AUTOIMMUNITY (AI)
Introduction: Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that affects approximately 1% of the worldwide population. Although the pathogenesis of the disease is not fully understood, several studies have suggested that IL-17A-secreting CD4+ T cells (Th17 cells) may play a key role in the development and severity of RA. Th17 polarization is promoted by stimulation of the aryl hydrocarbon receptor (AHR), a ligand-dependent transcription factor that responds to a wide range of ligands, such as the tryptophan metabolite 6-formylindolo [3,2-b] carbazole (FICZ). Exposure of CD4+ T cells to FICZ under Th17-polarizing conditions enhances Th17 development and Ahr deficiency in T cells inhibits Th17 cell generation and the onset of experimental arthritis. Here, we analyzed the AHR-related genes activation and Th17 cells frequencies on RA patients samples.

Methods and Results: Peripheral blood cells were isolated from healthy volunteers and RA patients for analysis of CD4+IL-17+ frequencies and to obtain CD4+ T cells. For analysis of Th17 frequencies, peripheral blood mononuclear cells were stimulated with PMA (50 ng ml⁻¹) and ionomycin (500 ng ml⁻¹) in the presence of brefeldin A for 4h. CD4+ T cells were isolated by negative selection and the cell purity was confirmed using flow cytometry (>95%). Fresh isolated CD4+ T cells were immediately collected in TRIzol for posterior RNA extraction. The mRNA expression of AHR, CYP1A1 and IL-17 were analyzed and normalized to the expression of GAPDH. Frequencies of blood circulating Th17 cells (CD4+IL-17+) were higher in RA patients when compared with healthy volunteers. In addition, we also observed an up-regulation of AHR and IL-17 mRNA expression in CD4+ T cells of patients. To evaluate AHR activity we analyzed the mRNA expression of the AHR-target gene CYP1A1. It was found higher frequencies of CYP1A1 expression in CD4+ T cells from RA patients when compared to controls. Conclusions: In fact, our results show an up-regulation of AHR related genes to the higher frequencies of Th17 cells in rheumatoid arthritis patients. Indeed, we endorse that AHR is being activated in RA showing that this receptor is important to autoimmune disease development according to previous description in experimental murine arthritis.
ARYL HYDROCARBON RECEPTOR MEDIATES SMOKING-INDUCED RHEUMATOID ARTHRITIS EXACERBATION

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Introduction: Rheumatoid Arthritis (RA) is a multifactorial autoimmune arthropaty with unknown etiology that affects ~1% of worldwide adult population. RA is characterized by joint pain, intense immune cells infiltration in articular tissue, synovial hyperplasy (pannus) and bone destruction. Genetic and environmental factors are associated with RA development, but the interaction of these factors is not well understood. Genetic polymorphisms that can influence the susceptibility to environmental factors may be crucial in RA development. Among the environmental factors, cigarette smoking is the most studied and has been associated with increased susceptibility to RA. However, the mechanisms by which smoking aggravates RA remain unknown. Results: Using a sex/smoking pared samples of Rheumatoid Arthritis Patients and Healthy controls we found an Aryl hydrocarbon receptor (AHR, a key receptor for environmental pollutants) haplotype over-represented in RA patients. This haplotype contains an AHR single nucleotide polymorphism (SNP; rs2066853, allele A) that enhances AHR transactivation function and was related to higher Th17 frequencies in RA. Furthermore, smokers with this AHR haplotype are highly prone to develop RA than non-smokers or smokers without this haplotype. In experimental arthritis, AHR agonists exacerbated antigen-induced arthritis (AIA) in mice, whereas mice genetically deficient in Ahr develop a less severe form of AIA. Furthermore, smoking aggravated AIA in mice in an AHR-dependent manner. Conclusion: These data demonstrate that genetic polymorphisms at AHR are closely linked to smoking-induced RA aggravation, and that individuals with this genotype should be strongly warned against smoking. Financial support: FAPESP (2011/02505-7) and TIMER (HEALTH-F4-2011-281608)
B-1 CELLS IN THE METABOLIC CONTROL OF STZ-INDUCED DIABETES IN MICE.

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Introduction: A regulatory role of CD5+ B cells has being described in diabetes model. Nevertheless, the role these cells play in the pathogeny of diabetes is controversial. For this reason, this study was developed to investigate the involvement of B-1 cells in murine streptozotocin (STZ)-induced diabetes.

Materials and methods: C57/BL, BALB/c and BALB/xid (B-1 cells-deficient mice) male mice strains were treated intraperitoneally (i.p.) with STZ (40mg/kg) for 5 days. Animals were evaluated for blood glucose levels (BGL) and cell populations in the pancreas by flow cytometry. Our findings confirm that BALB/c mice did not develop diabetes when this dose of STZ was used. Unexpectedly, the dose of 40mg/kg induced diabetes in BALB/xid mice having higher BGL than C57/BL or BALB/c mice. To evaluate the role of B-1 cells in diabetes induction, peritoneal B-1 cells obtained from BALB/c mice were purified based on expression of CD19+CD23- and adoptively transferred i.p. to BALB/xid mice before or after the STZ-treatment. BALB/xid mice adoptively transferred with B-1 cells were not diabetic after STZ-treatment. B-1 cells infiltrate pancreatic islets in these animals. The B-1 cells migration capacity to islets substrate was also confirmed in vitro. To investigate if any factor secreted by B-1 cells was responsible for the protection of BALB/xid in STZ-induced diabetes model, we treated BALB/xid diabetic mice with B-1 cell culture supernatants, intramuscularly. BGL decrease in these animals 2 hours after injection and begins to return to normal levels 4 hours after STZ injection.

Conclusion: In conclusion, our data shown that B-1 cell deficient mice have a higher reactivity to STZ-treatment. In addition, B-1 cells migrate to pancreatic islets of non-diabetic STZ-treated mice and the treatment with B-1 cells supernatants regulates circulating BGL of BALB/xid diabetic mice in an insulin-like manner.

Financial Support: CNPq, FAPESP.
CD28 MPEG PV1-Fab', A SPECIFIC CD28 ANTAGONIST, INHIBITS EXPERIMENTAL AUTOIMMUNE UVEITIS PROGRESSION IN MICE.

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Introduction and aim: Experimental Autoimmune Uveitis (EAU) is an organ specific T-cell mediated disease model that targets the posterior pole of the eye. As to the involvement of T-cell mediated inflammation, cellular features of EAU resemble those of the human disease, being mainly CD4+ T lymphocytes exhibiting a Th1 phenotype in vivo. The CD28:B7 interaction activates an important signaling pathway in T lymphocytes and is known to be involved in different autoimmune diseases. However, little is known about the involvement of CD28 in ocular autoimmune diseases. Therefore, in this study, we evaluated the efficacy of CD28 mPEG PV1-Fab', a specific CD28 antagonist, in the treatment of EAU. Methods: Female B10.RIII mice were immunized subcutaneously with interphotoreceptor retinoid binding protein [IRBP] in Complete Freund Adjuvant [CFA] plus B. pertussis toxin [PTX] followed by the intraperitoneal inoculation of PV1 or NHS (Control) Fab' fragments [10mg/Kg] at 9, 13 and 17 days post-immunization (n=10-13/group). At day 21, eyes were collected for histopathological evaluation, leukocyte phenotyping, and cytokine production. Results: A decrease in disease onset of PV1-treated groups compared to NHS-treated and control groups (63.6% vs. 100% vs. 100%; n=10-13/group) was observed. General disease score was also significantly lower in the PV1-treated group when compared with the untreated group (0.76 vs. 1.54; n=10-13/group; p<0.05). These changes in disease severity were accompanied by a modified frequency of T cell populations infiltrating the eye in both treated groups. NHS-treated animals showed a higher frequency of CD3+CD4+ cells in their eyes when compared to both untreated and PV1-treated mice (n=4-5/group; p<0.05). Surprisingly, PV1-treated animals had a significantly higher frequency of CD3+CD8+ cells infiltrating the eyes (n=4-5/group; p<0.05). However, all three groups had the same frequency of T cell populations in their lymph nodes. In addition, it seems that PV1-treated mice had less pathogenic Th1 cells in their lymph nodes, on day 21, when compared with the other groups (n=4-5/group). Conclusion: Taken together our data confirm a modulating effect of PV1 on T lymphocytes during the course of EA. Although not expected, the higher frequency of T CD8+ lymphocytes in the eyes of PV1-treated animals could be a consequence of pathogenic T CD4+ cell depletion by the PV1 antibody fragment. Financial support: UNIEMP and Seventh Framework Programme.
Introduction: Streptococcus pyogenes is cause disorders related to autoimmune events, as poststreptococcal glomerulonephritis (PEGN). Neutrophils are implicated in immunocomplexes formation, meaning that they are key cells in the pathophysiology of PEGN, and, consequently, are a natural target for research in studies aided by in silico experiments. The objective of this communication is to present a computational modeling of the immune system (IS) for performing in silico experiments on the pathophysiology of PEGN.

Methods and Results: We performed a literature review – conducted in the database PubMed – in order to identify the previously published IS simulation strategies – as applicable to the study of S. pyogenes / PEGN. The investigations performed point to the use of the following models: ordinary differential equations, cellular automata, and multi-agent systems (MAS). We chose to focus on MAS opening possibilities for the verification of hypotheses about the way in which cells and cytokines interact in the IS. The literature review provided subsidies for the construction of a computational model based on MAS, denominated AutoSimune, using the framework Repast Simphony. The following entities were modeled and implemented: (1) agents – antigen (S. pyogenes), antibody, bacteria, viruses, and cells; (2) zones – tissue, lymph node, circulation, bone marrow, thymus, air way tissue, and kidney tissue; and (3) diffusion of substances (cytokines).

Among the simulated cells are neutrophils, modeled on AutoSimune with the following characteristics: When entering a zone of air way tissue or kidney tissue, the agent follows the signaling substance PK1 (stress factor released by tissues that are suffering damage due to infection or immune response), circling to find the site of infection. The neutrophil agent looks for cells that are emitting PK1 (stressed cells and / or infected), then performs phagocytosis.

The simulation includes important aspects of IS such as the inflammatory process, dead cells, pathogens, and antigen-antibody complexes. When the neutrophil agent lifetime expires, it suffers apoptosis. Initial experiments performed to investigate autoimmune events showed that the model presents behavior coherent with the current biomedical literature. Conclusion: In silico research – focusing neutrophil – will contribute to increase the knowledge about the mechanisms of the disease in the PEGN.

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DECREASED SERUM IL-22 LEVELS IN BRAZILIAN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by autoantibody production, which can affect people of all ages, gender and race of the world. The etiology and pathogenetic mechanisms of this disease have not been elucidated. Cytokines are strongly implicated in the pathogenesis of SLE and IL-22 has been described in this context, however studies about its role in SLE remains incipient. The present study aims to assess serum IL-22 levels from SLE patients of the Hospital das Clínicas of UFPE and its correlation with clinical manifestations and disease activity. Methods and Results: Ninety SLE patients (89 women, mean age 38 ± 10.6 years) who fulfilled the American College of Rheumatology classification criteria for SLE were enrolled in this study. Thirty healthy individuals (19 men and 11 women, mean age 34.9 ± 9.9 years) were also included as a control group. Clinical and laboratory parameters were recorded. Serum IL-22 concentrations were determined by specific ELISA kits according to the manufacturer’s recommendation (eBiosciences). Statistical analyzes were performed by univariate comparisons using nonparametric tests (Mann-Whitney tests). The results are shown considering the mean value. The IL-22 serum levels were significantly decreased in SLE patients (mean 78.25 pg/mL) compared with control (mean 230.3 pg/mL) (p<0.0001). In addition, we found a significant correlation in patients with higher IL-22 levels and anti-dsDNA positive (mean 133.5 pg/mL) when compared with the anti-dsDNA negative group (mean 69.11 pg/mL) (p=0.0238). However, according to previous studies, correlation analysis between serum IL-22 levels and disease activity evaluated by SLEDAI score or other clinical and laboratory parameters showed no association. Conclusion: Our results suggest a possible involvement of IL-22 in the pathomechanisms of the SLE. Furthermore, these data set support new insights about IL-22 role in SLE disease and its potential use as therapeutic target.

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DENDRITIC CELLS MODULATED BY CHLOROQUINE SUPPRESS THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction: Chloroquine (CQ), an anti-malarial drug, has been largely used in the treatment of inflammatory conditions, despite the reported toxic effects due to its chronic use. We have recently shown that administration of CQ reduces the clinical signs of Experimental Autoimmune Encephalomyelitis (EAE) (PLoS ONE 8(6): e65913, 2013). Evidences suggest that Dendritic Cells (DCs) may play a role in the CQ-mediated control of inflammation. The aim of the present study was to investigate the possible in vitro modulation of DCs by CQ, and whether the adoptive transfer of CQ-modulated DCs into EAE mice reduces the disease severity.

Methods and Results: Bone marrow-derived DCs were treated with CQ (dose range 5-100µM), dexamethasone (50µM) or vehicle (PBS), pulsed with MOG\textsubscript{35-55} peptide and stimulated with lipopolysaccharides. The flow cytometric analysis of DCs showed a significant reduction in MHC-II, CD-80 and CD-86 expression compared with the PBS-treated cells. Also, the ultra-structural analysis showed that CQ-treated DCs present altered morphology with reduction in dendropoiesis. Bead-isolated CD4\textsuperscript{+} T cells co-cultured with CQ-treated DCs proliferated significantly less compared with lymphocytes cultured with PBS-DCs. Finally, when adoptively transferred into mice (n=5) before EAE induction, CQ-modulated DCs were able to reduce the clinical and immunological signs of the disease such as clinical score, cellular infiltration in the Central Nervous System, and reduction in MOG-specific cellular response. To our knowledge, this is the first study showing the therapeutic use of CQ-modulated DCs in the control of a chronic autoimmune inflammation.

Conclusion: Our data suggest that adoptive transfer of CQ-modulated DC may be an alternative approach to overcome CQ toxicity.

Footnote: Supported by São Paulo Research Foundation (2011/17965-3; 2011/13191-3)
INTRODUCTION: We demonstrated that delayed gastric emptying in inactive Crohn disease (CD) was associated with dyspeptic symptoms (Gastroenterology, 140:5, 2011). Binnewies et al proposed that the delayed gastric emptying in CD was only observed during the disease activity (Gastroenterology, 142:5, 2012). Serum ghrelin levels are elevated in active CD and could modify the GI motility. AIM: To investigate the correlation between the dyspeptic symptoms, gastric emptying or serum ghrelin with clinical disease activity in CD. METHODS: Crohn Disease Activity Index (CDAI) were calculated in twenty patients (10 women, mean age 44 years) with CD, then they were submitted a gastric emptying test by breath test using 13C octanoic acid coupled to a solid meal and answered a validated questionnaire (The Porto Alegre Dyspeptic Symptoms Questionnaire - PadyQ). In addition, fasting and postprandial plasma total ghrelin level were determined by a commercially RIA kit. Spearman coefficient was used to assess the correlation between total PadyQ scores, gastric emptying (t 1/2 or t lag) or ghrelin level (fasting and postprandial) with CDAI. RESULTS: in patients with CD, there was a significantly correlation between total dyspeptic symptoms (r=0.6806; p=0.0005), or specific dyspeptic symptoms (pain in upper abdomen, nausea, vomiting, upper abdominal bloating and early satiety), with the activity of the disease. On the other hand, there were not statistical correlations between t 1/2 (r=-0.2348; p=0.1595) or t lag (r=-0.1978; p=0.2016) with CDAI. Serum ghrelin levels were elevated by fasting (615.9+/- 93.21; N=15) and reduced 90 min after the test meal (r=0.4485; p=0.0407), but not for fasting (r=0.3099; p=0.1305), in patients with activity of the disease. CONCLUSION: Dyspeptic symptoms, but not gastric emptying, correlated with clinical activity in Croh’s patients. There was a persistence of high levels of serum ghrelin in CD with activity of the disease. More studies need to be performed in order to evaluate the role of the persistence of high post prandial ghrelin levels with the presence of dyspeptic symptoms in CD. Financial support: CAPES, CNPQ.
EFFECT OF ANTI-RHEUMATIC DRUGS OVER MUSCLE WASTING IN EXPERIMENTAL INFLAMMATORY ARTHRITIS

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**Background:** Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by chronic inflammation of joints, associated with progressive disability and systemic complications like weakness and muscle loss. Muscle loss affects 30-60% of RA patients. It is still unknown whether the drugs used to treat RA also reduce muscle loss. The aim of this research was to evaluate the effect of etanercept (ETN), a tumor necrosis factor alpha inhibitor, and methotrexate (MTX) over muscle loss caused by collagen-induced arthritis (CIA).

**Methods and Results:** CIA was induced in male DBA/1J mice (Nature Protocols 2: 1269-75, 2010) and animals were randomized in four groups (n=10): untreated CIA, CIA treated with ETN (5.5mg/kg), MTX (35mg/kg) and with both drugs (ETN+MTX). Treatments (twice a week) started one week after booster injection and lasted for six weeks. Data shows mean±SEM and p<0.05. Arthritis clinical score was evaluated daily and progressed in untreated CIA, while this development was significantly slower in treated mice from day 10 to day 30 after the booster. Spontaneous locomotion was evaluated weekly and did not differ among groups. Animal weight was also evaluated weekly and ETN group weight (21±1.0g) was greater than MTX (19±1.3g) at weeks 5 and 7. When considering the variation from initial weight, the difference between ETN (0.7±0.5g) and MTX (-1.5±0.5g) was confirmed at week 5. Gastrocnemius (GA) and tibialis anterior (TA) muscles were dissected and weighted and both muscles were heavier in ETN (80±10 and 25±2.1g, respectively) than MTX (80±10 and 25±2.1g, respectively) and ETN+MTX (83±10 and 25±2.2g, respectively). When GA and TA muscles weight were normalized with animal weight, the difference was confirmed, demonstrating that ETN animals (4.8±0.6 and 1.4±0.2g, respectively) had proportionally more muscle mass than MTX (4.0±0.7 and 1.2±0.1g, respectively) and ETN+MTX (3.9±0.2 and 1.2±0.1g, respectively). Untreated CIA body and muscles weights remained similar to both treatments.

**Conclusions:** This study demonstrated that both drugs, ETN and MTX, decreased experimental arthritis severity. However, only ETN seems to be able to protect animals from muscle loss, since ETN group demonstrated to have more body and muscle weights. More studies are needed to evaluate the process of muscle wasting caused by experimental arthritis and how these drugs affect these pathways.

**Financial support:** CAPES, CNPq, FAPERGS, FIPE-HCPA.
EFFECT OF GAMMA-TERPINENE ON EXPERIMENTAL MODEL ARTHRITIS

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Introduction: Rheumatoid Arthritis (RA) is an autoimmune disease responsible for articulation lesions. The long term conventional RA treatment is responsible for harmful side effects. Eucalyptus is a plant rich in essential oils that contain terpenoid compound with anti-inflammatory properties. Objective. The aim of this study was to analyse the anti-inflammatory effect of gamma-terpinene on ovalbumin (OVA)-induced arthritis. Methods: Female Swiss mice (n=5) were twice subcutaneously sensitized with OVA adsorbed in complete Freund’s adjuvant and on the 21st day, the animals were challenged with a single injection of OVA in incomplete Freund’s adjuvant (IFA) into the footpad. The edema formation was measured on day 12 after OVA challenge. Data were analyzed by Student’s t-test. Results: Daily oral administration of gamma-terpinene (25 mg/kg or 50 mg/kg) or dexamethasone (5 mg/kg, s.c.) since the first day of OVA challenge did not change the weight of liver, spleen and kidneys showing low or absence of toxicity. Also oral treatment with gamma-terpinene at both doses decreased significantly (P< 0.05) paw edema (2.9 mm± 0.11 mm and 3.49 mm± 0.82 mm respectively) as compared with control group (3.73 ± 0.093). Conclusion: These preliminary data indicate that gamma-terpinene was effective in decreasing inflammation observed in animals with arthritis like.

Financial support: CNPq.
EFFECT OF LENTIVIRAL HETEROLOGOUS EXPRESSION OF HSPAG11B ALTERNATIVE SPLICE VARIANTS ON INFLAMMATORY ARTHRITIS IN THE MICE KNEE JOINT

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Introduction: The antimicrobial gene SPAG11B (sperm-associated antigen 11B) is localized within a β-defensin cluster wherein complex alternative splicing mechanisms result in more than 20 distinct transcripts in mammals (mRNA variants A - W). Although little functional information is available regarding this highly conserved family of proteins, a study conducted in our laboratory evidenced that the expression of SPAG11B isoform C (SPAG11B/C) is dynamically regulated in chondrocytes during rat embryogenesis. Additionally, a recent study characterized hSPAG11B/D (full length and its N-terminus, conserved in hSPAG11C) as a competitive inhibitor of the serine-proteinase tryptase, a pivotal mediator of inflammatory diseases such as arthritis. Herein, we hypothesized that SPAG11B/C and D may confer protection against inflammatory arthritis via inhibition of tryptase.

Methods and Results: SPAG11B/C is constitutively expressed in synoviocytes and chondrocytes in the knee joint of adult C57BL/6 mice and upregulated during mBSA/IL-1β-induced arthritis, as evidenced by in situ hybridization and immunohistochemistry. The coding sequences of hSPAG11B/C, D and E were cloned into a bicistronic IRES lentiviral vector system containing the gene reporter eGFP (pWPXLd-IRES-eGFP). Subsequently to several optimizations of virus production in order to reach high titers of purified VSV-G pseudotyped lentivirus, functional integration of transgenes of interest was confirmed by RT-PCR and immunofluorescence in the knee joint of mice injected with titers ranging from 1 x 10^6 – 10^7 colony forming units (CFU/joint). Among animals transduced with 2 x 10^6 CFU/joint of lentivirus carrying the coding sequences of hSPAG11B/C, D or E, mice expressing hSPAG11B/C had a decrease in leukocyte infiltration to the synovial membrane and joint cavity, whereas other histopathological parameters, such as cartilage degradation and bone erosion remained unchanged. Conclusion: SPAG11B/C is upregulated during mBSA/IL-1β-induced arthritis and may have anti-inflammatory activity. Further experiments employing higher lentivirus titer (2 x 10^7 CFU/joint) and mechanistic approaches are under development.

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EFFECT OF MODERATE AEROBIC EXERCISE OVER MUSCLE WASTING IN EXPERIMENTAL ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease that primarily affects synovial joints. As a consequence of the disease, RA patients typically suffer from severe joint pain, reduced muscle strength, and impaired physical function. Chronic inflammation may lead to an imbalance between protein catabolism and anabolism and to deficits in muscle regeneration, resulting in significant skeletal muscle wasting. Exercise may improve immune function, due to its anti-inflammatory effect, and prevent muscle wasting caused by chronic inflammation. The aim of this research is to evaluate the effect of physical exercise on weight loss and muscle wasting signaling cascades in mice with induced arthritis.

Methods and Results: female DBA1/J mice with collagen-induced arthritis (CIA) (Curr Protoc Immunol. 15(15):1-25, 2010) were randomly divided into two groups: CIA treated (n = 7) and CIA non-treated (n = 7). After the onset of the disease, CIA treated group was submitted to moderate aerobic exercise on a treadmill, 30 minutes a day, 5 days per week for 4 weeks. CIA untreated group remained sedentary. Data was analysed with ANOVA Two-Way followed by Bonferroni and independent sample t-test and p<0.05 was considered significant. Hind paw swelling and clinical score of arthritis were evaluated daily and reached no differences between groups. Spontaneous exploratory locomotion and animal weight were assessed weekly and differences were not detected. After death, tibialis anterior muscle was collected for histological analysis and myofiber cross sectional area also had no difference between groups. Gastrocnemius muscle was collected for protein analysis of MuRF-1, PAX-7 and myogenin by immunoblot. Expression of MuRF-1 (muscle breakdown marker), PAX-7 (satellite cells proliferation marker) and myogenin (satellite cells differentiation marker) showed no difference between groups.

Conclusion: although physical exercise appears as an interesting approach to reverse muscle wasting caused by arthritis, its positive effect was not seen in this exercise protocol. Female animals were used in this exercise program and, thereby, hormonal influences must be considered due to the important role of testosterone on the generation of muscle mass. Further studies with male animals and different exercise protocols are necessary to elucidate the effect of this approach over muscle wasting.

Financial support: CAPES, CNPq, FAPERGS, FIPE-HCPA.
EFFECT OF RC-3095, AN ANTAGONIST OF GASTRIN-RELEASING PEPTIDE RECEPTOR, REGULATING SYNOVIAL FIBROBLASTS IN EXPERIMENTAL ARTHRITIS

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Introduction: The gastrin-releasing peptide (GRP) is the mammalian homologue of the bombesin (BN), and its receptor signaling is involved in several functions, including inflammatory response. Both have been found in synovial membrane and fluid of rheumatoid arthritis patients. RC-3095 is an antagonist of the GRP receptor. The objective is to evaluate the role of GRP and the effect of RC-3095 in synovial fibroblast proliferation and invasion.

Methods: Mouse DBA/1J fibroblast-like synoviocyte (FLS) were isolated from the tarsus of the hind paws of collagen-induced arthritis. FLS immunocitochemistry was performed to evaluate the presence of GRP-receptor, confirming the presence of these. Then, evaluated FLS (2x10⁴/96-wells) viability in 24h treated with RC-3095 (concentration from 0.05 to 10mM) observing that the concentrations used weren't toxic on FLS, maintaining cellular viability. Next, FLS proliferation stimulated with Lipopolysaccharide (LPS) (1 and 10µg/mL) or GRP (0.1, 1 and 10mM) was performed using the MTT assay in 24h. The dose of (1µM) was defined for other experiments because this was the highest dose with lower cell mortality (p<0.05). The GRP 10mM increased the fibroblast proliferation in 18%, while LPS 10mM increased 15% compared to FLS unstipulated (p<0.05). Finally, the invasion of FLS was assayed in a transwell system using Matrigel-coated inserts from BD (Franklin Lakes, NJ, USA) and treated with GRP (10mM), RC-3095 (1mM) and GRP+RC-3095 (GRP 10mM and after 30 min RC-3095 1mM) (n= 4 per group). Treatment of highly invasive DBA/1J FLS with RC-3095 (1934 ± 941 cells) significantly decreased the number of cells invading Matrigel over 24h period in 35.3% (p=0.003) compared to GRP (5371 ± 418.1 cells) and non-different to FLS treated with GRP+RC-3095 (3054 ± 794.5 cells). Differences between experimental groups were compared by ANOVA one-way test. Conclusion: RC-3095 was able to decrease synovial fibroblasts invasion stimulated by GRP, which increased FLS proliferation and invasion and could be involved in the development of experimental arthritis through FLS. These findings suggest that interference with the neuropeptide GRP pathway is a potential new strategy for the treatment of arthritis. Financial support:CNPQ, Fipe.
Introduction and Objective: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by diverse immunological alterations. Controversy in published data on function and frequency of Th1, Th2, Th17 and Treg cells in SLE is partially due to heterogeneity in defining phenotype markers and technical protocols used. Cryopreservation may contribute to imbalance between these subtypes by inducing cell apoptosis or affecting cell proliferation. In the present study we aimed to evaluate the effects of freeze/thawing cycles on the frequency, phenotype and functional aspects of Th1 (CD3⁺CD4⁺CCR4⁻CXCR3⁻CCR5⁺), Th2 (CD3⁺CD4⁺CCR5⁻CXCR3⁻CCR4⁺), Th17 (CD3⁺CD4⁺CCR6⁺CD161⁺) and Treg cells (CD3⁺CD4⁻CD25^{high}CD127⁻) in healthy controls (HC) (n = 18) and SLE patients (n = 20). Apoptosis was determined by staining with Annexin-V and cell proliferation by the expression of Ki67. Methods and Results: Th1, Th2, Th17 and Treg cells expressing annexin V were increased in culture of samples subjected to freeze/thawing when compared to fresh samples in patients and controls. In contrast, cell proliferation (Ki67⁺ cells) in cultures stimulated with anti-CD3 was increased in Th2 (p<0.013), Th17 (p<0.001) and Treg cells (p<0.002) in samples subjected to freeze/thawing when compared to fresh samples in SLE patients. The same was true for Th1 (p<0.036), Th2 (p=0.016), Th17 (p=0.006) and Treg cells (p=0.089) in HC. Conclusion: Our findings demonstrate the importance to validate technical protocols to evaluate functional and phenotypic characteristics on T cells. Cryopreservation can increase cell death rate in Th1, Th2, Th17 and Treg cells in samples from SLE patients and controls. In addition, cryopreservation increased the proliferation rate of some T cell subset in SLE and HC group. These results emphasize the importance of appropriate pre-analytical handling of samples for phenotypic, functional and apoptosis analysis of lymphocytes. Financial support: FAPESP and CNPq
EFFECTS OF HIGH SALT DIET CONSUMPTION IN EXPERIMENTAL COLITIS IN MICE

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Introduction: Intestinal mucosa is the major surface of contact with the external environment. The gastrointestinal tract is in constant interaction with the microbiota and the antigens from diet. Under normal conditions, this interaction would induce oral tolerance. However, any failure in homeostasis results in inflammatory reactions that include inflammatory bowel diseases (Crohn’s disease and ulcerative colitis). The typical symptoms of IBD are diarrhea, abdominal pain, rectal bleeding, weight loss and anemia. In Ulcerative colitis, lesions are restricted to colon and rectum and they are characterized by an infiltrate of inflammatory cells in the lamina propria. There are imbalanced frequencies of regulatory T cells (Treg), Th1, Th2 and Th17 cells. Dietary composition has been seen as a potential risk factor for the increased incidence of autoimmune diseases. A specific factor observed is NaCl intake. Studies have demonstrated an increase in Th-17 cells in inflammatory diseases in the presence of small amounts of salt. Thus, it is expected that the presence of salt in the diet of mice with colitis would lead to an aggravation of inflammation. Our objective is to test the effects of high salt intake in colitis and to analyze the mechanisms involved in the effects.

Methods and Results: Twenty two female mice were divided into 4 groups: naïve control group fed AIN93G diet (n= 5), DSS group fed AIN93G (n= 6), HS group fed high salt diet (4% NaCl) (n=5), DSS + HS group which had colitis and were fed high salt diet (n= 6). Colitis was induced by administration of 1.5% dextrana sodium sulfate (DSS) in the drinking water. Mice received the high salt diet for 7 days when it was replaced by the AIN93G diet and colitis was induced by DSS administration for 7 days. There was no difference in diet consumption among groups. The clinical score of colitis was higher in both colitis groups when compared with the naïve group. Levels of slgA were higher in all treated groups when compared with naïve. Conclusion: Further studies should be performed to demonstrate the influence of a diet rich in salt in the presence of colitis, but we found preliminary data showing an aggravation in the gut inflammation upon high salt diet consumption. We are currently analyzing other parameters, such as cytokine production and histology, that might help to confirm the effect of high salt in colitis. This data will be present at the congress.

Financial support: CNPq, FAPEMIG, Capes.
EVALUATION OF AHR AND ITS DOWNSTREAM TARGETS EXPRESSION IN T CELLS DURING THE INDUCTION OF ARTHRITIS IN DBA-1/J AND DBA-2/J MICE

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Introduction: AhR is a transcription factor that mediates toxicity of environmental contaminants and has emerged, over the last decade, as a critical regulator of the immune system. Its signaling is regulated by, among others, induction of AhR repressor (Ahrr). It is well known that AhR activation contributes to Th17 differentiation, which play an important role in the development of autoimmune diseases such Rheumatoid arthritis (RA). RA is a systemic autoimmune disorder mainly characterized by a chronic inflammation of synovial tissues, which the mechanisms involved in disease initiation and progression are still incompletely understood. The disease has a complex component induced by several genes that interact with environmental and stochastic factors. Collagen induced arthritis (CIA) is a mouse model that has clinical and pathological features of RA, and similar to human, susceptibility is associated with certain genes. The association of AhR, T cells and arthritis prompted us to investigate the AhR and its downstream targets expression in DBA-1/J (susceptible) and DBA-2/J (resistant) mice strains.

Methods and results: Both DBA-1/J and DBA-2/J mouse strains were immunized with collagen for induction of CIA (n=5). Control mice were immunized without collagen (n=5). T cells from spleen and lymph nodes of naïve, immunized and control mice were isolated by magnetic beads and used for total RNA extraction. These samples were then separately hybridized in triplicate with whole genome Agilent microarrays containing 44,000 oligos, and data were analyzed using bioinformatics programs (Agilent Genespring software). Our results show that AhR is highly expressed in naïve T cells from susceptible DBA-1/J mice compared to DBA-2/J and after CIA induction its expression does not change. As expected, IL-22 is highly expressed during CIA in susceptible DBA-1/J mice even before the disease induction, in naïve T cells. AhR repressor otherwise, is highly repressed in DBA-1/J T cells in all stages (P <0.05).

Conclusions: Interestingly, the data suggests that the presence of AhR repressor might has a pivotal role in the DBA-2/J mice strain and contribute to its resistance to arthritis induction.

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EVALUATION OF NATURAL PRODUCTS AGAINST AN IMIQUIMOD-INDUCED PSORIATIC MOUSE MODEL

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Introduction: Psoriasis is a chronic inflammatory, autoimmune skin disease often associated to many morbid states. About 2-3% of the Brazilian population show clinical evidences of the disease. Previous studies showed that Essential Fatty Acids (EFA's) such as linoleic, α- and γ-linolenic and oleic acids found in some natural oils are the main responsible for clinical benefits related to healing, inflammation and Psoriasis. The mucilaginous hydrophilic extract (MHE) of Aloe bardenensis sp. was approved in 2010 by the Brazilian government (GM/MS 4.217) for the treatment of psoriasis vulgaris. The present study evaluated the benefits of these natural products as alternative low cost therapies against mild psoriasis using an Imiquimod (IMQ) induced psoriatic mouse model.

Methods and Results: Four groups of 6-8 weeks old BALB/c male mice (n=3) were used in this experiment (CEUA 061/2012). To induce psoriasis, we applied topically 80mg daily dose of IMQ 5% cream six consecutive days to all groups. Twelve hours after the 1st IMQ application the treatment were initiated for the same period using Betametasone 0.5% (CT+ / G0); 0.5ml of Aloe MHE 1:1 Acetone vehicle(G1); 0.1ml EFA’s rich oil (G2); and vehicle (G3). The inflammation’s severity of the shaved back skin, was scored using an objective scoring system based on the clinical Psoriasis Area and Severity Index (PASI) adapted to the affected skin area of the mouse model. The mouse skin myeloperoxidase (MPO) levels were assessed using a double beam Evolution 300 UV/VIS spectrophotometer at 460nm. Data are represented as mean ± SD for 3 different time intervals: 60'' (G0, -0.0013 ± 0.0083; G1, -0.0070 ± 0.0046; G2, 0.0017 ± 0.0023; G3, 0.0100 ± 0.0080) 240''(G0, 0.0007 ±:0.0093;G1 - 0.0070±0.0046;G2, 0.0033±0.0021;G3 0.0107±0.0085) 600'' (G0, 0.0050±0.0098; G1,-0.0063 ±0.0045 ;G2, 0.0077 ±0.0025 ; G3, 0.0133 ±0.0080). An increased PASI index within G2 group (EFA’s rich oil) was observed possibly because of the enhanced effect of the oil upon IMQ skin’s absorption. G3 MPO levels were the highest but its PASI index was as low as in the G0 (CT+) group. Conclusion: Preliminary data indicates the IMQ induced psoriatic mouse model can be useful for mimicking hyperkeratosis phenotypically similar to human psoriasis. Both natural substances showed some influence either onto MPO levels or onto IMQ immunomodulatory effect. Further experiments using bigger groups must be conducted to achieve more conclusive results.
EXACERBATION OF AUTOIMMUNE ENCEPHALOMYELITIS IN MICE CURED FROM MALARIA INFECTION: EVIDENCE FOR INCREASED AUTOIMMUNE SUSCEPTIBILITY AFTER INFECTION

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Introduction: The thymus plays an important role in shaping the repertoire of T lymphocytes and preventing that auto-reactive cells reach the periphery. We have already reported that experimental Plasmodium berghei NK65 (that do not induce cerebral malaria) infection provokes thymic atrophy through apoptosis and the premature egress of immature thymocytes (CD4^+CD8^+) to the secondary lymphoid organs. In this context, we aimed to evaluate whether the presence of these immature cells in the periphery would facilitate the development of Experimental Autoimmune Encephalomyelitis (EAE).

Methods and Results: Experiments were approved by the institutional committee. C57BL/6 and BALB/c mice (n=10) were infected with NK65 parasites and at the peak of thymic atrophy the treatment with chloroquine (to cure the infection) started for five consecutive days (5mg/kg/day). Three days after the last dose of chloroquine, a mixture of 100µg of MOG_35-55 peptide and Complete Freund’s Adjuvant (1:1) was inoculated s.c. for the induction of EAE. We observed that malaria-cured EAE mice presented lower body weight and higher clinical score compared with the PBS-EAE mice. Our results also showed that NK65-EAE mice had higher infiltration of CD4^+CD8^+ cells in the CNS and these cells were high producers of IL-17 and were responsive to in vitro re-stimulation against MOG. EAE-resistant BALB/c mice developed EAE after these mice were cured from malaria.

Conclusion: Collectively, these data suggest that premature egress of thymocytes to the periphery (due to malaria infection) aggravates EAE in susceptible mice strain while increases susceptibility in resistant strains of mice. The implications of the data reported here for susceptibility/severity in human multiple sclerosis deserves further investigation.

FOOD PREFERENCES IN RATS WITH COLLAGEN INDUCED ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is an inflammatory disease with autoimmune manifestations characterized by chronic inflammation of the joints associated with systemic complications [1]. Systemic inflammation of RA causes metabolic changes and deregulation of leptin, adiponectin and insulin, leading to anorexia, changes in normal food intake and weight loss [2]. However, observational with human subjects have many potential bias and animal models can be a good alternative for studies on the effect of chronic arthritis in metabolic changes and feeding preferences. To evaluate the feeding preferences of rats with type II collagen induced arthritis (CIA).

Methods and Results: Female Wistar rats were separated in two groups: control (CO, n=10) and collagen-induced arthritis (CIA, n=11) performed accordingly to Rosloniec et al.[3]. Arthritis clinical score (0-16) of animals was registered three times a week [3]. All animals were offered four different diets at the same time: control diet (CD), high calorie diet (HCD), high protein diet (HPD) and high fat diet (HFD). The animals and ration leftovers were weighted every three days until the end of the experiment. Comparison between groups was performed by two-way ANOVA. The first signs of arthritis appeared by 14 days after induction (score 2.7±3.1) and the inflammatory peak appeared to be around day 17 to day 22 (score 9.8±4.1). During the peak of arthritis, CIA rats had a significant weight loss (-14.7±8.5g) while CO group maintained their weight (-0.2±4.6g, p<.01). At the same time, CIA had reduced total food intake (72.3±8.6g) compared to CO (107.3±5.7g, p<.01). Besides that, CIA changed the food preference, increasing the intake of HPD (16.3±5.3 g, p<.05) compared to CO (10.0±1.5 g).

Conclusion: CIA animals demonstrated changes in their food intake, especially during the inflammatory peak of arthritis, with concomitant reduction in weight. These changes include decreased total food consumption and different food preferences, like increased intake of high-protein diet, results never reported before.

Financial support: FIPE-HCPA

References:
**Aim:** To explore the role of γδ T cells in the pathogenesis of autoimmune anti myeloperoxidase (MPO) anti-neutrophil cytoplasmic antibody (ANCA) associated glomerulonephritis (GN). **Background:** Most circulating T cells express a TCR composed of αβ heterodimers, however a small population of T cells express the γδ TCR (unconventional γδ T cells). γδ T cells exhibit characteristics that enable them to participate in innate host defence and regulating αβ T cells in adaptive immune responses. **Methods:** We compared autoimmunity and GN between WT and TCRδ-/- (γδ T cell deficient) mice. Autoimmunity was induced by MPO immunisation in Freund’s Adjuvant and GN triggered using a subnephritogenic dose of anti-glomerular basement membrane antibody. **Results:** Renal injury was significantly attenuated in TCRδ-/- mice compared with WT mice (proteinuria; 3.2±0.3 vs 2.3±0.2mg/24hr, p<0.05 and abnormal glomeruli; 34.4±2.9 vs 20.0±3.1%, p<0.005). This was associated with decreased glomerular leukocyte accumulation (macrophage; 5.3±0.7 vs 1.3±0.7cells/glomerular cross section [c/gcs], neutrophils; 1.4±0.2 vs 0.3±0.06c/gcs and CD4 cell; 1.4±0.3 vs 0.3±0.06c/gcs, all p<0.05). Antigen specific draining node lymphocytes showed that the absence of γδ T cells resulted in decreased frequency of IFNγ producing CD4 T cells (Elispot 82.2±19.0 vs 20.1±7.1cells, p<0.05) and reduced dermal MPO induced delayed type hypersensitivity swelling (0.24±0.04 vs 0.02±0.01Δmm, p<0.05). No difference in the development of MPO ANCA Ig was observed (0.3±0.03 vs 0.3±0.05OD450nm, p=0.7). Analysis of dendritic cells (DC) in lymph nodes draining MPO immunisation sites showed significantly reduced DCs (6.3x10^4±7.4x10^3 vs 4.2x10^3cells/draining LN, p<0.05) with increased proportion of apoptotic DCs (1.6±0.3 vs 3.4±0.6%, p<0.05) TCRδ-/- mice. Reconstitution of TCRδ-/- with purified WT γδ T cells restores the development of systemic MPO autoimmunity (DTH; 0.15±0.004 vs 0.07±0.01Δmm, p<0.005) and GN (proteinuria; 12.4±1.1 vs 8.1±0.9mg/24hrs and abnormal glomeruli; 30.5±3.9 vs 19.4±2.5%, p<0.01). **Conclusion:** γδ T cells affect pathogenic adaptive autoimmune anti MPO responses by optimising the development of CD4 T effector adaptive autoimmune responses.
GENE EXPRESSION PROFILE OF PERITONEAL MACROPHAGES FROM AIRMAX MICE BEARING SLC11A1 R AND S ALLELES DURING ARTHRITIS

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Introduction: Macrophages play a central role in the pathogenesis of rheumatoid arthritis and Slc11a1 gene regulates macrophage and neutrophil activity. Mice homozygous for Slc11a1 S allele (AIRmax SS) selected from high inflammatory response AIRmax line are more susceptible than AIRmax RR to pristane-induced arthritis (PIA), suggesting that Slc11a1 or other closed-linked gene interacts with inflammatory loci to modulate this experimental arthritis. Methods and Results: AIRmax RR and AIRmax SS mice received two ip injections of 0.5 mL pristane on days 0 and 60; PIA incidence and severity of PIA were assessed for 180 days. PCR arrays were used to examine the gene expression profile of peritoneal macrophages during the chronic phase of PIA, in order to identify candidate molecules that may be involved in the development and progression of the disease. Eighteen genes of the chemokine/receptors network had higher constitutive expression in AIRmax SS than AIRmax RR macrophages, which may be favor arthritis susceptibility. Upregulation (p<0.05) of 7 and 12 genes were observed in the macrophages of pristane-treated AIRmax SS and AIRmax RR mice, respectively. Histological analysis of paws from arthritic AIRMax SS mice showed a moderate inflammatory process, characterized by mononuclear cells. Kidneys were infiltrated with mononuclear cells and presented glomerular basement membrane thickening and hyaline cylinders in renal tubules in both lines. Conclusion: These results reveal that Slc11a1 alleles modulate the expression profile of chemokine/receptor genes, which may be involved in the increased AIRmax SS mice susceptibility to PIA.

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Introduction: Concerning the evolutive point of view, it is interesting to note that human and mice share similar T1D autoimmune diabetes. The non-obese diabetic (NOD) mouse is the murine counterpart that represents an animal model for T1D. Accordingly, we raised the hypothesis that human-mouse synteny chromosomal regions located at T1D susceptibility may harbor functional genes that might be associated to disease. To test this, we compared the mRNA transcriptome of mononuclear blood cells of T1D patients and NOD mice focusing on genes located at T1D susceptibility regions. Methods and Results: Agilent Whole Genome Microarrays (4x44K) were used to profile the respective transcriptomes of 19 T1D patients and 8 controls and of pre-diabetic and diabetic NOD mice. The human or mouse gene expression signatures were defined by using the Agilent GeneSpring GX platform and Cluster-TreeView softwares. Human T1D susceptibility loci were defined using T1Dbase (http://t1dbase.org) and mouse counterparts established on Homology Maps database (http://www.ncbi.nlm.nih.gov/projects/homology/maps/). We defined homolog pairs located within human-mouse synteny regions by using synteny tool from Ensembl Genome Browser (http://www.ensembl.org/). Gene pairs located in synteny regions were checked for protein identity according to HomoloGene data (http://www.ncbi.nlm.nih.gov/homologene/). From a set of 463 human genes we identified 73 presenting murine counterparts. From these, 31 presented same fold change modulation for both species, 12 of them mapped at murine diabetes susceptibility regions (Idd). Four genes (APOM, COL11A2, HLA-DOB and PRR3) were up- and eight (CYP21A2, STK19, PHTF1, RSBN1, CDSN, TRIM39, VARS2 and IL21) down-regulated in patients. From the 73 homolog pairs, 59 were identified in human-mouse synteny regions with protein identity ranging from 61 to 99 %. Conclusion: The results revealed that human-mouse chromosomal T1D synteny susceptibility regions may or may not show the same pattern of expression. In contrast, we demonstrated that some genes with unfamiliar role in T1D may be involved in this disease. Also, most of the coded proteins presented high degree of human-mouse sequence identity, suggesting functional conservation and reinforcing their putative role on disease development and/or maintenance. Financial Support: FAPESP, CNPQ, CAPES.
Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease that is mainly characterized by chronic inflammation, increased production of pathogenic autoantibodies and multiple clinical manifestations. This disease is more prevalent in women, mainly African descents. The occurrence of cardiovascular diseases in SLE patients has been reported in several studies and has been associated with the presence of accelerated atherosclerosis in these subjects. The aim of this study was to investigate the presence of dyslipidemia in SLE patients and its association with bioactive molecules that have pro-atherogenic activity (C reactive protein, IL-6 and TNF-α) and anti-inflammatory effect (IL-10). Methods and Results: Were included in this study 80 women (mean age = 40.7 ± 12.2 years) whom had four and more ACR criteria for SLE. They had around 8.5 years of SLE diagnosis, presented a median lupus activity of 6.0 (SLEDAI) and used prednisone. After serum lipid analysis (total cholesterol, HDL and LDL-cholesterol and triglycerides), dyslipidemia was detected in fifty-nine out of 80 (73.7%) patients, being observed that 52 of these subjects presented type d dyslipidemia (low level of HDL-cholesterol, isolated or associated with high level of LDL-cholesterol or triglycerides). Twenty-one out of 80 SLE patients did not have dyslipidemia and were the control group. There was an association between lupus activity (SLEDAI) and non-HDL-cholesterol levels in the dyslipidemic group \((r = 0.41, P = 0.001)\), and between this activity and apoB/apoA ratio \((r = 0.34, P = 0.009)\). Dyslipidemic and non-dyslipidemic patients presented similar median levels of CRP \((0.519 \text{ mg/L vs. 0.543 mg/L})\), IL-6 \((7.3 \text{ pg/mL vs. 9.0 pg/mL}, P > 0.05)\), TNF-α \((10.2 \text{ pg/mL vs. 12.8 pg/mL}, P > 0.05)\) and IL-10 \((7.1 \text{ pg/mL vs. 6.4 pg/mL}, P > 0.05)\). However, it was verified a strong correlation between IL-6 and CRP-levels determined by ultrasensitive immunoassay, mainly in dyslipidemic patients \((r = 0.61, P < 0.0001)\). Conclusions: Brazilian SLE patients present a high prevalence of dyslipidemia. However, dyslipidemia is not associated with increased serum levels of CRP, IL-6 and TNF-α or decreased IL-10 level, suggesting that lupus activity and corticosteroid therapy may contribute for its development.

Financial support: CNPq and CAPES
Introduction: Rheumatoid Arthritis (RA) is one of the most prevalent rheumatic autoimmune diseases worldwide, and its clinical manifestations are mainly chronic synovial inflammation of multiple joints, leading to deformity and disability. RA development is considered multifactorial, associated with both genetic and environmental factors. When the cell receives signals of danger, the inflammasome is formed and acts as an early sensor, triggering internal reactions that activate the pathway of inflammation. The inflammasome NLRP1/NALP1 related protein (one of the major proteins in NLRP1 inflammasome) has been shown to have an important role in innate immunity in activation of DC as well as in adaptive immunity. The single nucleotide polymorphisms (SNPs) in the NALP1 gene can alter the function of the protein and are involved with increasing inflammation which might be associated with RA susceptibility. In this study the association of SNPs rs12150220 (A>T) and rs2670660 (A>G) with RA susceptibility in Northeast Brazilian population was investigated. Methods and Results: Seventy seven RA patients and 132 healthy controls from Pernambuco (Brazilian Northeast) were enrolled. Genotyping was performed using Taqman probes for the SNPs rs12150220 (A>T), which promotes a codon change, and rs2670660 (A>G) within an intragenic region, using Real time PCR 7500 (Applied Biosystems). Statistical analyses were performed using SNPStats tool. No statistically significant associations were found between tested SNPs and RA susceptibility. Conclusion: NALP1 polymorphisms are not associated to RA in Northeast population.

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INTERACTION BETWEEN SMOKING AND HLA-DRB1*04 GENE IS ASSOCIATED WITH A HIGH CARDIOVASCULAR RISK IN BRAZILIAN AMAZON PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction. Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints that affects approximately 1% of the population worldwide. The HLA-DRB1 gene locus plays a major role in genetic susceptibility to RA, a condition that has been associated with a high cardiovascular morbidity and mortality in many studies. Objective. The aim of this work was to investigate which types of HLA class II genes are associated with RA in patients from the Brazilian Amazon and their influence on high cardiovascular risk status in this population. Methodology. For this purpose, a case-control study was carried out with a total of 350 non-Indian individuals made up of a cohort of 132 consecutive RA sufferers and 218 healthy controls. A χ² test showed that HLA-DRB1*04 (p<0.0016; OR=1.89; 95% CI=1.29–2.79) and HLA-DRB1*10 (p=0.0377; OR=3.81; 95% CI=1.16–12.50) are the major HLA genes associated with susceptibility to RA. A logistic regression model also showed that the interaction between HLA-DRB1*04 (p=0.027; OR=6.02; 95% CI=1.21–29.7), age (p=0.0001; OR=1.26; 95% CI=1.13–1.39) and smoking (p=0.0001; OR=23.6; 95% CI=4.25–32.1) is associated with a probability of a high cardiovascular risk status at an early age. Conclusions. The results of this study show for the first time that HLA class II type is associated with RA in Brazilian Amazon populations and that a specific interaction between the HLA-DRB1*04 gene and smoking is associated with a high cardiovascular risk status, as initially reported in the European population. This study therefore contributes to an understanding of gene-environment interactions in RA patients.
INTERLEUKIN-12 GENE POLYMORPHISM IN NORTHEAST BRAZILIAN POPULATION WITH LUPUS ERITHEMATOSUS SYSTEMIC.

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Introduction: Systemic Lupus Erythematosus (SLE) is a multisystemic disease autoimmune of unknown etiology, characterized by damages in multiple organ systems which cause clinical manifestations. Genetics and environmental factors contribute to higher susceptibility of SLE. Several studies have showed that polymorphism in genes of cytokines are involved in predisposing to development of SLE. Interleukin-12 (IL-12) is a pro-inflammatory cytokine that induces the production of interferon-g (IFN-g), favours the differentiation of T helper 1 (Th1) cells and it is a connecting point for innate and adaptive immunity (J Clin Pathol 56:481–490, 2003).

Objective: The aim of this study was to investigate the functional SNP of IL-12 gene at position +1188 of the 3’ UTR region (rs3212227) in patients with SLE, compared with a control group, and to determine association with clinical manifestations.

Methods and Results: Forty-five Brazilian patients with SLE and 22 unrelated healthy control volunteers, all patients attended at Hospital das Clinicas from Pernambuco-Brazil, were genotyped by allele specific -polymerase chain reaction (PCR-FRLP) followed by visualization on 2% agarose gel electrophoresis on ultraviolet transilluminator to detect the genotype distribution and allelic frequencies of the polymorphisms. The homozygous CC genotypes was significantly increased in SLE patients compared to control group (p value=0.034). The odds ratio value for C allele was 2.62 with a 95% CI from 1.24 to 5.53. As regard the association of clinical manifestations to the genotype frequency. However, we did not find any statistical significant with the clinical manifestations.

Conclusions: This data reinforces the critical importance of the genetic variant (rs3212227) of IL-12 gene, as a genetic marker for susceptibility to SLE.

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INTERLEUKIN-2 GENE POLYMORPHISM IN NORTHEAST BRAZILIAN POPULATION WITH LUPUS ERITHEMATOSUS SYSTEMIC

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibody production mainly directed against nuclear antigens (ANAs). The variety of clinical manifestations and symptoms is a reflection of the complexity of this autoimmune disease, associated to several genes and environmental factors involved in its initiation. The interleukin-2 (IL-2) is a pro-inflammatory cytokine produced by T lymphocyte. Deficient production of IL-2 leads to an increased rate of infections and increased numbers of activated autoreactive cells. Objective: The aim of the present study is to investigate the possible association between susceptibility to SLE and SNP located in the promoter region of the IL-2 gene (rs2069763) in a sample of the Northeast Brazilian population and the contribution of this polymorphism in the clinical or immunological manifestation of the disease. Methods and Results: Forty-seven Brazilian patients with SLE and 22 unrelated healthy control volunteers were genotyped by allele specific -polymerase chain reaction (PCR-SSP) followed by visualization on 2% agarose gel electrophoresis on ultraviolet transilluminator to detect the genotype distribution and allelic frequencies of the polymorphisms. The homozygous TT genotypes was significantly increased in SLE patients compared to control group (p value=0.037). The odds ratio value for T allele was 3.5 with a 95% CI from 1.71 to 7.15. As regard the association of clinical manifestations to the genotype frequency, we found a statistical significant increase to serosite. Conclusions: The genetic variant (rs2069763) of IL-2 gene, appears to influence the susceptibility to SLE, suggesting to be a risk factor for serosite in SLE patients.

Financial Support: CAPES and FACEPE
INTRODUCTION: A therapeutic preparation of human IgG, intravenous immunoglobulin (IVIg), has been employed to treat several inflammatory and autoimmune diseases (J Allergy Clin Immunol. 127:315-23, 2011). B cells are supposed to be one of the targets of IVIg. However, the detailed molecular mechanism for it remains to be elucidated. In this study, we aimed at identifying the mechanistic features at the molecular level. **Methods and Results:** Murine B cells were cultured and stimulated with CpG for 48 hours with or without IVIg. IVIg down-regulated IL-10 production of CpG-activated B cells (CpG, 733 ± 57pg/mL vs. CpG+IVIg, 392 ± 25pg/mL, P<0.01), while it did not suppress the IL-6 production (CpG, 64 ± 7.7pg/mL vs. CpG+IVIg, 62 ± 7.0pg/mL, P>0.05). The responsible component of IVIg was identified as the F(ab’)2 portion. (CpG, 975 ± 19 pg/mL; CpG+IVIg, 272 ± 32 pg/mL; CpG+F(ab’)2, 481 ± 13 pg/mL; CpG+Fab, 881 ± 37 pg/mL; CpG+Fc, 955 ± 55 pg/mL; CpG vs. CpG+IVIg and CpG+F(ab’)2, P<0.01). Flow cytometric analysis revealed that IVIg up-regulated the expression of CD22, a B cell inhibitory receptor, on B cells. In addition, IVIg, bound to the surface of activated B cells, was found to be co-localized with intracellular SHP-1 under a confocal laser microscopy. To examine the IVIg-induced modulation of intracellular signaling, B cells were stimulated with CpG for 30 minutes in the presence or absence of IVIg, and then analyzed for TLR9 signaling by Western blotting. Signal intensities of each TLR9 signaling component were compared to those of the unstimulated sample. We found that IVIg attenuated several TLR9-initiated signaling pathways, such as phosphorylation of TAK1 (CpG, 1.48 ± 0.11 vs. CpG+IVIg, 0.99 ± 0.13, P<0.05), NF-κB (CpG, 2.42 ± 0.30 vs. CpG+IVIg, 1.57 ± 0.22, P<0.05) and ERK (CpG, 10.3 ± 2.88 vs. CpG+IVIg, 2.20 ± 1.09, P<0.05), but not IRAK-1 (CpG, 0.38 ± 0.12 vs. CpG+IVIg, 0.50 ± 0.05, P>0.05) and p38 MAPK (CpG, 1.84 ± 0.10 vs. CpG+IVIg, 2.00 ± 0.32, P>0.05). Finally, we confirmed that intraperitoneal injection of mice with CpG together with IVIg or anti-mouse IL-10 antibodies inhibited the activation of B cells (CpG, 1.9×10^6 cells; CpG+IVIg, 7.7×10^5 cells; CpG+αIL-10, 1.1×10^6 cells; CpG vs. CpG+IVIg and CpG+αIL-10, P<0.05). Data are shown as means for 3 or 4 samples ± SEM. **Conclusion:** We postulate a scenario, in which IVIg attenuates B cells by suppressing IL-10 production, a B cell growth factor, and thus down-regulates the production of pathogenic antibodies.

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METABOLIC DISORDERS IN A MURINE MODEL OF ANTIGEN-INDUCED ARTHRITIS

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Introduction: Rheumatoid arthritis is the most common form of autoimmune chronic inflammation in rheumatic diseases. However, little is known whether arthritis complications are associated to metabolic disorders. The aim of this study was to verify whether antigen-induced arthritis (AIA) modify the metabolism and inflammatory response of adipose tissue and liver in mice. Methods and Results: BALB/c mice were immunized with mBSA and complete Freund's adjuvant (CFA). After 2 weeks the animals were challenged with knee injection of PBS (control) or mBSA (antigen). The mice were killed at 1, 3, 6, 24 and 48 hours after injection (n=8 for each group). Nociceptive behavior (Von Frey filaments) and knee histology were analyzed to determine the arthritis index. Serum levels of total cholesterol, triglycerides, glucose, adiponectin, resistin and leptin were measured by enzymatic kits or ELISA. To evaluate the inflammation, the total and differential leukocytes knee count; the concentrations of CXCL1 by ELISA and Myeloperoxidase (MPO) activity in knee, adipose tissue and liver were evaluated. In a separate set of experiments, intravital microscopy in adipose tissue and liver of LysM-eGFP mice (eGFP-expressing neutrophils) was performed. Mice AIA exhibited an increase in nociceptive behavior when compared to control. The histological arthritis index was increased in AIA mice after 3 hours until the end of experimental period in relation to control mice. In general, AIA mice presented hyperlipidemia and hyperglycemia, observed in the analysis of serum total cholesterol, triglycerides and glucose. The adiponectin concentration was reduced after 24 hours, resistin was diminished at 3 hours and leptin was increased from 3 to 24 hours following mBSA challenge. There was a peak at 24 hours of total leucocyte knee count in AIA mice with neutrophilic predominance. The CXCL1 and MPO levels in knee, adipose tissue and liver were increasing throughout the experimental period. There was an increase in neutrophil infiltration after 3 and 24 hours in liver and after 24 hours in adipose tissue. Conclusion: These results suggest that rheumatoid arthritis may be associated with metabolic alterations which also include adipose tissue and liver inflammation associated with neutrophil infiltration in both tissues.

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MODULATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY THE SYMPATHTIC NERVOUS SYSTEM

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Introduction: The nervous system can interact with the immune system. One way by which this interaction occurs is via the sympathetic nervous system (SNS). It has been already described that lymphoid organs such as spleen and lymph nodes are richly innervated by sympathetic fibers, which exert their actions by releasing catecholamines. CD4+ T cells express adrenergic receptors, mainly the β2 adrenergic receptor (β2AR). β2AR signaling on CD4+ T cells seems to impair Th1 differentiation and function. Experimental autoimmune encephalomyelitis (EAE) is the animal model of multiple sclerosis, an inflammatory, demyelinating disease of the central nervous system mediated by Th1 and Th17 cells.

Objective: The aim of this study was to investigate the effect of increased sympathetic nervous system activity on the clinical course of the experimental autoimmune encephalomyelitis.

Methods: We immunized wild type (WT) and α2AC adrenergic receptor knockout (α2AC AR−/−) mice with MOG35-55/CFA and analyzed the clinical course of EAE, mononuclear infiltrating leucocytes and the INF-γ and IL-17 producing CD4+ T cells.

Results: The analysis of the clinical score showed reduced severity of EAE in mice lacking the α2AC AR−/− as compared to WT. Next, we evaluated the central nervous system inflammatory cells by flow cytometry in the peak of EAE. α2AC AR−/− mice had fewer active microglia/macrophages (CD11b+CD45hi) and in contrast a significant increase in resting microglia (CD11+CD45lo) when compared to WT mice. Furthermore, intracellular staining showed decreased frequency of IFN-γ-producing CD4+ T cells in the central nervous system of α2AC AR−/− and a similar number of IL-17-producing CD4+ T cells than that in wild-type mice.

Conclusion: Our data suggest that the sympathetic nervous system can modulate the experimental autoimmune encephalomyelitis and the central nervous system inflammation.

Financial support: CAPES and FAPESP
NITRIC OXIDE (NO) REGULATOR DRUGS EFFECTS IN COLLAGEN-INDUCED ARTHRITIS (CIA)

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Introduction: Rheumatoid arthritis is an autoimmune inflammatory disease of unknown etiology. NO is related with inflammation and its regulation leads to anti-inflammatory proprieties in CIA as well as muscle repair after muscle injury. NO role in muscle loss associated with arthritis has not been studied yet. The aim of this research was to evaluate the effects of the NO synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) and of the NO donor 3-morpholinosydnonimine (SIN-1) in muscle loss in CIA model.

Methods and Results: Female Wistar rats with CIA (Curr Protoc Immunol. 15(15):1-25, 2010) were radomized in 3 groups: Control (saline, n=10); L-NAME (30 mg.kg⁻¹, n=10); and SIN-1 (0.3 mg.kg⁻¹, n=13), after the onset of the disease, they were treated twice a day for 10 days. Data shows mean±SD and p<.05 was considered significant. Clinical score and hind paw edema was measured daily, spontaneous locomotion and body weight were analyzed in the onset of the disease and prior to euthanasia. These parameters showed no significant differences among groups. After death, liver was collected and hepatotoxicity was not observed. Soleus, tibialis anterior, and gastrocnemius muscles were weighted and used for histological analysis. Muscles weight showed no significant differences among treatments. Myofiber cross sectional area (CSA) of animals treated with L-NAME and SIN-1 showed no difference among each other, however both reached higher myofiber CSA than control group (1013±314; 1064±358; 759±209 μm² respectively).

Conclusion: Our data suggest that both SIN-1 and L-NAME have no effects over the development of experimental arthritis and weight loss. However, both treatments avoided muscle wasting, showing higher myofiber CSA. Muscle loss caused by arthritis causes fatigue, weakness, and impaired functionality, and anti-arthritis drugs that could prevent this complication would have great clinical importance. More studies, including analysis of molecular pathways involved in muscle degradation, are underway to elucidate NO regulation in arthritis muscle loss.

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NOD2 RECEPTOR ACTIVATION PROMOTES THE TREG/TH17 CELL AND M1/M2 MACROPHAGE PHENOTYPE IMBALANCE AND CONTRIBUTES TO TYPE 1 DIABETES PATHOGENESIS

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease that develops 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 effector mechanisms involved in the progression of T1D, little is known about the role of innate immunity receptors in the initiation of the disease. In this regard, NOD-like receptors (NLRs), which are intracellular receptors responsible for the recognition of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs), appear to be an interesting target in the context of the activation of innate immune response and induction of autoimmunity. Therefore, we investigated the role of NOD2 receptor in the development of T1D.

Methods and Results: NOD2 deficient mice and their wild-type (C57BL/6) were inoculated intraperitoneally with streptozotocin (STZ/40mg/Kg) or vehicle solution (sodium citrate, pH=4.5) for five consecutive days. Blood glucose levels and body weight were monitored weekly. The pancreatic lymph nodes (PLNs) were removed to assess the frequency and absolute number of both myeloid and lymphoid cells by flow cytometry. The cytokine expression was determined in the pancreatic tissue by ELISA and real time PCR. Our results demonstrate that NOD2−/− mice are more resistant to T1D than wild-type mice (87.5% vs. 50% diabetes incidence; p=0,0408). In agreement, these mice exhibited reduced inflammatory infiltrate in the pancreatic islets (insulitis) and augmented insulin levels in the serum. NOD2−/− mice also demonstrated an increase in the Treg:Th17 ratio and significant reduction in IL-1β, IL-6, IL-17, IL-23 (p19) and IFN-γ expression in the pancreatic tissue. On the other hand, we detected higher IL-4 and IL-10 production in the pancreatic tissue and an increase in the M2:M1 macrophage subtype ratio in the PLNs of these mice. Lastly, we detected a higher gene expression of NOD2 in the PLNs and pancreas of nonobese diabetic (NOD) mice with 8 weeks old compared to NOD mice with 20 weeks old, which is consistent with the hypothesis that the NOD2 receptor plays a pivotal role in the initial stages of the disease. Conclusion: These results suggest that the NOD2 receptor activation leads to a proinflammatory cytokine production, which drives the Th17/Th1 response, thus resulting in the pancreatic islet damage and T1D onset. Financial support: CNPq and FAPESP
PRECLINICAL EVALUATION OF FR104, AN ANTAGONIST ANTI-CD28 MONOVALENT FAB’ANTIBODY, IN A SKIN INFLAMMATORY DTH PRIMATE MODEL

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Introduction

Targeting CD28 costimulation without perturbation of CTLA-4; PDL-1 and B7-mediated inhibitory signals might favour immune tolerance mechanisms. We previously showed that anti-CD28 antagonists suppress effector T cells while enhancing regulatory T cells and immune tolerance in primate transplant models. So far, anti-CD28 antagonists devoid of any agonist activity and showing good pharmacokinetic profile have not been developed. Here we evaluated FR104, a novel monovalent humanized and pegylated Fab’ anti-CD28 antibody fragment; unable to induce human T cell activation and cytokine release.

Methods and Results

We developed a tuberculin-induced delayed-type hypersensitivity (DTH) model in baboons as a surrogate model of Th1-driven skin inflammatory responses occurring in psoriasis. 11 baboons were first immunized twice with the human BCG vaccine. Immunization was confirmed ex-vivo by IFN-g ELISPOT assay as well as in-vivo by antigen recall with intradermal tuberculin injection, which lead to the development of specific erythema (10-20 mm) from day 3 to day 9 after tuberculin injection. Skin biopsies performed at day 4 confirmed the presence of an intense inflammatory infiltrates composed mainly of CD3+ T-lymphocytes and CD68+ macrophages. 7 baboons were treated IV by a single administration of FR104 at 5 mg/Kg (n=4) or 0.05 mg/Kg (n=3) and 4 baboons received only the excipient. Pharmacokinetics analysis showed an elimination half-life of 9 days. Whereas all excipient-treated animals showed no modification of erythema development after a monthly tuberculin challenge for at least 5 months, administration of FR104 at 5 mg/Kg completely prevented erythema and skin infiltrates at least 2 months after injection, as long as blood CD28 receptor occupancy remained >80%. The effect was long-lasting since these animals recovered only 3 months post-injection (blood receptor occupancy <30%) but with weaker and shorter skin responses for at least 5 months after injection. Administration of FR104 at 0.05 mg/kg, showed a partial efficacy, since intensity of erythema was reduced but still present.

Conclusion

We showed that FR104 PK/PD was compatible with clinical development in autoimmune diseases, such as psoriasis. The persistent effect seen in this DTH primate model suggested promotion of immunoregulation that might lead to higher therapeutic indexes compared to B7 antagonists (Orencea) in term of prevention of relapse and rebound.
susceptible individuals. One of its main forms, ulcerative colitis (UC), is restricted to the colonic mucosa and is characterized clinically by weight loss, rectal bleeding and diarrhea. Such manifestations present periods of remissions and relapses, and there is no cure. The attention now to the treatment of UC is facing probiotics, among which Escherichia coli Nissle 1917 (EcN) deserves great prominence. The aim of this study was to evaluate the effect of the EcN probiotic in a murine model of UC induced by Dextran Sulfate Sodium (DSS).

Methods and Results: For this, we used female BALB/c mice (n=10/group) (Protocol nº 46/2011 - CEUA/UFMG) with induced colitis (3,5% DSS solution) for 7 days. We used the probiotic EcN administered intragastrically as a preventive therapy, beginning ten days before the induction of colitis. The mice were divided into 4 groups: control, colitis (DSS), EcN, EcN+DSS. In addition to the clinical signs observed during induction of disease, the following aspects were analyzed after euthanasia: recruitment of neutrophils and eosinophils by the analysis of MPO and EPO, respectively; analysis of cytokines KC, eotaxin and IL1β; oxidative stress, by analyzing the production of reactive oxygen species. Another experiment using the same experimental design was conducted to evaluate the intestinal permeability, using the technique of FITC-dextran. There was a significant improvement between the groups treated and not treated with the probiotic in clinical signs of disease. The inflammatory state generated was significantly reduced, with a decrease in the recruitment of neutrophils and eosinophils to the focus of inflammation, and reduction of the proinflammatory cytokines KC, eotaxin and IL1β. The intestinal permeability, which is typically increased during the onset of IBD, tended to a reduction after treatment with EcN. No difference was observed in the rates of reactive oxygen species produced in this model.

Conclusion: The analyzes showed that the probiotic EcN model has beneficial effect in acute UC, however further analyses are necessary in order to investigate the mechanisms by which this effect is realized.

Financial Support: Capes, CNPq, FAPEMIG
SIMVASTATIN INHIBITS CYTOKINES IN A DOSE RESPONSE IN PATIENTES WITH RHEUMATOID ARTHRITIS

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Introduction: Few studies report the effect of statins in the modulation of cytokines, particularly of Th17 pathway. The aim of the present study was evaluate the effects of simvastatin in peripheral blood mononuclear cell (PBMC) cytokines profiles of IL-22, IL-17A, INF-γ and IL-6 and associate with disease state of the RA patients. Methods: Peripheral blood mononuclear cells from 22 RA patients were purified and stimulated or not with phorbolmyristate acetate/ionomycin and were treated with Simvastatin in different doses. Supernatants were collected after 48 hours and cytokine levels were quantified by ELISA. Patients were assessed for clinical and laboratory variables and correlations of cytokine levels with disease activity measures [Clinical Disease Activity Index (CDAI), Disease Activity Score for 28 joints (DAS28)] and Health Assessment Questionnaire (HAQ). Results: IL-17A, IL-6, IL-22 and IFN-γ were significantly reduced in a dose response after simvastatin treatment (50µM, p=0.0005; p<0.0001; p<0.02; p=0.0005, respectively). IL-17A and IL-6 cytokines were also significantly reduced in lower concentrations of simvastatin (10 µM) compared to controls (p=0.018; p=0.04) and compared to standard drug (p=0.007; p=0.0001). The results also showed that only RA patients with severe disease (DAS28>5.1 and CDAI>22) had poor response to simvastatin in reduce cytokines levels, mainly for IL-17A and IL-22 cytokines (p=0.03; p=0.039, respectively). Conclusion: RA patients in clinical remission, mild or moderate had lower levels of all cytokines analyzed after simvastatin treatment, showing that these patients have better response to treatment. Our findings suggest that the simvastatin therapy modulate IL-17A, IL-6, IL-22 and IFN-γ in dose dependent manner and its effect is associated with stratification of patients according to disease activity. Supported by: CAPES, FACEPE, INCT_if
SPHINGOSIN-1-PHOSPHATE RECEPTOR 1 MODULATION OF NOD MOUSE INTHATHYMIC CELL MIGRATION

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Introduction: NOD mice spontaneously develop type 1 diabetes following T cell-dependent destruction of pancreatic beta cells. Several alterations are observed in NOD thymus, such as the presence of giant perivascular spaces (PVS) filled with mature CD4⁺, CD8⁺ and CD4⁺CD25⁺Foxp3⁺ regulatory T cells. Moreover, NOD thymocytes have a reduced expression of the integrin VLA-5 and decreased haptotatic migration towards fibronectin, suggesting that the VLA-5 defect could be involved in the retention of these cells inside the thymus. In contrast, some thymocytes are able to leave the organ, suggesting the involvement of other molecules related to cell migration. In this context, several reports show the role of the sphingosine-1 phosphate receptor 1 (S1P1) on T cell migration and exit from thymus in normal and pathological conditions. Thus, the aim of our work is to investigate the role of S1P1-mediated interactions in NOD mouse intrathymic migration disturbances.

Methods and results: We observed by flow cytometry a lower S1P1 expression on NOD mice thymocytes compared with C57BL/6 controls, including VLA-5⁻neg mature CD4⁺CD62Lhi and CD8⁺CD62Lhi subpopulations (80.2 mean ± 4.2 SEM vs. 93.4 ± 1.8 and 64.4 ± 8.2 vs. 89.5 ± 1.5, respectively), which bear the phenotype of the cells retained within giant PVS (n=3 C57BL/6; n=4 NOD). In functional transwell migration assays, we observed that NOD CD4⁺CD62LhiVLA-5⁻neg and CD8⁺CD62LhiVLA-5⁻neg thymocytes migrate less towards different sphingosine-1-phosphate (S1P) concentrations when compared with controls (1.88 ± 0.1 vs. 4.24 ± 0.8 and 1.8 ± 0.1 vs. 5 ± 0.7, respectively). In contrast, CD4⁺CD62LhiVLA-5⁺ and CD8⁺CD62LhiVLA-5⁺ NOD thymocytes have increased migratory ability towards S1P (4.3 ± 0.4 vs. 2.2 ± 0.2 and 4.2 ± 0.4 vs 2.2 ± 0.3) (n = 4). Interestingly, serum and intrathymic levels of S1P in NOD and control mice are similar, suggesting that migratory alterations were not due to ligands concentrations. These migratory modulations were no longer observed when fibronectin was added to the system.

Conclusion: S1P1 is less expressed on NOD mice mature thymocytes and consequently affects cell migration. Our data also suggest that VLA5-mediated interactions possibly modulate S1P1 activity. In this context, S1P1 can be a potential candidate for a better understanding of migratory disturbances observed in NOD mice as well as in type 1 diabetes pathophysiology.

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SUCCINATE ENHANCES CD11C+ CELLS INTO LYMPH NODES IN EXPERIMENTAL ARTHRITIS

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Introduction: Dendritic cells (DCs) are efficient antigen presenting cells owing to their capacity to acquire and process antigens. Another attribute is their potential to express high levels of co-stimulatory molecules that trigger the proliferation and activation of naïve T-cell. Recent results showed that DCs express high levels of GPR91, a previously described orphan G-protein-coupled receptor binding the citric acid cycle intermediate succinate. The same study showed that succinate can induce DCs-mediated chemotactic and proinflammatory responses. In the present work, using antigen-induced arthritis (AIA) model, we evaluated if succinate increases DCs migration to lymph nodes.

Methods and results: In AIA model the effect of succinate treatment was evaluated on DCs migration into draining lymph nodes. Male C57BL/6 mice were primed with an injection (s.c.) of 500 μg methylated bovine serum albumin (mBSA) emulsified in complete Freund's adjuvant and sterile saline. The treated group received succinate (3.0 mg) which was added in the emulsion; 7 days later, were evaluate the CD11c/MHC-II and CD11c/CD86 frequencies in draining lymph nodes. Succinate treatment induced an increase in the CD11c/MHC-II (t7=2.71; P<0.05) and CD11c/CD86 (t8=3.19, P<0.05) cells 7 days after the immunization. Furthermore, we evaluated if succinate treatment has effect in arthritic symptomology, such as nociception and cell migration to joint. For this, after being primed, animals received booster and, arthritis was triggered by intra-articular injection of mBSA (10 μg). Articular hypernociception (electronic Von Frey) and cell migration (Neubauer chamber) was evaluated. Succinate treatment enhanced nociception (t9=4.48, P<0.05), cell migration into joint (t7=1.75, P<0.05). Flow cytometry revealed that succinate increased the number of cells producing IL-17 in draining lymph nodes (t7=3.16, P<0.05) but did not change those producing IFN-γ (t7=0.31, P>0.05). All experiments were performed in accordance with protocols approved by the institutional Ethics Committee (protocol 146/2011).

Conclusion: We showed that succinate treatment enhanced the number of DC migrating into draining lymph nodes. Such augmentation may account for rises IL-17 producing cells and accentuation of arthritic symptomology.

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THE ROLE FOR A NON-RECEPTOR TYROSINE KINASE IN T CELL RECEPTOR SIGNALING AND CHRONIC INFLAMMATION

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Introduction: Multiple sclerosis (MS) affects more than 2 million people worldwide, and increased susceptibility involves both genetic and environmental factors. An animal model for MS, experimental autoimmune encephalomyelitis (EAE), allows studies of disease-controlling genetic regions. Linkage analysis has identified the genetic locus Eae27 for playing a role in EAE susceptibility in mice. A congenic mouse strain, in which Eae27 from an EAE resistant strain has been introduced to an EAE susceptible background, shows less progressive EAE compared to the background strain. Bioinformatics and SNP analysis indicate that the Abelson related gene (Abl2), encoding the non-receptor tyrosine kinase, Arg, is a strong candidate gene within Eae27. As key regulators of the actin cytoskeleton, and actors in the downstream signaling of the T cell receptor, the family of Abl kinases could play a role in the mechanisms causing autoimmunity. The aim of this study is to reveal the role of Arg in EAE, and whether an identified non-synonymous SNP in the Abl2 gene could be responsible for the disease and T cell phenotypes observed in the EAE susceptible strain.

Methods and Results: Cytokine measurements show decreased IL-2 levels (p=0.047) in in vitro stimulated splenic T cells from congenic mice compared to the EAE susceptible background strain. The therapeutic effect of the Abl kinase inhibitor, imatinib mesylate, was tested in the EAE susceptible background strain upon immunization with a myelin basic protein peptide. Mice receiving the inhibitor developed a significantly (p=0.049) less progressive EAE compared to placebo group, indicating that Abl2 could be the disease-causing gene within Eae27. The identified SNP gives rise to an amino acid change within Arg’s C-terminal F-actin binding domain. Therefore, the effect on Arg’s ability to bind F-actin fibers is currently being studied in vitro by co-sedimentation assays using recombinant proteins and purified actin from chicken muscle.

Conclusion: Eae27 congenic mice develop a less progressive EAE and express a differentiated T cell phenotype compared to background. Abl2 could be the disease-causing gene, and mice receiving an Abl kinase inhibitor develop less progressive EAE compared to placebo group. Further studies are about to reveal the role of Arg in EAE, and whether an identified non-synonymous SNP alters the function of the Arg protein leading to increased disease susceptibility.
Myeloid leukocytes such as neutrophils or macrophages are critical components of innate immunity but their improper activation may also lead to tissue damage during autoimmune inflammation. We have previously shown that certain neutrophil responses require Src-family kinases, Syk and PLCγ2. Therefore, we tested the role of tyrosine phosphorylation pathways in in vivo inflammatory reactions. Src-family kinases, Syk and PLCγ2 were all found to be required for autoantibody-induced inflammatory reactions such as the K/BxN serum-transfer arthritis or autoantibody-induced skin blistering disease in experimental mice. The genetic deficiency of those signaling molecules also prevented accumulation of myeloid cells at the site of inflammation. Given the role of tyrosine kinases in β2 integrin-mediated leukocyte activation, we hypothesized that Src-family kinases, Syk and PLCγ2 are also required for β2 integrin-mediated leukocyte migration. Surprisingly, neutrophil migration in a conventional Transwell assay did not require Src-family kinases, Syk or PLCγ2 even though it was strongly reduced by the genetic deficiency of the β2 integrin-chain CD18. In addition, the Src-family kinase inhibitor dasatinib did not affect in vitro neutrophil migration. In vivo competitive migration assays (in which wild type and knockout cells are allowed to migrate to the site of inflammation within the same animal) also revealed that Src-family kinases, Syk and PLCγ2 were not required for neutrophil or monocyte migration in sterile peritonitis or autoantibody-induced arthritis models. On the other hand, tyrosine kinases were required for immune complex-induced cytokine production by neutrophils and macrophages. Taken together, Src-family kinases, Syk and PLCγ2 are required for neutrophil activation and cytokine production but do not play any direct role in CD18-mediated migration of myeloid cells to the site of inflammation.
USE OF A MIMETIC PEPTIDE OF THE TRANSFORMING GROWTH FACTOR B1 (TGF-BETA) AS A POTENTIAL IMMUNOMODULATORY

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Introduction: TGF-β1 is a regulatory cytokine capable of inducing suppression of effector T cells by blocking activation and function of these lymphocytes. Thus, TGF-β1 is important in controlling the immune response to self antigens and non-self. In addition to the suppressive function of the target cell, this cytokine carries out the function of modulating the expression of Foxp3 by Treg cells being able to turn peripheral T cells CD4+CD25- into CD4+CD25+ becoming important target of research related to autoimmune diseases.

Methods and Results: The present study was performed to validate a synthetic bioactive peptide selected by Phage Display methodology which mimics the TGF-beta 1. Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers (n=11) to stimulation with pm1 peptide for cytokines after 24 hours of stimulation. Inflammation inhibition assay were performed on adult male C57BL/6 mice (n=35) where an acute inflammation was induced in the peritoneum and treated with the peptide pm1 for further analysis of the cell infiltrate. To confirm if the pm1 peptide was able to modulate an increase of T cells CD4 + CD25 + FOXP3 +, healthy volunteers PBMC (n=6) stimulation was performed and analyzed by flow cytometry.

Results: Our results showed that the peptide pm1 was able to decrease in vitro the amount of TNF-alpha (p<0.05) and simultaneously increase IL-10. In vivo test showed that the treatment with this peptide was able to decrease cell infiltration (p<0.05), which is responsible for the inflammatory response. And also, the stimulation in vitro with this peptide showed to be able to increase in the percentage of Treg cells compared to stimulation with TGF-β1 when analyzed by flow cytometry.

Conclusion: The peptide analyzed was effective in maintaining Treg cells, as well as effective in decreasing TNF-alpha. So, the use of peptide mimetics to TGF-β1 may be adopted to reduce the consequences of a severe response autologous, creating then a complementary therapy for self-immunity.

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