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

SBI
Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# TRANSPLANTATION AND IMMUNOGENETICS

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

Title: **WOUND HEALING GENES AND SUSCEPTIBILITY TO CUTANEOUS LEISHMANIASIS IN BRAZIL**

Author(s): LUCAS FREDERICO DE ALMEIDA[(1)]; LÉA CASTELLUCCI[(1)]; SARRA JAMIESON[(2)]; JOYCE OLIVEIRA[(1)]; LUIZ HENRIQUE GUIMARÃES[(1)]; MARCUS LESSA[(1)]; MICHAELA FAKIOLA[(2)(3)]; AMÉLIA RIBEIRO DE JESUS[(1)(4)(5)]; E. NANCY MILLER[(3)]; EDGAR M. CARVALHO[(1)]; JENEFER BLACKWELL[(2)(3)]

Institution: (1) National Institute of Science and Technology in Tropical Diseases, Brazil and Federal University of Bahia, Salvador, Brazil; (2) Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Subiaco, Western Australia, Australia; (3) Cambridge Institute for Medical Research and Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK; (4) Instituto de Investigação em Imunologia, São Paulo, Brasil; (5) Universidade Federal de Sergipe – Aracaju, Brasil

Text: **Introduction** *Leishmania braziliensis* causes cutaneous (CL) and mucosal (ML) leishmaniasis. In the mouse, *Fli1* was identified as a gene influencing enhanced wound healing and resistance to CL caused by *Leishmania major* (Infect Immun.78:2734-44, 2010). Polymorphism at *FLI1* is associated with CL caused by *L. braziliensis* in humans, with an inverse association observed for ML disease (Genes Immun. 12:589-94, 2011). Here we extend the analysis to look at other wound healing genes, including *CTGF*, *TGFB1*, *TGFBR1/2*, *SMADS 2/3/4/7* and *FLII*, all functionally linked along with *FLI1* in the TGF beta pathway. **Methods and Results** Haplotype tagging single nucleotide polymorphisms (tag-SNPs) were genotyped using Taqman technology in 325 nuclear families (652 CL cases; 126 ML cases) from Brazil. Robust case-pseudocontrol (CPC) conditional logistic regression analysis showed associations between CL and SNPs at *CTGF* (SNP rs6918698; CC genotype; OR 1.67; 95%CI 1.10-2.54; *P*=0.016), *TGFBR2* (rs1962859; OR 1.50; 95%CI 1.12-1.99; *P*=0.005), *SMAD2* (rs1792658; OR 1.57; 95%CI 1.04-2.38; *P*=0.03), *SMAD7* (rs4464148; AA genotype; OR 2.80; 95%CI 1.00-7.87; *P*=0.05) and *FLII* (rs2071242; OR 1.60; 95%CI 1.14-2.24; *P*=0.005), and between ML and SNPs at *SMAD3* (rs1465841; OR 2.15; 95%CI 1.13-4.07; *P*=0.018) and *SMAD7* (rs2337107; TT genotype; OR 3.70; 95%CI 1.27-10.7; *P*=0.016). Stepwise logistic regression analysis showed that all SNPs associated with CL at *FLI1*, *CTGF*, *TGFBR2*, and *FLII* showed independent effects from each other, but SNPs at *SMAD2* and *SMAD7* did not add

independent effects to SNPs from other genes. **Conclusion** These results suggest that TGFβ signalling via SMAD2 is important in directing events that contribute to CL, whereas signalling via SMAD3 is important in ML. Both are modulated by the inhibitory SMAD7 that acts upstream of SMAD2 and SMAD3 in this signalling pathway. Along with the published *FLI1* association, these data further contribute to the hypothesis that wound healing processes are important determinants of pathology associated with cutaneous forms of leishmaniasis.

**Financial support** We acknowledge the support of NIH Grant AI 30639 for the field work in Brazil, and The Wellcome Trust for supporting the laboratory work and statistical analyses carried out in the UK. LFA was funded by FAPESB.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# POLYMORPHISM OF CYTOKINES IL-6, IL-10, TNF, TGF-β1 and IFN-γ IN PATIENTS WITH PULMONARY TUBERCULOSIS

**VALDIRENE LEÃO CARNEIRO**[1,6,8]; ROSALINA GUEDES DONATO SANTOS[1]; MARIA TERESITA BENDICHO[8]; MARILDA CASELA BATISTA[2]; FILIPE SAMPAIO REIS DA SILVA[3]; JULIANA SACRAMENTO MOTA SOUZA[3]; CATARINA ALFAYA MOREIRA[4]; ROBERTO MEYER [5,6]; SONGELI MENEZES FREIRE [5,6,7]

1.PhD student PPGIm/UFBA 2.MSc student PIOS/UFBA 3.Undergrad. student/FAPEX 4.Undergrad. student/CNPq. 5. Professor UFBA. 6.INCT-DT/MCT/CNPq member 7.CNPq researcher 8. Professor UNEB

**Introduction:** Tuberculosis (TB) has been considered for decades a serious global public health problem. The immunological mechanisms which lead to the control of Mycobacterium tuberculosis (Mtb) are not fully understood. Cytokines may play a central role in the control, susceptibility and clinical forms of this disease. In order to control Mtb, it have been described the Th1 cytokine profile which involves the IL-12, INF-γ and TNF, among other proinflammatory molecules. In contrast, IL-10 and TGF-β1 inhibit macrophages and can compromise the immune response against Mtb. The genetics of the host with abnormal and ineffective immune response may complicate the course of resolution of TB. The aim of this study was to compare the allele and genotype frequencies of polymorphisms for IL-6, IL-10, TNF, TGF-β1 and IFN-γ from a sample of individuals with latent and active TB. **Methods and Results**: Blood samples from volunteers have been collected and divided into three groups: individuals diagnosed with TB (n = 48); PPD positive individuals without TB diagnosis (n = 65) and individuals with PPD negative and no history or symptoms of TB (n = 48). TNF polymorphisms (-308G/A), TGF-β1 (*codon 10C/T, codon 25C/G*), IL-10 (-1082 A/G; -819T/C; -592A/C), IL-6 (-174G/C) and IFN-gamma (+874 T/A) have been analyzed with the CYTGEN$^{TM}$ kit from One Lambda Inc. The allele, genotype and phenotype frequencies were obtained and compared for all groups using the Chi-square or Fisher's exact test provide by Prism 5$^{TM}$ from Graph Pad Software. The +874 IFN-γ AA genotype was the most frequent in TB patients (56.3%) when compared with positive PPD (46.3%) and negative PPD (40.9%) individuals, but the difference was not statistically significant. The distribution of allele and genotype frequencies for the polymorphism of the cytokine genes TNF, TGF-β1, IL-10 and IL-6 also showed no statistically significant difference between the groups. **Conclusion**: Our data show a higher proportion of TB patients with IFN-γ gene +874 (AA) whose expression is related to low production of this cytokine. Although recent studies report significant increase of genotype AA for the IFN-γ gene polymorphism +874 in TB patients, the preliminary data from the present study have not shown significant difference of such polymorphism in our population.

Further research, including some ethnic markers and increased sample size, has been carried out in order to better define these results.

**Financial Supports**: INCT-DT; CNPq; IBIT-FJS; LABIMUNO-UFBA; HEOM.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# HE TRANSCRIPTOME PROFILING OF THE MURINE CERVICAL THYMUS REVEALS A CLUSTER OF UPREGULATED GENES RELATED TO IMMUNE SYSTEM PROCESSES

AMANDA FREIRE DE ASSIS(1); CLAUDIA MACEDO(1); PAULA BARBIM DONATE(1); THAÍS CRISTINE ARNS(1); NATHÁLIA JOANNE BISPO CEZAR(1); ELZA T. SAKAMOTO-HOJO (1); EDUARDO A. DONADI (1); GERALDO ALEIXO PASSOS(1)

(1) Molecular Immunogenetics Group, Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo

**Introduction:** The thymus is a primary lymphoid organ where T cell precursors derived from bone marrow undergo a complex process of differentiation and maturation within the thymic microenvironment, which culminates in the development of self-tolerant T cells. The mouse *Mus musculus* possesses a small functional cervical thymus (in the neck region) that is colonized by T cell progenitors derived from bone marrow. Different from thoracic thymus, the cervical thymus produces T cells only after the birth but it is able to generate thymocytes that are selected by T cell receptor (TCR) repertoire, in accordance to positive and negative selections, and export mature immune competent T cells. Functional studies on this organ were conduced recently but gene expression data on a large scale approach comparing and/or contrasting possible differences between cervical and thoracic thymus is still lacking. Our aim is to trace gene expression signatures and functional enrichment of C57BL/6 cervical comparing to thoracic thymus. **Methods and Results:** To characterize the cervical thymus samples we performed histological (HE) and Foxn1 expression assays. The transcriptome profiling was analyzed using Agilent whole functional genome oligo microarrays. We show that the cervical thymus presents the typical thymic medulla-cortex architecture. The Foxn1 expression, used as an inclusion criterion, confirmed the thymic origin of the samples, since this transcription factor is thymus-specific it has an obligatory role in organogenesis of the thymus and is essential for epithelial differentiation of the fetal thymus and thymopoiesis. A cluster of upregulated immune system genes was also found. This enables us to infer the occurrence of the main thymic functions in this organ as for example; regulation of T cell proliferation (Cd274, Cd80, Il15, Il12b), antigen processing and presentation (Cd74, H2-T23, H2DMb1, H2DMb2), regulation of T cell activation (Thy1, Cd80, Cd83, Infg), adaptive immune response based on somatic recombination of TCR receptors (Mbl12, Cd74, Cd55, C1q, Icam1). **Conclusions:** The transcriptome profiling of the murine cervical thymus indicates that this organ is immunologically functional featuring a comparable set of transcripts as we observe in the thoracic thymus.

**Financial support:** CAPES, FAPESP and FAEPA**.**

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# THE IMPACT OF PP65 ANTIGEN DETECTION TO EXCLUDE EMPIRICAL TREATMENT FOR ACTIVE CYTOMEGALOVIRUS INFECTION IN KIDNEY TRANSPLANT PATIENTS.

SÓCRATES BEZERRA DE MATOS (PG)[1]; SÂMARA GRAPIÚNA PINHEIRO (IC)[1]; KELLY CRISTINE MOURA COSTA PEDROZA (PG)[1]; MARCO AURÉLIO SALVINO (PG)[2]; ROBERTO MEYER (PhD)[3]; **FERNANDA WASHINGTON DE MENDONÇA LIMA (PhD)[1].**

(1). Serviço de Imunologia de Doenças Infecciosas – SIDI / Universidade Federal da Bahia; (2). Centro de Transplante de Medula Óssea – CTMO / Hospital Universitário Prof. Edgard Santos; (3). Instituto de Ciências da Saúde / UFBA.

**Introduction and Objectives:** Cytomegalovirus (CMV) active infection is the most important infection observed during the post-transplant period, when clinical team must choose among three different strategies of drug therapy: universal prophylaxis, empirical therapy and preemptive therapy. This study aimed to evaluate the importance of pp65 antigen detection as a tool to exclude the empirical therapy against CMV in kidney transplant (KT) patients of Bahia state, Brazil.

**Methods and Results:** This study enrolled 72 KT patients. CMV active infection was monitored weekly (<100 days) and biweekly (> 100 days until 6 months) by pp65 fluorescent immunoassay (antigenemia). We selected all episodes in which patient had or not signs/symptoms commonly related to active CMV infection (e.g., fever, diarrhea, leukopenia, abdominal pain and others). It was possible to characterize patients considering episodes of pp65 detection (Ag+/Ag-) and sign/symptom (Si+/Si-). The distribution of episodes was 28 (Ag+/Si+), 66 (Ag-/Si+), 9 (Ag+/Si-), 7 (Ag-/Si-) (p=0.04). Among symptomatic patients, the distribution of signs/symptoms according to antigenemia (Ag+ vs. Ag-) was: Fever (13.0% vs. 15.5%; p=0.80), Diarrhea (19.6% vs. 20.6%; p=1.00), Urea/creatinine high levels (21.7% vs. 14.4%;

p=0.33), Epigastric pain (6.6% vs. 4.1%; p=1.00), Leukopenia (10.9% vs. 7.2%; p=0.52), Transaminases high levels (2.2% vs. 7.2%; p=0.43) and Others (21.7% vs. 24.8%; p=0.83).

**Conclusion:** The results show the importance of CMV antigenemia as a guide to antiviral therapy, since there was no sign/symptom more prevalent in individuals Ag+ than Ag- and, 70.2% (66/94) of symptomatic episodes suspects had no CMV as the etiological agent, enabling the exclusion of unnecessary drug therapy.

**Financial Support:** FAPESB / CAPES / SIDI / CTMO / HUPES

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# THE ROLE HEME OXYGENASE-1 IN ACUTE KIDNEY INJURY AND IMMUNE CELLS MODULATION

**MARIANE TAMI AMANO[(1)]; MATHEUS CORREA-COSTA[(1)]; GABRIELA CAMPANHOLE[(1)]; VINICIUS ANDRADE-OLIVEIRA[(1)]; ANGELA CASTOLDI[(1)]; MEIRE HIYANE[(1)]; CLÁUDIA SILVA CUNHA[(1)]; NIELS OLSEN SARAIVA CAMARA[(1)].**

(1)Institute of Biomedical Sciences, University of São Paulo, Immunology, São Paulo, Brazil.

**Introduction**: HO-1 has been associated to renal function regulation and protection during inflammatory diseases. Ischemia and reperfusion injury (IRI) is an acute inflammatory response considered to be the main cause of acute renal injury in kidney. T cells have been recently associated to have a role in IRI. We investigated if mice treated with hemin, a HO-1 inducer, were protected from IRI and the possible changes in dendritic cells (DC) and T cells in this models. **Materials and Methods:** We treated C57Bl/6 mice with 25 mg/kg of hemin 24h before surgery. We used sham treated and non-treated control groups. One day after surgery, we collected renal lymph nodes and kidneys and we analyzed cells and cytokines by FACS, quantitative PCR and bioplex. The renal function was evaluated by urea and creatinine levels in the serum. **Results**: After IR we observed that hemin treated animals were protected from IRI presenting lower levels (85±12mg/dL) of urea comparing to control (212 ±18mg/dL). The same difference was observed measuring the creatinine. The hemin group presented two-fold the levels of HO-1 by real-time PCR, corroborating with the idea that this enzyme is involved in renal protection in inflammatory insults. We checked the phenotype of DC and T cells and we observed that DC from hemin treated group expressed higher levels of CD86 and MHC class II then control group, but no difference was observed in CD80 and CD40 levels. The population of activated T cells ($CD4^+CD69^+$) was increased in hemin treated animals. Then, we measured the expression and levels of pro-inflammatory cytokines as IL-12p40, IL-6 and IFN-g which were increased in the hemin group. On the other hand we also have an increase in IL-10 cytokine in this group, which seems that it is not an exacerbation of a pro-inflammatory response only, it is a general augmentation of immune response, involving anti-inflammatory cytokines at the same time. **Conclusions**: Thus, we concluded that hemin is involved in IRI

protection, which is probably due to an increase of HO-1 expression. However, the HO-1 seems to activate DC and T cells more than in non treated mice after IR, providing a different environment that might have an influence in the protection of the injury. Support: FAPESP, CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# MICRORNA AS A POSSIBLE DOWN-REGULATOR OF T CD4$^+$ LYMPHOCYTES PROLIFERATION IN LUNGS OF *MYCOBACTERIUM TUBERCULOSIS*-INFECTED MICE.

THIAGO MALARDO[1]; BERNARDO P. MOREIRA[1]; ÉVERTON PADILHA[1]; JÚLIO C.C. LORENZI[2]; CÉLIO L. SILVA[1]; ARLETE AP. M. COELHO-CASTELO[1].

1. Immunology department, School of Medicine USP (RibeirãoPreto)
2. Genetics department, School of Medicine USP (RibeirãoPreto)

**Introduction:** Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb), remaining as the second leading cause of death from infectious diseases worldwide. The immune response in TB has shown a Th1 pattern with IFNγ production, wich have been described as microRNA target in dendritic cells, NK cells and CD4$^+$ T cells after different stimulus. Mature microRNAs are derived from a long primary transcript, from genome, which undergoes a series of maturation stages processed by endonucleases, until they become mature, inhibiting translation of mRNA target. Indeed, some works have demonstrated the differential microRNA expression in the peripheral blood of TB infected patients, suggesting that it could be a biomarker of disease. However, there is lacking of informations about the involvement of these microRNAs during the inflammatory process in the Mtb-infected lungs. **Objective:** To evaluate the differential expression of microRNAs in lungs of *Mycobacterium tuberculosis*-infected mice**.** So, BALB/c mice were infected by intranasal route (*i.n.*) using 100 µl of suspension containing 1x10$^5$ bacilli of Mtb H37Rv. After 30 and 60 days of infection, lungs total RNA of infected and controls mice were extracted using the TRIzol. The identification of microRNAs was accomplished through Agilent miRNA microarray system and analyzed by GeneSpring GX11.5 software. **Results:** We identified 27 and 24 microRNAs differentially expressed on 30 and 60 days, respectively, which were confirmed by qPCR analysis. Using specific prediction programs, we found direct interactions of some microRNAs with Peli-1, that participate in innate immunity activation and down-regulate the CD4$^+$ proliferation. **Conclusions:** Further studies are necessary to describe the consequences and the mechanisms of this interaction, contributing to understanding of the role of microRNAs expressed in the lung of infected mice.

**Financial Support:** CAPES, FAEPA and FAPESP.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# THE EFFECT OF *SLC11A1* GENE POLYMORPHISM ON MACROPHAGE ACTIVATION DURING PRISTANE-INDUCED ARTHRITIS

MARA ADRIANA CORRÊA (1,2); TATIANE CANHAMERO (1,2); ANDREA BORREGO (1); NANCY STAROBINAS (1); JOSÉ RICARDO JENSEN (1); WAFA HANNA CABRERA (1); ORLANDO GARCIA RIBEIRO (1); OLGA CÉLIA IBAÑEZ (1); MARCELO DE FRANCO (1,2)
(1)Laboratório de Imunogenética, Instituto Butantan, São Paulo; (2)Departamento de Imunologia, Universidade de São Paulo, Brazil

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

**Supported by FAPESP/CNPq**

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# Title: INFLUENCE OF CCR2-64I AND CCR5-DELTA32 POLYMORPHISMS IN HYPERPLASIA AND PROSTATE CANCER

FRANCIS MARIA BAO ZAMBRA(1); VANDERLEI BIOLCHI(2); ILMA SIMONI BRUM(2); JOSÉ ARTUR BOGO CHIES(1)

(1).Laboratory of Immunogenetics, Department of Genetics, Universidade Federal do Rio Grande do Sul, Brazil; (2).Departament of Physiology, Universidade Federal do Rio Grande do Sul, Brazil

**Introduction:** Benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are two chronic conditions very common in aged men and have been related to inflammatory processes (Eur Urol. 52:964–72, 2007). Chemokines play an important role in mediating or regulating inflammatory responses by regulating the migration of immune cells through the activation of chemokine receptors on the surface of these cells (Cancer 109(12):2392–404, 2007). Chemokines and chemokine receptors are implicated in tumor pathogenesis, but it is not yet well defined if these molecules can be considered as a positive or a negative factor on the progression of human cancer (J Exp Med. 198:1381–1389, 2003). Two interesting chemokine receptors that seem to be involved in prostate carcinogenesis are the CCR2 (CC chemokine receptor type 2) (J Cell Biochem. 101(3):676–85, 2007) and CCR5 (CC chemokine receptor type 5) (Ann N Y Acad Sci. 1155:289–292, 2009). The aim of this study was to examine a possible association of two chemokine receptor gene polymorphisms (CCR2-64I, rs1799864; and CCR5-delta32, rs333) with the development of BPH and PCa in humans.

**Methods and Results:** In this study we genotyped 385 genomic DNA samples from southernmost Brazilian men (119 healthy control, 130 BPH and 136 PCa), predominantly euro-descendants, by PCR-RFLP to CCR2-64I polymorphism and by conventional PCR to CCR5-delta32. Our data show that median of serum PSA (Prostate Specific Antigen) levels was different between groups: 0.79, 1.45 and 6.91ng/mL in control, BPH and PCa group, respectively (all $p<0.001$). The prostate volume median was 20.00cm$^3$ in the control group, thus, lower than BPH (35.35cm$^3$) and PCa (35.80cm$^3$) groups (both $p<0.001$), nevertheless no difference was observed between BPH and PCa patients ($p=0.172$). All genotypic frequencies were in Hardy Weinberg equilibrium. The

allele frequencies of CCR2-64I were 14.0%, 15.8% and 11.1% in control, BPH and PCa, respectively; while of CCR5-delta32 were 5.1%, 7.1% and 6.2% respectively. Interestingly, CCR2-64I was detected as a protective factor to PCa when compared with BPH (OR=0.550; $_{95\%}$CI=0.311–0.975), but not when compared with the control group. No significant associations of the CCR5-delta32 variant were observed with BPH or PCa (all $p \geq 0.072$), or with PCa clinicopathologic status (all $p \geq 0.253$).

**Conclusion:** Our data suggest an association of the CCR2-64I variant as a protective factor in the development of prostate cancer.

**Financial support:** CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# EXPERIMENTAL DIABETES EXACERBATES SKIN TRANSPLANTION REJECTION IN RATS

FLÁVIO POLA DOS REIS(1); ANGELO SEMENTILLI(1); ANTONIO RICARDO DE TOLEDO GAGLIARDI(1)

(1) Centro Universitário Lusíada – Unilus – Fundação Lusíada, Santos, SP

**Introduction:** Diabetic kidney disease is responsible for half of all new patients requiring renal replacement therapy, and combined pancreas transplantation is becoming more frequent recently. However no evidence in the literature of pathophysiological mechanism of rejection in pre-diabetes. We investigated the effect of chronic experimental diabetes on skin allografts in rats as a simple model that could clarify some basic aspects and mechanisms involved in transplant rejection in diabetes.

**Methods and Results:** Skin grafting was performed with fragments of tail skin from sex matched non diabetic Wistar rats engrafted onto the thoracic area of non diabetic (n=12) and diabetic (n=10) recipients. Grafts were scored for rejection every other day and were removed on day 14. Graft histology was performed after paraffin-embedding and staining with haematoxylin-eosin. An investigator blinded to experimental groups scored the graft tissue. The skin grafts were classified according to: no rejection; or rejection including acute, chronic and humoral and/or cellular rejection. Statistical analysis were performed using JMP 5.1 software with ANOVA test. Diabetes was induced with IV injection of alloxan 40 mg/kg, average glucose levels of the diabetic rats was 414 mg% (SD=94 mg%) and 111 mg% (SD=16 mg%) for the control rats.

Inflammatory vascular infiltrate compromising the endothelium showing areas of fibrinoid necrosis and thrombosis characteristics of acute humoral rejection and lymphocyte infiltrate in subendothelial as acute cellular rejection was significantly ($p<0.0033$) higher in diabetic recipients than in non diabetic recipients, as well the inflammatory infiltrate in the epidermis ($p<0.0026$).

**Conclusion:** In this study, skin transplant acute rejection from non diabetic donors to chronic alloxan diabetic rats was significantly much more intense than the skin transplant from non diabetic to non diabetic rats.

**Financial support:** Fundação Lusíada.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# COMPARISON OF GLOBAL GENE EXPRESSION FROM PERIPHERAL BLOOD MONONUCLEAR CELLS BETWEEN TYPE 1 DIABETES PATIENTS AND CONTROLS

THAIS CRISTINE ARNS [(1)]; RENATA DOS SANTOS ALMEIDA [(1)]; ADRIANE FEIJÓ EVANGELISTA [(1)]; AMANDA FREIRE ASSIS [(1)]; NATHALIA JOANNE BISPO CEZAR [(1)]; CLAUDIA MACEDO [(1)]; ELZA TIEMI SAKAMOTO-HOJO [(1)]; EDUARDO ANTONIO DONADI [(1)]; GERALDO ALEIXO PASSOS [(1)].

[(1)] Molecular Immunogenetics Group, Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo (USP);

**Introduction:** Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease, during which the pancreatic β-cells (which secrete insulin) are selectively destroyed. The development of T1DM is under polygenic control and it's a cell-mediated disease, involving T $CD4^+$, T $CD8^+$ and innate immune cells. Metabolic derangements associated with diabetes potentially affect all cells in the body, so the resulting changes in gene expression may be sampled in peripheral blood mononuclear cells (PBMCs). **Methods and Results:** the PBMC transcriptome from T1DM patients (n = 19) and controls (n = 3) was screened using Agilent Whole Genome Cy-3 mono-color 4X44K oligo-microarrays and analyzed using Agilent GeneSpring GX software. The microarray data were normalized by quantile and gene expression was filtered by intensity signal values and flags. Each sample was regarded as a matrix of gene expression values and the Pearson Correlation Coefficient was calculated for 22 × 22 permutations of the submatrices; for each pair of submatrices, a mean correlation coefficient was taken and placed in the 22 × 22 matrix. Clustering of the samples in the matrix was performed by R function heatmap.2. We also analyzed the whole transcriptome expression of PBMCs of T1DM patients and controls, using the analytical strategy of Gene Set Analysis (GSA), designed to detect modest but coordinate changes in the expression of groups of functionally related genes. GSA ($p<0.05$) was performed and yielded 196 gene sets. Hierarchical clustering using Pearson uncentered distance metrics was applied to the selected gene sets using Cluster and Tree View software. **Conclusion:** Pearson's correlation allowed the observation of both distinct and similar expression profiles among controls and patients. Unbiased transcriptome analysis of PBMCs using Gene Set Analysis allowed identifying T1DM associated gene sets and their modulation patterns.

**Financial Support:** FAPESP, CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# HYPEROXALURIA LEADS TO AN INCREASE IN INFLAMMATION IN EXPERIMENTAL MODEL OF RENAL ISCHEMIA AND REPERFUSION

REINALDO CORREIA DA SILVA[1], MATHEUS CORREA COSTA[2], CRISTHIANE FAVERO AGUIAR[1], MARINA BURGOS DA SILVA[2], MEIRE IOSHIE HIYANE[2], MARCOS ANTONIO CENEDEZE[1], ALVARO PACHECO SILVA [1], NIELS OLSEN SARAIVA CÂMARA[1,2]

[1]Laboratory of Clinical and Experimental Immunology. Department of Medicine. Federal University of São Paulo, São Paulo, Brazil. [2]Laboratory of Transplantation Immunobiology. Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.

**Introduction:** Acute kidney injury (AKI) is defined as a rapid loss of renal function due to damage to the organ, resulting in the retention of products of metabolism and uremic toxins that are normally excreted by the kidney. AKI caused by ischemia and reperfusion (I/R) induces renal dysfunction associated with specific markers of inflammation such as TNF-α, interleukins and interferons. On the other hand, the injury I/R may contribute to crystal deposition of calcium oxalate (CaOx) renal tubules, causing additional damage in tubular epithelial cells, inducing necrosis and leading to progressive tubular atrophy and interstitial fibrosis. **Objective:** The objective was to assess whether the deposition of calcium oxalate crystals increase renal damage in rats with acute kidney injury and to analyze how animals exposed to ischemia and reperfusion evolve when subjected to an overload of CaOx. **Materials and Methods:** The rats received a solution with 0.8% ethylene glycol (EG) and 1% ammonium chloride (NH4Cl) in drinking water, for a period of 4 weeks. Then, they were submitted to 60 minutes of renal ischemia. The reperfusion injury were analyzed 24 hours after the re-establishment of renal blood flow. Serum creatinine, urea and renal tissue histology were evaluated. **Results:** Addition of EG increased urine volume and led to reduced urine pH. Serum creatinine and urea levels in animals subjected to renal ischemia and reperfusion increased compared to control group. EG treatment showed a further increase in these levels, with a significant increase compared to group I/R. Treatment also induced higher gene and protein expression of inflammatory cytokines for

CINC2, CINC3,TNF-α, IL-6 and IFN-γ, glomerular alteration and increased crystals in tubules after I/R, a characteristic of calcium oxalate deposition.

**Conclusions**: Renal ischemia and reperfusion injury is increased after deposition of crystals in renal tubule, also increasing inflammation in acute kidney injury. FAPESP, CNPq, Complex Fluids INCT.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# THE PROFILE REACTIVITY ANALYSIS OF IgM REPERTOIRE IN HUMAN KIDNEY TRANSPLANT WITH DIFFERENT LEVELS OF TOLERANCE USING PEPTIDE MICROARRAY.

RAFAEL TRINDADE BURTET(1)(3); VICTOR GREIFF (4); NICOLE WITTENBRINK (4); JULIANE LÜCK (4); HERNANDEZ M. SILVA (2)(3); JORGE KALIL(2)(3); VERÔNICA COELHO(2)(3); MARCELO MACEDO BRIGIDO(1)(3); ANDRÉA QUEIROZ MARANHÃO(1)(3); MICHAL OR-GUIL (4).

(1) Laboratory of Molecular Biology, Department of Cell Biology, IB-UnB, Brasília / DF;
(2) Laboratory of Immunology, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil;
(3) National Institute of Science and Technology for Investigation in Immunology, INCT;
(4) Systems Immunology Lab, Department of Biology, Institute for Theoretical Biology, Humboldt University Berlin and Research Center ImmunoSciences, Charité – Universitätsmedizin Berlin, Germany.

**Keywords:** renal transplantation, operational tolerance, random peptide array, antibody binding profile, machine learning

**Introduction**: Organ transplantation is a widely used therapy, but allograft rejection is still a limiting factor for this type of intervention. However, a rare group of transplanted individuals spontaneously develops a state of operational tolerance (OT), characterized by a stable graft function without use of immunossupressors. This tolerogenic state may be associated with specific humoral determinants, where the reactivity allows us to "see" the antigens world, and the set of responsive antigens could be used as a "sorter" to the immune status. We aim to evaluate whether the OT state involves differential antibody repertoire reactivity in relation to other clinical status. For this purpose we used a random peptide array microchip to search for antibody repertoires among the clinical study groups. **Methods:** Each microarray was split into three subarrays displaying the same library of 1,000 random peptides (942 15-mers). Median spot signal intensity (SI) were read into R using the function readData and the mean SI across subarrays (n=3) was calculated for each peptide (n=942). Supervised data classification, was carried out using an R implementation of the Potential Support Vector Machine (P-SVM) algorithm. **Results:** Comparison of sorted group mean peptide SI revealed that OT samples SI tend to be higher than those in CR but lower than those in HE

samples; OT and ST samples do not separate. The variance of peptide SI is tendentially lower for study groups under immunosuppression (ST and CR). The estimated average variance within the ST and CR groups is about three times lower compared to OT or HE. Principal Component Analysis (PCA) indicates reduced biological variability within study groups under immunosuppression. Although the first two principal components were found to explain 77.2% of the data set's variance, PCA failed to separate individual study groups. However, samples from patients under immunosuppression (ST, CR) form a very tight cluster while OT and HE samples are widespread in the biplot. This is in line with the reduced peptide SI variance observed for CR and ST. In leave-one-out cross-validation, the classifier discriminated three pairs of study groups (OT vs. CR, ST+CR vs. HE+OT, CR vs. HE) with a balanced accuracy (BACC) of more or equal to 66.0% . The P-SVM algorithm extracted 16 unique features for the subproblem OT vs. CR (BACC=73.8), that is, selective peptides that occurred in each fold.

**Financial support:** CNPq/CAPES

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# GENETIC VARIATIONS ON *IL10* GENE, EXPOSURE TO ENDOTOXIN AND ASTHMA

SHIRLEIDE ASSIS[(1)];CINTIA RODRIGUES MARQUES[(1)];RYAN SANTOS COSTA[(1)]; NEUZA M ALCANTARA-NEVES[(1)];MAURICIO L BARRETO[(2)];KATHLEEN C BARNES[(3)];AND CAMILA A FIGUEIREDO[(1)]

[(1)]Instituto de Ciências da Saúde, UFBa, Salvador, BA, Brasil; [(2)]Instituto de Saúde Coletiva, UFBa, Salvador, BA, Brasil; [(3)]Division of Allergy and Clinical Immunology, The Johns Hopkins University, Baltimore, MD, USA.

**Introduction**: Endotoxin is a component from gram-negative bacteria being a common environmental pollutant described to protect against the development of allergic disease especially through the induction of *IL-10.* Studies have shown that single nucleotide polymorphisms present in the *IL10* gene can alter the concentration of this cytokine. The aim of the study was to evaluate the association between *IL10* genetic variants, exposure to endotoxin and a gene x environment effect on asthmatic children. **Methods and Results**: We genotyped 12 *IL10* SNPs in 1,353 children aged 4-11 years living in a poor urban area in Salvador, Brazil, using TaqMan probe based, 5' nuclease assay minor groove binder chemistry. We measured endotoxin in dust from 1m2 of beds of the subjects using Limulus Amebocyte Lysate (LAL–QCL1000) assay (Cambrex do Brasil Ltda) following the manufacturers' instructions. Association tests were performed by logistic regression for *IL10* polymorphisms and having detectable (>2500EU/mL) or undetectable levels of endotoxin in children's homes including sex, age and principal components for informative ancestry markers as covariates, using PLINK. Our results shown that SNPs rs1554286 (OR: 0.73; 95% CI:0.56-0.97), rs1800871 (OR: 0.75; 95% CI:0.567-0.99), rs1800872 (OR: 0.76; 95% CI:0.58-0.99) of *IL10* were associated with exposure to endotoxin. The gene x environment analysis shown that the A allele for rs1554286 marker might protect against asthma for individuals with low exposure to endotoxin (OR: 0.52; 95% CI:0.24-1.14), but may be a risk factor for individuals with high endotoxin exposure (OR:1.02; 95% CI:0.82-1.27). **Conclusion**: *IL10* variants rs1554286, rs1800871 and rs1800872 are negatively associated to endotoxin exposure however the gene x environment

analysis have shown no significant associations between *IL10* gene SNPs on asthmatic children exposed or non-exposed to endotoxin.

**Financial support:** The Wellcome Trust Foundation and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# INFLUENCE OF SLC11A1 *R* AND *S* ALLELES ON DMBA-INDUCED SKIN CANCER SUSCEPTIBILITY

MARIANA PERLATI-SANTOS[1], WAFA HANNA KOURY CABRERA[1], TATIANE CANHAMERO GASPARELO[1], ANDREA BORREGO[1], JOSÉ RICARDO JENSEN[1], ORLANDO GARCIA RIBEIRO[1], NANCY STAROBINAS[1], OLGA CELIA MARTINEZ IBAÑEZ[1]; MARCELO DE FRANCO[1].

[1]Laboratório de Imunogenética, Instituto Butantan, São Paulo, Brasil.

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

**Financial support:**Fapesp and CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# Role of laminin in alloreactive T-cell migration during acute rejection

ARIANY OLIVEIRA SANTOS, WILSON SAVINO AND INGO RIEDERER

Laboratory on Thymus Research, Oswaldo Cruz Institute,
Fiocruz, Rio de Janeiro.

**Introduction:** During rejection process, recipient T cells are activated in secondary lymphoid organs and migrate to the graft, being able to destroy it. Extracellular matrix proteins (ECM), as laminin (LM), are important in lymphocyte positioning and effector function in alloreactive responses. **Objectives:** The aim of this work is to identify the cells from inflammatory infiltrate during graft rejection and evaluate the role of LM in supporting T lymphocyte migration. **Methods:** hearts from neonatal C57Bl/6 (allogeneic graft) or BALAB/c (syngeneic graft) mice were transplanted to the ear of BALB/c adult mice and fifteen days after transplantation the cells were recovered and analyzed by flow cytometer and ex vitro migration assay were performed, using LM as substrate, to evaluate the migratory capacity of cells from draining lymph nodes. **Results:** within the allografts, we found an enrichment of CD8+ T cells in the inflammatory infiltrate (Syngeneic (Syn): 4.16% ± 0.93, Allogeneic (AllO):18.82% ± 4.74), increased LM deposition (Syn: 5.14 ± 0.34, Allo: 7.76 ± 0.33 N=7) and higher numbers of CD4 activated T cells(Syn: 1.513 x 103 ± 0.49, Allo: 11.20 x 103 ± 3.68 N=4) and CD8 activated T cells (Syn: 0.25 x 103 ± 0.06, Allo: 5.61 x 103 ± 1.61 N=3). In allograft draining lymph nodes, we verified an augmented LM deposition in basement membrane of blood vessels(Control: 15,42 ± 2,61 N= 6, Syn: 16,0 ± 2,82 N= 4, Allo: 22,0 ± 3,50 N= 10), an enhancement of activated T cell numbers expressing CD49f (alpha6 chain of the laminin receptor VLA-6) in high densities. To check the migratory capacity of T cells, we performed *ex-vivo* T cell migration. We verified an enhanced LM111-driven migratory response of CD4 and CD8 T cells from allograft draining lymph nodes when compared to controls (CD4 CO: 1,0 ± 0,90, CD4 Syn: 4,600± 1,99, CD4 Allo: 8,07 ± 1,40 N= 4; CD8 CO: 1,27 ± 0,84, CD8 Syn: 4,700± 1,70, CD8 Allo: 8,0 ± 0,27 N= 4). **Conclusions:** our data suggest that the predominance of activated T cells in allografts can be related to an enhanced laminindriven migration of these cells towards the allografts, thus favoring their destruction. Since LM is a family of glycoprotein heterotrimers with many isoforms presenting distinct distribution and function, we decided to study their expression on the allografts and also evaluate the role of these isoforms on T cell migration.

**Financial support**: POM/Fiocruz, Faperj, CNPq.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# Mechanisms of inhibition of acute rejection by Mycobacterial Hsp70

THIAGO DE JESUS BORGES[1]; FELIPE D. MACHADO[1]; RAFAEL L. LOPES[1]; NATHANA G. PINHO[1]; RAFAEL F. ZANIN[1]; ANA PAULA D. DE SOUZA[1]; CRISTINA BONORINO[1].

[1]Faculdade de Biociências e Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

**Introduction:** Heat shock proteins (Hsps) are stress induced proteins with immunomodulatory properties. We have previously observed that TBHsp70 inhibited maturation of dendritic cells (DCs) and induced IL-10 production by these cells. Recently, we have demonstrated that extracellular Mycobacterial Hsp70 (TBHsp70) could inhibit allograft rejection in two murine skin allograft models and this effect was dependent on Tregs. Thus, we wanted to further elucidate the mechanisms involved in this process. We investigated if TBHsp70 can modulate donor DCs in vivo and the involvement of TLR2 in inhibition of allograft rejection induced by TBHsp70. **Methods and Results:** We performed a regular skin allograft model which allografts from B6 mice (I-$A^b$) were immersed in a solution containing OVA or TBHsp70, and then grafted on BALB/c mice (I-$A^d$). 24 or 96h post transplantation (Tx), host skin dLNs were excised and cells stained for I-$A^b$, CD11c, B220, CD103, CD11b, CD4, CD8 and Ki67. We saw no difference in I-$A^b$+ cells between the two groups. We observed that about 60% of the cells were DCs and no difference between treatments. We gated on DCs population depending on their MHC II expression (CD11c+I-$A^b$(hi) and CD11c+I$A^b$(dim)). We saw that after 24 and 96h, the percentage of CD11c+I-$A^b$(hi) cells – mature DCs – were lower in TBHsp70-treated animals. In addition, after 96h post-Tx, the percentage of CD11c+I$A^b$(dim) cells – immature DCs - was higher in TBHsp70 group. In addition, we identified two donor DCs subtypes that migrated to host dLNs: CD103+ DCs and pDCs. No difference in percentage of both subtypes was observed between OVA and TBHsp70 treatments. However, in both subtypes the percentage of CD11c+I-$A^b$(hi) cells were lower in TBHsp70 group. Also, percentage of CD11c+I$A^b$(dim) cells, in both subtypes, was higher in TBHsp70 group when compared with OVA. Also, we observed a diminished number of proliferating (Ki67+) CD4+ and CD8+ T cells in TBHsp70 group. Finally, TBHsp70-prolonged allograft survival was dependent on TLR2 expression in the graft (*p=0,0067*), but not in the host. **Conclusion:** TBHsp70 can inhibit acute allograft rejection through downregulation of donor DCs MHC II. Donor migrating cells are CD103+ DCs and pDCs. In these two subtypes, cells had lower MHC II expression. In accordance with MHC downregulation, T cell proliferation was diminished in dLN of animals that received allografts treated with TBHsp70.

Financial Support: FAPERGS and FINEP.

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

SBI Sociedade Brasileira de Imunologia
www.sbicongressos.com
Campos do Jordão SP Brazil
October 20 - 24, 2012

# ASSOCIATION OF AN *HLA-G* 3'UTR HAPLOTYPE WITH SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS: A NORTH-SOUTH ANALYSIS IN TWO INDEPENDENT BRAZILIAN COHORTS.

TIAGO DEGANI VEIT[1]; CLAYTON PEREIRA SILVA DE LIMA[2]; LAURA CORSO CAVALHEIRO[3]; CLAITON VIEGAS BRENOL[3]; JOÃO CARLOS TAVARES BRENOL[3]; RICARDO MACHADO XAVIER[3]; EDUARDO JOSÉ MELO DOS SANTOS[2]; JOSÉ ARTUR BOGO CHIES[1].

[1] Universidade Federal do Rio Grande do Sul - Laboratório de Imunogenética;

[2] Universidade Federal do Pará - Laboratório de Genética Humana e Médica

[3] Hospital de Clínicas de Porto Alegre - Serviço de Reumatologia

**Introduction:** Despite the efforts in unfolding the genetic component for rheumatoid arthritis (RA), the genetic elements that contribute to the onset of this disease remain largely unknown. HLA-G is a non-classical HLA class I molecule whose expression is induced in the course of inflammatory diseases and has been suggested as a possible mechanism of tissue protection against autoimmune inflammatory responses, therefore acting as a mechanism of immune surveillance. It is possible that gene polymorphisms may be playing a role in HLA-G levels and therefore in the susceptibility to this disease and in disease course. Our group has previously observed a positive association of a 3'UTR haplotype encompassing the 14bp locus and the +3142C/G (rs1063320) – the deletion/G (D/G) haplotype – and susceptibility to systemic lupus erythematosus. The objective of this study was to investigate the genetic influence of the two *HLA-G* 3'UTR polymorphisms – the 14 bp insertion/deletion (rs1704) and the +3142C>G (rs1063320) – in the susceptibility to RA in a double-center study comprising a Southern-Brazilian cohort from Porto Alegre and Northern-Brazilian cohort from the city of Belém.

**Methods and Results:** A total number of 529 RA patients and 470 controls was PCR genotyped for the two polymorphisms. We observed an increased frequency of the D/G haplotype carriers among female patients as compared to female controls in Porto Alegre (0.234 vs. 0.133), and in the Belém cohort (0.382 vs. 0.271). After performing a logistic regression model controlling for gender, age and city of origin, we observed a significant association between the D/G haplotype and risk for arthritis and, importantly, a significant interaction between gender and the presence of the allele. The risk conferred by the D/G

haplotype was shown to be sex-specific: while women carrying the allele presented a relative risk of 1.85, in men the allele was not significantly associated with risk or protection. We then sought to investigate whether the risk conferred by the D/G haplotype was specific to a subgroup of patients. In this sense, female carriers presented 2.05-fold risk for rheumatoid factor (RF)-positive arthritis, while the risk for having RF-negative arthritis was non-significant.

**Conclusion:** Our results suggest a differential influence of *HLA-G 3'UTR* polymorphisms in disease susceptibility, with the D/G haplotype as a risk factor for RA in RF+ females.

**Financial support:** CNPq.