was to evaluate if the association of VitD3 with proteoglycan (PG) is able to establish a state of specific tolerance.
Methods and Results: Female BALB/c retired breeders (n=6 per group) were immunized with three different doses of PG (10, 20 and 50 mg) in the presence of VitD3. 0,1 mg of vitamin was injected every other day during 15 days and PG was co-injected at the days 3 and 11. One week later, the animals were injected with PG associated with a strong adjuvant (dimethyldioctadecyl ammonium bromide). Seven days after immunization, the specific immune response was checked by a delayed type hypersensitivity reaction (DTH) and also by *in vitro* cytokine production by spleen cells. Association of VitD3 with 50 mg of PG, but not with the lower antigen concentrations, induced specific tolerance characterized by a less intense DTH reaction [275 (110-770) nm] compared to positive control group [505 (350-1175) nm]. Spleen cells from tolerized group stimulated with PG also produced higher amounts of IL-5 [597 (172-1124) pg/ml] and IL-10 [412 (64-1087) pg/ml].
Conclusion: The highest amount of PG associated with VitD3 was able to induce specific immunological tolerance.

Financial support: FAPESP.

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ALLELE FREQUENCY OF THE HCV^{+} PATIENTS ATTENDED AT FUNDAÇÃO DE MEDICINA TROPICAL OF AMAZONAS - BRAZIL

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Introduction: IL-28B (Interferon-3λ) was discovered as a member of the IFN-λ family comprising IFN- 1λ and IFN-2λ, which are encoded by IL-29 and IL-28A, respectively. This group of endogenous cytokines has potent antiviral properties and has been related to inhibition viral replication. According to this polymorphism several independent studies presented on chromosome 19q13 in which is located above the 3 kb gene IL28B. This study aimed to determine the allelic frequency of the cytokine gene of *IL28B* (rs 8103142) of HCV^{+} patients untreated with ribavirin and interferon alpha and assisted at Fundação de Medicina Tropical of Amazonas ribavirin and interferon alpha. **Methods and Results:** The sample consisted of 114 individuals, 70 HCV^{+} individuals and 44 controls. The DNA was extracted according Brazol protocol, followed by real-time PCR (Applied Biosystems). For the association analysis was performed Fisher's exact test, using the statistical software *BioEstat* 5.0 with 5% significance level. The results revealed that the HCV^{+} patients group is 25, 71% C/C, 51, 42% C/T and 22, 85% T/T. On the other hand, the group control is 29, 54%C/C, 42, 22% C/T and 27, 27% T/T. **Conclusion:** We conclude the C/T allele was more frequent in both groups followed by the alleles CC e TT in the individuals of Amazonas.

Financial Support: FAPEAM, CNPq, HEMOAM, FMTAM, UFAM, UFPA.

Key word: *IL28B*, Hepatitis C, inhibition viral replication.

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PROTECTION AGAINST EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY PREVIOUS *Staphylococcus aureus* INFECTION: POSSIBLE CONTRIBUTION OF IL-10

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Introduction: Multiple sclerosis (MS) is a devastating autoimmune inflammatory disorder of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS. Environmental factors, in particular viruses, bacteria and other pathogens are thought to play a pivotal role in the development of autoimmune diseases. The objective of this study was to evaluate the production of IL-10 by brain infiltrating cells in mice infected with *S. aureus* before EAE induction. **Methods:** Female C57BL/6 mice (n=7) were infected with *Staphylococcus aureus* (ATCC 43300 strain) by intraperitoneal route. Three days later the animals were immunized with 200 µl of an emulsion containing 150 µg of myelin oligodendrocyte glycoprotein (MOG) in Complete Freund´s Adjuvant containing 5 mg/ml of BCG. Mice also received 2 intraperitoneal doses of *Bordetella pertussis* toxin, 0 and 48 h after immunization. Animals were daily evaluated for clinical score and body weight loss. Brain inflammation was assessed during acute phase. At the chronic phase (30th day after immunization), the animals were euthanized and cytokine (IFN-gamma and IL-10) production by spleen and brain infiltrated mononuclear cells were assayed. **Results:** Previously infected animals developed a less severe disease characterized by reduced weight loss, much lower clinical scores and also a smaller degree of inflammation in the brain. Similar levels of IFN-gamma (EAE: 26±5ng/ml; *S. aureus*/EAE: 30±10ng/ml) and IL-10 (EAE: 282±77pg/ml; *S. aureus*/EAE: 228±37pg/ml) were produced by spleen cells stimulated with MOG. However, the production of these cytokines by cells stimulated with SAC was significantly higher in previously infected mice: IFN-gamma (EAE: 2096±610pg/ml; *S. aureus*/EAE: 5138±115pg/ml) and IL-10(EAE:2142±236pg/ml; *S. aureus*/EAE: 7297±802pg/ml). Production of IFN-gamma by brain mononuclear infiltrating cells stimulated with MOG was similar in both groups (EAE: 1598±1468pg/ml; *S. aureus*/EAE: 1254±497pg/ml). However previously infected mice produced more IL-10 (EAE: 22±3pg/ml; *S. aureus*/EAE: 36±7pg/ml). **Conclusion:** Protection against EAE development

triggered by *S. aureus* infection is possibly associated with the local IL-10 production.

Financial support: FAPESP

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TOLERANCE TOWARDS MOG CAN BE TRIGGERED BY HIGHER PEPTIDE DOSES IN THE PRESENCE OF VITAMIN D3

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Introduction: The active form of vitamin D3 (vit D3) has known immunomodulatory effects on a wide range of immune cells, including T, B and dendritic cells (DCs). Tolerogenic properties are described in DCs treated with vit D3. These cells express lower levels of MHC class II, produce less IL-12 and more IL-10. The aim of this study was to evaluate if the association of vit D3 with myelin oligodendrocyte glycoprotein (MOG), a myelin peptide of the central nervous system, is able to establish a state of specific tolerance. **Methods and Results:** Female C57BL/6 mice were intraperitoneally injected every other day for 15 days (on days 1, 3, 5, 7, 9, 11, 13 and 15) with vitamin D (0, 1μg). On days 3 and 11, MOG (low dose: 2 μg; intermediate dose: 50 μg or high dose: 150 μg) was coinjected with vit D3. One group received only vit D3. One week later mice were subcutaneously immunized with 200 μg of MOG in Complete Freund's Adjuvant (CFA). Two weeks after the mice were challenged at the right footpad with 50 μg of MOG. Delayed type hypersensitivity reaction (DTH) and cytokine production by spleen cells were assessed 24h later. Association of vit D3 with MOG 150, but not with MOG 2 or 50, determined a decrease in DTH response (positive control group = 0.4 ± 0.1; vit D + MOG 150 = 0.1 ± 0.1mm)

and also in the production of IFN-gamma (positive control group = 4807±1802; vit D + MOG 150 = 1955 ± 1478 pg/mL) and IL-17 [positive control group = 215 (108-288); vit D + MOG = 22 (8.5-29.5) pg/mL] by spleen cells stimulated with MOG. These differences were statistically significant. The control group that received only vitD3 before immunization with MOG + CFA showed no alteration at the DTH response and only a non significant decrease in IFN-gamma and IL-17 production. **Conclusion:** The specific tolerance induced by the highest MOG dose plus vit D3 was associated with downmodulation of IFN-gamma and IL-17.

Financial support: FAPESP

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Effects of costimulatory molecules of conventional and regulatory CD4$^+$ T cells in the modulation of acute imune response to *Plasmodium chabaudi* AS

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Introduction: Malaria is one of the most prevalent and widely distributed infectious diseases in the world, affecting approximately 500 million individuals yearly; one million of them (mostly children) dying from complications of the disease. Material: Female C57BL/6Foxp3$^+$GFP$^+$ mice were infected intraperitoneally with 10^6 infected red blood cells (iRBC). The phenotype of conventional and regulatory CD4$^+$ T cells was analyzed by flow cytometry until day 10 post-infection. CDFour and 5 days after infection, splenocytes were labeled with the CellTrace Violent Proliferation Kit and stimulated with iRBC. Anti-ICOS and anti-OX40 blocking antibodies were added in the cell cultures at a concentration of 10 ug/ml. After incubation for 48 and 72 h, cells were stained with monoclonal antibodies to identify costimulatory molecules by flow cytometry. Results: We observed that infection with *P. chabaudi* induces *in vivo* an increase in the number of conventional and regulatory CD4$^+$ T cells per spleen during the early infection. The expansion of T cell populations is followed by an increased expression of ICOS, OX40, and GITR, which are co-expressed in activated cells. The inhibitory molecules PD-1 and PDL-1 have a low expression in conventional CD4$^+$ T cells during the early infection, with increased expression of PDL-1 occurring on day 7 post-infection and of PD-1 from the 10th day on. In regulatory CD4$^+$ T cells, the inhibitory molecule FAS-L has increased expression on day 10 post-infection, showing 3-fold higher expression compared to conventional CD4+ T cells on days 5 post-infection (peak value for this population). We also found that the inhibition of ICOS results in 2-fold reduction of the proliferation of conventional and regulatory CD4$^+$ T cells after 72 h of culture. Moreover, the inhibition of OX40 reduces the proliferation of conventional CD4$^+$ T cells in 3 times and that of regulatory CD4$^+$ T cells up to 7 times after 72 h of culture. Conclusion: The results suggest that regulatory CD4$^+$ T cells are activated during the early phase of malaria and that

ICOS and OX40 are directly involved in the response of these cells to *P. chabaudi* infection.

Financial support: CAPES and CNPq.

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NATURAL KILLER T CELLS IMPAIR THE ACCUMULATION OF T REGULATORY LYMPHOCYTES IN THE AIRWAYS OF ALLERGIC MICE

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Introduction: Invariant "Natural Killer" T lymphocytes (NKT) represent a distinct subpopulation of T lymphocytes with the ability to rapidly produce pro-Th2 cytokines, such as IL-4. Therefore, these cells has been extensively associated with the pathogenesis of allergic asthma. Recent works demonstrated a cross-talk between NKT cells and T regulatory lymphocytes (Treg); thus, we hypothesized that during asthma development NKT cells could modulate Treg and *vice versa*. **Objective:** investigate the physiological and/or pathological interaction between NKT cells and Treg lymphocytes during the development of allergic asthma. **Methods:** C57Bl/6 *Wild type* (WT) or NKT deficient mice (Jα18$^{-/-}$) were immunized subcutaneously with ovalbumin (OVA) adsorbed onto aluminum hydroxide, at days 0 and 7, and challenged via intranasal administration of an OVA/PBS solution, at days 14 and 21. At day 22, animals were euthanized; the bronchoalveolar lavage (BAL), draining lymph nodes, lungs and spleen were collected and analyzed by flow cytometry. **Results and conclusion:** we found that the Th2 lung inflammation was impaired in Jα18$^{-/-}$ mice, corroborating the idea that NKT cells are, somehow, involved in the pathogenesis of allergic asthma. The flow cytometry analysis revealed an increase in the frequency of CD4^{+}CD25^{high}Foxp3^{pos} in the lungs and BAL of Jα18$^{-/-}$, in comparison to WT animals. Thus, in addition to the current concept that NKT cells promote asthma development through the direct polarization of type 2 response, our data suggest that NKT cells impairs the influx or *in situ* differentiation of Treg lymphocytes, which in turn can lead to Th2 polarization.

Financial Support: FAPESP

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THE EFFECT OF ORAL ADMINISTRATION OF HSP65-PRODUCING *LACTOCOCCUS LACTIS* IN AUTOIMMUNE DIABETES IN NOD MICE

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Introduction: Heat shock proteins (HSP) play a double role in the immune system as antigens for lymphocytes and agonists of innate immunity. They are able to control inflammation and enhance regulatory T cell function. Type 1 diabetes (T1D) is an autoimmune disease that affects 25 million people worldwide and can cause serious health complications, even under conventional treatment. Considering the need to develop new therapies for T1D, we investigated the efficacy of a strategy that associates the immunomodulatory potential of HSP65 and the tolerogenic properties of the gut mucosa in disease prevention. **Methods and Results**: The innocuous bacteria *Lactococcus lactis* was engineered to secrete HSP65 so that it would deliver low doses of HSP65 in the gut when orally administered. This system mimics a continuous feeding protocol, known as the most effective regimen for oral tolerance induction. Female NOD mice were treated four consecutive days for three times and thereafter blood glucose was monitored. NOD mice treated with multiple doses of HSP65/*L.lactis* presented a reduction in the incidence of diabetes. This modulatory effect disapeared when a single dose of bacteria was administered. To test the effect of HSP65/*L.lactis* in Treg induction, frequency of spleen CD4+CD25+Foxp3+ T cells were analyzed after treatment. There was a significant increase in Treg frequency 72h after treatment followed by a decrease 7 days later suggesting that HSP65/*L.lactis* had an early but not sustained effect in either Treg induction or expansion. Indeed, HSP65/*L.lactis* did not affect diabetes development in cyclophosphamide-treated NOD mice, probably due to the known toxic effect of this drug on regulatory T cells. **Conlusions**: Therefore, administration of HSP65-producing *L.lactis* induced disease modulation in NOD mice and it might represent an alternative therapeutic tool for T1D. Further improvement in dosage and administration protocols as well as on the mechanisms of its modulatory effect will be the next steps of our study.

Financial suport: FAPEMIG, CNPq and CAPES.

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TRANSFERENCE OF IgG Abs BY THE BREASTFEEDING PREVENT ALLERGY IN EARLY LIFE OF MICE

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Introduction and objectives: Maternal immunization with allergen in mice has been demonstrated to be an important strategy to prevent the development of allergy in offspring. The mechanisms involved in the regulation of allergic response are crucial to improve maternal vaccination protocols. We evaluate the effects of breastfeeding by OVA-immunized mothers in the development of offspring allergy. **Materials and methods:** Females BALB/c mice were immunized with OVA by subcutaneous route and mated on 21º dpi. The amniotic fluid was collected on 21º day of gestation by cesarean section, and cytokines measured by flow cytometry. Milk samples were obtained directly from the stomach of 10 days-old (do) offspring. To assess the contribution of breastfeeding, neonates from OVA-immunized mothers were nursed with non-immunized mothers and vice-versa. Offspring were immunized at 25 do. Presence of anti-OVA IgA and IgG in milk samples by ELISA and anti-OVA IgE Ab was determined by passive cutaneous anaphylaxis reaction. **Results: I**mmunization before conception inhibits anti-OVA IgE Ab production in the offspring immunized at neonatal period. To verify whether this inhibition could be related to antibodies and cytokines transfer/changes, we analyzed the amniotic fluid and neonatal serum, respectively. An active maternal anti-OVA IgG Ab and IFN-g, TNF-a, IL-12p70 and IL-10 were transferred to fetus compared to non-immunized mothers. Moreover, in milk samples we detected and increased anti-OVA IgA and IgG Ab levels. To verify the importance of breastfeeding in this experimental model, the offspring from immunized mother nursed non-immune mother, when immunized showed a significant decreased in the IgE response. **Conclusion:** The findings showed that maternal immunization with OVA prevent the development of IgE response of offspring, due to the intense transference of IgG Abs by the breastfeeding.

Financial support: FAPESP, LIM 56- HCFMUSP

IMMUNO 2012
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HSP65-PRODUCING *LACTOCOCCUS LACTIS* PREVENTS INTESTINAL INFLAMMATORY DISEASE BY IL-10- AND TLR2-DEPENDENT PATHWAYS

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Introduction: To maintain intestinal health, the immune system must faithfully respond to antigens from pathogenic microbes while maintaining a state of tolerance to commensals and food antigens that confront it every day. However, disruption of this thin balance can cause inflammatory bowel disease (IBD) in genetic susceptible hosts. Heat shock proteins (Hsp) are phylogenetically conserved chaperones that are important for the survival of eukaryotic cells. The heat shock proteins (Hsps) are good antigen candidates for the therapeutic use of oral tolerance in inflammatory diseases. The aim of this study was investigate the effects of Hsp65-producing *Lactococcus lactis* in intestinal inflammatory disease and characterized the mechanisms involved in such intervention.

Methods and Results: In this study, we used a strain of *Lactococcus lactis* genetically engineered to deliver Hsp65 from *Mycobacterium leprae* in the gut mucosa. C57BL/6, IL-10-/-, TLR2-/- and TLR4-/- mice received oral treatment with Hsp65 producing-*Lactococcus lactis* during four consecutive days. After ten days of interval, the colitis was induced by 1,5% dextran sulfate sodium (DSS). Oral administration of Hsp65-producing *L. lactis* completely prevented DSS induced colitis; abolishing the weight loss, diarrhea; fecal bleeding and inflammation in the colon. The mucosal protection was accompanied by diminished levels of TNF-α, IL-6 and IL-4 and by enhanced IL-10 in colonic

mucosa. In addition, mice pre-treated with Hsp65-producing *L. lactis* had increased frequency of regulatory T (Treg) cells expressing the latency-associated peptide (LAP- that is associated with surface TGF-β) and the forkhead box P3 (Foxp3) in the spleen. Moreover, this protection requires IL-10, since in IL-10 deficient mice Hsp65-*L. lactis* did not improve the inflammatory histological score. The same was observed in TLR2-/- mice but not in TLR4-/-. In accordance with this, in vitro experiments, reveled that Hsp65-*L. lactis* supernatant induced the expression of TLR2 in levels similar to the synthetic TLR2 ligand (PCSK). Finally, CD11b+ cells expressing TLR2, which produces IL-10, are involved on the protection.

Conclusion: Hsp65 delivered by *Lactococcus lactis* prevents intestinal inflammation via IL-10 and TLR2 pathways. This approach may lead to long-term management of IBD patients.

Financial support: FAPEMIG **(**PRONEX-2009), CNPq, Instituto de investigação em Imunologia (iii)

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EVALUATION THE HYDROETHANOLIC EXTRACT OF *LAFOENSIA PACARI* ON IMMUNE RESPONSE

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Introduction: *Lafoensia pacari* St. Hil. (Lythraceae) is a specie of the “cerrado” flora, traditionally used in Mato Grosso for loosing weight, healing external wounds, treatment pain and gastric ulcers and as an anti-inflammatory agent. The objective of this study was to evaluate the effect of the hydroethanolic extract of *L. pacari* on the number of peripheral blood leukocytes, spleen cells and the production of IgG1 in mice immunized or not with a low dose of antigen in the presence or absence of incomplete Freund’s adjuvant. **Methods and Results:** For this purpose, we used inbred mice of the C57BL/5 strain, adult, females, randomly distributed into six groups with 4-5 animals per group which received 200 mg/kg/day of the hydroethanolic extract of *L. pacari* for 21 days. The results show that the hydroethanolic extract of *L. pacari* administered for 21 days does not alter the total number of white cells and also the number of subpopulations of these cells in peripheral blood and spleen. Our results indicate that the hydroethanolic extract of *L. pacari* does not alter the kinetics or the levels of anti-OVA IgG1, suggesting that *L. pacari* does not have significant effects on mature cells of the immune system, including those that are responding to ovalbumin.

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MK801, an Ionotropic NMDAr Antagonist Ameliorates EAE by Reducing Encephalitogenic T CD4 Cells. Jean Pierre Schatzmann Peron[1], Wesley Nogueira Brandão[1], Andira Fickinger[1], Matheus Correa Costa, Vinícius Andrade Oliveira[1], Tárcio Braga Ribeiro[1], Enio Bassi[1], Niels Olsen Saraiva Cãmara[1].

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ABSTRACT

Glutamate is widely known as one of the most important and also most abundant neurotransmitter of the central nervous system. Glutamate may act through different types of receptors, namely metabotropic and ionotropic receptors. Not so long ago, several researches have demonstrated a great abundance of glutamate receptors in immune cells, for example macrophages, dendritic cells and mainly T cells. However, there is still a wide gap in the knowledge of the biology glutamate over immune cells. Thus, we decided to evaluate the role of MK 801, a specific MK801 antagonist for the glutamate ionotropic receptor NMDAR. For that, C57 BL6 female mice were immunized with 150 μg of MOG 35-55 and treated or not with 0,3 mgKg of MK801 intraperitoneally on days 0,1,2 and 3. At the peak of disease animals were submitted to euthanasia and the percentage of Th1 and Th17 were analyzed by flow cytometry after extraction from the CNS by percoll gradient centrifugation. Interestingly, MK801 treated animals had much milder disease when compared to control group. To corroborate that, this group had reduced frequency of both Th1 and Th17 cells infiltrating brain and spinal cord. To further evaluate the pathways involved in this activation, DO 11.10 T cell clones were activated in vitro in the presence of anti-CD3 with or without MK801 or NMDA. Total and p-ERK 1-2 were evaluated further by western blot. Our data demonstrated that NMDA activation of T CD4 cells boosts ERK phosphorylation and MK801 was able abrogate the phenomenon. Further, in order to evaluate whether glutamate was involved in Th17 or Th1 promotion, we performed in vitro differentiation of T CD57Bl6 T $CD4^{+}CD25^{-}$ cells. Our experiments demonstrated that in fact, glutamate signaling through NMDAR on T cells boosts the commitment of both interferon-γ and IL-17 secreting T CD4 cells. Conclusion Our data demonstrates that the neurotransmitter and aminoacid glutamate may greatly modulate T cell response in vitro and in vivo. These findings not only contribute to the field of neuroimmunology but also for a new point of view concerning its role neuroinflammatory diseases.

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T CELL AND MONOCYTE SUBSETS IN RESPONSE TO *S. MANSONI* ANTIGENS IN CUTANEOUS LEISHMANIASIS

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Introdution: We demonstrated previously that the *S. mansoni* antigens used in the current study down-modulate the Th1 inflammatory response in a group of cutaneous leishmaniasis (CL) patients. We evaluated the effects of these antigens on lymphocyte and monocyte phenotype activation status in response to the soluble *Leishmania braziliensis* antigen (SLA) in cells of CL patients.
Methods and Results: The study included the first 30 individuals living in the endemic area in tegumentar leishmaniasis Corte de Pedra, Bahia, Brazil. PBMC of CL patients were stained with fluorochrome conjugated antibodies to CD4, CD8, CD25, CD28, CTLA-4 and Foxp3 in T lymphocytes and CD14, CD16, HLA-DR, CD80 and CD86 in monocytes. The Ethical Committee of the Maternidade Climério de Oliveira, Federal University of Bahia approved the present study, and an informed consent was obtained from all study participants or their legal guardians.The addition of rSm29 antigen to the cultures stimulated with SLA enhanced the frequency of $TCD4^+$ cells (from 34.8 ± 2.8 to 40.8 ± 2.8%), being this antigen able to enhance the expression of $CD28^+$ in $TCD4^+$ and $TCD8^+$ cells (from 85 ± 17 to 113 ± 22 MFI and from 85 ± 14 to 91 ± 14 MFI, respectively). SmTSP-2 and PIII antigen were able to increase the expression of CTLA-4 (from 49.6 ± 4 to 58 ± 5 MFI and from 49.6 ± 4 to 53 ± 4.6 MFI, respectively) in $TCD4^+$ cells. The addition of rSm29 and SmTSP-2 to the cultures led to an increase in the frequency of $CD4^+CD25^+$ cells expressing Foxp3 (from 7.5 ± 1.7 to 10.2 ± 2.1% and from 7.5 ± 1.7 to 10.4 ± 2.3%, respectively). We also evaluated the frequency of monocyte subtypes (classical, intermediate and non-classical) expressing costimulatory molecules. The addition of the Sm29 and PIII to the cultures expanded the frequency of non-classical monocytes ($CD14^+CD16^{++}$) moreover these cells expressed lower

levels of HLA-DR in the presence of rSm29 (from 718 ± 188.4 to 547 ± 140.5 MFI). The addition of SmTSP-2 to the cultures lead to a decrease in the expression of CD86 in intermediate monocytes ($CD14^{++}CD16^{+}$) (from to 562 ± 149.7 to 447.8 ± 112.5 MFI).

Conclusions: The addition of rSm29, rSmTSP-2 and PIII to the PBMC culture stimulated with SLA up-regulated the frequency of Treg cells and expression of CTLA-4 by $TCD4^{+}$ cells. These antigens also alter in a lesser extent the expression of costimulatory molecules in different subtypes of monocytes.

Financial support: MCT/CNPQ, Grant 479417/2008 3 Universal.

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MURINE MODEL OF ALLERGIC AND ATOPIC DISEASE AND ITS TREATMENT WITH POLYCLONAL IMMUNOGLOBULIN

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Introduction: Allergic patients maintain high levels of allergen specific IgE, and higher titers of total IgE than the average of the normal population. In 1970, Vaz and Levine showed that a persistent formation of IgE can be obtained by intermittent immunization of high-responder mouse strains with low doses of antigen. Since immunoglobulin therapy (polyclonal Ig) offers a chance to permanently change the immune system, we aimed in this study to test its effect in IgE production by high-responder mice.

Methods and Results: We studied three different mice strains (BALB/c, C57BL/6 and B6D2F1). Animals were immunized with 1µg of OVA with aluminum hydroxide as adjuvant at days 1, 21 and 42. OVA aerosol challenge was performed a week after the last immunization or a month after the treatment with IVIg. Test groups were treated with human polyclonal Ig by intraperitoneal injections (IVIg) before challenge. Mice were deeply anesthetized before blood, bronchoalveolar lavage and lungs were collected, and animals euthanized. Levels of total serum IgE and specific serum IgE and IgG1 were detected by ELISA assays. Cytokines from pulmonary tissue were also measured by ELISA assays. Pulmonary inflammation was assessed by broncoalveolar lavage (BAL) cell count and by histologic analysis. All sensitized animals presented persistent synthesis of IgE at different levels and patterns. B6D2F1 had the highest IgE levels all the time, whereas C57BL/6 mice had intermediate levels, BALB/c mice had the lowest levels, but they were the only ones that showed a rise after aerosol challenge. Treatment with IVIg had no significant effect on IgE synthesis. However, had a clear inhibitory effect on the cellular infiltrate (total cells and eosinophils) as well as on the lung IL-5 production.

Conclusion: The protocol developed by Vaz and Levine was able to keep animals sensitized for more than one year and the pattern of IgE synthesis of different strains may serve as a model of both allergic and atopic diseases. The

effect of IVIg was observed up to one month after application and it can be regarded as of long duration.

Financial support: CNPq and FAPEMIG.

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FAS-FASL RESCUES FUNCTIONAL CD8 T CELLS AND HELP MACROPHAGES CONTROL *TRYPANOSOMA CRUZI* INFECTION.

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Introduction: Apoptotic lymphocytes impair protective immune response during *Trypanosoma cruzi* infection. Inhibition of Fas-FasL signaling in the course of *T. cruzi* infection prevented premature apoptosis of CD8 T cells. CD8 T cells are crucial to control infection cells but they are also implicated in tissue injury. How apoptosis affects the cooperation between CD8 T cells and macrophages remains unclear.

Methods: Macrophages and CD8 T cells were obtained from infected mice during acute phase. Macrophages were re-infected in vitro and co-cultured with CD8 T cells. Cultures were treated with anti-CD3 for activation in the presence of anti-FasL or control IgG. We evaluated the production of cytokines, nitric oxide (NO) and parasite infection. Apoptosis and purification of CD8 T cells were evaluated by flow cytometry.

Results: CD8 T cells undergo apoptosis upon re-stimulation with anti-CD3, reducing NO production and parasite control, even in the presence of high IFN-γ levels. Blockade of Fas-FasL death pathway reduced apoptosis and increased

the number of CD8 T cells. The production of IFN-γ by CD8 T cells was not affected by treatment with anti-FasL. Nonetheless, treatment wiyh anti-FasL upregulated the pro-inflammatory profile of macrophages; increasing the production of IL-6, TNF-α, NO and the control of parasite replication. When we evaluated the effector mechanisms of CD8 T cells we observed that cytotoxicity was not modulated by the Fas-FasL pathway.

Conclusion: These results indicate that it is possible to modulate the interaction between CD8 T cells and macrophages infected with *T. cruzi* through inhibition of apoptosis mediated by Fas and FasL molecules.

Financial support: CAPES, CNPq and FAPERJ.

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October 20 - 24, 2012

ENDOGENOUS IL-10 IS RELATED TO INCREASED IMMUNOPATHOLOGY AND REDUCED CONTROL OF *Brucella abortus* INFECTION

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Introduction: *B. abortus* is a Gram-negative pathogenic bacterium which causes a chronic disease in humans and domestic animals called brucellosis. The host ability to mount a Th1/pro-inflammatory response against this bacterium is crucial to control and to solve the infection. It is suggested that IFN-γ produced by Th1cells is critical to control the infection in mice. The interleukin IL-10 is considered to be responsible to decrease the IFN- γ production *in vitro* interfering directly in the pathogen elimination. During *B. abortus* infection, IL-10 acts limiting inflammatory response favoring the establishment of persistence infection in mice. The goal of this study was evaluated the role of endogenous IL-10 in control the immunopathology during *B. abortus* infection. **Methods and Results:** To assess the role of IL-10 *in vivo* IL-10 KO mice or 129 Sv/Ev mice were infected with *B. abortus* and the number of viable bacteria recovery from spleens was evaluated. IL-10 KO mice showed lower bacterial load in the spleen when compared to wild type mice during all the time points. Furthermore, the IFN-γ and TNF-α production were measured in the supernatant from spleen cell culture *in vitro* when the cells were stimulated with *B. abortus*. IL-10 KO cells showed greater increase in pro-inflammatory cytokines when compared to wild type cells. In bone-marrow dendritic cell supernatant greater levels of TNF-α and IL-12-p40 were observed in IL-10 KO cells when compared to wild type mice cells when they were stimulated with this bacterium. Descriptive histopathology analyses were performed in livers demonstrating a gradual diminishment of granuloma in IL-10 KO and control animals. However, decreased pathology was more effective in IL-10 KO livers considering the granuloma area measured by digital morphometry and the hepatic parenchyma recovery. **Conclusion:** Taken together, the data provided by this work support that the lack of IL-10 is related to higher resistance to *B. abortus* infection and increased production of pro-

inflammatory cytokines culminating with better bacteria elimination and a quicker tissue pathological resolution leading to a more effective control of this infection.

FINANCIAL SUPPORT: CAPES and CNPq

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REDUCTION OF LUNG INFLAMMATION IN MITE ALERGEN-SPECIFIC EXPERIMENTAL RESPIRATORY ALLERGY BY INOCULATION OF TOLEROGENIC BONE-MARROW – DERIVED DENDRITIC CELLS

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Introduction: It is estimated that respiratory allergies affect about 20 to 30% of the world population. Dendritic cells (DCs) are professional antigen presenting cells that possess a unique potential to initiate primary, including tolerogenic, immune responses. Antigen-presenting DCs in a state of partial maturation have a tolerogenic profile, being able to induce immunological tolerance. The prevalence of allergic diseases, as well as the severity of their symptoms, are becoming increasingly high, what encourages the studying of new immunotherapy methods. In this work, the effects of tolerogenic dendritic cells (tolDCs), presensitized with an aqueous extract of *Blomia tropicalis* mites (BtE), on the development of respiratory allergy in mice, were studied.
Methods and Results: Syngeneic 4- to 8-week old A/J mice were utilized. TolDCs were obtained by culturing bone marrow cells with dexamethasone and granulocyte-macrophage colony stimulating factor. The tolDCs were immunophenotyped by flow citometry and its cytokine-producing profile was evaluated by ELISA. The tolDCs presented with low MHC class II and co-stimulatory molecules expressions, and low IL-12 secretion ($P<0.05$). Groups of five mice were intraperitoneally immunized with BtE in aluminium hydroxide and intranasally challenged with BtE. These mice presented with high levels of eosinophil peroxidase in the bronchoalveolar lavage fluid and lungs, high total cells leukocyte numbers in the lungs, and lung inflammation ($P<0.05$ or $P<0.01$). The previous inoculation of BtE-sensitized tolDCs significantly ($P<0.05$) reduced the lung inflammation.
Conclusion: The tolDCs significantly reduced the lung inflammatory infiltrate in an experimental model of allergy to *Blomia tropicalis*. Experiments should be carried out to find out if the tolDCs are able to ameliorate ongoing respiratory allergic responses. The data from the effect of the inoculation of tolDCs on other immunological parameters in the experimental model is still undergoing analysis.

Financial support: Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq); Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB - PRONEX), Rede Nordeste de Biotecnologia (RENORBIO).

IMMUNO 2012
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MODERATE ACUTE EXERCISE CHANGE INDUCES IMMUNOLOGICAL PARAMETERS IN SALIVA AFTER RECOVERY

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Introduction: Exercise promotes changes in the salivary IgA and can be associated with a high incidence of upper respiratory tract Infections. However, the effect of exercise on other immunological parameters in saliva is unknown. This study determined the effect of moderate acute exercise upon immunological salivary parameters, such as the levels of cytokines (TGF-β and IL-5), IgA, α-amylase and total protein, over a 24 h period.
Methods : Ten male adult subjects exercised for 60 minutes at an intensity of 70% VO2peak. Saliva samples were collected before (“basal”) and 0, 12 and 24 h after an exercise bout. The statistical differences were determined by a one-way ANOVA and Tukey post hoc tests, with the significance level set at $p < 0.05$.
Results: The salivary TGF-β concentration increased 184% 12 hours (6.32 ± 0.07 pg/µg protein) and 136% 24 hours after exercise (5.26 ± 0.21 pg/µg protein) relative to the levels observed immediately after exercise (2.22 ± 0.12 pg/µg protein) ($p<0,05$). The total proteins were lower, 26 % and 18 % respectively, after 12 hours (1.25 ± 0.05 µg/µl) and 24 hours (1.40 ± 0.07 µg/µl) than immediately after exercise (1.71 ± 0.07 µg/µl) whereas the α-amylase increased 22% 12 hours (474.11 ± 21.30 U/µg protein) and 35 % 24 hours after exercise (524.11 ± 17.10 U/µg protein) compared with the immediately after exercise (387.26 ± 22.50 U/µg protein) ($p<0,05$). The IgA concentration was increased 23,79% 24 hours after exercise (20.81 ± 1.04 mg/µg protein) relative to the levels immediately after exercise(16.81 ± 0.80 mg/µg protein) ($p<0,05$), and there is no difference in the IL-5 concentration.
Conclusion: We conclude that moderate acute exercise (70% VO2peak) does not induce changes immediately after exercise, but that 24 h after exercise, favourable effects are induced in immunological parameters that can be modulated for increases in TGF-β but not IL-5.

Financial support: Luana Rosa is the recipient of FAPESP scholarships (# 2010/16770-1). This study was financially supported by FAPESP (# 2009/09107-7).

IMMUNO 2012
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AMINO ACID-BASED DIET EXACERBATES DSS-INDUCED COLITIS IN MICE

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Introduction: Nutritional therapy is an important tool in the management of inflammatory bowel disease (IBDs). However is not yet clear which dietary formulation is better and studies comparing elemental and polymeric formulations are conflicting. A leading hypothesis for the use of elemental diets, which contain amino acids as sole nitrogen source, is the removal of antigen stimulation in the inflamed intestinal environment. However, it is not clear whether these antigens are relevant to the activity of inflammation.
Objective: Investigate the role of an amino-acid based diet in experimental colitis.
Methodology: Colitis was induced in C57BL/6 mice by three cycles of 1% DSS administration in the drinking water for 7 days, alternating with 7-day periods of recovery. Mice were fed a protein-free amino-acid-based diet (AA group), or a diet containing whole protein (Casein group) during colitis induction.
Results: Mice that were fed a protein-free diet showed greater weight loss, bleeding, diarrhea and shorter length of the colon than animals fed the standard diet. Histological analysis showed increased cellular infiltration in the mucosal layer, destruction of the mucosal architecture and thickening of the muscular layer in these mice. Concentrations of secretory IgA were higher in small intestine and colon of AA group. After the first cycle of DSS, the AA group had lower concentrations of IL-4 and TGF-β and, after the second cycle, higher concentration of inflammatory cytokines such as IL-6 and IL-12 in the colon. In spleen of AA mice, we found a lower concentration of IL-17 after the second cycle and of IL-6, IL-17 and TGF-β after the third cycle. In the duodenum, the AA group also had lower levels of IL-10 throughout the experiment.
Conclusion: Administration of amino-acid-based diet was associated with exacerbation of colitis severity. Reduction of anti-inflammatory cytokines and increase of proinflammatory cytokines contributed to this effect.

Financial Support:FAPEMIG, CNPq

IMMUNO 2012
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IMPACT OF STRESS MEDIATORS ON THE FUNCTION OF DENDRITIC CELLS

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Introduction: It is known that the immune system is regulated directly and indirectly by central nervous system (CNS), mainly during response to stress. Recent studies published by our group demonstrated that dopamine (DA) and substance P (SP) modulate cytokine release from policlonally-activated T cells from healthy individuals. In this context, the cathecolamine DA attenuated the production of Th1-related cytokines while enhanced IL-6 release from activated T cells. With regard to SP, this neuropeptide enhanced TNF-α and IL-17 production in the same system of activation.
Objective: As many of the T cell effector function is driven by signals delivered by dendritic cells (DC), our objective was to investigate the impact of different stress mediators on the function of myeloid and plasmocytoid DCs.
Methods: Peripheral blood was collected from healthy individuals and maintained for 6h in the presence or absence of stress-related doses of DA (1 x 10^{-6} M), SP (1 x 10^{-6} M), serotonin (5-HT, 2 x 10^{-6} M) and hydrocortisone (HC, 1 x 10^{-6} M). In some wells, 100 ηg/mL of LPS were added. The cells were stained with different monoclonal antibodies to determine, by cytometry, the frequency of mDC and pDC, and the cytokine production was also quantified by cytometry.
Results: The frequencies of mDC(CD11c+) and pDC(CD123+) were similar in the peripheral blood from healthy individuals and the addition of 5-HT and HC for 6h up-regulated the expression of CD86 on mDC, while the SP elevated the expression of this co-stimulator molecule on pDC. Concerning cytokine profile, SP, DA and 5-HT enhanced IL-6 production, while DA e 5-HT elevated IL-12 release. The production of IL-23 was observed following addition of DA. Concerning anti-inflammatory cytokines, HC addition induced IL-10 release. Furthermore, LPS-induced production of IL-1β, IL-6, IL-23 and IL-12 was elevated by SP, DA and 5-HT. Interestingly, DA diminished the IL-10 release induced by LPS. And besides HC inhibits the production of pro-inflammatory cytokines, this glucocorticoid enhanced the production of IL-10 in LPS-activated cell cultures.
Conclusions: Our results, although preliminary, suggest that DA, 5-HT and SP modulate differently the function of DC, mainly by up-regulating its immunogenic function, while HC favors the induction of tolerogenic DC.

Financial support: FAPERJ, CAPES, UNIRIO, CNPq

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NEGATIVE ASSOCIATION BETWEEN ADIPONECTIN AND INFLAMMATORY BIOMARKERS IN EXPERIMENTAL DIET INDUCED OBESITY

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Introduction: Adipokines are hormones and cytokines produced by adipose tissue which have an important role in the regulation of immune response. Changes in the production of these molecules by adipose tissue have been involved in promoting a state of low-grade chronic inflammation shown in obesity. The aim of our study was to evaluate the association of adiponectin levels with inflammatory markers in an experimental model of obesity.
Methods and Results: Female ICR-CD1 mice were fed with a high-fat diet (60% calories ; obese group, n=8) during 14 weeks and were compared with mice fed with a standard chow (control group, n=8). Plasma concentrations of adiponectin, soluble E-selectin, vascular cell adhesion molecules (sVCAM-1), intercellular adhesion molecules (sICAM), fibrinogen, TNF-α, IL-1β, IL-6 and IL-10 were determined by Luminex-100 IS, multiplex assay kits. Soluble E-selectin levels were higher in obese mice than in the control group (39.48±2.38 ng/mL *vs* 20.36±3.50 ng/mL, P=0.0012). Levels of adiponectin, sVCAM-1, sICAM, fibrinogen, TNF-α, IL-1β, IL-6 and IL-10 were similar between both obese and control groups. However, it's important to highlight that adiponectin plasma concentration had a negative correlation with sE-selectin (r=-0.70, P=0.049) and sVCAM-1 (r=-0.88, P=0.0006).
Conclusion: These results showed that feeding a hypercaloric diet for 14 weeks to ICR-CD1 mice caused an elevation of sE-selectin. In our model of obesity a negative association between adiponectin and some inflammatory markers (such as sE-selectin and sVCAM-1) was found. In conclusion, adiponectin has an important role in the regulation of inflammatory response promoted by a pathological situation such as obesity.

Financial support: MICINN (BFU2011-30336), UCM (910379ENEROINN) grants, RETICEF (RD06/0013/0003) from ISCIII and BTSA (The PRONAOS Study, CDTI 2008 1114).

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B AND T LYMPHOCYTE POPULATIONS ARE REGULATED DIFFERENTLY BY OUABAIN IN MICE

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Introduction: Ouabain (OUA), previously known as a cardiotonic steroid capable of inhibiting Na^+ K^+-ATPase, has been identified as a endogenous hormone produced by the adrenals and hypothalamus and found circulating in mammalian plasma (Hypertension 30:886,1997). It has been suggested that ouabain with others glucocorticoids is released by the adrenal under stress situations. As glucocorticoids, the ouabain is also able to regulate immunological functions. Here, the main objective was to study whether doses of ouabain achieved during stress can regulate B and T lymphocyte populations. **Methods and Results:** We used C57BL/6 or Balb/C mice (male or female 4-12 weeks old) injected intraperitoneally (i.p.) with 0.56 mg/kg ouabain for 3 days. In the fourth day the subpopulations of B and T lymphocytes were analyzed by flow cytometry, 24 hours after the last injection. Our results show that, in the bone marrow, there was a decrease in the total number of cells ($22,5 \times 10^6 \pm 1,1$ CTR vs $17,3 \times 10^6 \pm 0,6$ OUA) but, among B lymphocytes, there was a reduction only in mature B cells. In the spleen was also observed a decrease in mature B lymphocytes ($55,4 \times 10^6 \pm 1,7$ CTR vs $32,3 \times 10^6 \pm 2,8$ OUA), mainly affecting follicular B cells. We observed no effect in regulatory B cells in the spleen. Ouabain regulates B lymphocytes inducing apoptosis in spleen and partially inhibiting the proliferation of mature B lymphocytes in response to LPS. In addition, ouabain increased the migration of B lymphocytes to mesenteric lymph nodes. In the thymus, ouabain had no effect *in vivo* on T

cell subsets, but the number of $CD4^+$ $Foxp3^+$ were significantly reduced in the spleen (10,6 $x10^6$ ± 1,6 CTR vs 7,7 $x10^6$ ± 0,5 OUA), whereas there was no significant modulation in the total number of T $CD8^+$ lymphocytes. In the case of T lymphocytes, an increase in apoptosis or inhibition of proliferation in response to anti-CD3 induced by ouabain cannot be observed. **Conclusion:** Ouabain seems to use different mechanisms in regulation of B and T lymphocytes subpopulations.

Financial Support : PROPPI/UFF ; FAPERJ ; CNPq .

IMMUNO 2012
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Enhanced Treg-mediated suppression following Beta2 adrenergic receptor signaling is associated with PKA-dependent increase in CTLA-4 expression and iTreg generation.

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Considering modulation of CD4+ T cell responses, beta2 adrenergic receptor (B2AR) signaling is known to impair Th1 differentiation and function in a cAMP-dependent way, leading to inhibition of cell proliferation and decreased production of IL-2 and IFN-γ. Foxp3+ CD4+ regulatory T (Treg) cells play a key role in the regulation of immune responses and are essential for maintenance of self-tolerance. Nevertheless, very little is known on adrenergic receptor expression in Treg cells or either on the influence of noradrenaline on their function. **Objective**: The aim of this study was to investigate the effect of B2AR stimulation on Treg function. **Methods and Results:** Treg and CD4+ CD62L+ GFP- naïve T cells from Foxp3^{gfp} mice were sorted in a FACSAria II. We first confirmed B2AR expression in Treg cells by qPCR. To test whether or not B2AR in Treg cells are functional, we measured intracellular cAMP using a commercial kit. As expected, Treg intracellular cAMP levels showed a 4 fold increase after b2AR agonist treatment. The cAMP increase was completely reversed by pre-treatment with a B2AR antagonist. For suppression assays, sorted naïve T cells and Treg cells were cultured in a proportion of 1:0,25 with irradiated splenocytes and soluble anti-CD3. Before setting up the cultures, Treg cells were treated or not with B2AR agonist, or with B2AR agonist plus B2AR antagonist. After 3 days, naïve T cell proliferation was analyzed by proliferation dye dilution in a flow cytometer and quantified using FlowJo. We found that B2AR agonist was able to increase suppressive activity of Treg cells since naïve T cell proliferation was about 40% lower than the one observed in control cultures. Pre-treatment with B2AR antagonist reverted B2AR agonist-mediated increase in Treg suppression. We also found increased CTLA-4 expression in Treg cells that were pre-treated with B2AR agonist. Moreover, Treg cells treated with B2AR agonist were able to induce more iTreg cells from naïve T cells than non-treated Treg cells. Since cAMP activates PKA, we investigated if B2AR agonist-mediated increase in Treg suppression and CTLA-4 expression could be reverted by inhibiting PKA. We found that pre-treatment with PKA inhibitor prevented the effect of the B2AR agonist. **Conclusion:** Our data suggest that sympathetic fibers could be able to regulate Treg suppressive

activity in a positive manner through B2AR signaling. This increased Treg suppressive function is dependent on the cAMP-PKA pathway.

Financial support: FAPESP, CNPq

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Title: GLUTAMATE SIGNALING THROUGH NMDAr BOOSTS PROINFLAMMATORY RESPONSE IN MACROPHAGES AND LYMPHOCYTES ACTIVATED IN VITRO.

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Introduction: Several reports have indicated that Glutamate may be directly related to the deterioration of the nervous system tissue, as it was found that patients with neuroinflammatory diseases such as Multiple Sclerosis, Parkinson´s and Alzheimer´s Disease have an excessive amount of this neurotransmitter in their nervous system tissue. Recent studies revealing the presence of glutamate receptor in immune cells indicates that glutamate affects the local immune response and the immune modulation by this neurotransmitter could be important for the disease outcome. NMDA receptor is an ionotropic type of Glutamate receptor. MK-801, a NMDA receptor antagonist, has been used in EAE studies and its administration reduced neuronal lesions and symptoms in treated animals, but its direct effect on the immune response was not evaluated.

Methods and Results: DO 11.10 and J774 lineage cells were cultured in a 48 well plate and stimulated or not with anti-CD3 or LPS respectively. Moreover, cells were incubated in medium alone or with 10μM of NMDA or 10μM of MK. After 48h cells were prepared for qPCR and Western Blot analysis. Our results show that glutamate has a proinflammatory role through the stimulation of NMDA receptor. Stimulation of DO 11.10 and J774 lineage cells with specific NMDA receptor agonist reduced the expression of SOCS3 and IL-10, whereas MK-801 reversed these results. Analysis of the NFkB pathway to measure cell activation revealed that NMDA stimuli stimulated the mentioned pathway through decrease of IkappaB and increase of IKK in J774 lineage cells. On DO 11.10 cells it was observed increased phosphorilated Erk1 and Erk2.

Conclusion: The results obtained reveal that Glutamate has a proinflammatory effect on immune cells through NMDA receptor. This results show an important interaction of neurotransmitters with the immune response, resulting in neuroimmunomodulation. The knowledge of neuroimmunomodulatory mechanisms may be helpful in the

research and treatment of immune diseases in the nervous system.

Financial Support: CNPQ 480827/2010-9 and CAPES

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T_{REG} CELLS INCREASE IN THE PERITONEAL CAVITY OF NON DIABETIC STZ-INDUCED MICE AFTER ADOPTIVE TRANSFERENCE OF B-1 CELLS

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Introduction: Type 1 diabetes is an organ-specific autoimmune disease in which T cells mediate damage of pancreatic islet β cells. Cumulative evidences indicate that B cells play an important role in promoting diabetes. B cells are subdivided into B-1 cells and conventional B-2 cells based on their origin, anatomical distribution, surface markers, subtypes of antibodies produced and capacity of self-renewal. It has been shown that B-1a cells can be involved in various autoimmune diseases and recently, a regulatory role of $CD5^+$ B cells was described in diabetes model, regulating pathogenic and Treg T cells function. This study aims to investigate the involvement of B-1 cells in the development of murine streptozotocin (STZ)-induced diabetes.

Methods and Results: BALB/c and BALB/*xid* (B-1 cells-deficient mice) male mice were treated intraperitoneally with STZ (40mg/kg) for 5 days. It is important to note that BALB/c mice do not develop diabetes when this dose of STZ was used. After STZ treatment, BALB/*xid* glucose levels were higher than that of BALB/c ($p<0,001$). To evaluate the role of B-1 cells in diabetes induction, peritoneal B-1 cells from BALB/c mice were purified based on expression of $CD19^+$ $CD23^-$ by FACS Aria III Cell Sorter and adoptively transferred intra-peritoneally to BALB/*xid* mice (BALB/*xid* + B-1). STZ-treatment was performed before or after B-1 cell transference. Despite of STZ-treatment induces diabetes in BALB/*xid* mice, we observed that BALB/*xid* + B-1 mice did not become diabetics in both protocols adopted. Histological sections of the pancreas showed a lower number of pancreatic islets and a lower amount of insulin labeled β cells in diabetic mice (BALB/*xid*) than in other groups. We also observed that B-1 cells decrease in the peritoneal cavity of BALB/c and BALB/*xid* + B-1 mice 10 days after STZ-treatment ($p<0,05$). However, an increase in peritoneal Treg cells in these mice ($p<0,01$), but not in BALB/*xid* mice was observed.

Conclusion: Our data show that B-1 cell-deficient mice showed higher reactivity to STZ-treatment with more severe symptoms, intensive pancreas damage, insulin deficiency and high BGL. In addition, B-1 cells decrease in peritoneal cavity of BALB/c mice and migrate to pancreatic islets of STZ-treated mice. Based on these data, we hypothesized that B-1 cells may be involved in the protection to STZ-induced diabetes, by affecting Treg cell population.

Financial Support: CNPq, FAPESP.

IMMUNO 2012
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October 20 - 24, 2012

OUABAIN INHIBITS HUMAN MONOCYTE ACTIVATION *IN VITRO*: PREVENTION OF THE PROINFLAMMATORY mCD14+/CD16+ SUBSET APPEARANCE AND CELL SIZE PROGRESSION

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Introduction: Classically described as a potent inhibitor of Na^+,K^+-ATPase, ouabain was further shown to act as an effective immunomodulator in mammals. Recently, our group showed that this hormone downregulates membrane CD14 (mCD14) in human monocytes, though it is not known if monocyte activation status could modify ouabain influence. The aim was to study whether ouabain may influence *in vitro* activation/differentiation of human monocytes and whether, in turn, monocyte activation status could modulate ouabain effects. **Methods and Results:** Blood samples were obtained from healthy donors and parameters were analyzed via flow cytometry ($n \geq 6$). Increment in cell size (indicative of activation) and mCD14, CD16 and CD69 expression were analyzed in total monocytes or in monocyte subpopulations displaying small or large sizes after two distinct periods of adhesion (2h and 24h) before treatment with ouabain for 24h. Immediately after 2h adhesion, only 15% of monocytes presented increased cell size, contrasting with 40% of large monocytes observed after a period of 24h adhesion, thus confirming that cells undergo *in vitro* activation induced by adhesion. In this context, 100nM ouabain induced a 60% decrease in the percentage of large monocytes in culture only when added to cells immediately after 2h adhesion. Moreover, ouabain induced a significant decrease in the percentage of monocytes expressing high levels of mCD14 (nearly 40%) and also in the amount of CD16+ monocytes (around 15%) after 2h adhesion. However, no alteration was observed in monocytes treated with ouabain after 24h adhesion. Analyzing small and large cells separately, mCD14 was slightly less modulated in large cells, compared to small cells, and CD16 was not modulated at all in large cells. Since monocytes after 24h adhesion showed no lack of ouabain binding and no ouabain-induced CD69 upregulation, as seen in monocytes after 2h adhesion (2-fold increase in

CD69+ cells), it seems that ouabain is somehow incapable of triggering an appropriate cell signaling induction once monocytes become activated. **Conclusions:** Our data suggest that ouabain inhibits monocyte activation *in vitro*, preventing both cell size increase and the appearance of the proinflammatory mCD14+/CD16+ subpopulation. Thus, our findings recommend more attention for the regulation of either inflammation or infection in individuals suffering from disorders commonly associated with high ouabain plasma levels, like hypertension.

Financial s**upport:** CAPES, CNPq and FAPERJ.

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STUDY OF THE EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) ON HIV-1 REPLICATION IN MACROPHAGES.

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Introduction: In the course of HIV-1 infection several host factors are implicated with the modulation of viral replication. The Vasoactive Intestinal Peptide (VIP) and the Pituitary Adenylate Cyclase Activating Peptide (PACAP) have many physiological functions, including: the regulation of inflammatory responses, cell differentiation and hormonal regulation. VIP and PACAP binds to receptors coupled to G protein (VPAC1, VPAC2 and PAC1) and have a wide tissue distribution. In the immune system, VIP and PACAP, so as its receptors, are expressed in T-cells and macrophages, and act as regulators of immune function. In this study we investigate the mechanisms by which VIP and PACAP interferes with the HIV-1 replicative cycle and modulates viral replication in macrophages. **Methods and Results:** In brief, monocyte-derived macrophages, obtained from healthy donors by density gradient centrifugation, were infected *in vitro* with HIV-1 and treated with different concentrations of VIP, PACAP and/or receptor-specific agonists or antagonists. After 12 days, replication was evaluated by ELISA for HIV-1 p24 Ag in supernatants. We found that VIP and PACAP inhibit the replication of HIV-1 in macrophages in a non-dependent dose manner, with the best inhibition up to 50% (n=5) for both neuropeptides, and that the inhibition promoted by VIP is dependent of VPAC1 and VPAC2, whereas PACAP can act through the three receptors (n=5). We observed that VIP and PACAP can act in a synergistic or additive manner, dependent of concentration, to inhibit HIV-1 replication (n=3). VIP and PACAP increased the production of RANTES, MIP1-α and IL-10 in macrophages (up to 2-fold over control, n=4, 6 and 4), and neutralization of these molecules reverted the HIV-1 inhibition (up to 40%, n=5). We also observed that VPAC1 is more prominent than VPAC2 to induce the β-chemokines, while the induction of IL-10 by VPAC2 is more robust than its counterpart, whereas, the increase of both modulators can be achieved by PAC1 (n=3). **Conclusions:** Our results

show that VIP and PACAP inhibit the replication of HIV-1 in macrophages, and this action is dependent of the distinct activation of its receptors. The induction of the β-chemokines and IL-10 shows clues about some mechanisms involved, inhibition at the viral entry step and inhibition of viral transcription, respectively.

Financial support: IOC/Fiocruz, CNPq and Faperj

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THE ROLE OF TLR-2, TLR-4 AND MyD88 IN THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE DIABETES

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Introduction: Autoimmune type 1 diabetes (T1D) is an autoimmune disease characterized by pancreatic beta cell destruction. During T1D development, several cytokines produced by activated macrophages and Th1 lymphocytes such as IFN-γ, IL-1β and TNF-α, have a role in the autoimmune inflammatory process in the pancreatic islet. Recently, the role of Toll-like receptors (TLRs) has been demonstrated in this disease; however the mechanisms involved in this process have not yet been fully explained. In this study, we evaluated the role of TLR-2, TLR-4 and MyD88 adaptor molecule in the development of experimental autoimmune diabetes induced by multiple low-doses of streptozotocin (STZ). **Methods and Results:** Experimental autoimmune diabetes was induced by intraperitoneal administration of STZ (5 consecutive daily doses of 40 mg/kg) in C57BL/6 wild type (WT), TLR-2 KO, TLR-4 KO and MyD 88 KO mice. Body weight and glucose levels were monitored twice a week for 21 days. Histological analysis of the pancreas was performed to quantify the number of islets, inflammatory cellular infiltrate and immunohistochemistry for caspase-3. The frequency of CD4+ cells, CD8+ cells, CD4+CD25+Foxp3+ (regulatory T cells) and macrophages (F4/80) in spleen and pancreatic lymph nodes were determined by flow cytometry. Interestingly, a more severe disease has been developed in diabetic TLR-2 KO mice, since higher blood glucose levels and a severe weight loss could be observed in these mice compared to WT group. Furthermore, both an increased inflammatory cellular infiltrate and caspase-3 expression could be detected in the pancreatic islets of diabetic TLR-2 KO group compared to the WT group, suggesting a greater destruction of β pancreatic cells. Interestingly, a significant decrease in the frequency of regulatory T cells was observed in the spleen of diabetic TLR-2 KO animals compared to the WT group 21 days after the first dose of STZ. **Conclusion:** Hence, TLR-2 receptor deficiency resulted in exacerbated development of experimental autoimmune diabetes, thereby suggesting an important role for

this receptor of innate immunity in the modulation of the immune response developed in this disease.

Financial support: FAPESP (2011/03833-8).

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IMMUNE REPONSE MODULATION AND DEVELOPMENT OF EXPERIMENTAL CEREBRAL MALARIA: EFFECTS OF AGARICUS BLAZEI MURRIL TREATMENT

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Introduction: The *Agaricus blazei* Murrill (AbM) is a Brazilian originated mushroom, and it is being used as an alternative medicine and functional food, which preventing various diseases, such as infection, allergy, and cancer. AbM was described to contain bioactive compounds related to an immunomodulatory activity. Malaria is a disease caused by *Plasmodium* species reaching 106 countries, affecting approximately 216 million people and leading to deaths of 655,000, and that has been exacerbated by the emergence of drug-resistant parasites. The most important complication of *Plasmodium falciparum* infection in human is the development of cerebral malaria (CM). Here, we investigated the effect of AbM in modulation of immune response and development of CM in mice infected with *P. berghei* ANKA (PbA), parasite strain faithfully recapitulate many of the characteristics of human CM.
Methods and Results: C57Bl/6 mice were pretreated (3 days) with extract or fraction of AbM and then infected with $1x10^5$ parasited red blood cells (pRBCs), followed by treatment with AbM or chloroquine, and the parasitemia, survival, body weight, development of CM and immune response were evaluated. Mice treated with AbM demonstrated lower parasitemia, longer survival, reduced weight lost and protection against CM development. There was also a reduction pro- and anti-inflammatory cytokines production (TGF-β/IL-10, IFN-γ, IL-1β, IL-6 and IL-17), when compared with untreated PbA-infected mice. In addition, the pre-treatment of pRBCs with AbM in vitro following to infection in vivo, resulted in lower parasitemia, longer survival, reduced weight lost and protection against CM.
Conclusion: These findings indicate, for the first time, that AbM has an important protective role in the development of experimental CM and modulation of immune response during PbA infection.

Financial support: CNPq and FAPEMIG.

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TREATMENT WITH HSP65-PRODUCING *LACTOCOCCUS LACTIS* PREVENTED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY LAP$^+$ REGULATORY T CELL.

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Introduction: Recent findings have shown that heat shock proteins (HSP) display an important role in immune regulation, as a ligand for both innate and adaptative immune receptors. Furthermore, it's known that HSP can prevent experimental autoimmune encephalomyelitis symptoms, a rodent model of multiple sclerosis. We aimed to investigate if Hsp65-producing *L. lactis* prevents lesions in spinal cord of EAE in mice, the main organ affected in this model. **Methods and results:** C57BL/6 Foxp3-GFP$^+$ or Foxp3-GFP$^-$ mice were continuously fed with water (control group), wild type *L. lactis* (WT group) or Hsp65-producing *L. lactis* (HSP group) for five consecutive days. Ten days later mice were immunized s.c. in the base of tail with 100 µg of MOG_{35-55} in CFA plus 4mg/ml of *Mycobacterium tuberculosis*. Pertussis toxin (300 ng) was injected i.p. at the day of immunization and 48 h later. Spinal cords and lymphoid organs were removed 4 and 14 days post-immunization for histological or flow cytometry analysis. Pretreatment with Hsp65-producing *L. lactis* rendered mice less susceptible to EAE induction when compared to other groups. Flow cytometry analysis showed less lymphocytic infiltration in spinal cord of mice from HSP group 4 and 14 days after EAE induction, although the frequency of LAP$^+$ Treg cells was higher than in the other groups. Among these Tregs, CD4$^+$Foxp3$^-$LAP$^+$ Treg cells (Th3) were present in higher frequencies than CD4$^+$Foxp3$^+$LAP$^+$ Tregs. We also found that treatment with Hsp65-producing *L. lactis* reduced CCR6 expression in effector spleen T cells 4 days after immunization and increased CXCR3$^+$ on them. Interestingly, LAP$^+$ T cells expressed CXCR3 in a much higher intensity than others cell types. **Conclusion:** Treatment with HSP65-producing *L. lactis* modulates the T cell compartment in mice spinal cord, leading to a predominant regulatory pattern. This phenomenon occurs mainly through recruitment of Th3 cells from secondary lymphoid tissues and down-regulation of CCR6 on encephalitogenic T cells.

Financial Support: CNPq, CAPES and FAPEMIG.

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THE EFFECT OF GLUCOCORTICOID IN THE DIFFERENTIATION PROCESS OF THE B CELL LINEAGE IS INDEPENDENT OF P-GLYCOPROTEIN ACTIVITY

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Introduction: Glucocorticoids (GC) are produced and released by the adrenal gland and become elevated in response to a variety of stress situations. Although GC are well known for their immunosuppressive effects, there is little information regarding the effect on immune cells, especially in B cell precursors. P-glycoprotein (Pgp) is a efflux pump involved in the transport of several lipophilic compounds, including GC. Thus, the aim of this work was to assess the *in vivo* effect of GC on the differentiation process of the B cell lineage in murine bone marrow (BM). Moreover, we also investigated Pgp activity profile and whether GC-induced effects are dependent on Pgp function impairment. **Methods and Results:** Two months-old C57BL/6 female mice were injected i.p. with hydrocortisone (70, 140 or 200 mg/kg/day), for one or 3 consecutive days. In the next day, mice were sacrificed and cells from BM were counted, stained with specific antibodies and analyzed by flow cytometry. To analyze the *in vivo* activity of Pgp, hydrocortisone treated mice were injected i.p. with the Pgp inhibitor cyclosporine A (100 mg/kg). One hour later, mice were injected with the fluorescent Pgp substrate rhodamine 123 (2.5 mg/kg) and then sacrificed after one hour. Values are expressed as mean ± standard deviation ($x10^5$) of viable cells ($n \geq 9$). A single injection of different concentrations of hydrocortisone did not change the amount of any of the subsets in the BM. However, 3-day treatment with 70mg/kg hydrocortisone was able to reduce the number of precursors $sca1^+ckit^+$ (0.21 ± 0.09 to 0.09 ± 0.04), $ckit^+$ (1.42 ± 0.48 to 0.86 ± 0.38) and B lineage cells such as Pre/Pro B (14.49 ± 6.04 to 5.38 ± 2.79), immature B (3.77 ± 1.37 to 2.27 ± 0.90). Subsets $sca1^+$ (2.48 ± 0.96 to 1.36 ± 0.45) and $ckit^+$ $B220^+$ (0.40 ± 0.30 to 0.12 ± 0.05) were reduced only in the concentration of 200 mg/kg hydrocortisone. Nonetheless, mature B cells were resistant to treatment, even at the highest concentration used. All subsets showed PgP activity, although rhodamine 123 extrusion profile was not altered by treatment. **Conclusion:** According to other authors, GC are able to induce apoptosis in precursors and immature B cells in BM, while mature B cells are resistant to treatment. However, the activity PgP is not related to susceptibility to exposure to GC.

Financial support: CNPq, FAPERJ.

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DAIRY LACTOBACILLI WITH ANTI-COLITIS PROPERTIES

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Introduction: The gastro-intestinal microbiota plays an important role in human health through the modulation of immune responses. While selected bacteria from this environment are marketed in specific probiotic products to stimulate these responses, relatively little is known about the immune modulation potential of dairy bacteria that have principally been selected for their fermentation properties. *Lactobacillus delbrueckii* is one of the lactic acid bacteria used for the production of yogurt, a product with a long standing reputation as a healthy diet component and has been implicated in immune modulation effects. Seemingly contradictory results have also been reported. However, it is known that the modulation of immune responses by probiotics may reduce chronic inflammation in inflammatory bowel diseases. In this context, we studied the anti-colitis potential of a collection of *L.delbrueckii* strains.

Methods and Results: A collection of *L.delbrueckii* strains was screened for anti-inflammatory effects *in vitro*, measuring their effect on NF-κB activation by TNF-α. For this purpose, an NF-κB dependent reporter gene was used in human gut epithelial HT29 cells. All the strains tested reduced TNFα-induced NFκB activation in a strain-dependent manner by reducing the phosphorylation of IκB. Two of the most effective strains completely lost their ability to suppress NF-κB after trypsin treatment, indicating that the bacterial effectors involved in the NF-κB modulation are proteinaceous in nature. Based on our *in vitro* results, we selected three strains for tests in a DSS model of experimental colitis. One of the strains tested, *L. delbrueckii* subsp. *lactis* CNRZ327, improved the DSS-induced damage, thus confirming its anti-inflammatory properties.

Conclusion: We showed that dairy lactobacilli that often are part of a regular diet can modulate immune responses and may thus affect health more than generally thought.

Financial support: FAPEMIG; CNPq.

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TITLE: TREG CELLS SUBPOPULATIONS IN HUMAN THYMUS

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INTRODUCTION: Regulatory t cells (Treg), either natural or induced, are essential in the maintenance of organism homeostasis. Natural Treg cells are produced in the thymus, however, Tregs can be also induced in the periphery. Several subsets of Treg cells, including the Foxp3 negative ones, have been described as working collectively to maintain immune homeostasis. Interestingly, some authors have currently demonstrated the existence of Treg Foxp3+ subsets that suppress properly Th1, Th2 or Th17 immune responses. These Treg cells subsets, express some molecules, such as transcription factors and chemokine receptors, typical of these different responses. It is believed that these Treg cells subsets are induced in response to Th1, Th2 or Th17 immune response but it is not clear if they are early produced in the thymus. **OBJECTIVE:** To study whether thymus presents different Foxp3 Treg subpopulations. **METHODS:** Thymic specimens (n=10) were obtained from children who underwent corrective cardiac surgery at Heart Institute of São Paulo. We obtained CD4+ thymocytes by performing magnetic cell sorting. The molecules expression was evaluated by flow cytometry, and analyzed using FlowJo. Cells were stained for CD4, CD8, CD25, CD73, CXCR3, CCR4 and CCR5, then fixed and permeabilized before staining with anti-Foxp3. **RESULTS:** All thymic samples (n=10) presented positive expression of several markers in CD4+CD25+Foxp3+ subpopulation ($p<0.05$). Thymic lymphocytes presented expression of CXCR3 (% in Foxp3+ 32.35±4.07), CCR4 (62±6.59), CCR5 (22.3±1.13), CD73 (32.88±3.2). **CONCLUSION:** Our results indicate that the different Treg cells subsets could be early induced in thymus, during Tcells selection. Moreover, it is possible that natural Treg cells leaves thymus with the commitment to suppress properly the different immune responses, but it is necessary performing the functional assays to confirm these conclusions.

Support: Fapesp. **Contact:** vecoelho@usp.br, schanoskialessandra@gmail.com

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ATLa, AN ASPIRIN-TRIGGERED LIPOXIN A4 SYNTHETIC ANALOG, IN THE TREATMENT OF FIBROTIC EFFECTS OF BLEOMYCIN-INDUCED LUNG DAMAGE

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Introduction: Pulmonary fibrosis is characterized by diffuse chronic interstitial inflammation, increased fibroblast proliferation, and enhanced extracellular matrix synthesis and deposition. Lipoxins (LXs) are endogenously produced eicosanoids. Among the various ATL analogs studied, ATLa have been shown to be active in vivo in several models of inflammatory disease. Our aim is to evaluate the mechanisms underlying the anti-fibrotic effect of ATLa on bleomycin-induced pulmonary fibrosis in mice.
Methods and Results: Pulmonary fibrosis was induced in C57Bl/6 mice by bleomycin (BLM) (0.06 U/mouse i.t.) and control mice was instilated with sterile saline. ATLa treatment was performed at days 7 and 10 after BLM. The lungs were obtained for histological analysis, morphometry, immunohistochemistry, flow cytometry and cytokine detection. Lung function was evaluated by mechanical ventilation. The quantification of cells in the lung with flow cytometry showed enhanced leukocyte population in BLM group and treatment with ATLa diminished Macrophages (BLM: 9.463% ± 1.951 N=8 and ATLa: 4.813% ± 0.6800 N=8), Neutrophils (BLM: 26.30% ± 3.351 N=7 and ATLa: 14.72% ± 2.525 N=6), Dendritic cells (BLM: 15.96% ± 3.524 N=7 and ATLa: 7.333% ± 1.573 N=7), T CD4 (BLM: 16.02% ± 2.093 N=8 and ATLa: 9.601% ± 1.446 N=8) and B (BLM: 7.760% ± 1.105 N=9 and ATLa: 17.29% ± 3.752 N=9) lymphocytes. The cytokines quantification demonstrated an increase in TNF-α (BLM: 2.945 ng/mg ptn ± 0.4699 N=7 and ATLa: 1.560 ng/mg ptn ± 0.2890 N=6), TGF-β (BLM: 12.63 ng/mg ptn ± 1.447 N=9 and 4.629 ng/mg ptn ± 0.5640 N=8), IL-1β (BLM: 1.515 ng/mg ptn ± 0.1326 N=8 and ATLa: 0.9218 ng/mg ptn ± 0.1113 N=6) and IL-17 (BLM: 2.225 ng/mg ptn ± 0.4495 N=6 and ATLa: 1.088 ng/mg ptn ± 0.3302 N=5) and low levels of IL-10 (BLM: 1.983 ng/mg ptn ± 0.6108 N=5 and ATLa: 5.710 ng/mg ptn ± 1.113 N=5) secretion after BLM, ATLa reversed these responses. ATLa administration reversed BLM-induced collagen deposition, α-SMA and TGF-β expression in the lungs, change the inflammatory phenotype of macrophages, shifting the M1 to M2 activation and enhanced the maker of pneumocytes type 2 (SP-C). The resistance (BLM: 2.202 ± 0.09156 N=5 and ATLa: 1.674 ± 0.05356 N=5) and elastance (BLM: 80.50 ± 3.253 N=5 and ATLa: 56.70 ± 2.213 N=5) were higher with the BLM and ATLa treatment reduced this effect.

Conclusion: These data strongly suggest a potential therapeutical effect of ATLa as an anti-fibrotic drug.

Funding support: CNPq and FAPERJ

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IMMUNODOMINANCE AS A PATHOGENIC DETERMINANT IN CD4 T CELL MEDIATED REACTIONS

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SalomaoZoppi Diagnósticos.

Objectives: In murine schistosomiasis, H-2k CBA and C3H strains differ from H-2b C57BL/6 for developing severer CD4 T cell(CD4) mediated liver pathology(LP) and a high Th1/Th17 CD4 response against the schistosome egg antigen Sm-p40 and its immunodominant epitope 234-246. Low pathology strains do not react significantly against this antigen. This correlation between high Th1/Th17 Sm-p40$_{234-246}$ reactivity and severe LP suggests that the former may polarize the anti-egg response and lead to the latter.A possible explanation for how one epitope may determine the course of the entire anti-egg response could be restrictive immunodominance(RI), a phenomenon induced when vast epitope availability combines with a high affinity for a selective major histocompatibility class II haplotype(MHCII), to allow that epitope to dominate presentation by antigen presenting cells. RI strongly favors the development of a vigorous Th1 response against that dominant epitope.
With Sm-p40 comprising at least 10% of all egg's proteins and 234-246 being its highest affinity epitope on the unusually stringent H-2k haplotype, high pathology strains present conditions perfect for the establishment of RI of Sm-p40$_{234-246}$ that hypothetically, could generate such an intense Th1 response that it thwarts all attempts at modulation and the result is severe LP.
To test this hypothesis we: a) analyzed the LP of non-H-2k mice whose MHCII restriction is likely to produce RI of Sm-p40 and b) analyzed the LP and cytokines of CBA mice treated with an epitope cocktail designed to neutralize Sm-p40$_{234-246}$ RI and c) attempted to induce severe pathology in C57BL/6 mice by inducing RI
Methodology: Non-H-2k strains bound to develop RI of Sm-p40 were identified by studying Sm-p40 MHCII restriction with the RANKPEP algorithm. SJL mice were chosen for presenting a single Sm-p40 epitope. For analysis of SJL LP, infected mice were euthanized 7 weeks-post-infection(wpi) and their livers submitted to granuloma morphometric analysis(MA).
To study Sm-p40$_{234-246}$ RI neutralization, we infected 3 groups of CBA mice. Group 1 received complete Freund's adjuvant(CFA), 2 and 4 wpi. Group 2 received on the same dates, CFA and a cocktail of 4 synthetic egg epitopes chosen for their ability to compete for I-Ak presentation with Sm-p40$_{234-246}$, and group 3 received nothing. All mice were euthanized at 7wpi for MA of liver granulomas and CD4 cytokine profile.

Results: Analysis of SJL mice showed they develop as severe a LP as CBA mice. Analysis of RI neutralization revealed that treatment with the cocktail emulsion allowed CD4 modulation resulting in reduced LP severity.
Conclusions: These results provide evidence that RI might be a pathogenic force in the establishment and severity of CD4 mediated diseases and that its neutralization might be an effective therapeutic strategy that deserves further exploration.

Financial support: FAPESP, SalomãoZoppi Diagnósticos.

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IMMUNOMODULATORY EFFECTS OF HSP65-PRODUCING *LACTOCOCCUS LACTIS* ON THE POPULATION OF CD8 T CELLSDURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE

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Introduction: Heat shock proteins (Hsps) were firstly described as participants in the cellular response to several types of stress. Currently, it is already known that Hsps plays a major role in immune system modulation, controlling, for instance, autoimmune diseases such as multiple sclerosis (MS), ademyelinating autoimmune inflammation of the central nervous system. Current treatments are partially effective in suppressing MS symptoms and they are associated with many side effects.Our group has shown that HSP65-producing *Lactococcuslactis*(Hsp-*L. lactis*)prevented experimental autoimmune encephalomyelitis (EAE) manifestations in mice by expanding CD4+LAP+ regulatory T cells in both lymphoid organs and spinal cord. Herein we aimed to investigate whether Hsp-*L. lactis* treatment could interfere in the CD8+ T cell population, since these cells are also involved in EAE development.
Methods and Results: C57BL/6 Foxp3-GFP knock-in mice were continuously fed with water (control group), wild type *L. lactis* or Hsp65-producing *L. lactis*for five consecutive days. Ten days later mice were immunized s.c in the base of tail with 100 µg of myelin oligodendrocyte glycoprotein (MOG_{35-55}) in CFA. Pertussis toxin (300 ng) was injected i.p. at the day of immunization and 48 h later. After 2, 4, 10 and 14 days of EAE induction, spinal cord, spleen (SPL), mesenteric (MLN), cervical (CLN) and inguinal (ILN) lymphnodeswere removed and cells were stained with appropriated antibodies for flow cytometry analysis.As we have shown previously, pretreatment of mice with Hsp-*L. lactis* prevented the development of EAE. Moreover, we found increased frequencies of CD8+ T cells expressing the latency-associated peptide (LAP), a membrane-bound TGF-beta, 2 and 4 days after EAE induction in SPL and MLN of Hsp65-L.lactis-treated mice. Although some cells also expressed Foxp3, we are currently performing experiments to better characterize these cells and their real importance in this model.
Conclusion: We propose that not only CD4+LAP+ T cells are responsible for the Hsp-*L. lactis*immunomodulatory effects previously observed in EAE model. CD8+LAP+ T cells may also play an important role in such effect.

Financial Support: FAPEMIG, CNPq, CAPES, Instituto de Investigação em Imunologia (iii).

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CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN THE INHIBITORY EFFECT OF INTERLEUKIN 27 ON HIV-1 REPLICATION IN MACROPHAGES AND IN PERIPHERAL BLOOD MONONUCLEAR CELLS.

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Introduction: Interleukin 27 (IL-27), a member of the IL-6/IL-12 family, is endowed with a powerful anti-HIV-1 activity. For example, it is known that, secondary to cell exposure to IL-27, viral production by HIV-1-infected lymphocytes or macrophages is inhibited up to 80%. It was recently described that IL-27-mediated type 1 Interferon (IFN) production is critical in this phenomenon, through increasing the expression of the cell antiretroviral factor APOBEC3G. However, the IL-27 anti-HIV-1 mechanisms are yet to be fully understood. Considering these findings, it is possible that the protein kinase R (PKR) is involved, since this kinase participates in the signaling pathways leading to type I IFN synthesis. We also believe that the signaling protein Stat3 and the cytokine IL-10 may have a role in the IL-27-mediated anti-HIV-1 inhibition, because these molecules are up-regulated by IL-27. In this study we aimed to analyze additional molecular and cellular mechanisms underlying the anti-HIV-1 effects of IL-27, searching for the role of PKR, Stat3, IL-10, APOBEC and BST-2 in this phenomenon.
Methods and Results: We used cells from healthy donors that were infected or not with HIV-1 and then treated with recombinant human IL-27. Our results show a increased amount of IL-27 in the serum of patients infected with HIV-1 (10,6ng/ml ±1,3; n=29), regardless of the viral load of each patient. We found that the expression of APOBEC3G or APOBEC3F is not up regulate in PBMCs in response to IL-27 (n=5), the opposite of what has been described in macrophages. On the other hand, PBMCs exhibit a large activation of PKR, which is dependent of the Stat3 activity (n=4). IL-27 can also induce an increase in the production of IL-10 by macrophages (22,5 pg/ml ±8,8; n=3) and, specially, by PBMCs (148,9 pg/ml ± 47,0; n=6). We observed a significant increase in the expression of BST-2 in PBMCs treated with rhIL-27 (n=5). We could not demonstrate the association between the IL-27-mediated inhibition of HIV-1 replication and the production of IL-10 (n=4), as well as with the activation of PKR(n=5).

Conclusion: Thus, we believe that further investigation in this field must be

done, including the impact of the increased expression of BST-2 on the IL-27-mediated inhibition of HIV-1 replication in PBMCs.

Financial support: POM/IOC; CNPq; FAPERJ

Effect of BJcuL, a lectin purified from the venom of *Bothrops jararacussu*, on the activation of monocyte cell lines

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Introduction: Lectins are non-immune origin glycoproteins that interact with a specific and reversible manner with membrane carbohydrates through hydrophobic and non-covalent interactions and are able to induce the cell agglutination phenomenon. BJcuL is a lectin isolated from *Bothrops jararacussu* venom, known to have antitumoral activity and recently as immune response inductor as it is able to induce migration, polarization and adhesion of human neutrophils to the extracellular matrix. The aim of this study was to identify BJcuL immunomodulator action upon THP-1 and U937 monocytic cell lines.
Methods and Results: The citotoxicity of BJcuL upon cells were analyzed by flow cytometry using 7AAD marker and by the MTT colorimetric test. To evaluate the macrophage activation upon lectin the NBT method was used, measuring the anion superoxide production through NBT reduction to formazan. The expression of the surface molecular cells markers CD80, CD86, HLA-DR and HLA-ABC were determinated by flow cytometry. For the controls were used PMA and LPS associated to the lectin or isolated. The viability marker 7AAD stains non-viable cells through intracellular penetration by cell membrane porosity. The MTT consists in a reduction of this salt by mitochondrial of viable cells forming the formazan product. Our results showed that BJcuL did not present macrophage citotoxicity. At the concentrations of 0.8 to 7.5 µg/ml BJcuL was able to promote increase of anion oxide production in a dose-dependent manner when compared with control cells. In the major concentration there was an increase of approximately 50%. The cellular activation marker analysis showed that BJcuL is able to induce the increase of all markers expression and there is still higher when associated to LPS, except for HLA-DR.
Conclusion: The lectin showed no citotoxity and over all it was able to induce cell activation by increase of anion superoxide production and also induce the increase of cellular surface molecules responsible for effective immune response, suggesting its action as immunomodulator.

Financial support: PUCPR, CAPES, CNPq.

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CRITICAL ROLE OF NOD2 IN THE TH17 IMMUNE RESPONSE IN TYPE 1 DIABETES

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease that occurs when immunological tolerance to self tissues fails, resulting in the autoimmune destruction of pancreatic beta cells in genetically predisposed individuals. Despite extensive studies about the progression and effector mechanisms in the pathogenesis of T1D, little is known about the initial steps in the development of the disease. In this regard, NOD-like receptors (NLRs) appear as an interesting target in the context of this initial interaction between beta-cells and the innate immune response. NOD-like receptors (NLRs) are intracellular receptors responsible for the recognition of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). Recent studies have demonstrated a role of NOD2 in the regulation of adaptive immunity, since its activation leads to the production of cytokines related to the differentiation of IL-17-producing helper T cells (Th17), even though its precise role in autoimmune diseases remains elusive. Therefore, we investigated the role of NOD2 in the pathogenesis of T1D, with focus on the regulatory T cells (Treg)/Th17 balance. **Methods and Results:** NOD2 deficient mice and their wild-type (C57BL/6) counterparts were inoculated intraperitoneally with streptozotocin (STZ/40mg/Kg) for 5 consecutive days. Blood glucose levels and body weight were monitored weekly. The pancreatic lymph nodes (PLN) were removed to assess the frequency and absolute number of Th17 and Treg cells by flow cytometry. IL-1b, IL-6 and IL-17 expression was determined in the pancreatic tissue by ELISA and RT-PCR. Our results demonstrate that diabetic mice had a significant increase in NOD2 expression in the pancreatic tissue. In addition, NOD2 deficient mice developed a less severe hyperglycemia with only 28% of them becoming diabetic, associated with a reduced inflammatory infiltrate (insulitis) and augmented insulin content within the pancreatic islets. Corroborating with these data, NOD2 deficiency caused a decrease of Th17 cells without interfering on the Treg cell population in the PLNs. In parallel, we observed a significant reduction in IL-1b, IL-6 and IL-17 gene and protein expression in the pancreas of NOD2 deficient mice. **Conclusion**: These results suggest that the NOD2 receptor induces a Th17 proinflammatory phenotype and thus, possibly contributes to the pancreatic islet damage during T1D onset.

Financial support: CNPq.

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SKIN INJURY AND PPAR-α ACTIVATION MODULATES THE SYSTEMIC METABOLISM AND INFLAMMATORY IMMUNE RESPONSE IN EXPERIMENTAL WOUND HEALING

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Introduction: Peroxissome proliferator-activated receptor alpha (PPAR-α) is a nuclear transcription factor involved in the regulation of lipid metabolism and inflammation. PPAR-α may be associated to the modulation of wound healing, which is a multifactorial process dependent on mechanisms of cell signaling and inflammation.

Objective: to evaluate the inflammatory response at the wounds and its relationship to the systemic metabolism of mice treated with a PPAR-α agonist. Methods: Skin wounds were performed in the dorsal region of 129/Sv mice, treated daily with the PPAR-α agonist, Gemfibrozil, by oral or topical route. Mice were followed for 240h post-surgery (p.s.) for skin repair and metabolic changes that could be induced by PPAR-α activation. Neutrophils and eosinophils activity was evaluated by myeloperoxidase (MPO) and eosinophil peroxidase (EPO) respectively.

Results: There was improved wound healing in mice treated with 100 or 50 mg/Kg of PPAR-α agonist by oral or topical route respectively. The oral treatment induced a better repair in the early 24h, 48h and 72h p.s. while mice treated by topical application of Gemfibrozil presented faster healing in all times evaluated. Wound's induction affected the systemic metabolism of mice leading to significant weight loss. PPAR-α agonist did not alter glucose, triglycerides or liver function, although all injured animals had a significant decrease on triglycerides levels in the early times p.s., independent on the treatment. Regarding the leukocytes at wounds' site, untreated mice presented a peak in

MPO activity 24h p.s., which was stabilized later at the end of the 10th day, while Gemfibrozil treated groups showed a greater activity at 120h and 240h p.s., when compared to the earlier period p.s. On the other hand, EPO activity was similar in both groups, with higher levels at 120h p.s.

Conclusion: The triglycerides serum level was altered in the course of wound healing and may be associated to skin lesion, while PPAR-α agonist acts in wound repair by accelerating healing and modulating neutrophil influx to the skin. Finally, our results suggested that PPAR-α may be an important target for novel therapies aimed at improved wound healing, especially if the receptor agonists are used as topical formulations in extensive lesions.

Financial support: FAPESP, CNPq, FCFRP-USP.

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INVOLVEMENT OF SOCS2 IN THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Introduction: The experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating is the commonly employed model for the study of the immunopathogenesis of human multiple sclerosis. EAE is characterized by a Th17 response in its initial phase, following by a Th1 profile during later phases. Suppressors of Signaling Cytokines (SOCS) proteins family regulate the production of several cytokines in various models of inflammation. However, their involvement in EAE is unclear. The objective of this study was to analyze and characterize the involvement of SOCS2 in the immunopathogenesis of EAE.

Method: EAE was induced both in C57Bl/6(WT) and $SOCS2^{-/-}$ mice using Myelin Oligodendrocyte Glycoprotein (MOG) and Freund adjuvant and the clinical scores and body weight were monitored. In addition, at 14 and 28 days after injection (dpi), the inflammatory levels in the spinal cord samples were analyzed.The production of cytokines and chemokines (IFN-g, IL-17, TNF-a, RANTES and MCP-1) in the brain were assessed by ELISA.

Results: $SOCS2^{-/-}$ mice displayed decreased severity of EAE at 14dpi, the peak phase of the disease, when compared to WT, which correlated with a lower percentage of weight loss. However, knockout mice showed lower remission rates at the late phase. Histopathological analysis of the spinal cord of deficient animals demonstrated a reduction in parenchymal inflammation, meningitis and demyelination. Levels of proinflammatory cytokines (FN-g, IL-17, IL-6, TNF-a) and chemokines (RANTES and MCP-1) were reduced in the brains of $SOCS2^{-/-}$ mice at the early and late phase when compared with WT. While the production of chemokines in the late phase was reduced it was significant when compared with WT counterparts.

Conclusions: In summary these results indicate that SOCS2 protein has a role in the pathophysiology of EAE.

Financial support: CAPES, CNPq, FAPEMIG, NIH

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TITLE: EVALUATION OF THE MECHANISMS DERIVED FROM TLR2 ACTIVATION INVOLVED IN THE INHIBITORY EFFECT OF HIV-1 REPLICATION

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Introduction: HIV-1 infected patients have increased intestinal permeability, which grants a significant amount of microorganisms passage to the blood circulation, event known as microbial translocation. Various Toll like receptor (TLR) ligands can be found among the translocated products. These receptors are activated by certain microorganism's components and, therefore, may be activated by a large variety of pathogens. TLR activation induces the synthesis of a wide variety of cytokines and chemokines, modulating the function of HIV-1 target cells. Studies carried in our lab show that TLR2 activation results in potent inhibition of HIV-1 replication in lymphocytes and macrophages. This phenomenon is mediated by the cytokine IL-10 and β-chemokines. However, facing the potent innate immune response triggered by TLR2 activation, with a large variety of inflammatory products, it is possible that other cellular factors may be involved in the inhibition of HIV-1 replication by this receptor in infected cells. Thus, we intend to evaluate if other mechanisms take part in the TLR2 mediated anti-HIV-1 effect.
Methods and Results: Human primary macrophages, obtained from healthy donors, were treated with different TLR2 agonists (Zymosan, Pam2CSK4 and Pam3CSK4) in order to analyze the cell production of IL-27 (qPCR and ELISA) and other soluble mediators by ELISA. Preliminary results suggest that uninfected human primary macrophages exposed to TLR2 ligands for 24 hours presented increased production of IL-27 (200,8 ± 114,9 pg/mL; n=4 for Pam3CSK4 and 216,4 ± 123,9 pg/mL; n=4 for Pam2CSK4), which is a cytokine capable of potent inhibition of HIV-1 replication. In the same way, IL-10, another cytokine endowed with anti-HIV-1 properties, had it's synthesis increased (201,1 ± 91,3 pg/mL; n=4 for Pam3CSK4; 82,3 ± 13,7 pg/mL for Zymosan and 128,4 ± 40,8 pg/mL for Pam2CSK4) by the same stimulus. Finally, our results also show that CCL5, a β-chemokine that binds to the same co-receptor used by HIV-1 to infect cells, thus impeding virus attachment, had increased concentrations (245,3 ± 84,2; n=4 for Pam3CSK4; 112,7 ± 22,3 pg/mL for Zymosan and 268,7 ± 138,5 pg/mL for Pam2CSK4) after the treatment.
Conclusion: It's probable that the TLR2 mediated HIV-1 inhibition is total or partially dependent on these soluble factors. We intend to perform functional essays to evaluate the role of these molecules in the HIV-1 inhibition mediated by TLR2 activation.
Funding: CNPq, Faperj, POM/IOC

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Conjugated Linoleic Acid ameliorates mucosal damage and prevents weight loss in a murine model of colitis

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Introduction: The inflammatory bowel diseases (IBD) include ulcerative colitis and Crohn's disease, if they are characterized by an intense inflammatory response in the gastrointestinal tract.
There is strong evidence that inflammatory bowel diseases are the result of an imbalance between the commensal bacterial flora and immune apparatus of the intestinal mucosa where several molecules present are altered, involving various aspects of innate immunity, inflammatory response and mucosal barrier. Among the proposed therapeutic and preventive to IBD, is the Conjugated Linoleic Acid (CLA), which consists of a fatty acid isomers that have been studied extensively at the expense of their promising actions linked to human health. In-vitro studies with the use of immune cells and animal models have shown that CLA can modulate immune function and inflammatory response **Objetive:** This study aims to determine the effect of administration of CLA through dietary supplementation for 4 weeks at 0, 1,0% of CLA isomers (50:50) in mice with inflammatory bowel disease induced by model of experimental colitis by dextran sulfate sodium (1,0% 7 days) **Results:** Supplementation of CLA in the diet before the induction of colitis decreased mucosal damage; maintained cytokine profiles (IL-4 & IL-10) resembling those of naive mice, preventing decrease of serum and secretory IgA and weight loss. An anti-inflammatory profile of macrophages in the inflamed tissue of the CLA-fed mice may be one of the mechanisms involved in this prevention. **Conclusion:** CLA mediated protection against experimental colitis and might represent a novel tool in IBD prevention.

Financial Support:FAPEMIG, CAPES and CNPq.

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SEPSIS-INDUCED IMMUNOSUPPRESSION IS INCREASED IN TNFR1$^{-/-}$ MICE

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INTRODUCTION AND AIM: TNF is considered the main proinflammatory cytokine released during sepsis. It is well established that sepsis predisposes developmentof long-term immunosuppression, characterized by expantion of Tregs.We aimed to investigate the role of TNF and TNFR1 in development of sepsis-induced immunosuppression. **METHODS AND RESULTS:**C57BL/6 and TNFR1$^{-/-}$ (p55$^{-/-}$) mice were subjected to severe sepsis by cecal ligation and puncture (CLP) model and treated with antibiotic (20 mg/Kg for 3 days) to improve survival 6 h after CLP. Sepsis-induced immunosuppression was evaluated by susceptibility of sepsis-surviving mice to secondary infection,using a non-lethal pneumonia model induced by intranasal inoculation with *Legionella pneumophila* at 15th day after CLP .All naïve WT mice survived secondary infection, whereas 100% of the WT sepsis-surviving mice died within 7 days after secondary infection. Notably, TNFR1$^{-/-}$sepsis-surviving mice succumbed to secondary infection earlier than WT mice. In accordance, TNFR1$^{-/-}$ sepsis-surviving mice showed higher bacterial load than WT mice in 24 and 72h after secondary infection. FACS analysis revealed that the frequency of CD4^{+}CD25^{+}Foxp3^{+}Tregs in spleen were considerably higher in WT sepsis-surviving (11.0±1.1%) than naïve mice (6±1.02%). Moreover,Treg frequency in TNFR1$^{-/-}$ sepsis-surviving mice was higher (15±2.22%) than WT sepsis-surviving mice. Analysis of proliferation of CD4 T cells *in vivo* (BrdU assay) reveled that TNFR1$^{-/-}$ sepsis-surviving mice exhibit lower proliferation than WT mice. Finally, we examined IL-10 and TGF-β, a Treg-related cytokines, in both mice. TGF-β levels in lung tissue, but not IL-10, were considerably increase of TNFR1$^{-/-}$ and WT sepsis-surviving mice compared (80 and 85pg/mg) their naives. **CONCLUSION:** The results suggest that TNF via TNFR1 is involved in control of expansion of Tregs during sepsis-induced immunosuppression.

FINANCIAL SUPPORT: CNPq, FAPESP, FAEPA.

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CHRONIC EXPERIMENTAL MODEL OF RESPIRATORY ALLERGY TO *BLOMIA TROPICALIS*

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Introduction: Chronic exposure to inhaled allergen can causes airway inflammation, remodeling and dysfunction typical features of allergic asthma. The mite *Blomia tropicalis* is the more frequent sensitizer agent in tropical regions, including in cities of Northeastern Brazil. The aim of the present study was to investigate the impact of exposure to repeated doses of *Blomia tropicalis* extract on airway inflammation and humoral responses in sensitized mice.
Methods and Results: Groups of 5-6 BALB/c male mice were sensitized with B. *tropicalis* extract (BtE) (100 µg/animal), adsorbed into alum (4 mg/animal), intraperitoneally, on days 0 and 7. The challenge was carried out by intranasal instillation with BtE (25µg/animal) four times/week during 4 weeks. The negative control group was sensitized with alum and challenged with sterile PBS. After 33 days of exposure to repeated doses of BtE, animals were euthanized and the following parameters were evaluated: a) total leukocytes count in the bronchoalveolar lavage fluid (BALF); b) IL-5 level in the BALF; c) serum levels of specific IgE, IGg1 and IgG2a antibodies obtained by indirect ELISA. Animals challenged with 25µg/animal of *B. tropicalis* extract showed an infiltrate of inflammatory cells ($p<0.05$) and increased levels of IL-5 ($p<0.05$) in BALF and high levels of specific IgE ($p<0.05$), IgG1 ($p<0.001$) and IgG2a ($p<0.001$) anti-BtE in serum, compared to non-allergic mice.
Conclusion: The chronic exposure to BtE have shown be able to stimulate an IL-5 mediated airway inflammatory process as well as humoral responses representative of allergic process. Additional experiments are in progress in our laboratory in order to further characterize other parameters such as hyper-reactivity and lung remodeling of this model.

Financial support: CNPq and FAPESB

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EVALUATION OF THE EFFECT OF ACETONE EXTRACT of Lafoensia pacari *IN VIVO*

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Introduction: *Lafoensia pacari* (Lp) is a typical tree from Western Central of Brazil whose stem barks have been traditionally used for the treatment of human gastric ulcers and skin wounds. Phytochemical studies of extracts of *L. pacari* revealed the presence of tannins, free sterols, saponins, triterpenes and ellagic acid. As part of a general study on the activities of *L. pacari* we have shown that the ethanol extract of this plant stimulates the synthesis of antibodies against a thymus-dependent antigen and inhibits the carrageenan-induced cellular migration. In the present work, we evaluated the effect of the acetone extract of *L. pacari* on body, liver and spleen weight as well as on total and differential peripheral leucocytes and spleen cells.

Methods and Results: To elucidate the activity of the acetone extract of *L. pacari* on body, liver and spleen weight as well as on total and differential peripheral leucocytes and spleen cells, ten adult C57BL/6 mice were randomly divided into two groups: group 1: the animals were left without treatment (control); group 2: mice were treated with acetone extract of *L. pacari* (AELp) (200 mg/kg daily p.o) for three weeks. Blood samples were collected from the retro orbital plexus before starting treatment and on days 7, 14 and 21 for the measurement of peripheral blood leukocytes. At the end of the experiment, the animals were euthanized, spleen and liver were removed and weighed individually, and the spleen cells were obtained. The animals were weighed on day zero and at the end of the experiment. The total peripheral blood leukocytes

and spleen cells were counted manually using a Neubauer chamber and the subpopulations of leukocytes were counted in blood smears stained by Panoptic fast. A phytochemical screening showed that the acetone extract of *L. pacari* contains tannins, saponins, fixed acids and steroids - phenolic hydroxyl and aliphatic groups without carbonyl.

There was no statistical difference in body, liver and spleen weights between the groups or within each group. The acetone extract of *L. pacari* did not alter the spleen cell number, total leukocytes counts, and subpopulations of peripheral leucocytes between the groups nor between each group.

Conclusion: We concluded that the acetone extract prepared from stem barks of *L. pacari* does not alter the amount of mature cells of the immune system, neither peripheral blood nor in the spleen.

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STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH (SHED) DECREASE THE ABILITY OF MONOCYTE-DERIVED DENDRITIC CELLS (mo-DC) TO INDUCE T CELL PROLIFERATION

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Introduction: Mesenchymal stem cells have immunomodulatory properties, which could have clinical applications, which are, however, limited by the restricted availability of these cells. On the other hand, SHED are readily accessible, but their immunomodulatory properties have not been studied. This study was designed, therefore, to evaluate SHED immunomodulatory effects on DC differentiation, maturation and ability to activate T cells.

Methods and Results: SHED and peripheral blood mononuclear cells (PBMC) were obtained from unrelated donors (n=4). PBMC were induced to differentiate into mo-DC by culture in the presence of IL-4 and GM-CSF for 7 days. LPS, added at day 5 of culture, was used to induce maturation of mo-DC. Effects of SHED were analyzed by flow cytometry, after their addition to the cultures from day 0 or fifth day of culture, at a 1:10 ratio. Mo-DC exposed to SHED since day 1 showed decreased median fluorescence intensity (MFI) of BDCA-1 (70 ± 38%) and CD11c (32,5 ± 16%) markers, in comparison to control; and when activated by LPS, decreased MFI levels of CD40 (52 ± 28%), CD80 (35 ± 34%),

CD83 (67,4 ± 32%) and CD86 (50 ± 56%), in comparison to control. Mo-DC exposed to SHED from the fifth day of culture did not show changes in markers' expression. To assess the ability of day 0 SHED-exposed mo-DC to activate T cell responses, mo-DC (HLA-DR$^+$) were separated from SHED (HLA-DR$^-$), by magnetic beads, and were cultured (1:10 ratio) with non-adherent PBMC (labeled with CFSE). After 5 days, cell proliferation was assessed by dilution of CFSE. CD4$^+$ T cell proliferation induced by SHED-exposed mo-DC decreased by 63% (immature mo-DC) and 50% (LPS-activated mo-DC) in comparison to control mo-DC. Likewise, CD8$^+$ T cell proliferation was decreased in 40% and 26% in culture with mo-iDCs and mo-mDCs, respectively, when differentiation occurred in the presence of SHED.

Conclusion: This study shows that SHED affects mo-DC differentiation, a phenomenon that is reflected on the reduction of DC maturation markers and by a decreased ability of SHED-exposed mo-DC to induce T cells proliferation. The further characterization of this phenomenon may support the use of SHED in immunomodulatory approaches using dendritic cells in clinical settings.

Financial support: FAPESP

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GENETIC ASSOCIATION STUDY OF POLYMORPHISMS FOXP3 AND HLA-G IN WOMEN WITH CERVICAL LESIONS

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Introduction: Cervical intraepithelial neoplasia (CIN) comprise a number of dysplastic changes, or abnormal cell maturation. It is observed is the probability that these lesions progress to cervical cancer. Some molecules have been framed as diagnostic markers and has been a central theme of several studies. The Human leukocyte antigen-G (HLA-G) is a non-classical major histocompatibility complex class Ib molecule. This peptide acts in immune regulation, acting in the phenomenon of immune tolerance. In cancer, this molecule seems to contribute to an escape of tumor cells to immune response. Futhermore, the forkhead box P3(FOXP3) is a transcription factor that acts in the suppression of regulatory T cells and in the repression of oncogens. Some studies have demonstrated the importance of FOXP3 and HLA-G in cancer cases and detection of polymorphisms of these molecules in body fluids may be an indicator of prognosis in cases of cervical lesions. The aim of the study was determination the presence of genetic polymorphism of FOXP3 and 14-bp insertion/deletion (Ins/Del) polymorphism in exon 8 of the 3' untranslated region of the HLA-G gene in blood samples, and its relation to the progress of the disease and prognostic data involved in cervical lesions. **Methods and Results:** Thirty-eight women with cervical lesions were initially selected to take part in this genetic association study. They were diagnosed with chronic cervicitis (n = 7), CIN I (n=17), CIN II (n=2), CIN III (n= 12) by pathology and classified according with the Richart (1986) to diagnostic cervical intraephitelial neoplasia (CIN I, CIN II and CIN III). For the detection of the FOXP3 (C-2383T, rs3761549) and HLA-G (14-bp deletion/insertion) polymorphisms, the polymerase chain reaction (PCR) system was used, and digested with

restriction. The distribution of HLA-G del/del, del/ins and ins/ins in patients was 23.7%, 47.4% and 28.9%. When comparing cases with control group (n=192) results significant were not detected. The distribution of genotypes transcription factor FOXP3 (C-2383T, rs3761549) CC, CT and TT was 65.8%, 28.9% and 5.3%. Significant results (p=0.006) were observed when comparing the genotype frequency between cases and controls (n=81). **Conclusion:** Considering the critical role of both molecules in the immune response, future investigations should be focused on understanding the exact role of HLA-G genetic variants and of transcription factor FOXP3 in the cervical lesions.

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INTERPLAY BETWEEN *HELICOBACTER PYLORI* INFECTION AND POLYMORPHISMS 14PB INSERTION / DELETION OF THE GENE HLA-G AND FOXP3 2383 C / T

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Introduction: Human leukocyte antigen-G (HLA-G) is a non-classical HLA class Ib molecule shown to exhibit immunomodulatory function in a wide range of immune-based disorders. Recent reports indicate that the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene influences the HLA-G mRNA stability and isoform splicing patterns, thus modulating the levels of HLA-G expression. Some studies have demonstrated which a 14-bp insertion/deletion (Ins/Del) polymorphism in exon 8 of the 3' untranslated region of the HLA-G gene seems to be involved in autoimmune diseases and viral infections. The Treg cells are characterized by expression of the transcription factor forkhead box P3 (FoxP3). The 2383C/T polymorphism is located in the promoter region of the FOXP3 gene. Polymorphisms in the FOXP3 gene can alter the FOXP3 factor either quantitatively or functionally, leading to a dysfunction of Treg cells and consequently to the development of autoimmune diseases. However, neither was found about the relationship of these polymorphisms in bacterial infections, specifically those related to *Helicobacter pylori*.

Methods and Results: Genomic DNA was extracted from the peripheral blood of patients and controls by salting out technique, as described by Miller et al (1988) with some modifications. The present study analyzed by conventional PCR the 14-bp insertion/deletion polymorphism of exon 8 HLA-G gene and transcription factor FOXP3 (C-2383T, rs3761549) in 60 and 50 patients with infection by *Helicobacter pylori,* respectively and 192 healthy controls. The distribution of HLA-G del/del, del/ins and ins/ins in patients was 30%, 50% and 20% while the genotypes transcription factor FOXP3 (C-2383T, rs3761549) CC,

CT and TT was 80%, 14% and 6%. We did not find any significant difference between genotypes 14-bp ins/Del and the genotypes transcription factor FOXP3 (C-2383T, rs3761549) the frequencies of polymorphisms in relative when compared to controls group. Nonetheless, our findings are initials and interpreting our findings, important limitations must be considered. We carried out a single-center case-control study, and additional investigations with larger sample size are warranted to validate our findings.

Conclusion: In conclusion, future investigations should be focused on understanding the exact role of HLA-G genetic variants and of transcription factor FOXP3 in the *Helicobacter pylori* infections.

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IL-10 DEFICIENCY BLOCKS DIFFERENTIATION OF REGULATORY T CELLS AND DECREASES SEPSIS-INDUCED IMMUNOSUPPRESSION

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Introduction and Aim: Sepsis evolution is regularly accompanied by the development of a pronounced immunosuppression, which is characterized by a predominance of regulatory T cells (Treg) and an increased susceptibility to secondary infection (JMM. 85:495-506, 2008). IL-10 is a potent anti-inflammatory cytokine secreted by macrophages, Th2 cells, stimulated B cells and Treg. IL-10 is associated with Treg function (Nat. Rev. Immunol. 10:170-81, 2010). In the present study we investigated the role of IL-10 during immunosuppression post sepsis. **Methods and Results:** WT mice were subjected to severe sepsis induced by cecal ligation and puncture (CLP–100% of mortality without treatment) and treated with antibiotic (20mg/kg of ertapenem sodium by 3 days), resulting in 50% of survival (all experiment n=5-10). A high serum concentration of IL-10 was observed in lung of mice CLP-surviving mice at day 3 and 15 after CLP-lethal. To examine the role of IL-10 in the development of susceptible the secondary infection post-CLP, we used IL-$10^{-/-}$ mice. We did not detect any differences significant between IL-$10^{-/-}$ and WT mice in survival rate after CLP. At 15^{th} day after CLP, all WT sepsis-surviving mice succumbed to nonlethal secondary infection induced by *Legionella pneumophila* and increased of bacteria in spleen and lung, which were accompanied by enhanced number of M2 macrophages. Interestingly, sepsis-surviving IL-$10^{-/-}$ mice submitted to nonlethal pneumonia induced by *L.*

pneumophila showed reduced levels of bacteria in spleen and lung, and a strongly improved survival (60% of survival rate), although an increase of M2 macrophages number has also been observed in sepsis-surviving IL-$10^{-/-}$ mice. However, sepsis-surviving IL-$10^{-/-}$ mice showed a decreased of TGF-β production. Moreover, an increased number of Tregs markers ($CD4^{+}CD25^{+}FOXP3^{+}$) were observed in spleen from WT CLP15d mice, which this increase was not present in IL-$10^{-/-}$ CLP15d mice. Finally, we observed the conversion of $CD4^{+}CD25^{-}Foxp3^{-}$ T cells into $Foxp3^{+}$ Tregs by M2 macrophages is dependent on IL-10. **Conclusion:** These results point to an important role of IL-10 in the development of Tregs and establishment to sepsis-induced immunosuppression, but the mechanisms involved in this increase of Tregs is still unclear.

Financial support: CAPES, FAPESP.

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THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND EXOGENOUS GLUCOCORTICOIDS IN THE MODULATION OF EXPERIMENTAL INFLAMMATORY BOWEL DISEASE

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Introduction: Inflammatory immune responses may be modulated by the hypothalamic-pituitary-adrenal axis (HPA) through neuroimmunoendocrine interactions and cortisol secretion. However, even in the presence of intact adrenal glands patients may develop chronic diseases such as Inflammatory Bowel Disease (IBD), which may be caused by an imbalance between regulatory and effector responses in the intestinal mucosa. On the other hand, although glucocorticoids (GC) are used to treat IBD, cortisol-producing adrenal glands are also involved in stress response, which may predispose to uncontrolled inflammatory diseases. Then, our objective was to investigate the role of the HPA axis and exogenous glucocorticoid treatment in experimentally induced IBD.
Methods and Results: C57BL/6 mice were subjected to bilateral adrenalectomy and after a 15 day-surgery recovery period the colitis was induced by oral intake of water containing 3% Dextran Sulfate Sodium (DSS) for 6 consecutive days. Animals were daily assessed for weight loss and clinical signs of disease. Mice were sacrificed at 6^{th} day of colitis induction and colon samples were collected to assess cytokine production by ELISA. Blood samples were also obtained for evaluation of circulating leukocytes. Our results showed that colitis was more severe in mice subjected to adrenalectomy, which showed greater weight loss, increased disease clinical score and earlier mortality when compared to colitis group. The absence of adrenal glands was also related to an increase in pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-17 in the gut, along with an augmentation of IL-10, probably in an attempt to compensate for the exacerbated inflammatory response. Moreover, these local alterations were accompanied by diminished circulating eosinophils in the blood of adrenalectomized mice when compared to colitis, which had eosinophilia. On the other hand, mice with colitis and treated exogenous GC showed worse severity of the disease, despite a lower production of proinflammatory cytokines and decreased peripheral blood eosinophils, indicating that corticoids and neuroimmune interactions may be involved in the maintenance of the peripheral leukocyte pool and control of exacerbated responses in the gut mucosa.
Conclusions: Taken together, our results showed HPA axis and GC play an important role in the regulation of systemic leukocytes and especially local inflammatory response in the gut. **Financial support:** CAPES/FAPESP

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DIFFERENTIAL EXPRESSION PROFILE OF 2'5'OAS GENES ON SYSTEMIC SCLEROSIS:UPREGULATION OF OASL AND OAS2

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Introduction: Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular damage, production of autoantibodies against nuclear antigens and excessive fibrosis which occurs on skin and internal organs of the patients. It is a complex disease with multifactorial causes that can be triggered by several putative factors, ranging from infections to toxin exposure. Genetic factors and problems in homeostatic immune regulation are considered to be major contributors to SSc predisposition. As in other autoimmune diseases, immune system homeostasis is compromised in SSc patients. Unique profiles of circulating cytokines, chemokines and altered lymphocyte populations are detected on patients when compared to healthy subjects. Similar to what happens in other diseases, several alterations on the type I interferon (IFN) system are found on SSc patients. The 2'5'OAS gene family comprises four different genes named OAS1, OAS2, OAS3 and OASL. These genes are induced by IFN stimulation, the expression of these genes can be regulated by different stimulus and happen in an orderly fashion. Participation of 2'5'OAS gene products on regulatory processes probably explains why these genes can have impact on autoimmune diseases. In a study related to Systemic Lupus Erytemathosus (SLE), it was shown that OAS1, OAS2 and OAS3 are upregulated in patients with active SLE. The mRNA levels of OAS2 are shown to be increased in SSc patients, but so far there is no information regarding other 2'5'OAS genes expression on the disease. Our goal in this study are evaluated the expression to of all four human 2'5'OAS gene family members in patients with SSc.

Methods and Results Quantitative PCRs were designed to evaluate the levels of each 2'5'OAS gene in PBMCs obtained from patients or healthy donors. Patients' cells were also treated with type I or type III IFNs, in order to evaluate if these cells are able to induce all four 2'5'OAS genes after stimulation. We observed thar OASL and OAS2 genes are upregulated ($p < 0,005$) on SSc patients, while OAS1 and OAS3 are found in normal levels. All 2'5'OAS genes are induced after treatment of patients cells with type I or type III IFNs.

Conclusion Increased levels of OASL and OAS2 probably are a result of cell exposure to specific inductors of these genes. Their regulatory activities on patients' cells can be an important factor to SSc immune alterations.

Financial support: CNPq, CAPES and FAPEMIG

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ROLE OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELL THERAPY IN A MODEL OF NEPHROTOXIC INJURY

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Introduction: Acute and chronic kidney injury (AKI) and (CKI) are responsible for a significant percent all of acute kidney failures and continue to be associated with high rates of mortality and the need of long term replacement therapy. In view of a lack of more efficient therapies, studies have focused on the potential of mesenchymal stem cell treatment and its mechanisms. Our study aimed towards the understanding of the role of adipose tissue-derived stem cell (AdSC) treatment in a murine model of acute and chronic kidney injury induced by folic acid (FA).

Methods and Results: AKI and CKI models were induced by FA (200mg/kg, i.p.) administered into male FVB mice of 8 weeks and after 24h, AdSC (1.10^6 cells/animal, i.p.) were injected. Animals were euthanized 48h and 4 weeks after FA to evaluate AKI and CKI (ctr, FA and FA+AdSC n= 3,5 and 5 mice respectively). Blood and tissue samples were harvested for function (serum urea), protein (multiplex) and histology (immunostaining and PicroSirius) assays. Significance was determined by ($p<0,05$) and values are shown as (mean±SD). Acute serum urea levels showed that AdSC treatment induced significant functional protection in comparison to FA-only treated animals (FA+AdSC, 106,9 mg/dL ± 29,03 vs. FA, 271,8 mg/dL ± 107,9). This data correlated with lower protein expression of inflammatory cytokines and chemokines, significant for KC (FA+AdSC, 76,28 ug/mL ± 52,07 vs. FA, 280,20 ug/mL ± 42,14) and tendency for GM-CSF, MIP-1a, MCP-1 and IL-6. Chronic assays demonstrated that at long term, AdSC treatment reduced kidney fibrosis, seen through PicroSirius staining (FA+AdSC, 0,0037 (% of total area) ± 0,0014 vs. FA, 0,0105 (% of total area) ± 0,0013%) . These animals also had reduced kidney protein expression of inflammatory cytokines and chemokines, significant for Eotaxin (FA+AdSC, 3,90 ug/mL ± 0,60 vs. FA, 5,80 ug/mL ± 0,64) and IFNg (FA+AdSC, 5,23 ug/mL ± 2,81 vs. FA, 13,04 ug/mL ± 3,06) and a tendency for, IL-2 and MIP-1a. Together, AKI data revealed that AdSC preserved tissue function and reduced acute tissue inflammation induced by FA. In addition, CKI results revealed that AdSC treatment protected against kidney fibrosis and chronic inflammation.

Conclusion: In summary our study demonstrated that AdSC were able to protect against AKI and CKI by preserving kidney function, modulating tissue inflammation, and reducing kidney fibrogenesis.

Financial Support: CNPq, FAPESP, Ethics Committee: (CEP: 0664/10)

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ANTI-INFLAMMATORY EFFECT OF A FIBRONECTIN-BINDING PROTEIN EXPRESSING *LACTOCOCCUS LACTIS* CONTAINING A EUKARYOTIC DNA VECTOR CODING FOR INTERLEUKIN 10 USING A MURINE MODEL OF CROHN´S DISEASE

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Introduction: Interleukin-10 (IL-10) is the most important anti-inflammatory cytokine at intestinal level and its absence is involved in inflammatory bowel diseases (IBD), such as Crohn's disease. However, oral treatment with IL-10 is limited due to its low survival in the gastrointestinal tract (GIT) and systemic treatments lead to undesirable side effects. In this context, the aim of this work was to evaluate the anti-inflammatory effect of the administration of an invasive *Lactococcus lactis* strain (*L. lactis* FnBPA+ that expresses the fibronectin-binding protein A of *Staphylococcus aureus*) capable of delivering a eukaryotic expression vector (pValac) containing the gene coding for IL-10 of *Mus musculus*, using a Trinitrobenzenesulfonic acid (TNBS) induced Crohn´s disease mouse model, as a new strategy for the prevention and treatment of IBD.

Methods and Results: For this purpose, the pValac:*IL-10* plasmid was firstly constructed and the expression of IL-10 by cells of the Chinese Hamster Ovary cell line, after transfection with the constructed plasmid, was confirmed by

Confocal Microscopy and Flow Cytometry. Conventional BALB/c mice received an intra-rectal inoculation of TNBS to induce intestinal inflammation similar to human Crohn's disease. These mice then either received bacterial supplementation (10^8 UFC/mouse/day) of *L. lactis* FnBPA+ (pValac) (Wt-group), *L. lactis* FnBPA+ (pValac:*IL-10*) (pValac:*IL-10* group) or saline solution (control group). Large intestines were removed, visually inspected for macroscopic evaluation and prepared for histological evaluation and immunohistochemistry using standard methods. The liver was aseptically homogenized and serial dilutions were plated in different growth media to determine microbial translocation. Mice from the pValac:*IL-10* group showed lower damage scores in their large intestines (at both macroscopic and microscopic levels), decreased numbers of IL-17 producing cells and lower microbial translocation to liver, compared to mice from the Wt-group or those that did not receive bacterial supplementation (control group).

Conclusions: Administration of *L. lactis* FnBPA+ containing the pValac:*IL-10* was effective in the prevention of inflammation in a murine model of Crohn's disease, confirming the potential use of therapeutic plasmids delivered by lactic acid bacteria, such as *L. lactis*, for the prevention and treatment of a diverse array of diseases.

Financial support: CAPES, CNPq, CONICET, ANPCyT and CIUNT.

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STUDY OF THE RELATIONSHIP BETWEEN *Toxocara canis* INFECTION AND THE DEVELOPMENT OF THE ALLERGIC PROCESS.

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Introduction: Several studies are investigating the protective effect of infectious agents against allergic reactions. Current researches demonstrate the ability of some human helminth of modulating the immune response of their hosts. The objective of this study was to investigate the relationship between parasitism by *T. canis* and the development of an allergic response in mice immunized with ovalbumin (OVA).

Methods and Results: The number of leukocytes and eosinophils was determined in the blood, peritoneal cavity (PC) and bronchoalveolar lavage fluid (BALF) of 20 Balb/c mice. These animals were divided into four groups (G1, G2, G3 and G4) all of which were challenged (intranasally) with OVA at 12 and 17 days of the experiment. G2 and G4 were also immunized (subcutaneously) with a mixture of OVA and aluminum hydroxide at day zero and 7, and G3 and G4 were infected (intragastrically) with embryonated eggs of *T. canis* on day zero. On the 18t^{h} day, absolute leukocytes counts were assessed in a Neubauer chamber and the absolute number of eosinophils was derived from relative differential leukocyte counts on smears stained with Panotic. Statistical analysis were made by one-way ANOVA ($p < 0.05$). The G4 showed an increase in the number of total blood leukocytes when compared with G1 and G3. Although the number of leukocytes in the BALF did not present any significant change, we could observe a decrease when comparing the G4 with G2. It was observed an increase of total leukocytes in the PC just in G3 comparing with G1 and G2. The eosinophil counts in the blood became clear that the experimental conditions can induce increases in the number of these cells and that this increase is more significant in the presence of both, infection and OVA (G4). In the BALF, G2 and G4 presented a significant increase in the eosinophils numbers when compared with G1 and G3. This suggests that the eosinophilia in this fluid is due to the OVA and not to infection. Though the comparison between G2 and G4 haven't been significant, we could note a mild decrease in the eosinophils of

G4, what could indicate a immunomodulatory role by the parasite in the lung. The experimental conditions did not affect the eosinophils influx in the PC. **Conclusion:** At the 18° day post-infection, the parasite *T. canis* corroborates with the increase of the eosinophils in the blood and BALF, although the results with the BALF suggest a possible inhibitory role on the eosinophils influx in the lungs in a more late phase of infection.

Financial Support: None.

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IL-22 RESTRAINS THE CONTRACTION OF ACTIVATED IMMUNE RESPONSE AND THE DEVELOPMENT OF REGULATORY T CELLS IN NON-PATHOLOGICAL SKIN INFLAMMATION

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Introduction: Adult skin consists of a keratinized stratified epidermis and an underlying thick layer of collagen-rich dermal connective tissue that provides support and nourishment to the resident cells. The skin is a protective barrier against the outside environment and any injury in it must be rapidly and efficiently repaired. Wound healing is a complex process requiring the collaborative efforts of many different tissues, cells and a plenty of cytokines like IL-22, which promotes epithelial cell proliferation and repair, thereby improving tissue healing. However, the involvement of IL-22 in cutaneous wound closure is still not well understood. Then, this work aimed to understand the role of IL-22 in the inflammation and repair of skin wounds. **Methods and Results**: Surgical skin lesions were induced in C57BL/6 wild type (WT) or IL-22 deficient (IL-22$^{-/-}$) mice which were followed during 120h for clinical and wound closure evaluation. The results showed that IL-22 deficiency caused a delay in wound closure. It was noted that in the absence of this cytokine the lesions were apparently less inflamed with reduced edema and a thinner layer of fibrin, as visualized in macroscopic analyses. In addition, mice submitted to skin lesions presented loss of weight independent on IL-22 in the first 4 days post surgery, showing that the stress to which these animals were exposed or the extension of the cutaneous wounds may affect systemic metabolism as observed in various clinical conditions. Furthermore, in the absence of IL-22 there was a total higher number T lymphocytes, in special CD4^{+} T cells in the draining lymph nodes when compared to WT mice. Most interestingly, IL-22 seems to restrain the inflammatory response, since IL-22$^{-/-}$ mice presented a significant increase of CD4+CD25+CTLA-4+ cells and an apparent augmentation of this population expressing GITR. Moreover, although IL-22$^{-/-}$ animals had higher number of CD4^{+}CD25^{-} T cells in the lymph nodes, there was a significant increase in the

transcription factor Foxp3 and in the number of CD4+CD25-PD-1+ cells in these leukocytes when compared to WT animals.

Conclusion: These results suggested that the initial inflammation dependent on IL-22 is essential to a faster closure of surgical wounds. Moreover, IL-22 seems to restrain the development of regulatory T cells or the contraction of the activated immune response in the context of non-pathological skin inflammation.

Financial support: CNPq, FAPESP.

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
V Extra Section of Clinical Immunology - ESCI

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October 20 - 24, 2012

EFFECT OF INHIBITION OF MMP-13 GENE EXPRESSION BY RETROVIRAL- DELIVERED shRNA IN A MODEL OF EXPERIMENTALLY INDUCED PERIODONTAL DISEASE.

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Introduction and Objective: Targeted inhibition of MMP-13 has been actively studied as a therapeutic approach to diverse inflammatory conditions, including cancer, liver disease, rheumatoid arthritis and periodontal disease; however the use of biochemical inhibitor compounds has serious potential side effects due to the lack of specificity. This proposal intends to use a gene therapy approach using retroviral-delivered shRNA to specifically inhibit MMP-13.

Methodology and Results:

Murine models of experimentally-induced periodontal disease were used and inhibition of MMP-13 was achieved by injecting the affected gingival tissues with the retroviral vectors encoding shRNA for MMP-13. It was used 30 animals in an experimental period of 15 days. Injection of empty vector was used as control. The inhibition of the MMP-13 in the gingival tissues was analyzed by western blot. The effect of this inhibition on bone destruction and inflammatory gene expression at protein levels was evaluated by micro-computed tomography and ELISA, respectively. The inhibition of MMP-13 mediated by retroviral-delivered shRNA in these tissues was confirmed by western blot and decreased the expression of IL-6 and TNF-α in the periodontal tissues, as well as significantly reduced inflammation-induced alveolar bone resorption.

Conclusion: Targeted inhibition of MMP-13 reduces the severity of the inflammation associated with periodontal disease.

Financial Support: FAPESP (07/05583-3)

IMMUNO 2012
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October 20 - 24, 2012

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MMUNE MECHANISMS OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN NEWLY DIAGNOSED TYPE 1 DIABETES PATIENTS

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Introduction: High dose immunosuppression (HDI) followed by autologous hematopoietic stem cell transplantation (AHSCT) has emerged in last past years as a therapeutic alternative for newly diagnosed type 1 diabetes mellitus (T1D) patients. After transplantation, C-peptide levels increased significantly and the majority of patients achieved durable insulin independence with good glycemic control for variable time periods. **Objective:** To address the immune mechanisms of HDI/AHSCT in T1D patients, we evaluated the reconstitution of recent thymic emigrants, $CD4^+$ and $CD8^+$ regulatory T cell subsets and islet-specific autoreactive $CD8^+$ T cells after treatment. **Methods:** Blood samples were collected from healthy controls (N=16) and newly diagnosed T1D patients (N=25) at pre-transplantation (baseline) and at various time points after transplantation. Immunophenotyping was performed by flow cytometry. The frequencies of islet-specific $CD8^+$ T cells were quantified on PBMC samples using Qdots-labeled islet-peptide/HLA-A2 monomers and flow cytometry analyses. T1D patients were divided into two groups representing the upper and lower 50th percentile of cumulative frequencies of autoreactive $CD8^+$ T cells at baseline period. **Results:** We found increased ($p>0,05$) absolute numbers of regulatory $CD8^+CD28^-$ T cells at 3, 6, 9, 12, 18, 30, 36 and 42 (360,1±271,5

cells/µL) months post-HDI/AHSCT when compared with pre-transplantation period (166,2±95,8 cells/µL). The reconstitution kinetics of recent thymic emigrants ($CD3^+CD4^+CD45RA^+CD31^+$ T cells) and regulatory $CD4^+CD25^{hi}FoxP3^+$ T cells was faster than of total $CD4^+$ T cells. The $CD3^+CD4^+CD45RA^+CD31^+$ and $CD4^+CD25^{hi}FoxP3^+$ T cells reached baseline numbers at 18 and 54 months after transplantation, respectively. Patients with relatively high cumulative frequencies of autoreactive CD8 T cells at baseline relapsed rapidly after HSCT, resuming insulin-dependence to achieve good glycemic control. Conversely, patients with fewer autoreactive CD8 T cells at baseline remained free from exogenous insulin significantly longer ($p<0.006$). **Conclusions:** Our results suggest an improvement of the peripheral immunoregulatory mechanisms after HDI/AHSCT, which may contribute to reestablishment of self-tolerance and control of autoimmunity on these T1D patients. In addition, the frequency of $CD8^+$ T cells autoreactivity before HDI/AHSCT proved to be an immune correlate predicting clinical efficacy.

Financial support: FAPESP, CNPq, FAEPA

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
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October 20 - 24, 2012

CHLOROQUINE TREATMENT REDUCES THE SEVERITY OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS THROUGH THE STIMULATION OF REGULATORY T CELLS

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Introduction: Chloroquine (CQ), a drug-of-choice for the treatment of malaria, has shown to have some immune-modulatory properties, and it is been used as an adjuvant for HIV-infection therapy lately. We aimed to access whether CQ treatment is able to reduce the severity of experimental autoimmune encephalomyelitis (EAE).

Methods and Results: Mice (n=14/group) were treated with CQ (10mg/kg) for five consecutive days prior to EAE induction. Clinical signs were analyzed daily for thirty days. Ten days after the antigen administration half of the animals were killed and Ag-specific cellular proliferation was analyzed in cultures of splenic leukocytes stimulated with MOG35-55. At the end of the experiment, mice were killed and brains were collected for analysis of inflammatory cells of the CNS. Our data show that upon CQ treatment a significant reduction in EAE clinical score was observed. It was accompanied with a decrease in T cell proliferative response against MOG (EAE: 60%±7 vs CQ+EAE: 22%±5) as well as a significant reduction of infiltration of inflammatory cells in the CNS, which consisted mainly by Tregs and IL-10-producing cells.

Conclusion: The data presented here corroborate with previous findings showing that chloroquine may have an immune-modulatory function, and we show for the first time that chloroquine treatment suppresses the clinical signs of EAE through the stimulation of regulatory T cells.

Financial support: FAPESP (#2011/13191-3 and #2011/17965-3).

IMMUNO 2012
XXXVII Congress of the Brazilian Society of Immunology
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October 20 - 24, 2012

Schistosoma mansoni-derived lyso phosphatidylcholine (LPC) modulates macrophage function through TLR2 and PPARγ dependent mechanisms

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Objectives: Mansonic schistosomiasis is a disease caused by parasitic trematode *Schistosoma mansoni*, endemic to tropical countries, that causes pronounced modulation of the immune system enabling their chronic establishment in the host. We have recently demonstrated a role for parasite lipid-derived molecules, mostly lyso phosphatidylcholine (LPC), in the parasite-induced host immunomodulation, however the mechanisms involved are not well-understood. In the present study we aimed to investigate the role of *S. mansoni*-derived LPC to modulate the macrophage response, and the role of the nuclear receptor Peroxisome proliferator-activated receptor gamma (PPARγ) and the cytokine Macrophage migration inhibitory factor (MIF) in mediating these processes.
Methods and Results: Peritoneal macrophages obtained from wild type, TLR2-/- or MIF-/- mice were stimulated in vitro with lipids extracted from adult worms of *S. mansoni*. After 24 h we quantified the induction of lipid droplets by osmium staining and cytokines levels by ELISA. Pretreatment with the PPARγ inhibitor, GW9662, were performed in selected groups. We observed that lipids extracted from *S. mansoni*, mostly LPC, are capable to promote an increased formation of lipid droplets and release IL-10 and PGE_2 but not nitric oxide in macrophages in vitro after 24 h. The formation of lipid droplets induced by LPC was not significant different in $MIF^{-/-}$ compared to wild type macrophages after 24h. Treatment with LPC leads to a TLR-2 dependent increased PPARγ expression. In addition, schistosomal LPC-induced lipid droplet formation and IL-10 and PGE_2 production in macrophages were reduced by the treatment with GW9662, suggesting a role for PPARγ.
Conclusions: The results suggest that lipids of *S. mansoni* have imunomodulatory activity in macrophages capable to promote an increase of lipid droplet formation, PGE2 and IL-10 production by a mechanism partially dependent of PPARγ increased expression and function
Financial Support: FAPERJ, CNPq and IOC/FIOCRUZ