TRANSPLANTATION AND IMMUNOGENETICS (TR)
ACTION OF REGULATORY T CELLS (TREGS) IN ORGAN TRANSPLANTS

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Introduction: The immune system serves as a barrier against the pathogen and the growth of abnormal cells. However, it becomes a major obstacle to the success of organ transplantation. Immunosuppressive drugs are often used to protect cells from organ transplant rejection and graft, despite a significant reduction in the incidence of rejection, leads to morbidity and mortality caused by non-specific immunosuppression. The infusion of TREGs therapy has the potential to induce long term donor specific tolerance without preventing immune responses against pathogens. In this context, TREGs research has shown satisfactory results in experimental models. Thus, this study aims to evaluate the action of regulatory T cells in immunomodulatory therapy for transplant patients.

Methods and Results: We conducted research in databases SciELO, PubMed and Web of Science using the terms “regulatory T cells”, “organ transplantation” and “graft rejection”. The results highlight the need for further research. Although until now the progress in immunology transplants are numerous, several issues remain unresolved on the performance of rational immunosuppressive drugs and new ways to induce tolerance in humans. The challenge will be to understand the mechanisms involved in immune regulation and find a safe way of handling these data, allowing tolerance protocols to move from animal models to humans, effectively reducing the possibility of rejection and improving quality of life of transplant patients. Recently, the description of regulatory T cells (TREGs) brought new possibilities for understanding this phenomenon, with major therapeutic implications. The future manipulation of these cells in vitro for therapeutic purposes may lead to accomplish the induction of tolerance in vivo in clinical transplantation.

Conclusion: Thus, we conclude that despite the regulatory T cells are important modulators at the time of transplantation avoiding rejection, there are few studies on the subject, being mostly from rodents, therefore, the data should be better studied in humans.

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AIRMAX AND AIRMN LINES SELECTED FOR ACUTE INFLAMMATORY RESPONSE ALSO DIFFER IN CHRONIC INFLAMMATORY REACTIVITY AFTER A SUBCUTANEOUS BIOGEL INJECTION.

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

Support by Fapesp and CNPq.
Introduction: The Toll-Like Receptors (TLRs) were first identified in Drosophila in the late twentieth century. TLRs are receptors from a gene family (11 members) which are expressed in cells of the innate immune system such as macrophages and dendritic cells. Among these stands out the TLR4 recognizes various microbial components such as lipopolysaccharides (LPS) present in Gram-negative bacteria and anchors fosfatil-Glycosyl-linositol (GPI) from parasites such as Plasmodium falciparum and Trypanosoma cruzi. The human gene encoding the protein forming the TLR4 receptor was mapped to chromosome 9 (9q, q32-33). From this mapping was possible to identify two polymorphic changes Asp299GlyTRL-4 e Thr399IleTRL-4. Thus the aim of this study was to describe the frequency of polymorphic Asp299GlyTRL-4 polymorphisms and Thr399IleTRL-4 in samples from 77 patients infected with Plasmodium vivax treated at the Institute of Tropical Medicine Coari-AM and 58 samples from healthy controls individuals in Coari-AM. Methods and Results: The DNA was extracted according to the Accuprep® protocol followed by PCR amplification of the DNA fragment, subsequently visualized on agarose gel stained with 2.5% DNA Saber Safe. The genotyping revealed that 10% of patients with malaria have the Asp299Gly polymorphism in heterozygous (A/G). In the control group, this rate was lower, estimated at 2%. The frequency of polymorphism Thr399Ile heterozygous (C/T) was similar between groups, with 5% and 3% in individuals infected and uninfected, respectively. When applied to the chi-square (χ2) was observed that only the Asp299Gly polymorphism is not in Hardy-Weinberg equilibrium (χ2 = 4.7030, p = 0.0457). Conclusion: In conclusion, it was observed that the frequency of this polymorphic variation is larger than in other population, however further analysis should be conducted to refine the present study.
ASSOCIATION OF HLA-B*52 WITH NON-PROGRESSION TO AIDS IN BRAZILIAN HIV-1 INFECTED INDIVIDUALS

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Introduction: Among the genetic factors involved in immune response against HIV, Human Leukocyte Antigens (HLA) exert a strong influence in viral control, immune escape and aids progression. Several HLA Class I alleles have been associated with aids progression, but HLA-B alleles impose substantially greater selection pressure on HIV-1 and are considered the strongest genetic determinant of disease outcome. We evaluated the impact of host genetic in aids progression based on the distribution of HLA-B alleles among HIV-1+ individuals with distinct patterns of disease progression: rapid progressors (RP), typical progressors (TP) and long term non-progressors (LTNP).

Methods and Results: HIV-1 seropositive individuals followed at IPEC/FIOCRUZ, Rio de Janeiro, Brazil, between 1986-2010 were classified in RP, TP and LTNP, based on clinical/laboratorial data (Ethics Committee CAE: 0002.0.009.000-08). DNA was extracted from whole blood and HLA-B locus was typed using PCR-SSO. Allele frequency data were obtained using PyPop 0.6.0. Chi-square test or Fisher's exact test were used to compare the groups. Cox modeling was used to analyze the time until aids progression. Among the 496 classified patients (182 RP, 289 TP, 25 LTNP), 218 had their HLA-B alleles typed: 86 RP, 115 TP, 17 LTNP. The most frequent HLA-B alleles found were B*15 (11.2%), B*35 (11.0%), B*44 (9.9%) and B*14 (7.1%). Significant differences in HLA-B frequencies among the three groups were observed, as follows: B*49 was greater in TP (4.0%) than in RP (0.6%) (p<0.05, OR 0.14); B*48 was greater in LTNP (5.9%) than in TP (0%) (p<0.03, OR 0.00); and, noteworthy, B*52 was greater in LTNP (11.8%) than in RP (2.9%) or TP (2.6%) (p<0.05, OR 0.22 and p<0.05, OR 0.27, respectively). Controlling for factors associated with aids progression, the presence of B*52 allele was shown as a significant protector factor for aids progression (HR 0.46 [IC95% 0.25-0.85] p<0.02). Although no direct association has been observed for B*27 or B*57 alleles and the LTNP profile compared to TP and RP groups, in the adjusted model these alleles were confirmed as a protector factor for aids (HR 0.59 [IC95% 0.37-0.93] p<0.03), as previously described.

Conclusion: These data corroborate the existence of significant differences in HLA-B allele frequencies among distinct aids progression profiles, contributing to the investigation of the role of HLA in determining the outcome in HIV infection.

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ASSOCIATION OF HLA-G GENE POLYMORPHISM (3003 T>C) WITH RISK FOR SLE

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease that may affect multiples organs or system. Although the factors that contribute to pathogenesis of the disease are not completely understood, it is known that SLE is a multifactorial disorder that involves autoreactive B and T cells and immunoregulatory factors in disarray. Susceptibility to SLE is linked to immunological and genetic factors and several genes have been associated with the disease. Since HLA-G gene has immunoregulatory functions, most often suppressing the immune response, this gene was considered to be involved in SLE development. The present study investigated the association between genetic variants in HLA-G gene and SLE susceptibility in a population from the Brazilian Northeast.

Methods and Results: The study group comprised 114 SLE patients and 128 healthy controls from Pernambuco, Brazil. Identification and genotyping of polymorphisms were performed using Sanger’s Method of DNA sequencing with specific primers comprising the whole gene. The Hardy-Weinberg equilibrium was verified using the software Genotype Transposer and Fisher’s test was used for comparison of genotype and allele frequencies (p-value < 0.05). A total of 25 genetic variants were analyzed: 16 in 5’UTR, 1 in exon 3 and 8 in 3’UTR of HLA-G gene. Significant differences were observed between patients (T = 88%, C = 12%) and controls (T = 94%, C = 6%) for SNP (Single Nucleotide Polymorphism) 3003 T>C (rs1707) located at 3’UTR of HLA-G with C allele (OR = 2.1, CI = 1.06 - 4.27, p = 0.026) and T/C genotype (OR = 2.4, CI = 1.13 - 5.3, p = 0.015) conferring an increased risk for SLE development. When comparing a dominant and recessive genetic model, it was observed that effect of C allele was more consistent with a dominant genetic model (OR = 2.34, CI = 1.12 - 5.02, p = 0.017).

Conclusions: Our findings indicate an association between 3003 T>C (rs1707) polymorphism located in HLA-G gene and SLE, suggesting its possible involvement in disease susceptibility.

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EFFECTS OF HYPERTONIC SALINE ON MICROCIRCULATION AND LEUCOCYTE-ENDOTHELIAL INTERACTION AFTER BRAIN DEATH INDUCTION IN RATS

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Introduction: The pathophysiological changes triggered by brain death (BD) include hemodynamic instability, hormonal disorders and immune system activation. A previous study demonstrated that BD induces hypoperfusion of rat mesenteric microcirculation leading to local inflammation (Clinics 2012; 67:69). Hypertonic Saline (HS; NaCl 7.5%) has been shown to be effective against ischemia-reperfusion injury. This study aimed to investigate HS on mesenteric microcirculation in an experimental model of BD.

Methods and Results: Male Wistar rats (250-350g) were anesthetized with isoflurane and maintained on mechanical ventilation. BD was induced by intracranial balloon inflation. HS (4ml/Kg) or normal saline (NS; 4ml/Kg) were infused immediately after BD induction. There was no difference in mean arterial pressure behavior between groups. Perfusion of microvessels (<30 μm diameter) and leucocyte-endothelial interactions at post-capillary venules (20-30μm diameter) were analyzed by intravital microscopy 3 h thereafter. The expression of intercellular adhesion molecule (ICAM)-1 on mesenteric microcirculation was evaluated by immunohistochemistry, and serum cytokines, chemokine and corticosterone levels determined by enzyme-linked immunosorbent assays. Relative to NS, treatment with HS increased the percentage of perfused microvessels (39±7% vs 67±5%, p=0.0001) and reduced the number of rolling (163±9 vs 120±14 cells/10min, p=0.036), adhered (4.0±0.6 vs 2.0±0.3 cells/100μm, p=0.003) and migrated leucocytes (3.0±0.4 vs 0±0.3 cells/5000μm2, p=0.002), accompanied by a reduction of ICAM-1 expression. There were no differences in cytokines, chemokine and corticosterone levels. Conclusion: Despite the absence of any influence on hemodynamic behavior and on systemic markers of inflammation, infusion of hypertonic saline improves mesenteric microcirculatory perfusion and decreases inflammatory cells migration after BD induction in rats. Supported by CNPq and FAPESP
EXPERIMENTAL ACUTE GVHD MODULATION BY DONOR B CELL IS MHC-II-DEPENDENT

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Introduction: Acute Graft-versus-Host disease (aGVHD) is the major limitation of the allogeneic bone marrow transplantation. Although T cell is directly involved in promoting disease, its activity can be modulated by a variety of other cells, mainly APCs. In fact, B cells have been targets of immunotherapy with the use of specific monoclonal antibodies even with conflicting results.

Objective: To study the role of B lymphocytes in GVHD.

Methods and Results: Lethally irradiated hosts (BALB/c) received bone marrow (BM) and splenocytes from C57BL/6 (B6) with (Allo-w/B) or without B cells (Allo-wo/B). After 4 weeks, allo-w/B reached 90% survival while less than 20% was observed in allo-wo/B mice, indicating that B cells inhibited disease development. In fact, message for IFN-γ, TNF-α and CD3ζ was around three times higher in the allo-wo/B group in colon and liver, although IL-10 was 4 times higher in the colon. To evaluate the role of B cell derived IL-10 in aGVHD inhibition, B cells from IL-10KO or control animals were transferred to irradiated allogeneic chimeras which had been transplanted with B-depleted spleens plus BM cells. B cells deficient in IL-10 still protected from aGVHD (survival and clinical scores) as well as control B cells indicating that IL-10 is not involved in aGVHD prevention. Next we asked if T-B interaction was important for the B cell dependent blockage observed. When B cells lacking MHC class II were used, aGVHD was as bad as in animals receiving no B cells, indicating the need for T-B interaction. B cells had been shown to induce Tregs, both, in vivo and in vitro. We checked if allo-wo/B chimeras had less Tregs. For that, spleens, mesenteric (mLN) and peripheral lymph node (pLN), colon and liver were studied. No difference in the number of CD4+Foxp3+ cells was observed in any of the organs analyzed, suggesting that these cells are not the mediators nor effectors of the B cell induced inhibition.

Conclusion and perspectives: Our data show a modulatory role of donor B cell in aGVHD which does not depend on IL-10 or Treg induction although it depends on T-B interaction. The mechanism underlying this modulation is not clear yet. Importantly, we believe these results put in check the use of anti-B cell monoclonal antibody as an alternative therapy for treating aGVHD.
FETAL BOVINE SERUM X ALLOGENEIC HUMAN SERUM: CHOICE OF SERUM IS DETERMINANT OF RELIABLE CELL CULTURE FOR TRANSPLANTATION

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FETAL BOVINE SERUM X ALLOGENEIC HUMAN SERUM: CHOICE OF SERUM IS DETERMINANT OF RELIABLE CELL CULTURE FOR TRANSPLANTATION

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Introduction:
Human mesenchymal stem cells (hMSC) are an attractive and abundant cell source for regenerative medicine and have significant therapeutic potential via their ability to secrete immunomodulatory and trophic cytokines. All current protocols for ex vivo expansion of hMSC before clinical application include fetal bovine serum (FBS) which poses a potential risk for infections as well as immunological reactions due the xenogeneic compounds. In this context, to avoid the immunological reactions once stem cells are transplanted, the culture medium should be animal components free. Therefore in the present study we investigated if a pool of allogeneic human serum (aHS) could replace FBS for in vitro expansion of human adipose-derived stem cells (hASC). We discovered that the choice of serum had a significant effect on hASC.

Methods and Results:
For this study hASC were expanded in Dulbecco’s modified Eagle’s medium with 10% aHS or 10% FBS. Immunophenotypic characterization by flow cytometry showed similar surface marker expression from cells in both culture conditions as well as capacity of differentiation in adipogenic, chondrogenic and osteogenic lineage demonstrated by Oil Red, Alcian Blue and Von kossa staining. The first difference observed was hASC in aHS medium results in a distinct cytokine secretion profile with higher levels of IL-12 and INF-gama. Replicative senescence of hASC was a continuous process, as shown by β-galactosidase expression histochemically, but had an increase from the 8th passage onwards in FBS cultures. Importantly, telomerase expression was not detected in hASC evaluated by PCR, the expression of C-Fos decreased in hASC cultured in aHS and the capacity of teratoma formation was not detected in hASC cultured in aHS (in vivo study). Therefore, spontaneous cell transformation was not observed in cells cultured in aHS medium although they have higher proliferation.

Conclusion:
All these results indicate that hASC in aHS may be rapidly expanded in vitro maintaining their phenotypic stability. In conclusion aHS seems to be a suitable and reliable cell culture medium supplement xeno-free, off the shelf product, for hASC culture.

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G-CSF TREATED GRANULOCYTES PROMOTES GUT, SKIN AND LIVER PROTECTION AGAINST THE GRAFT VERSUS HOST DISEASE AND MAINTAINS THE GRAFT VERSUS LEUKEMIA EFFECT

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is used to treat a series of hematological diseases. While the presence of T cells mediates the graft-versus-leukemia (GVL) effect and improves engraftment, it is also responsible for the graft-versus-host disease (GVHD), which is the main barrier of HSCT. Patients transplanted with cells from G-CSF treated donors show an unexpected low rate of acute GVHD given the high numbers of T cells present. We have previously shown that blood from G-CSF treated donors have high numbers of inhibitory granulocytes. Experimentally these granulocytes are able to prevent aGVHD in a semi-allogeneic mouse model. Here, our goal is to characterize the granulocytes and its mechanism of action.

Methods and Results: Indeed, the spleens from 5 day G-CSF treated donors together with control bone marrow cells (G-B6) injected into irradiated F1(B6×BALB/c) mice shows survival rate of 100%, against 10% in non-G-B6 transplants. This protection depends on Gr1+ cells and presents milder pathological and clinical manifestations in gastrointestinal mucosa, skin and liver. However, when these G-B6 granulocytes are from IL-10 KO mice, protection is abolished both in survival curve and in clinical score. Transmission Electron microscopy (TEM) showed that this granulocytes have a degranulated profile, 83% less granules than control granulocytes. Surprisingly, 25 days after transplantation the percentage of the subtype of T regulatory Foxp3+ cells in protected chimeras is increased in all lymphoid organs analyzed and this justifies the long term specific tolerance observed. Finally, the anti-tumoral effect in G-B6 quimeras is as potent as the B6 control chimera and this effect was maintained for more than 20 weeks after HSCT.

Conclusion: Treatment with G-CSF generates degranulated granulocytes that reduce GVHD in an IL-10 dependent manner while keeping GVL effect opening a promising road in the prevention of human aGVHD.

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INCREASED INFLAMMATION AND FIBROSIS CAUSED BY HYPEROXALURIA IN AN EXPERIMENTAL MODEL OF RENAL ISCHEMIA AND REPERFUSION

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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.

Methods and Results: Male 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. 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. EG treatment also induced higher gene and protein expression of inflammatory cytokines, like CINC2, CINC3,TNF-α, IL-6 and IFN-γ, with subsequent higher collagen and α-SMA expression, glomerular alteration and increased crystals presence in tubules after I/R, a characteristic of calcium oxalate deposition.

Conclusions: Renal ischemia and reperfusion injury is increased after crystals deposition in renal tubule, leading to increased inflammation and facilitating renal fibrosis. FAPESP, CNPq, Complex Fluids INCT.
INFLUENCE OF THE GENDER ON THE INFLAMMATORY PROCESS IN A MODEL OF BRAIN DEATH IN RATS.

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Introduction: Activation of the immune system by brain death (BD) is characterized by the expression of inflammatory mediators, cell infiltration and edema. There is evidence that the immune response differs between men and women, what could influence on the organ viability and the results of the transplant. We investigated differences between genders regarding the evolution of the inflammatory process in different organs and mesenteric microcirculatory changes in a model of brain death in rats. Methods and Results: BD was induced by a sudden increase in intracranial pressure by rapid inflation of a ballon catheter in the extradural space. Groups of female in proestrus (time of maximal estradiol secretion) or estrus, and male rats were used throughout the experiments. Vascular permeability (VP) and myeloperoxidase activity (MPO) were assessed at 6 h as markers of organ inflammation. White blood cell counts and estradiol levels were analyzed. Mesenteric microcirculatory perfusion was observed after 3 h using intravital microscopy. Lung and kidney VP were increased in proestrus female (L=172±10.3, K=274±16.8; P<0.05) compared to male rats (L=105±8.3, K=154±27.8). Regarding MPO, liver of female proestrus (1.03±0.15; P<0.05) presented higher activity when compared to male (0.55±0.09). Male rats showed pronounced leukopenia (7880±689 cell/mm³), while proestrus female rats maintained the circulating cell number after 6 h (11567±1628 cell/mm³). Despite organ inflammation, proestrus female rats maintained the proportion of perfused small vessels (78.6±3.5%) 3 h after BD, whereas estrus female (50.4±8.9%, P<0.05) and male rats (39%±7, P<0.01) presented hypoperfusion. Conclusions: Our results evidenced important differences between genders after BD, suggesting that estradiol may exert a role on the inflammatory events triggered by BD and deserve, therefore, attention as a potential factor influencing the organ status. Supported by CNPq and FAPESP.
INTRANAGRAFT TRANSCRIPTIONAL PROFILING OF RENAL TRANSPLANT PATIENTS WITH PROXIMAL TUBULAR DYSFUNCTION UNDER MYCOPHENOLATE MOFETIL OR AZATHIOPRINE IMMUNOSUPPRESSION REGIMEN

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Background: Treatment with mycophenolate mofetil (MYF) is associated with less acute rejection than azathioprine (AZA) after kidney transplantation. However, little is known about the effectiveness of MYF and AZA in patients at a higher risk to develop tubular atrophy and kidney fibrosis. We sought here to verify whether MYF or AZA therapy improve the first-year outcomes of renal transplant patients with high urinary levels of RBP (uRBP), a biomarker of proximal tubular dysfunction (PTD). Furthermore, we investigated their intragraft transcriptomic profiles to disclose the molecular mechanisms linked with PTD and immunosuppressive interventions.

Methods and Results: Renal transplant patients (n=33) were screened for increased uRBP levels. They were divided into AZA or MYF-treated groups and submitted to renal allograft biopsies. Total RNA was extracted from the samples at 0 and 12 months and submitted to gene expression profiling. Differential transcriptomic data was interpreted through functional enrichment and interaction network analyzes. We observed that patients with severe PTD (uRBP > 1000mg/L) displayed different molecular landscapes due to high uRBP levels at the post transplant periods examined. At 0 months, gene expression changes were associated with immune response, kidney aging, proteolysis and injury protection, while at 12 months these changes were related to development and tissue remodeling. We also noticed that MYF-treated patients had a better clinical outcome compared to AZA-treated patients. In addition, intragraft transcriptomic signatures were distinct between the groups. These differences could also be observed in terms of highly interacting genes (hubs) depicted in each network. The former group showed genes associated with phagosome, cell-adhesion molecules, differentiation and autoimmune disorders. Conversely, the later exhibited genes related to endocytosis, proteolysis, calcium reabsorption, mitochondrial function and steroid hormone signaling.

Conclusion: Severe PTD is dynamically characterized by a set of genes that determine the biological phenomena occurring in the graft. Moreover, intragraft molecular changes in patients under distinct immunosuppression regimens may unravel the mechanisms underlying the enhanced renal graft tolerance induced by MYF therapy.

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PHENOTIPICALLY SELECTED MICE AS A MODEL OF SUSCEPTIBILITY AND RESISTANCE TO INTESTINAL ISCHEMIA/REPERFUSION INJURY

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Introduction: Intestinal Ischemia/reperfusion (IR) injury produces a systemic inflammatory state which can lead to severe organ dysfunction. The physiopathological mechanisms arising from that process have been studied in a variety of experimental models. Objective: The aim of this study was to evaluate the regulatory mechanisms of local and systemic inflammation after intestinal IR in two lines of mice phenotypically selected for maximal (AIRmax) or minimal (AIRmin) local Acute Inflammatory Response to polyacrylamide beads. Methods: Mice were subjected to 45 min of superior mesenteric artery occlusion followed by different periods of reperfusion. Control group were sham operated and basal. The local (gut) and systemic (lung) inflammatory reaction due to ischemia was evaluated by Myeloperoxidase (MPO) activity and gene and protein expression. Intravital microscopic (IM) observation was performed in mesenteric venules and the intestinal bacterial translocation (BT) was measured in mesenteric lymph nodes (MLN) and spleen. Results and Discussion: We observed by IM that the cell adhesion levels in I/R AIRmax mice were significantly higher (p<0.001) than other groups at 1h of reperfusion with consequent high intestinal cellular infiltration measured by MPO activity. Moreover, the local expression of Icam1, Tnfa and Vhl mRNA were also higher in I/R AIRmax line (p<0.05). The systemic effects of the IR were more pronounced in AIRmax mice. The BT to the MLN in I/R AIRmax mice was 3-fold higher than IR-AIRmin. The lung inflammatory reaction measure by MPO activity showed a progressive increase up to 4 h. A higher Hif1a, Vhl and Il6 gene expression was detected in the lung parenchyma mostly AIRmax mice. The proteomic analysis revealed a variety of proteins differently expressed in lung from AIRmax and AIRmin mice such as Profilin-1, Tropomyosin beta chain, Keratin type II cytoskeletal1, S100A9, Anexin1, Eukaryotic Translation Factor 5, Rho GDP-dissociation inhibitor 2. All these proteins were involved in adhesion, migration, or apoptosis. Conclusion: Our results show that AIRmax mice are more sensitive than AIRmin mice to intestinal IR injury with intense local/systemic inflammatory reaction, differential related gene/proteins expression and bacterial translocation. This interline difference is according to selection phenotypes indicating these lines as an appropriated model for the study of IR regulation concerning to inflammatory mechanisms.

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PRECLINICAL EVALUATION OF FR104, AN ANTAGONIST ANTI-CD28 MONOVALENT FAB’ANTIBODY, IN KIDNEY ALLOTRANSPLANTATION 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 rodents. 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

PK/PD study in monkeys revealed that FR104 elimination half-life ranged between 6 to 9 days and allowed for complete blood receptor occupancy over at least a month after a single iv injection. FR104 was evaluated in a baboon kidney allograft model at the dose of 5 mg/kg at day 0; 4; 14 and then every two-week until 3 months. In monotherapy, FR104 prolonged allograft survival and delayed acute rejection [MST: 18.5 days for monotherapy (n=4) vs 6 days for untreated recipients (n=3)] and induce accumulation in the blood and in the graft of Helios-negative regulatory T cells expressing CD25, CTLA4 and Foxp3. FR104 treatment synergized with a calcineurin-free regimen of therapeutic doses of MMF [MST: 103 days for FR104 + MMF (n=4) vs 18 days for MMF (n=4)], however half of these recipients still developed acute rejection during the period of treatment. In contrast, association of FR104 with low doses of tacrolimus or therapeutic doses of Rapamycin, prevented efficiently acute rejection in all recipients [MST: >90 days for FR104 + LowTAC (n=4) or FR104 + RAPA (n=3) vs 15 days for LowTAC (n=4) or 16 days for RAPA (n=3)]. FR104 prevented also totally alloantibodies development in association with MMF (0/4) or low doses of tacrolimus (0/4), while association with rapamycin was not optimal (1 out of 3 animals developed IgG alloantibodies). Finally, mRNA transcripts of Foxp3, PD-1 and TGF-beta were found to be upregulated in the graft of FR104-treated recipients as compare respective controls.

Conclusion

We showed that PK/PD was compatible with clinical development and FR104 reinforced immunosuppression in CNI low or free protocols. Accumulation of intragraft regulatory T cells suggested promotion of immunoregulatory mechanisms dependent of CTLA-4 availability that might lead to higher therapeutic indexes compared to B7 antagonists.
PROLINE RICH PEPTIDES FROM BOTHROPS JARARACA SNAKE VENOM IMPROVE ANGIOGENIC STEM CELLS FUNCTIONS AND THERAPY ON A HINDLIMB ISCHEMIA MODEL

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Introduction: Stem cells (SC) therapy is a promising treatment for ischemic diseases. Our group has shown that proline rich peptides from Bothrops jararaca snake venom (Bj-PRO) have angiogenic effects in a hindlimb ischemia model. Here, we evaluated the effects of Bj-PRO-7a and -10c on angiogenic SC functions in vitro and in vivo.

Methods and Results: Ischemia was induced by permanent femoral artery occlusion (FAO) of C57Bl/6 mice. Animals received intraperitoneal (i.p.) daily injection of 7a or 10c at the dose of 71 nmol/Kg and the control group received PBS vehicle. Treatment started 30 min after FAO. Flow cytometry analysis showed that 10c, but not 7a, treatment stimulated the mobilization of populations of bone marrow progenitor cells (c-Kit+Sca-1+, 0.06±0.02, p<0.05; c-Kit+VEGFR2+, 0.12±0.04, p<0.05; c-Kit+CD31+, 0.11±0.03, p<0.05; Sca-1+CD31+, 0.25±0.07, p<0.05; Sca-1+VEGFR2+, 0.31±0.09, p<0.01; c-Kit+Sca-1+VEGFR2+, 0.05±0.02, p<0.05; n=3-5) to the bloodstream when compared with vehicle. We then hypothesized that preconditioning of angiogenic SC with Bj-PRO could improve their therapeutic effects. 7a or 10c pre-treated human cord blood endothelial progenitor cells (CB-EPC) were then tested for in vitro adhesion by colorimetric assay. 10c-treated group, but not 7a, showed higher adhesion capacity compared with control (gelatin/vitronectin: p<0.001; fibronectin: p<0.01; n=5). In transplantation studies in vivo, GFP transgenic mice bone marrow mononuclear cells (BM-MNC) were pre-treated with Bj-PRO (71 nM) for one hour and injected 24h post-FAO, just before intravital microscopy recording. A greater number of rolling BM-MNC was found on both 7a and 10c-treated groups (2.70±0.26, p<0.01; 4.3±0.42, p<0.05 respectively; n=5-6). However, the firm adherence to the ischemic endothelium was increased only by the 10c treatment (0.93±0.65, p<0.05, n=5). Both treatments enhanced the BM-MNC-induced increase in blood flow in ischemic limbs (evaluated by laser Doppler perfusion imaging) (7a: 0.99±0.02; 10c: 0.96±0.08; both p<0.05, n=5). Histological analysis showed that both groups had capillary density raised (7a: 2396±255.1, p<0.001; 10c: 2804±509.3, p<0.05; n=4) compared to PBS-treated BM-MNC; the same occurred with arteriole density (1.63±0.49, p<0.05, n=3-4) on 10c group.

Conclusion: Our data suggest ex vivo angiogenic SC pre-conditioning with 7a or 10c is able to increasing its therapeutic efficiency.

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TEGUMENTARY LEISHMANIASIS IN BRAZIL AND WOUND HEALING GENES

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Introduction

Previous studies from our group had found association between FLI1 polymorphisms with CL caused by L. braziliensis in humans, with an inverse association observed to ML disease. The objective of this study was extend our analysis to look at other wound healing genes, including CTGF, TGFBR1, TGFBR1/2, SMADS 2/3/4/7 and FLII, all functionally linked to FLI1 in the TGF beta signaling 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 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. These data further contribute to the hypothesis that wound healing processes are important determinants of pathology associated with cutaneous forms of leishmaniasis. Financial support NIH Grant AI 30639; The Wellcome Trust. JMO was funded by CAPES.
THE ROLE OF ADIPONECTIN IN A MURINE MODEL OF ALLOGENIC SKIN TRANSPLANTATION

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Introduction: Studies show that excess fat has many harmful effects on human physiology and it is associated with multiple increased comorbidities in recent decades. Moreover, it is now known that in addition to modulating different systems related to energy metabolism, adipose tissue is also an important endocrine regulator. Among the bioactive products it produces, adiponectin (APN) discovered almost 15 years ago, has received great attention. APN is a factor secreted primarily by adipose tissue and plays an important role in regulating energy metabolism and the immune system. Research indicates that it is capable of inhibiting the pro-inflammatory activation of several cells, such as monocytes, dendritic cells and lymphocytes. Although several researchers have focused on the role of this molecule in the cardiovascular system, little is known about its action on pathologies related to allogeneic reactivity and immune rejection. Thus, we propose to study the role of obesity and, in particular, adiponectin in the process of organ rejection using an allogeneic murine skin transplant model with C57Bl/6 and APN knockout (APN KO) mice as receptors.

Methods and Results: The skin transplant model was implemented by the engraftment of CBA (H2k) tail skin onto the dorsal region of gender-matched C57Bl/6 (H2b) or APN KO (H2b) mice (n=5). Digital images of the graft were taken daily and the percentage of graft necrosis was evaluated to determine graft survival. Skin rejection was defined when tissue displayed 100% necrosis. This study was approved by the institution's ethics committee (CEUA). APN KO mice displayed a tendency towards reduced graft survival in comparison to wild-type mice, indicating a possible anti-inflammatory role for this adipokine in immune rejection. Real-time PCR studies confirmed these results with reduced gene expression of FOXP3 in APN KO mice draining lymph nodes (1.19±0.49) (mean±SD) (n=4) in comparison to C57Bl/6 wild-type mice (1.74 ±0.72) (n=3)(p<0.05). Conclusion: These results suggest APN may possess a considerable role in modulating transplant rejection. Future studies shall elucidate the role of APN in allogeneic immune activation and the modulatory mechanisms of APN in the rejection process.

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THE ROLE OF INTERLEUKIN-6 GENE POLYMORPHISM IN THE INFECTION OF CYTOMEGALOVIRUS IN RENAL TRANSPLANT PATIENTS

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Introduction: The cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in solid organ transplant patients. CMV reactivation from latency occurs after immunosuppression, thus immune surveillance is required to maintain constant persistent infection under control. The single nucleotide polymorphism (SNP) located at position -174 in the promoter region of interleukin (IL)-6 gene is associated with differential expression of this cytokine. The aim of this study was to investigate the role of IL-6 -174C/G with CMV infection in patients undergoing renal transplantation at the University Hospital Onofre Lopes (HUOL), Natal, RN, Brazil.

Methods and Results: From August 2012 to June of 2013 samples were collected from the peripheral blood of organ recipients after transplantation. Separation of peripheral blood mononuclear cells (PBMC) was performed before DNA extraction from serum and PBMC samples of organ receptors. The polymerase chain reaction (PCR) was performed in two stages (nested-PCR) for the amplification of a conserved region of CMV genome. Genotyping of IL-6 -174C/G was performed using allele specific PCR in real time using SYBRÒ Green. Of the 28 patients included in the study, 54% were male and 46% female (median age=47y). Viral DNA was detected in PBMC of 10/28 (35.7%) patients and in both serum and PBMC of 5/28 (17.8%) patients. The frequencies of genotypes CC, CG and GG were 60.7%, 39.3% and 0%, respectively. The population was in Hardy-Weinberg equilibrium (c² = 1.67, p = 0.19, df = 1). Of the 10 patients with CMV infection in PBMC, 6 (60%) carried a heterozygous genotype while 13 from the 18 (72.2%) patients negative for the virus in PBMC carried the C allele in homozygosis, although this difference was not statistically significant (p = 0.125, Fisher test). Similar results were found with respect to the presence of CMV in serum. Conclusion: In this group of patients, the viral status of CMV was not related to SNP-174C/G genotypes, although it was possible to observe a higher positivity for CMV in heterozygotes patients, which suggests that a higher production of the cytokine, characterized by the presence of G allele, might interfere in pathogenesis of infection. The CC genotype presented a possible protective effect against CMV infection therefore a larger number of cases is needed to confirm the association.