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Congenital Disorders of Glycosylation: Expect the Unexpected.

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The Congenital Disorders of Glycosylation (CDG) comprise ~140 genetic disorders that affect biosynthesis, recognition and function of glycans. One series of CDGs is caused by recessive bi-allelic mutations in 7 of the 8 members of the Conserved Oligomeric Golgi (COG) complex, which maintains ER-Golgi homeostasis. Mutations cause improper glycosylation because they disrupt normal Golgi organization and trafficking. Patients typically have hypotonia, developmental delay, intellectual disability, variable skeletal problems and dysmorphology. Many die early.

Our first surprise: We identified 14 patients with a characteristic skeletal abnormality and progeroid dwarfism, but normal intellectual development. All patients have the identical *de novo*, dominant mutation in only a single allele of *COG4*. This generates an abnormally small Golgi and changes trafficking, but it has negligible effects on glycosylation in cells or patients. However, various collagens accumulate within the ER, leaving a collagen-depleted extracellular matrix.

The mutant protein accumulates due to slower turnover which alters normal COG4 interactions with a plethora of cytoplasmic proteins. Our goal is to determine how this single mutation produces these cellular abnormalities and profound clinical presentations.

Our second surprise: We discovered severely disabled patients with mutations in fucose kinase that activates diet-derived or salvaged fucose for glycosylation. That pathway was considered insignificant, contributing <10% to fucosylation. Instead, the *de novo* pathway from GDP-Mannose is known as the major contributor. We found that very low concentrations (0.5-20 μM) of exogenous fucose progressively inhibit the *de novo* pathway, completely by ~50 μM. Surprisingly, GDP-Fucose derived from Glc is much more sensitive to this inhibition, than that derived from Man. Cells seem to distinguish multiple pools of GDP-Fuc based on their origin. At low concentrations, the glucose transporter (GLUT1) delivers fucose, but at higher ones amiloride-sensitive micropinocytosis is the major contributor. Labeling of multiple cells lines with 13C- fucose shows that different glycans can selectively choose de novo or salvaged fucose. The cell knows about different pools, but it is not clear how it knows this.

What is clear is that paying attention to patients with rare diseases can enrich and enlighten our basic science.

Study of expression of specific glycans on platelet membrane in congenital disorders of glycosylation patients.

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Introduction: Congenital Disorders of Glycosylation (CDG) are human genetic diseases due to defects in the pathway of glycoconjugates. Most of these patients present thrombo-hemorragic events. **Objective:** To contribute to the knowledge of the physiopathological bases associated with thrombo-hemorrhagic alterations in CDG patients. **Methodology:** The expression of total glycans in platelet membrane we studied in a PRP population of normal controls (6) and PRP from a PMM2-CDG. The platelet population (PRP) was identified by flow cytometry labeling using the anti CD-41-PE antibody. A panel of 10 biotinylated lectins labeled with Stretavidin and conjugated with an Alexa-647 antibody were tested to detect glycans. We compared the value of MFI observed in the control group for each lectin studied in relation to the values obtained in the patient PMM2-CDG, by means of a Student's T test considering a significant difference the lower values $p = <0.05$. **Results:** The MFI values showed no significant differences between the controls and the patient with PMM2-CDG in the following readings: AAL, CON-A, UEA-I, RCA-I, MAA-I, WGA**.** In addition, we were able to corroborate that expression of PNA, GSL-I and GSL-II lectins was not observed, indicating very low levels of O-glycans in the platelet membrane proteins. However, this study showed significant differences ($p <0.05$) in the expression of the SNA lectin between the controls and the patient. **Conclusion:** For the first time we have characterized the profile of glycans present in platelets of a PMM2-CDG patient, observing a decrease in the expression of SNA lectin ($p <0.05$), consequently, a lower Sialic Acid content. The decrease in the expression of Sialic acid could increase the platelet clearence, (thrombocytopenia) and as a result, a potential risk of hemorrhage in these patients. We observed that the platelet proteins are mainly N-glycosylated. CONICET, FONCyT, UCC.

Mucopolysaccharidosis: The clinical experience in the northeast of Mexico.

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Mucopolysaccharidoses (MPS) are a group of clinically heterogeneous diseases due to the deficiency of lysosomal enzymes required for the breakdown of mucopolysaccharides or glycosaminoglycans (GAGs). The storage products cause progressive damage of cells, tissues, and organ systems, leading to a wide range of clinical manifestations and early death.

There are 7 types of MPS, with 11 enzyme deficiencies. Each of them shares some clinical features like joint and skeletal dysplasia, coarse facial features, corneal clouding, inguinal or abdominal hernias, recurrent upper respiratory tract infections, heart valve disease, carpal tunnel syndrome, and variable neurological involvement. Each one has severe and attenuated phenotypes, so the clinical diagnosis is not as easy as it may seem.

Enzyme replacement therapy is available in Mexico for MPS I, II, IV and VI. A good number of patients have received this treatment in some medical institutions in the country, with different results because of the heterogeneity of the disease, even within the same type of MPS.

There are still many challenges in the diagnosis and treatment of MPS.

I will present our clinical experience with MPS in the last 13 years in the Northeast of Mexico: the clinical features and phenotypes, the genotypes, the journey to diagnosis, the challenges, the response to treatment, the outcome, and the lessons that we have learned.

Application of genomic technologies in genetic diagnosis.

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The completion of the Human Genome Project in 2003 was the beginning of genomic medicine era, in which information from genomes would guide clinical decision and contribute to the achievement of personalized medicine. Personalized medicine also requires the availability of appropriate genetic diagnosis for individual patients. With the use of next-generation sequencing (NGS) technology, identification of genetic variations related to disease causality is progressing very fast. Big efforts are being performed for understanding the rare monogenic and complex traits and revealing the genetic basis of the disease based on exome sequencing. These efforts are improving diagnosis, increasing the knowledge about the mechanism of disease and genotype-phenotype correlation, helping to new drugs development, and disease management either by available treatments or genetic counseling.

The application of NGS to clinical diagnosis has different scales depending on the size of the targeted region and the application purpose. Complexity and cost increase from the analysis of a single large gene to a group of genes (panel), to the whole exome (1–2% of the genome, ~20,000 genes). The genes included in a panel are selected based on a common pathway, a defined phenotype, shared or similar phenotypes, or known complex disorders. The 85 % of the pathogenic disease-causing variants identified so far are located in highly conserved coding regions of the genome, therefore, is expected that exome sequencing could elucidate at least 78% of causative variants. Copy number variations (CNVs) are significant contributors to the inherited genetic disorders. When a CNV is present in genes matching the clinical phenotype is most likely pathogenic due to the change in gene dosage or gene disruption. CNV can now be predicted from the NGS sequencing data further increasing the diagnostic power of this technology. Since some deleterious variations are present in non-coding regions of the genome, which cannot be called by exome sequencing is expected that whole-genome sequencing will be increasingly used in clinical settings to circumvent these difficulties.

NGS-base molecular testing is especially useful for Mendelian disorders where genetic and allelic heterogeneity and lack of clearly defined genotype–phenotype correlations exist. In these diseases direct testing to specific candidate gene is very difficult and the gene by gene approach, using Sanger sequencing, is expensive and time-consuming. Good examples are autosomal recessive ataxias (at least 50 causal genes identified) and congenital disorders of glycosylation (>100 causal genes identified). At LDG of INMEGEN clinical exome (includes only genes in OMIM), whole-exome sequencing and smaller panels are implemented for routine clinical testing. Since October 2016 until September 2019, more than 1200 individuals, searching molecular diagnosis for common or rare Mendelian disease or risk evaluation for inherited cancer risk, have been studied. The diagnosis efficiency depends on the disease phenotype, the panel used and the available patient information. In spite of the extended application of NGS for research and genetic diagnosis, many challenges are still present. The lack of knowledge about Latinoamerican populations, which are under-represented in public

“normal” and “disease” databases affects variant interpretation. Medical professionals are not familiarized with the correct use and limitations of NGS. Ethical concerns related to privacy issue, discrimination, genomic data protection and management of unexpected finding need to be addressed in our countries. Finally, most patients in need of genetic diagnosis are unable to afford the cost; therefore, equal access to genetic testing need to be considered in the public health system.

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Role of O-GlcNAc glycosylation in inflammatory processes in macrophages.

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Background and aims: -

O-GlcNAc glycosylation (O-GlcNAcylation) is a reversible post-translational modification catalysed by O-GlcNAc transferase (OGT). This modification regulates the activity of cytosolic and nuclear proteins according to glucose availability. This modification, which corresponds to the addition of N-Acetylglucosamine (GlcNAc) on serine and threonine residues of proteins, participates in several hyperglycemia-associated complications. An important feature of metabolic diseases such as diabetes and obesity is the presence of a low-grade chronic inflammation that causes numerous complications. However, the relationships between O-GlcNAcylation and inflammatory processes remain poorly explored. The aim of this work was to evaluate the potential involvement of O-GlcNacylation in pro-inflammatory signalling in macrophages.

Materials and methods:

The study was performed using the RAW264.7 murine macrophage cell line, peritoneal or bone marrow-derived mice macrophages as well as macrophages differentiated from human monocytes. O-GlcNAcylation of proteins was evaluated using a BRET-based biosensor targeted to different cell compartments, or by western blot using anti-O-GlcNAc antibody.

Results:

Using the BRET-based biosensor, we observed that activation of Toll-like receptor 4 (TLR4) by LPS increased O-GlcNAcylation at the plasma membrane, in the cytosol and the nucleus of RAW264.7 macrophages. Similar results were observed when O-GlcNAcylation was evaluated by western-blotting with an anti-O-GlcNAc antibody, in RAW264.7 cells. These effects were also confirmed in primary macrophage such as bone marrow-derived and peritoneal mouse macrophages, as well as in human monocyte derived macrophages. Then we evaluated the consequences of OGT deletion on the production of cytokines by macrophages. Peritoneal-derived macrophages were prepared from OGT Lox/lox LysM-Cre mice. We observed that OGT deletion significantly increased the effect of LPS on the expression of IL1β and IFNΥ.

Conclusion:

Our results indicate that activation of the O-GlcNAcylation pathway may be an integral part of the TLR4-induced signal, and suggest that this pathway is involved in some of the proinflammatory effects of LPS in the macrophage.

Exploring the pleiotropic roles of *O*-GlcNAc Transferase from the inside to the outside of the cell.

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O-GlcNAcylation is the addition of a single *N*-acetylglucosamine (GlcNAc) to numerous proteins catalyzed by *O*-GlcNAc Transferase (OGT) and modulates many cellular processes in close connection with the availability of intracellular nutrients. Elevated OGT and *O*-GlcNAcylation levels are frequently reported in various cancers, supporting abnormal biological behaviors of cancer cells, including proliferation and adhesive properties. It is therefore important to deepen the understanding of the molecular mechanisms regulated by OGT. In this talk, I will present our recent works on how OGT can interfere with cell cycle regulation. I will also show some of our last data regarding the effect of OGT silencing on cell surface complex glycosylations in human colon cells.

Expression changes of Galectin-4 and Galectin-9 in cervical cancer.

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Galectins are a family of proteins that bind to specific glycans, with high affinity for beta-galactosides. Expression changes of these proteins have been associated with tumor progression.

Our research group has been interested in analyzing the expression changes of Galectin-9 and Galectin-4 and the association with clinic-pathological parameters in cervical cancer. These galectins have two carbohydrates recognition domains connected by a linker peptide. They have both, intracellular and extracellular localization. Their functions vary with respect their localization, concentration and cell type. Galectin-9 participate in a variety of biological functions, such as adhesion, aggregation, proliferation, apoptosis and immune response. Extracellular Galectin-9 participate in cell adhesion and signal transduction

In cervical cancer tumors has been reported low expression of Galectin 9, and those tumors with lower o null expression were related with poor prognosis. Furthermore, serum concentration of Galectin 9 has been reported increased in some cancer types, including cervical cancer. On the other hand, Galectina-4 has been poorly studied, and its expression in cervical cancer has not been evaluated.

Our research group have determined the serum Galectin-9 levels of healthy women, with premalignant lesions and cervical cancer. The results showed an increased concentration related to the tumor transformation, by contrast the tumor expression of Galectin-9 showed a decreased in cervical cancer with respect normal tissue. Galectin-4 analysis also showed and increased concentration in serum of women with cervical cancer. And the preliminary results showed that the expression increases in cervical cancer tissue, contrary to Galectin-9. The results suggest that Galectins -9 and -4 could be used as markers and as target gene therapy in the future.

_O_-GlcNAcylation of Thymidylate Synthase: a new regulatory mechamism mediating 5-fluorouracil chemotherapy response in colorectal cancer.

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5-Fluorouracil (5-FU) based-chemotherapy is the gold standard treatment for advanced-stage colorectal cancer (CRC). However, the response and the 5-year survival rates are only about 57% and 12,5% respectively for patients with advanced-stages due to resistance to 5-FU. An increased risk of CRC recurrence in response to 5-FU-based treatment is associated with metabolic disorders. Emerging evidence accumulated during the last decade highlight the role of altered glycosylations in CRC drug resistance mechanisms. In light of these observations, we explore the relationship between 5-FU response and a "nutrition-dependent" dynamic post-translational modification (PTM), *O*-linked N-acetylglucosaminylation (*O*-GlcNAcylation). This PTM consists in the addition of a single residue of β-D-N-acetylglucosamine (GlcNAc) to the hydroxyl group of serine and threonine residues of cytosolic, nuclear and mitochondrial proteins to regulate their subcellular localization, activity, stability or protein/protein interactions. *O*-GlcNAcylation is regulated by a single couple of antagonist enzymes: *O*-GlcNAc Transferase (OGT) and *O*-GlcNAcase (OGA). In this study, we realize comparative analysis on normal, and 5-FU sensitive and resistant colon cancer cell lines. First, by adsorption on succinyl-wheat germ agglutinin and Click-chemistry labelling-based approaches, we show that Thymidylate Synthetase (TS), the major target of 5-FU, is *O*-GlcNAcylated on several residues in a cell line-dependent manner after 5-FU treatment. Co-immunoprecipitation experiments show that OGT and TS interact physically. Interestingly, by using Western-Blot and flow cytometry analyses, OGT and OGA knockdown strategies reveal a correlation between global *O*-GlcNAcylation level, TS expression and 5-FU response in HT-29 sensitive cancer cell line. Finally, we demonstrate that *O*-GlcNAcylation up-regulates directly TS protein stability in an ubiquitin-proteasome pathway-independent manner. Taken together, our results suggest that *O*-GlcNAcylation could modulate 5-FU response by regulating stability of TS enzyme, the 5-FU major target and a clinical predictive biomarker of 5-FU response. Previous clinical studies demonstrated that TS expression is negatively correlated to patient 5-FU response and survival. This work could thus provide new therapeutic strategies associated with an adapted patient diet to foil cancer resistance.

_O_-GlcNAcylation is involved in the regulation of stem cell markers expression in colon cancer cells.

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The dynamic *O*-linked-N-acetylglucosamine posttranslational modification of nucleocytoplasmic proteins has emerged as a key regulator of diverse cellular processes including several hallmarks of cancer. However, the role played by this modification in the establishment of CSC phenotype has been poorly studied so far and remains unclear. In this study we confirmed the previous reports showing that colon cancer cells exhibit higher *O*-GlcNAc basal levels than non-malignant cells, and investigated the role played by *O*-GlcNAcylation in the regulation of CSC phenotype. We found that the modification of *O*-GlcNAcylation levels by pharmacological inhibition of the *O*-GlcNAc-transferase enzyme that adds *O*-GlcNAc (OGT), but not of the enzyme that removes it (OGA), increased the expression of all stem cell markers tested in our colon malignant cell lines, and induced the appearance of a double positive (CD44+/CD133+) small stem cell-like subpopulation (which corresponded to 1-10%) that displayed very aggressive malignant phenotype such as increased clonogenicity and spheroid formation abilities in 3D culture. We reasoned that OGT inhibition would mimic in the tumor the presence of severe nutritional stress, and indeed, we demonstrated that nutritional stress reproduced in colon cancer cells the effects obtained with OGT inhibition. Thus, our data strongly suggests that stemness is regulated by HBP/*O*-GlcNAcylation nutrient sensing pathway, and that *O*-GlcNAc nutrient sensor represents an important survival mechanism in cancer cells under nutritional stressful conditions.

CD4+ T cells: Looking out for that α2,8 sialic acid.

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Human CD4+ T cells orchestrate the adaptive immune response after being activated by antigen presenting cells. Activation of these cells is characterized by specific cytokine secretion, but also by glycosylation changes that participate in their function and homeostasis. Activation of human naïve CD4+ T cells is accompanied by downregulation of ST3Gal I and ST6Gal I sialyltransferases that causes a reduction in sialic acid (Sia) expression defined as an asialophenotype, characterized by a reduced binding of SNA (α2,6 Sia) and MAL II (α2,3 Sia) lectins and increased binding of PNA (asialo Core-1-*O*-glycans) lectin. Nonetheless, experimental work performed in our group showed that anti-CD3/anti-CD28 antibodies mediated activation is in fact accompanied by hypersialylation, as evidenced by Sia metabolic labeling that showed increased expression of surface Sia*N*Az. To reconcile both findings, and in view that activation was found to also increase expression of genes coding for ST8Sia1 (ganglioside synthesis), ST8Sia2 and ST8Sia4 (protein polysialylation) sialyltransferases, we hypothesized that activation increased expression of α2,8 Sia glycoconjugates, not previously reported in these cells. Subsequent works exploring ganglioside synthesis, have allowed us to identify that activation of human naïve CD4+ T cells causes increased expression of the ganglioside GD3 [(Sia(α2,8)Siaα(2,3)]Gal(β1,4)Glc(β1,1)Cer) and neoexpression of ganglioside GD2 (GalNAcβ(1,4)[Sia(α2,8)Siaα(2,3)]Gal(β1,4)Glc(β1,1)Cer), the latter being associated to TCR clustering. Also, the GM2/GD2 synthase was found to be required for efficient activation. In our most recent work, we were able to also detect by using anti-polysialic acid antibodies 12E3 and 735, the presence of polysialic acid in these cells. Silencing of ST8Sia2 and ST8Sia4, was observed to cause increased IFN-γ and IL-2 expression, indicating that polysialic acid has a role in negatively regulating the activation response. Currently, we are pursuing the identification and functional characterization of the polysialylated proteins in human T CD4+ cells.

References. 1. Activation of human naive Th cells increases surface expression of GD3 and induces neoexpression of GD2 that colocalize with TCR clusters. 2. Polysialic acid is expressed in human naïve CD4+ T cells and is involved in modulating activation.

Galectins are associated with the neuroinflammation response in neurodegenerative diseases.

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Neurodegeneration is a pathological condition of the nervous system (NS), in which nervous cells (neurons) lose their function, structure or both, leading to the development of diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Alzheimer's disease (AD) is a neurodegenerative disorder characterized by damage to the brain regions associated with learning and memory, such as the hippocampus. The aggregation of Amyloid-β (Aβ) is an early and critical event in the pathogenesis of AD, in which the abnormal production as well as the auto-aggregation of Aβ may trigger neuronal death and cognitive impairment. The use of $A\beta_{25-35}$ peptide in animal models has contributed to the understanding of its effects on Aβ toxicity related mechanisms and its detrimental effect on spatial memory. The administration of $A\beta_{25-35}$ into the temporal cortex, dorsal hippocampus or intracerebroventricular increases the nitric oxide pathway, oxidative stress, membrane lipid peroxidation, glycosidic patterns changes and inflammation, processes which may contribute to synaptic dysfunction and neuronal death. Galectins are animal lectins that bind to β-galactosides, such as lactose and N-acetyllactosamine, contained in glycoproteins or glycolipids. Galectin-1 (Gal-1) and Galectin-3 (Gal-3) are involved in pathologies associated with the inflammatory process, cell proliferation, adhesion, migration, and apoptosis. Recent evidence has shown that the administration of $A\beta_{25-35}$ into the hippocampus of rats increases the inflammatory response that is associated with memory impairment and neurodegeneration. Galectins could participate in the modulation of the neuroinflammation induced by the $A\beta_{25-35}$. The aim of this study was to evaluate the presence of Gal-1 and Gal-3 in the neuroinflammation induced by administration of $A\beta_{25-35}$ into the hippocampus and to examine spatial memory in the Morris water maze. The animals were tested for spatial learning and memory in the Morris Water Maze. This study found memory retrieval impairment occurring at day 17, a cognitive deficit which had increased significantly at day 31 after the administration of $A\beta_{25-35}$ peptide in the CA1 of the hippocampus. Behavioral performance showed that $A\beta_{25-35}$ didn't affect spatial learning but did impair memory, with animals taking longer to find the platform. On the day 32, hippocampus was examined for astrocytes (GFAP), microglia (Iba1), Gal-1 and Gal-3 via immunohistochemical analysis, and the cytokines IL-1β, TNF-α, IFN-γ by ELISA. This study's results showed a significant increase in the expression of Gal-3 in the microglia and astrocytes, while Gal-1 didn't increase in the dorsal hippocampus. The expression of galectins is associated with increased cytokines in the hippocampal formation of $A\beta_{25-35}$ treated rats. These findings suggest that Gal-3 could participate in the inflammation induced by administration of $A\beta_{25-35}$ and could be involved in the neurodegeneration progress and memory impairment.

Heparan sulfate as key regulators of stem cells properties:
Toward new strategies for the treatment of degenerative diseases as osteoarthrosis.

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Sulfated glycosaminoglycans (GAGs) such as heparan sulfate (HS) and chondroitin sulfate (CS) are important macromolecules involved in homeostasis regulation of cell niches in numerous tissues. They are associated to core protein and constitute the superfamily of heparan sulfate proteoglycans (HSPGs) and CSPGs present on the cell surface and in the Extracellular Matrix (ECM). They play fundamental roles on self-renew, clonogenicity, proliferation, differentiation, migration and survey of numerous cell types. Such functionality is linked to their ability to interact with heparin binding proteins (HBPs), including growth factors, cytokines, and chemokines. Interactions of sulfated GAGs with HBPs ensure the link to their high affinity receptors and then participate to crucial cell signaling pathways. The specificity of these interactions is associated to precise sulfation pattern of the disaccharides that constitute GAGs. According to the tools of the CRRET lab glycomic plateform, we are able to purify GAGs species, as well as to characterize their chemical signature and functionality on HBP and cells.

Since many years, our team demonstrated that GAGs structural modifications are involved in regulation of physiological processes during aging and of pathological ones during degenerative diseases associated to aging, such as Alzheimer Disease and OsteoArthritis. The characterization of such glycanic targets in these pathologies permit to propose new innovative therapeutic strategies. Some of these strategies can be based on the use of glycanic products to optimize current limiting steps of therapeutic uses of stem cells: mobilization for purification, clonogenicity for amplification, and survey for engraftment in basically deleterious degenerative context. We propose to illustrate such strategies by examples of our results focusing on osteo-articular system.

Alzheimer´s disease and blindness

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Alzheimer´s Disease (AD) is a neurodegenerative disorder and is the main cause of dementia in the world. The pathology of AD includes the deposition in the brain of abnormal aggregates of β-amyloid (Aβ) in senile plaques (SP) and abnormally phosphorylated tau in neurofibrillary tangles (NFT). In patients with AD several changes in the retina, lens, and in the vasculature have been demonstrated that inducing visual symptoms. In this study we show the effect of Aβ peptide administered in CA in the visual pathway and the correlation between inflammation and glycosidic changes in an animal model of AD (3xTg-AD) and compared with the effect in wild type mice. The immunoreactivity to GFAP, TSPO, IL-6, and TNF-α was evaluated to evaluate changes in the inflammatory process. The changes in the glycosidic patterns were evaluated by the uses of lectins from Vicia villosa (VVL, α or β N-LACNH2), SNA (Neu5Acα2-6); ConA (α-Man); Lotus tetragonolobus (LTL, Fucα1-3GlcNAc) and Amaranthus leucocarpus (ALL, Galβ1,3GalNAcα1,O-Ser/Thr). Our results show an intracellular accumulation of Aβ peptide in the retina, optic nerve and visual cortex of mice 3xTg-AD; they also show increased expression of GFAP, TSPO, IL-6, and TNF-α in these areas. The glycosidic patterns were modified decreasing the immunoreactivity of this lectins in the retina, optic nerve and visual cortex of the mice 3xTg-AD. Our results provide a comprehensive framework of the Aβ42 peptide in visual loss due to inflammation present in the AD and offer a new possibility of a new therapeutic target.

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Fructans and fructooligosaccharide (FOS) synthesis with *Bacillus subtillis* levansucrase: mechanism and applications.

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In this conference we review some of the natural environments in which fructans play an important role in nature and its importance in human health, to highlight the importance of fructan and FOS synthesis in industry. In particular, research carried out in our group regarding B*. subtilis* levansucrase (SacB), a widely studied glycoside hydrolase from 68 family will be discussed. Although reports on SacB properties date back to last century, questions regarding levan synthesis mechanism are still open. These questions refer mainly to structure-function relationships as well as the effect of environmental factors and reaction conditions on product specificity, sucrose and/or levan hydrolysis vs transfer and more interestingly, levan molecular weight.

Advances in various aspects of SacB mechanism are discussed, as well as how these properties have been applied in our group for the synthesis of different FOS or fructans. We demonstrated, through an extensive characterization of the levan hydrolysis reaction by SacB, that the wide diversity of products derives also from fructosyl transfer to free sugars available from sucrose and levan hydrolysis. Actually, levan is an efficient fructosyl donor for fructosylation reactions, in which FOS such as levanbiose, inulobiose, blastose are formed. The efficiency of SacB fructosylation with levan as donor was applied for the synthesis of blastose, a sucrose analogue with potential prebiotic properties.

Up to now there is not an efficient enzyme for the synthesis of levan-type FOS, in spite of intensive efforts to modify SacB or other levansucrases specificity by site directed mutagenesis, which will be reviewed. For this purpose, after a complete characterization of a combined bi-enzymatic reaction between SacB and an endolevanase produced by *B.licheniformis*. ($LevB_1$), we designed a fusion enzyme containing both activities. This fusion enzyme is able to produce levan-type FOS from sucrose, with molecular weights in the range of DP2 to DP10 including mainly 1-kestose, 6-kestose, neokestose, levanbiose and blastose, as will be finally discussed.

Recombinant *O*-glycoantingens production from *Mycobacterium tuberculosis* in *Pichia pastoris* as a tool to study tuberculosis infection.

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The *O*-mannosylation of recombinant proteins (PR) is a post-translational modification (PTM) that impacts on their structure and function. Interestingly, the *O*-mannosylation pathways between actinomycetes like *Mycobacterium tuberculosis (Mtb)* and yeast organisms are evolutionarily conserved, although the modification happens in different places, in the bacteria it occurs in the membrane, while in the yeast in the endoplasmic reticulum and in the Golgi apparatus. In fact, *O*-mannosylation in actinomycetes, occurs in a manner such that mannose residues are α-1,2-linked to serine or threonine residues. Similarly, in *Pichia pastoris*, the *O*-linked glycans produced are linear chains of four α-1,2-linked mannose residues. Then in biotechnological processes, *P. pastoris* is a promising host for the production of *O*-mannosylated antigens of *Mtb* due to the similarity in the PTMs performed by both systems.

This was demonstrated during the production and characterization of the two recombinant glycoproteins produced in *P. pastoris* that were recognized by antibodies from sera of patients with active tuberculosis, at least 2 times better compared to that observed with that produced in *E. coli* without PTM. The recombinant glycoantigens were produced without the native signal or tags. Glycoproteins expression was under the control of the methanol-inducible promoter pAOX1, with secretion being directed by the α-mating factor secretion signal. Production of glycoproteins was carried out in baffled shake flasks (BSFs) and controlled bioreactors. A production up to ~ 40 mg/L of the recombinant proteins was achieved in both production systems. The recombinant proteins were recovered from the supernatant and purified in three steps, achieving a preparation with 98% electrophoretic purity. The primary structures of the recombinant proteins were characterized, as well as its *O*-mannosylation pattern. As well as, two glycoantigens were tested in a cross-reactivity analysis using serum antibodies from patients with active tuberculosis. We demonstrated recognition of the recombinant glycoproteins, indicating indirectly the similarity between the recombinant proteins produced with the native protein from *Mtb*, and validating that *P. pastoris*, is a useful cell system to produce glycosylated *Mtb* antigens.

Acknowledgements

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Enzymatic synthesis of fucosylated lactose to mimic human milk oligosaccharides

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Fucosylated oligosaccharides are found in human milk and play a very important biological role as natural prebiotics that promote the development of a healthy gut microbiota, stimulate the innate immune system, and avoid enteric infections blocking pathogen adhesion, among other functions. Efforts have been made to synthesize fucosylated oligosaccharides, due to the difficulty to purify them from natural sources, such as human milk, epithelial tissue or blood. Within the strategies for their in vitro synthesis, the employment of fucosidases is remarkable, enzymes that normally cleave the fucosyl residue from the non-reducing end of fucosylated compounds, but also are capable of synthesize them by means of transfucosylation reactions. This work summarizes the progress of our group in the use of fucosidases for the synthesis of fucosyl-glucosides that have potential for industrial and commercial applications, particularly in the milk formula substitutes to mimic natural human milk oligosaccharides.

In the first step, glycosylhydrolases from various origins were used to produce fucose-containing disaccharides with prebiotic potential using different donor substrates and L-fucose as the acceptor substrate. Eight different disaccharides were synthesized as follows: three β-D-galactosyl-L-fucosides with the glycosidase CloneZyme Gly-001-02 using lactose as donor substrate; one β-D-galactosyl-L-fucose with β-D-galactosidase from Aspergillus oryzae using lactose as donor substrate; and four α-D-glucosyl-L-fucosides with α-D-glucosidase from Saccharomyces cerevisiae using maltose as donor substrate. All disaccharides were purified and hydrolyzed; in all cases, L-fucose moiety was present, and the synthesis of β-D-galactosyl-L-fucose was confirmed by mass spectrometry. High concentrations of L-fucose as the acceptor substrate enhanced the synthesis of the oligosaccharides in all cases. The three enzymes were able to synthesize fucose-containing disaccharides when L-fucose was used as the acceptor substrate, and the highest yield was 20% using β-D-galactosidase from A. oryzae.

The capability of six lactic acid bacteria to produce α-L-fucosidase was also demonstrated; α-L-fucosidases were cell-associated enzymes and produced constitutively in different carbon sources. The highest activity was observed in Lactobacillus rhamnosus GG (0.16 U/mg). The release of α-L-fucosidase by L. rhamnosus GG, and its use in the synthesis of fucosyl-oligosaccharides were investigated. Since α-L-fucosidase is a membrane-bound enzyme, its release from the cells was induced by addition of 4-nitrophenyl-α-L-fucopyranoside (pNP-Fuc); 78% of total enzyme activity associated to the cell was recovered. Fucosyl-oligosaccharides where synthesized using α-L-fucosidase extract and pNP-Fuc as donor substrate, and either lactose or lactulose as acceptor substrates, reaching a yield of up to 25%. Fucosyl-lactose was

obtained as a reaction product with lactose, and its composition was confirmed by mass spectrometry (MALDI-TOF MS).

Finally, the effect of lactose as acceptor substrate and pNP-Fuc as the donor substrate on the synthesis of a fucosylated trisaccharide was studied in a transglycosylation reaction using α-L-fucosidase from Thermotoga maritima. Through a MALDI-TOF mass spectrometry, it was demonstrated that the synthesized oligosaccharide corresponded to a fucosylated trisaccharide, and HPLC analyses of the hydrolyzed compound confirmed that it was fucosyl-lactose. As the concentration of the acceptor substrate increased, the concentration and synthesis rate of the fucosylated trisaccharide also increased, and the highest concentration obtained was 0.883 mM (25.2% yield) when the higher initial lactose concentration was used (584 mM). Furthermore, the lower donor/acceptor ratio had the highest synthesis, so at the molar ratio of 0.001, a concentration of 0.286 mM was obtained (32.5% yield). The hydrolytic and transfucosylation activities were assessed in presence of either NaCl or $CaCl_2$ at different aw; the presence of 1.1 M $CaCl_2$ favored the rate of transfucosylation, and improved the yield of synthesis, and did not affect the hydrolysis rate in these reactions. The combination of the activating effect of $CaCl_2$, the aw and lactose concentration had a synergistic effect favoring the synthesis of fucosylated oligosaccharides.

Crucial role of N-glycans in protecting the vaccine candidate E2CD154 against the classical swine fever virus

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Classical swine fever is a highly contagious and often fatal hemorrhagic disease of swine and is caused by Classical Swine Fever Virus (CSFV), and its structural E2 glycoprotein has a significant role in swine virulence. A new candidate for the eradication of the classical swine fever was developed by CIGB due to the limitations of the CSFV vaccines developed so far. This vaccine is based on the fusion of the E2 glycoprotein of CSFV to the extracellular domain of the swine CD154 molecule that can act as a molecular adjuvant for modulating the immune response (E2-CD154). The expression system used to obtain this protein was the HEK293 cell line. The chimeric protein E2-CD154 contains 7 potential N-glycosylation sites and it was demonstrated by mass spectrometry (MS) that only 6 of them are occupied by N-glycans. Analysis by NP-HPLC and MS showed that in total N-glycans of E2-CD154, 84% correspond to complex structures, 12.7% to oligomannoside structures and 3.3% to hybrid structures. In addition, complex N-glycans contain two or three antennas that may be modified by fucosylation and only contain residues of α2.6 sialic acid. E2-CD154 deglycosylation with PNGase F under non-reducing conditions dramatically reduced immunogenicity and therefore the protection generated by this candidate against infection with classical swine fever virus.

Aloe vera – from medicinal plant to an emergent source of bioactive polysaccharides.

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Nowadays, Aloe vera (*Aloe barbadensis* Miller) is, probably, one of the herbal remedies most frequently used throughout the world due to a myriad of health benefits. For several industries such as pharmaceutical, cosmetic and food, Aloe vera has offered new opportunities for the development of products with significant added value and high acceptance by consumers demanding a healthier lifestyle. In particular, for food industry, the potential use of Aloe vera gel either as a food ingredient or as a functional food has mainly been due to its beneficial properties in treating of different diseases, such as ulcers, diabetes, immune-system deficiencies, among others. Most of these biological properties have been related to the presence of bioactive polysaccharides present in the gel. In particular, two main types of bioactive polysaccharides can be distinguished in the *Aloe vera* gel, an acetylated mannose-rich polymer which has a function as storage polysaccharide, and a galacturonic acid-rich polymer as the main component comprising the cell walls of the parenchymatous tissue. Interestingly, acetylated mannose-rich polysaccharide, also known as acemannan, has been considered as the main responsible of the beneficial properties related to Aloe plant. However, the composition and structural features of these polysaccharides as well as the beneficial properties associated to them may be altered by different factors such as the climate, soil, postharvest treatments as well as processing. Thus, it is important to carry out an accurate identification for most of the changes, either structural or compositional, that could take place during the crop or after processing in order to provide a raw material with excellent biological properties.

Protein glycosylation in medically relevant fungi.

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The cell wall of the *Candida albicans* is composed of chitin, β-glucans, and glycoproteins, which are enriched with *N*- and *O*-mannans, and these are considered the main pathogen-associated molecular patterns that the innate immune system recognizes to establish a protective anti-*Candida* immune response. It has been demonstrated that *C. albicans O*-mannans are recognized by TLR4, *N*-mannans by mannose receptor, DC-SIGN, and dectin-2, and β1,3-glucan by dectin-1. Despite this knowledge, little is known about the relevance of cell wall components during the immune recognition of other fungal pathogens, such as other species of the *Candida* genus, and the causative agents of sporotrichosis. Here, we studied the relevance of cell wall components of *Sporothrix* spp. and *Candida non-albicans* during cytokine stimulation and phagocytosis by human monocytes and monocyte-derived macrophages, respectively. Our results showed that these organisms have similar wall composition, but different degrees of cell wall porosity and this physical parameter correlated with the ability to stimulate cytokine production. Glycan removal affected the stimulation of cytokines in a species-specific manner. As previously reported in *C. albicans*, *Sporothrix* cells stimulated cytokine production in a morphology-dependent way.
Moreover, we are moving forward to the study of *Sporothrix* spp., generating tools useful for the study and the genetic manipulation of these organisms. We have optimized the strategies for gene silencing, and as a proof of the concept, we have generated *S. schenckii* mutants with defects in the *OCH1* gene. This encodes for an essential enzyme for the elongation of *N*-glycans. The phenotypical characterization of these strains has shown, for the first time, the relevance of *N*-glycans during the interaction of *S. schenckii* with the host.

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The search fro a cryptic L-rhamnosyl transferase on the *Sporothrix schenckii* genome: probabilistic models of diverging domains.

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Polysaccharides such as α- and β-glucans, chitin and glycoproteins extensively modified with both *N*- and *O*- linked carbohydrates are the major components of fungal surfaces. *Sporothrix schenckii,* the etiological agent of a subcutaneous mycosis disease, secretes a heavily glycosylated glycoprotein which is thought to be needed in the adhesion process of the fungus to dermal tissue (mannose and rhamnose being the components of the *N*-linked glycan portion of it, Ruiz-Baca et al., 2009). A bioinformatic analysis of the *Sporothrix schenckii* genome has allowed the identification of genes required for the biosynthesis of UDP-L-rhamnose, the donor of the required monosaccharide for the synthesis of rhamnoconjugates (Martínez et al., 2012; Texeira et al., 2014), but the gene of the L-rhamnosyl-transferase needed on the subsequent step hasn't been uncovered this way. Monosaccharide L-rhamnosyl-transferases are multidomain proteins that show great diversity in their primary sequences; their catalytic and recognition sites are the most conserved residues, but these are shared with most members of the glycosyl-transferase superfamily. The high similarity between the shared conserved residues hinders the use of traditional homology searches (e.g. BLAST) for identifying likely L-rhamnosyl-transferases within a genome. To overcome these limitations and uncover candidate L-rhamnosyl-transferases genes on the *Sporothrix schenckii* genome, we are using more flexible and sensitive homology search strategies based on a combination of Regular Expressions (search for highly variable motifs) and "Hidden Markov Models" (HMM, from residue-probability matrices).

Galectin-glycan interactions in viral infection: The good, the bad, and the ugly…

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Galectins are a family of ß-galactoside-binding lectins characterized by a unique sequence motif in the carbohydrate recognition domain, and wide taxonomic distribution, from fungi to invertebrates and vertebrates, including mammals. Since their discovery in the 1970s, our understanding of their biological roles, initially limited to recognition of endogenous ("self") carbohydrate ligands in embryogenesis and early development, dramatically expanded in later years by the discovery of their key functions in tissue repair, cancer, adipogenesis, and regulation of immune homeostasis. In recent years, however, evidence has also accumulated to support the notion that galectins can bind exogenous ("non-self") glycans on the surface of potentially pathogenic microbes, and function as recognition and effector factors in innate immunity. Thus, this evidence has established a new paradigm by which galectins can function as pattern recognition receptors, by binding to surface glycans of viruses, bacteria and parasites. In doing so, they can also function as and as effector factors, as they can directly kill, or inhibit adhesion and entry of the potential pathogen into the host cell, or function as opsonins, by promoting phagocytosis and pathogen clearance from circulation. Our studies on the zebrafish and mouse model systems have shown that some galectins can bind to the viral envelope and inhibit viral attachment to the host epithelial cells. However, the binding of these galectins to the host cell surface that has been desialylated by the viral sialidases, can also be detrimental to the host. Further, some viruses appear to have co-evolved with their hosts to subvert the recognition roles of selected host galectins for successful attachment and infection (Supported by grant 5R01GM070589 from the National Institutes of Health, and grants IOS-0822257 and IOS-1063729 from the National Science Foundation).

SELECTED ORAL PRESENTATIONS

Flavivirus NS1s, glycans and endothelial cells: finding the sweet spot for viral pathogenesis and disease.

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Flaviviruses can cause systemic or neurotropic-encephalitic pathology in humans mainly associated to the disruption of biological barriers (e.g. pulmonary microvascular endothelium and the blood-brain barrier). The flavivirus nonstructural protein-1 (NS1) is a secreted glycoprotein involved in viral replication, and immune evasion. However, the contribution of secreted NS1 from related flaviviruses to viral pathogenesis remains unknown. Here, we demonstrate that NS1 from dengue, Zika, West Nile, Japanese encephalitis, and yellow fever viruses selectively bind to and alter permeability of human endothelial cells from lung, dermis, umbilical vein, brain, and liver in vitro and cause tissue-specific vascular leakage in mice, reflecting the pathophysiology of each flavivirus. The interaction NS1-endothelial cell was mainly mediated by specific glycans expressed on the surface of endothelial cells. Glycan arrays indicate that sulfation status may modulate this interaction. Further glycan analyses by mass spectrometry and confocal microscopy suggest that endothelial cells from distinct tissues poses a differential glycan expression. Mechanistically, each flavivirus NS1 leads to differential disruption of endothelial glycocalyx components (EGL), a network of proteoglycans, that maintains the homeostasis of endothelial barrier. Analyses of EGL components in vivo and human samples indicate that increased circulation of these components may be useful as biomarker for disease prognosis. Our findings reveal the capacity of a secreted viral protein to modulate endothelial barrier function in a tissue-specific manner both in vitro and in vivo and how relevant glycan-NS1 interaction can lead to viral pathogenesis, potentially influencing virus dissemination and pathogenesis, and providing targets for antiviral therapies and vaccine development.

Analysis of the cell wall peptidorhamnomannan during the interaction of *Sporothrix schenckii* with the host.

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Sporotrichosis is the most frequent subcutaneous mycosis in Latin America, caused mainly by *Sporothrix schenckii*. The fungal cell wall (CW) is the first point of contact with the host immune system, playing an important role in directing the immune responses. One well-known *S. schenckii* CW component is the peptidorhamnomannan (PRM), a complex of different proteins. The PRM is recognized by specific antibodies in patient's sera and by host cell receptors. This complex is composed of 50% mannose, 33% rhamnose, 1% galactose and 16% protein, and presents O-linked tetra- and pentasaccharides, that carry important antigenic epitopes.

The aim of the project is the generation of mutants that lack some of the proteins from the PRM complex, to assess their contribution during the pathogen-host interaction.

The PRM fraction was extracted from yeast, by means of binding to concanavalin A. This fraction was further analyzed by mass spectrometry, and a total of 336 proteins were identified. Based on different characteristics, three proteins were chosen for further analysis: a chaperonin GroEL, an uncharacterized protein, and the heat shock 70kDa protein 1/8.

For mutant generation, gene silencing and Agrobacterium-mediated transformation is being used. The phenotypic characterization will consist of measurement of the copy number and expression levels, evaluation of growth and morphology, analysis of the cell wall composition, and interaction with the immune system. In addition, recombinant proteins of the three gene products are being produced in a prokaryotic model, for the generation of antibodies that will help localize the proteins on the yeast cell.

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Role of the KEX2 protease in the composition of the cell wall and inmune recognition of *Candida albicans*.

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Candida albicans is a diploid, asexual and dimorphic fungus that is part of the normal commensal microbiota of mucosal surfaces. However, when local or systemic host defense mechanisms are affected, it can cause candidiasis. The most important structure during tissue invasion is the cell wall, fulfilling essential functions in maintaining cell integrity and interacting with the environment. The cell wall is composed of mannose in its outermost layer and of glucans and chitin in the inner layer. During the process of pathogenicity, the protein transport to the extracellular space plays an important role, the hydrolytic enzymes released at the end of this event must be secreted to allow remodeling in the cell wall and for its subsequent activation. The Kex2 protease participates in this transport process and takes care of essential events in the cell, such as cell wall formation and remodeling, processes virulence factors and proteins that are essential for the integrity of the cell surface and helps in the establishment of polarized growth. It has been reported that mutant strains of this gene show aberrant cell morphology and lose the ability to form hyphae. Therefore, we think that these pleiotropic effects could affect the ability to interact with cells of the immune system and the composition of the cell wall. Thus far, we have found the mutant cells lacking KEX2 show a decrease in phosphomanosylated levels and an increase in mannose levels, as well as an increment in the cell wall protein concentration. Moreover, the mutant cells have a different ability that the wild type strain to stimulate pro and anti-inflammatory cytokines. From the results obtained, it can be concluded that loss of KEX2 generates severe changes in the composition of the cell wall and the in the recognition of C. albicans cells by mononuclear cells and macrophages.

Molecular study of L-rhamnose synthesis in *Sporothrix schenckii sensu stricto.*

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Sporotrichosis is a subcutaneous mycosis caused by the dimorphic fungus *Sporothrix schenckii*. The cell wall is the first point of contact between the fungus and the innate immune cells. A well-known cell wall component of *S. schenckii* is the peptidorhamnomannan. The yeast cell wall peptidorhamnomannan is composed of 33.5% rhamnose, 57% mannose and 14.2% protein. The L-rhamnose is synthesized by bacteria, plants, and fungi in a similar process. In bacteria, its precursor dTDP-L-rhamnose is synthesized from α-D-glucose-1-phosphate and dTTP from a pathway that requires four different enzymes: RmlA, RmlB, RmlC, and RmlD. RmlD catalyzes the final step in this pathway converting dTDP-6-deoxy-L-lyxo-4-hexulose to dTDP-L-rhamnose. In some fungi, the rhamnose synthesis is carried out by three enzymes, the last one having a double activity of epimerase and reductase. Using a bioinformatics approach, we identified the putative orthologue of rmlD in *S. schenckii*, which encodes for a dTDP-4-deoxyrhamnose reductase. Also, the orthologue expression of the gene rmlD of *S. schenckii* in a mutant of *Streptococcus mutans* lacking RmlD. The expression of *S. schenckii* RmlD in *S. mutans* restores the phenotype of the mutant that has a low growth rate and cell aggregation. Using the ATMT method, we obtained eighteen transformants with the silencing construction for rmlD gene corroborated by PCR. The preliminary results of the Real-Time PCR showed a reduction in the expression levels of the gene in the transformants. Alcian Blue binding assays show an increment in the binding of the dye to the cell wall of the transformants.

Human adenovirus type 5 E1A increases alpha 1-2 fucosylation and FUT1 and FUT2 expression in infected cells.

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It has been previously reported that some viruses (ej. HCV, HZV, HIV) induce changes in the glycosylation profile of infected cells, particularly sialylation and fucosylation that are involved in their pathogenic mechanisms. The aim of this work was to determine if infection with Adenovirus type 5 (HAd5) causes changes in the glycosylation profile of A549 cells used as a model of lung epithelium. HAd5 is an infectious agent of the respiratory tract that can be life-threatening in immunocompromised individuals; these viruses have been used for many decades as a model to better understand viral biology and to develop vaccines as well as gene and cancer therapy vectors. Identifying if HAd5 infection induces changes in glycosylation could help identify novel mechanisms through which HAd5 promotes a favorable cellular context for its replication. Through metabolic labeling of sialylated and fucosylated glycans, it was observed that HAd5 infection significantly increases de novo fucosylation as early as 8 hours post infection (h p.i.), without affecting de novo sialylation. Interestingly, this change was dependent on viral gene expression. Through the use of a panel of fucose-specific lectins, it was determined that increased fucosylation occurs through a specific increase in alpha 1-2 linked fucose (Fuc), which was further associated by real-time PCR to an increase in the expression of the alpha 1-2 fucosyltransferases FUT1 (9 fold) and FUT2 (2 fold) and by western blot increase FUT1 protein at 16 h p.i. Because fucosylation increased since 8 h p.i. and was dependent on viral gene expression, this suggested the implication of early viral genes. Cells transfected with a plasmid that expresses E1A, the first early viral gene to be expressed during adenovirus replication, showed increased expression of FUT1, pointing to a relevant role of E1A in regulating host FUT genes. Additionally, it was found that HAd5 infection modifies the expression of the bi-fucosylated antigen Lewis y, a well-known glycoantigen involved in promoting proliferation and inhibiting apoptosis, suggesting its involvement in promoting viral replication.

Determination of neuraminidase activity in human macrophages during *in vitro* infection with dengue virus 2.

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Introduction: Dengue disease is a worldwide health problem that has been aggravating in the last years. Presence, abundance and diversity of sugars in cell´s surface are elements studied in the last few years that associate cell to cell communication. In the case of viral infections, neuraminidase, enzyme who removes sialic acid residues from receptors, allows a correct signal activation into the cell.

Objective: Determine transcriptional activity levels of neuraminidase during Dengue virus 2 infection in human macrophages.

Methodology: It was performed human macrophages infection with lab strain Dengue virus 2 New Guinea C, expression levels of neuraminidase-1 and proinflammatory cytokines were determined through qPCR.

Results: Macrophage infection was detected earlier as 12, 24- and 48-hours post-infection. Neu-1 expression was lower compared to non-infected cells. Effect of neuraminidase-inhibitor its in process of evaluation.

Preliminary conclusions: Dengue virus infection its capable to inhibit neuraminidase expression. This may reduce the ability to mount an antiviral strategy. Neuraminidase levels restore in late times may allows a swift in sugars to cover up viral proteins and favor new virions assembly.

Bacterial agglutination activity of glycosylated amino sugar-binding lectin domain of PIRABvp toxin from *Vibrio parahaemolyticus*.

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ABSTRACT

Lectins are carbohydrate-interacting proteins that play a crucial role in multiple physiological and developmental aspects of all organisms. They can specifically interact with different bacterial and viral pathogens through carbohydrate-recognition domains. In this study, the lectin subunit from marine and estuarine bacterium *Vibrio parahaemolyticus* was produced as recombinant protein and the wild-type protein was purified and identified. The ability to agglutinate and a growth inhibition of bacterial both native and recombinant protein were determinate. The wild-type was purified as a complex bound to the PirAvp toxin (12,000 Da) using affinity chromatography on stroma from glutaraldehyde-fixed rat erythrocytes showing an experimental molecular mass of 62,000 Da and one oligomeric form of 125,000 Da as determined by SDS-PAGE. The recombinant protein was identified as a 50,000 Da monomer and probably a 200,000-250,000 Da tetra- or pentameric form. Both were specific for binding D-galactosamine, glucosamine and glycoproteins such as ovoalbumin and fetuin showed a greater specificity compared with mono- and disaccharides. The lectin has the ability to agglutinate only rat erythrocytes and was also determined that lectin has N-glycosylations. A positive microbial agglutination and a growth inhibition activity were observed against Gram-negative and Gram-positive bacteria. This is the first report of a lectin glycosylated secreted for marine bacterium with bacterial agglutination activity and strong tendency to form oligomers.

Expression of sialic acid in MCF-7 cells stimulated with LPS.

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During the tumor progression changes occur in glycosylation, especially in the stage of elongation of the O-glycans, one of them is the incomplete elongation of saccharide chains. Among the important molecules related to aberrant glycosylation is sialic acid and its derivatives, which are located at the terminal positions of the oligosaccharides in glycoproteins. The increase in sialic acid in the α-2,6 bond is a consequence of an increase in the expression of sialyltransferase ST6Gal I, an enzyme that transfers the sialic acid in the α-2,6-glycoprotein bond. Said glycosylation is associated with the increase or decrease in the activity of different sialyltransferases. Sialyltransferase alfa 2.6 is overexpressed in several types of cancer, such as breast, colorectal, hepatocarcinoma and cervical cancer. In this work with evaluate the expression of sialic acid in MCCF-F cells, stimulate with. METHODOLOGY: MCF-7 cells were cultured in DMEM medium, 10% SBF, 7% CO2 at 37 ° C and were stimulated with LPS at a concentration of 20 ng / ml for 2, 4 and 6 hours. They are subsequently incubated with the *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA) lectins, with FITC at 20 µg / ml. The expression of sialic acid, was evaluate using flow cytometry in ATUNE NXT cytometer and immunofluorescence microscopy. RESULTS: the expression of sialic acid is observed by immunofluorescence and cytometry showed that in conditions without stimulus, sialic acid α-2.6 is expressed more in MCF-7 cells. When MCF-7 cells are stimulated with LPS at two, four and six hours they increase. sialic acid expression in both positions with respect to baseline determination (cells without stimulus). Cells stimulated with LPS have higher expression of sialic acid α-2.6 compared to sialic acid in position α-2,3. The expression of sialic acid α-2,3 recognized by MAA of six hours with respect to that of two and four hours decreases, and the expression of sialic acid α-2,6 recognized by SNA of six hours increases in comparison to the Two and four hours. CONCLUSION: There are increase of sialic acid α-2,6 in MCF-7 cells stimulated or not with LPS, while sialic acid α-2,3 decrease after six hour, our results suggest that sialic α-2,6, could participate in cells proliferation.

Expression of TF antigen and GALNT in MCF-7 cells stimulated with LPS

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INTRODUCTION: Expression of antigens associated with cell proliferation processes, such as TF antigen, correlates with the increase in metastasis. There are differences in GALNT expression, depending on cell type. LPS on MCF-7 cells increases metastasis, however its effect on the expression of TF antigen and GALNT is unknown. METHODOLOGY: MCF-7 cells are grown in DMEM, 10% FBS, at 37 °C, 5% CO2. Cytochemistry: Cells were treated with *Amaranthus leucocarpus lectin* (*ALL*) labeled with FITC and DAPI. Stimulation with LPS at a concentration of 20 ng/ml for diferent hours. Flow cytometry: We used the Attune Nxt. RT-PCR: Obtaining total RNA from MCF-7 cells, cDNA synthesis and carrying out PCR by Master Mix PCR kit from Thermo Scientific. RESULTS: There is expression of TF antigen recognized by *ALL* in MCF-7 cells, which decreases when cells are stimulated with LPS. There is expression of GALNT4 in MCF-7 cells not stimulated with LPS and there is expression of GALNT14 in stimulated and unstimulated MCF-7 cells. CONCLUSIONS: TF antigen expression is altered in MCF-7 cells when stimulated with LPS, while there is expression of GALNT4 and 14.

Selective detection of aberrant *N*-glycans associated to carcinomas through a *Phaseolus vulgaris* leucoaglutinin biosensor.

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Glycans play important roles in cellular processes via glycan-protein interactions, such as in tumor metastasis. The use of lectin biosensors offer advantages in glycan detection, comparing with other techniques. The proposed label-free biosensor aims the detection of aberrant *N*-glycans, synthesized by tumor cells, by *Phaseolus vulgaris* leucoaglutinin (PHA-L) acting as the biorecognition element. The lectin is immobilized via amine coupling on a self-assembled alkanethiol monolayer, which is formed on gold screen printed electrodes. When put in contact with the sample, the PHA-L biosensor allows the selective detection of cancer-associated *N*-glycans with increased β1,6 branching, since a complex between the lectin and the glycan is formed, owing to the lectin affinity for this aberrant structure. Complex formation is monitored through electrochemical impedance spectroscopy, where the amount of aberrant *N*-glycans can be related with the impedance increase after sample incubation.

Concentrations/quantities of reagents were optimized on each step of biosensor construction. Percentages of variation in charge transfer resistance ($\%\Delta R_{CT}$) were calculated using the charge transfer resistance before and after sample incubation, measured from the respective *Nyquist* diagrams. Results obtained for a blank solution (PBS buffer) and for a 10 mg ml^{-1} bovine thyroglobulin solution (used as model glycoprotein) were compared. A $\%\Delta R_{CT}$= 7.54 was obtained for the blank assay (value considered as acceptable noise, typical of materials and construction process) and a $\%\Delta R_{CT}$= 117.06 was obtained for the bovine thyroglobulin assay (Fig. 1). Impedance variations greater than $\%\Delta R_{CT}$=10 are attributed to PHA-L-*N*-glycan complex formation.

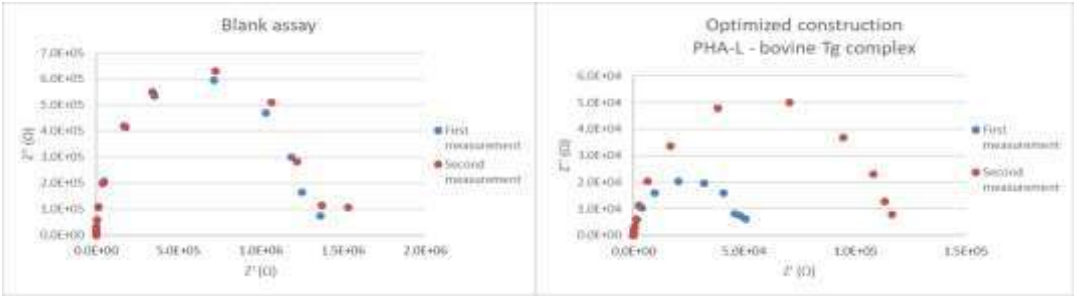

Fig. 1. *Nyquist* diagrams obtained for a blank assay (incubating the PHA-L biosensor with PBS buffer solution) and for a positive control assay (incubating with a 10 mg ml^{-1} bovine thyroglobulin solution).

Lactosylated albumin nanoparticles: potential drug nanovehicles with selective targeting toward an *in vitro* model of hepatocellular carcinoma.

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Hepatocellular carcinoma (HCC) ranks fifth in occurrence and second in mortality of all cancers. The development of effective therapies for HCC is urgently needed. Anticancer drugs targeted to the liver-specific asialoglycoprotein receptors (ASGPRs) are viewed as a promising potential treatment for HCC. ASGPRs facilitate the recognition and endocytosis of molecules, and possibly vehicles with galactose end groups, by the liver. In this study, bovine serum albumin (BSA) was conjugated with lactose using a thermal treatment. The formation of lactosylated BSA (BSA-Lac) was confirmed by a change of the chemical structure, increased molecular mass, and *Ricinus communis* lectin recognition. Subsequently, the low-crosslinking BSA-Lac nanoparticles (LC BSA-Lac NPs) and high-crosslinking BSA-Lac nanoparticles (HC BSA-Lac NPs) were synthesized. These nanoparticles presented spherical shapes with a size distribution of 560 ± 18.0 nm and 539 ± 9.0 nm, as well as an estimated surface charge of −26 ± 0.15 mV and −24 ± 0.45 mV, respectively. Both BSA-Lac NPs were selectively recognized by ASGPRs as shown by biorecognition, competition, and inhibition assays using an *in vitro* model of HCC. This justifies pursuing the strategy of using BSA-Lac NPs as potential drug nanovehicles with selective direction toward hepatocellular carcinoma.

Specific capturing of glycosylated graphene oxide by asialoglycoprotein receptor: a strategic approach for liver targeting.

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In this work, we report the evaluation of lactosylated graphene oxide (GO-AL) as a potential drug carrier targeted at an asialoglycoprotein receptor (ASGPR) from hepatic cancer cells. Structural-modification, safety evaluation, and functional analysis of GO-AL were performed. The structure and morphology of the composite were analyzed by scanning electron microscopy (SEM) and atomic force microscopy (AFM), while Raman and FTIR spectroscopy were used to track the chemical modification. For the safe application of GO-AL, an evaluation of the cytotoxic effect, hemolytic properties, and specific interactions of the glycoconjugate were also studied. SEM and AFM analysis of the GO showed graphene sheets with a layer size of 2–3 nm, though a few of them reached 4 nm. The Raman spectra presented characteristic peaks of graphene oxide at 1608 cm^{-1} and 1350 cm^{-1}, corresponding to G and D bands, respectively. Besides, Si–O peaks for the APTES conjugates of GO were identified by FTIR spectroscopy. No cytotoxic or hemolytic effects were observed for GO samples, thus proving their biocompatibility. The interaction of *Ricinus communis* lectin confirmed that GO-AL has a biorecognition capability and an exposed galactose structure. This biorecognition capability was accompanied by the determination of the specific absorption of lactosylated GO by HepG2 cells mediated through the asialoglycoprotein receptor. The successful conjugation, hemolytic safety, and specific recognition described here for lactosylated GO indicate its promise as an efficient drug-delivery vehicle to hepatic tissue.

The altered expression of Siglecs profile by monocytes and neutrophils in patients with type 2 diabetes (T2D), as a possible mechanism of immune response impairment.

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Abstract

Introduction. Sialic acid-binding immunoglobulin-type lectins (Siglecs) are transmembrane receptors expressed on the surface of immune cells, which regulate immune responses through of ITIMs/ITAMs activation. The expression of these molecules can be altered under certain pathological states such as hyperglycemia, a hallmark of individuals with T2D. **Objective.** To assess Siglec-3, Siglec-5/14 and Siglec-9 expression by monocytes and neutrophils from T2D patients and healthy individuals by flow cytometry. **Methods.** Cross-sectional study with healthy individuals (controls, n=17) and patients with T2D five or more years since diagnosis without complications (n=22). They were residents from Leon, Guanajuato and had acceptable or moderate glycemic control. A blood sample was obtained to measure fasting blood glucose, HbA1c and lipid profile. Mononuclear cells and neutrophils were separated by density gradient for flow cytometry analysis. **Results.** Siglec-5/14 expression by intermediate monocytes and neutrophils from T2D patients was lower compared to controls ($p<0.03$ and $p<0.009$, respectively). Siglec-9 expression by neutrophils was higher in T2D patients as compared with controls. Siglec-3 expression in immune cells was similar in both groups. **Conclusion.** Due to Siglec-5/14 inhibits the monocyte and neutrophils activation, the low expression of this molecule permits their activation promoting an inflammatory state. Instead, Siglec-9 expression by neutrophils, lead to its death causing a decrease in the amount of these cells. This could explain the susceptibility inflammation frequently found in T2D subjects. This mechanism yet poorly studied may be a subject for better understanding of diabetes complications.

Glucosamine protects against hydrogen peroxide in HMEC-1 cells.

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Introduction: Glucosamine (GlcN) is a nutraceutical product employed to alleviate paint, restore articular cartilage in patients with damaged or osteoarthritic joints. The primary product of this pathway, UDP-N-acetylglucosamine (UDP-GlcNAc), serves as the substrate for the addition of *O*-linked *N*-acetylglucosamine (O-GlcNAc) to cytosolic and nuclear proteins. GlcN enters cells via the glucose transporter system, is phosphorylated to glucosamine-6-phosphate by hexokinase, and thereby selectively increases hexosamine biosynthesis flux and UDPGlcNAc levels. By other hand, it has been described that GlcN is an antioxidant that scavenge several ROS including hydrogen peroxide (H_2O_2). H_2O_2, a reactive oxygen species with unique chemical properties, is produced endogenously in living systems as a destructive oxidant to ward off pathogens or as a finely tuned second messenger in dynamic cellular signaling pathways. H_2O_2 initiates signaling responses that include enzyme activation, gene expression, oxidative stress, programmed cell death and cellular damage. The aim of this study was identify if GlcN protects against dead induced by H_2O_2 and the relation to *O*-GlcNac. **Methods**: HMEC-1 were cultured with EBM medium supplemented with epidermal growth factor (EGF), hydrocortisone and Glutamine and 10% FBS. Cell are treated with or without glucosamine 20 mM and co-incubate with H_2O_2 300 µM (IC_{50}) for 24 h. After treatment the cells are incubate with MTT. Cell are also treated with GlcN 5, 10 and 20 mMol for 2, 4, 6, 12 and 24 h. The *O*-GlcNac it has been evaluated by immunocytochemistry and cytometry. **Results**: Preliminary results, indicates that GlcN prevents dead induced by H_2O_2 and GlcN by itself is no cytotoxic. There is an *O*-GlcNac increase after 6 h with GlcN 10 mM compared to the control group. This has been confirmed by cytometry. **Conclusion**: Our results suggest that GlcN prevents dead induced by H_2O_2 these effect has been related to *O*-GlcNac.

Production of an chitosanase from *Aspergillus kawachii* to obtain hydrolyzed chitosan.

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Abstract

Introduction: Chitosanase catalyzes the hydrolysis of chitosan glycosidic bonds. Based on the amino acid sequence, the chitosanases associated with the family of glycoside hydrolase (GH) 75 families. These enzymes can be used effectively for the biotransformation of chitosan to chito-oligosaccharides. The genus *Aspergillus* has species that produce glucosides hydrolases, for example *A. kawachii*, which can generate enzymes of interest in the food and pharmaceutical industries. The aim of this research was to produce a chitosanase of *Aspergillus kawachii* to obtain hydrolyzed chitosan.

Methodology: The production was by fermentation in liquid medium of *A. kawachii* in GT medium modified carbon source (Glc, QSA, GlcN, GlcNAc, Dextrin). The inoculum concentration was $1x10^6$ spores/mL, it was incubated for 48 h at 30 °C, 150 rpm. The enzymatic extract was concentrated by lyophilization. The pre-purification process was by chromatography, protein concentration and enzymatic activity was determined. Chitosan hydrolysates analyzed by gel filtration chromatography and HPAEC-PAD**.**

Results and Discussion: The fermentation that presented greater relative enzymatic activity (viscosimetry) was with a carbon source glucose. The process of pre-purification of chitosanase by chromatography (FPLC). Gel filtration fraction 4 presented greater relative enzymatic activity, this process establishes a discrimination by molecular weight eliminating biomolecules from fermentation. The degree of polymerization of the hydrolysates are varied, but without the generation of monomers.

Conclusion: *Aspergillus kawachii* is capable of produce a chitosanase constitutively by presenting an endo-type mechanism of action. This chitosanase can produce hydrolyzed chitosan.

_O_-GlcNacylation in dental pulp's stem cells: a potential clinical application.

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Introduction: *O*-GlcNAcylation is a reversible post-translational modification consisting of the addition of GlcNAc by OGT to Serine/Threonine residues of a protein and its remotion by OGA, it modification is located in nucleus, cytoplasm and mitochondria. It participates in cellular homeostasis, transcription, migration, proliferation and resistance to apoptosis. *O*-GlcNAcylation has been identified in almost all tissues and cell types. Dental pulp's Stem Cells (DPSC) are a population with a great potential for tissue regeneration, however, this modification nor its function or application has been identified in DPSC. The objective of the present work is to identify *O*-GlcNAcylation and its enzymes in DPSC in pulp tissue and in vitro. **Methodology:** 30 pulp tissues were obtained from healthy enamel organs, one part of the tissue was processed with the immunohistochemical technique and another fragment was used to isolate DPSC for in vitro culture. *O*-GlcNAc, OGT, OGA and STRO-1 were evaluated. **Results:** *O*-GlcNAc, OGT and OGA were found in different regions of dental pulp, interestingly the STRO-1 positive DPSC in pulp tissue and in vitro presented the modification and its enzymes. **Conclusions:** In embryonic stem cells it has been found that *O*-GlcNAcylation participates in cell viability, in this study *O*-GlcNAcylation was identified in DPSC of healthy pulp samples, suggesting that this modification may play a relevant role in survival of pulpal cells in different physiological and pathological conditions.

Selective Oxidation of *N*-Glycolylneuraminic Acid Using an Engineered Galactose Oxidase.

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Abstract: *N*-Glycolylneuraminic acid (Neu5Gc) is a common cell surface ligand in animals which is not biosynthesized in humans but can be acquired in human tissue from dietary sources such as red meat. It is important to understand the relevance of this potentially immunogenic glycan on human health, and selective detection methods are needed that can distinguish Neu5Gc from its biosynthetic precursor common in humans, i.e. *N*-acetylneuraminic acid (Neu5Ac). Here, we demonstrate that Neu5Gc can be selectively oxidized by an engineered variant of galactose oxidase without any reaction towards Neu5Ac. Oxidation of Neu5Gc itself allowed for the full spectroscopic characterization of the aldehyde product. In addition, we show that Neu5Gc is also oxidized when part of a typical animal oligosaccharide motif and when attached to a protein-linked *N*-glycan. Oxidation of Neu5Gc introduces biorthogonal functionality that can be exclusively labelled. We demonstrate that in combination with sialidase mediated hydrolysis, this two-enzyme system can provide a useful tool for the selective detection of Neu5Gc in complex biological samples such as the biopharmaceutical alpha acid glycoprotein.

POSTERS

Identification of *O*-GlcNAcylated proteins in *Trypanosoma cruzi*

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Originally an anthropozoonosis in the Americas, Chagas disease has spread from its previous borders through migration. It is caused by the protozoan *Trypanosoma cruzi.* Differences in disease severity have beenattributed to a natural pleomorphism in *T. cruzi*. Several post-translational modifications (PTMs) have been studied in *T. cruzi,* but to date no work has focused on *O*-GlcNAcylation, a highly conserved monosaccharide-PTM of serine and threonine residues mainly found in nucleus, cytoplasm, and mitochondrion proteins. *O*-GlcNAcylation is thought to regulate protein function analogously to protein phosphorylation; indeed, crosstalk between both PTMs allows the cell to regulate its functions in response to nutrient levels and stress. Herein, we demonstrate *O*-GlcNAcylation in *T. cruzi* epimastigotes by three methods: by using specific antibodies against the modification in lysates and whole parasites, by click chemistry labelling, and by proteomics. In total, 1271 putative *O*-GlcNAcylated proteins and 6 modification sequences were identified by mass spectrometry (data available via ProteomeXchange, ID PXD010285). Most of these proteins have structural and metabolic functions that are essential for parasite survival and evolution. Furthermore, *O*-GlcNAcylation pattern variations were observed by antibody detection under glucose deprivation and heat stress conditions, supporting their possible role in the adaptive response. Given the numerous biological processes in which *O*-GlcNAcylated proteins participate, its identification in *T. cruzi* proteins opens a new research field in the biology of Trypanosomatids, improve our understanding of infection processes and may allow us to identify new therapeutic targets.

Analysis of membrane glycosylation expression patterns on $CD4^+$ and $CD8^+$ T lymphocytes during acute *Toxoplasma gondii* infection.

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T cells play a key role during immune responses. Post-activation protein expression has been long studied in these cells providing important information to determine functional checkpoints. However, while post-translational modifications are known to be important for T cell maturation and Treg function, membrane post-activation glycosylation dynamics are not fully understood. Herein we aimed to evaluate whether membrane glycosylation patterns are modified after T cell activation. To accomplish this, Foxp3-EGFP Balb/c mice were infected with *T. gondii* cysts; on days 3,7 and 10 post-infection, splenocytes were obtained to analyze $CD4^+$ and $CD8^+$ regulatory ($Foxp3^+$) and conventional ($Foxp3^-$) populations by flow cytometry. Cells were stained with anti-CD4,anti-CD8,anti-CD69 and anti-CD25mAbs and with lectins from *Maackia amurensis* (MAII,α2,3 linked sialic acid), *Sambucus nigra* (SN,α2,6 predominantly and α2,3 to a lesser extent, both linked sialic acid), *Amaranthus leucocarpus* (ALL,SiAGalβ1,3GalNAc-α1-O-Ser/Thr) and peanut agglutinin (PNA, Gal-β(1,3)-GalNAc-α1-O-Ser/Thr). Results show that after infection, glycosilation patterns remained unmodified in both $CD4^+$and $CD8^+$ regulatory populations. However,in conventional T cells, the$CD4^+$ cell phenotype changed from ALL^{low} PNA^-MAII^+ SN^{High} to ALL^{low} $PNA^+MAII^+SN^{low/high}$;while the $CD8^+$ phenotype changed from ALL^+ PNA^+MAII^+ SN^{High} to ALL^{High} $PNA^{low/high}MAII^+$ $SN^{Low/high}$ after infection. This data demonstrates that $CD4^+$ and $CD8^+$ subsets have distinct glycosylation patterns, which are differentially modified after infection-induced T cell activation. Our analyses suggest glycan membrane analysis could reveal new functional checkpoints in the T cell activation process, previously overlooked by analyzing only protein expression.

Immunostimulation with LPS and β-glucan modulates the expression of a lectin and its receptor in *Cherax quadricarinatus.*

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Introduction.

In crustaceans, it has been suggested that specific protection against pathogens could be triggered by biological response modifiers. Lectins participate in all immunological mechanisms. In *Cherax quadricarinatus*, a humoral lectin (CqL) binds its own granular hemocytes through a specific glycosylated receptor (CqLR) and increases the production of reactive oxygen species (ROS). The objective is to identify the effect of LPS and β-glucan in lectin expression and its hemocyte´s function.

Methodology.

We challenged *in vivo* crayfishes with immune stimulants, β-glucan or LPS. We performed Total (THC) and differential (DHC) hemocytes count. We identified CqLR and CqL in the hemocytes using immunofluorescence. We determinate hemagglutinating activity in the serum and ROS production in hemocytes with NBT test for each crayfish groups.

Results.

After 2 h, the largest value in THC was observed in either challenged crayfishes. Hyaline hemocytes were the most abundant subset; however, after 6 h, granular hemocytes were the most abundant hemocyte. After 2 and 6 h of the β-glucan challenge, high CqLR expression was observed in the three hemocyte subsets; also, an increase of CqL expression was detected in a granular hemocytes sub-population. The hemagglutinating activity of the serum lectin challenged with β-glucan was 250% higher than in the LPS-treated-group. Hemocytes were stimulated *ex vivo* with CqL, ROS production was 180% higher in β-glucan+CqL group than LPS+CqL.

Conclusions.

The results suggest that, in presence of immunostimulants, CqL and its receptor play an important role in the regulation of immune cellular functions in *Cherax quadricarinatus*. UNAM-PAPIIT IN213818 and scholarship grant CONACYT 376926.

Potential role of O-GlcNAcylation on *in vitro* infection by influenza A virus H1N1 on A549 cells: preliminar study.

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Introduction: Flu is febrile and inflammatory disease caused by Influenza A type virus (IAV), which could evolve in respiratory complications and even death. Otherwise, O-GlcNacylation have shown an important role in multiple cellular and metabolic processes, however, little is known about its effect on viral infections, even if some viral studies have been done (HIV, HPV, etc.). To our knowledge, its impact on the IAV infection is completely unknown. **Methodology:** *In vitro* infection of A549 cells with IAV, H1N1 (strain New Caledonia 99) was used employing a MOI 2 and a 24 hr-infection period. Infection was confirmed by detection of the M1 viral protein by western blotting and immunofluorecence microscopy. To inhibit the O-GlcNAc transferase (OGT), [50 µM] of OSMI-1 inhibitor was used (2 hrs before virus addition). Levels of M1 and O-GlcNAcylation was evaluated by western blotting (RL2 antibody), adjusting by the level of β-actin. **Results:** In a total of 5 independent trials, the effect of the OGT inhibitor on LV infection was evaluated. In all cases, a significant decrease in M1 protein was observed, compared to infected cells; even after adjusting with β-actin levels. We are confirming the selective inhibition of O-GlcNAcylation by immunodetection. **Conclusion:** Our results suggest that the inhibition of OGT in A549 cells infected by type A VI, H1N1, causes a significant decrease in viral replication levels.

Non-enzymatic glycation of bovine serum albumine with triacetylchitotriose for the analysis of bacterial anti-adherence effect.

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The investigation of molecules that work by blocking the recognition of pathogenic bacteria towards the host cells is on the rise because this can contribute to diminish the indiscriminate use of antibiotics and with it the generation of resistant strains. The strategy is based on the use of glycoconjugates analogous to cell surface molecules, and which can competitively bind to pathogens and, with this, prevent the development of an infection. In the present study, glycoproteins were synthesized from bovine serum albumin (BSA) and the triacetyl chitotriose oligosaccharide, by means of the Maillard reaction under conditions of 40% humidity, temperature of 60 ° C, during 12 and 72 incubation. Glycoprotein characterization was carried out by electrophoresis under denaturing and reducing conditions (SDS-PAGE), intrinsic tryptophan fluorescence and Fourier transform infrared spectroscopy (FT-IR). The biological recognition was carried out by lectins and bacteria E. coli K99, K88 +, K88ab and K88ac. In conclusion, the characterization showed that the binding of the oligosaccharide to the protein was carried out and the recognition tests showed that bacterial adhesins can recognize the synthesized glycoproteins.

Antioxidant and prooxidant effects of polisaccharides from submerged culture of *Ganoderma sp.*

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Abstract

Introduction. Ganoderma is a genus that has been used for the treatment of many diseases, for thousands of years. Polysaccharides are one of the bioactive compounds isolated from this genus.

The aim of the present study was to determine the antioxidant effect of a polysaccharide fraction (GPS-2) isolated from *Ganoderma sp.*

Methodology: A Ganoderma species collected in a tropical forest of Morelos, Mexico. The submerged culture of *Ganoderma sp*. Afforded soluble (GPS-2) and insoluble polysaccharide fractions. The antioxidant effect of GPS-2 was determined on Human Peripheral Blood Mononuclear Cells (PBMCs) or Cordon Blood Mononuclear Cells (CBMCs). The reactive oxygen species (ROS) effects were evaluated by: flow cytometry, reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH), and with red mitochondrial superoxide indicator (MitoSOX™).

Results: The characterization of GPS-2 indicated that consists of a mixture of β-glucans (90%) and α-glucans (10%). GPS-2 induces a concentration-dependent antioxidant and pro-oxidant effect in CBMCs, but with PBBCs only a pro-oxidant effect can be observed.

Conclusion: GPS-2 with a prooxidant effect could be a promising natural anticancer agent.

Kefir exopolysaccharides as emulsifying agents of biologically active molecules.

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Kefiran is a polysaccharide produced by the flora of kefir grains. Recently, its application in the food industry has become more common as an emulsifying and texturing agent since the addition of this polysaccharide improves stability during processing and on final products. Emulsification is a useful tool to increase the content of bioactive molecules in foods, such as phenolic compounds, and even living cells (probiotics). The objective of the present work is to obtain exopolysaccharides from three kefir-fermented beverages and use these polysaccharides as emulsifying agents. First, the kefiran was produced from inoculation of kefir grains in three different media: skim milk, and plant-based beverages of coconut and soy. Following purification and lyophilization, kefirans physicochemical characterization was carried out. Next, emulsifying stability was studied for the subsequent tests with phenolic compounds. The obtained results indicated on a variety in physicochemical properties of the polysaccharides depending on the fermentation media used. For example, the molecular weight of the exopolysaccharide obtained from coconut drink was greater than $1.0x10^6$ Da, followed by the kefiran from soybean drink with molecular weight of $1.0x10^6$ Da, and skim milk (9.8x105 Da). In case of thermal properties, an outstanding result was observed for the milk polysaccharide which showed the highest glass transition temperature (100.43 °C) in comparison to soybeans and coconut saccharides that stood at 87.2 and 81.4 °C, respectively. To conclude, the chemical composition of the lyophilized kefirans is adequate for the correct formation of emulsions and therefore, they can act as the potential carrier agents of the bioactive molecules such as phenolic compounds.

Synthesis of fuco-oligosaccharides by α-L-fucosidase from *Thermotoga maritima* in organic solvents.

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Introduction. Fuco-oligosaccharides (FUCOs) are the most abundant human milk oligosaccharides and have beneficial effects on health like prebiotic, immunomodulator and antiadhesive. FUCOs can be synthesised by *α*-L-fucosidases, which have hydrolytic and transferase activity. Catalysis in the organic media favours transglycosylation activity.

The aim of this work was to evaluate the solvents effect on the hydrolytic/ transglycosylation activity and thermostability of the *α*-L-fucosidase from *Thermotoga maritima* (*Tm*Fuca).

Methods.

Hydrolytic activity in presence of DMSO, acetone and acetonitrile (10-50%) was measured by adding 4-nitrophenyl-*α*-L-fucopyranoside (*p*NP-Fuc) 3.5 mM and *Tm*Fuca (0.0065 U/mL) dissolved in phosphate buffer (0.1 M, pH 8) at 60 °C for 15 min. Transglycosylation was performed for 12 min under the same conditions but adding 438 mM D-Lactose, synthesis of FUCOs was measured in 10% of DMSO.

Thermostability was determined for 3 h at 60 °C in 10% of solvents.

Results and Discussion.

Residual activity of the fucosidase in acetone was better in all concentration respect the control assay, reaching the highest activity in 40% of acetone.

*Tm*Fuca has good thermostability preserving 75% of their activity at 3 hours with no significance difference between solvents.

Best transglycosilation/hydrolysis ratio (r_s/r_h) was obtained with 10% of DMSO (0.77) and acetonitrile (0.88), due to the advantages of DMSO on the synthesis of FUCOs was performed in 10% of DMSO reaching a yield of FUCOS of 0.71 mM (19.14%).

Conclusion. Solvents don't have any effect on the activity and stability of *Tm*Fuca allowing to use DMSO in the synthesis of FUCOS.

Study of the production of α-L-fucosidase using bifidobacteria.

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Introduction. Bifidobacteria have HMO´s hydrolytic enzymes, such as α-L-fucosidase, which catalyzes hydrolysis of fucooligosaccharides, but under certain conditions, catalyzes the exchange of carbohydrate residues forming new oligosaccharides.

The objective of this work was to evaluate the extraction of α-L-fucosidase from Bifidobacterium longum, using ultrasound.

Materials and methods. B. longum was inoculated in MRS medium with inulin, at 37 ° C for 24 h. The biomass was resuspended in phosphate buffer at a cell concentration of 6% and ultrasound was applied with a Sonics VCX 130 sonicator, varying the volume from 10 to 20 ml, the power from 50 to 80 W and the time from 2 to 8 minutes in working cycles of 30%. Enzymatic reaction was performed using 4-nitrophenyl □-L-fucopyranoside as a substrate. Enzymatic activity and biomass concentration were quantified by spectrophotometry.

Results.

A maximum of 38% of the residual activity was released, with an 8 minute sonication time, a power of 65 W and a volume of 10 mL.

The variable with the greatest significance was time, having a correlation between the sonication time and the enzymatic activity obtained, with respect to the interactions, the only significant was that of time-volume.

Conclusions. The use of ultrasound as an enzyme extraction method proved effective in releasing the fucosidase enzyme from B. longum, allowing up to 38% of residual activity to be released. The highest residual activity is obtained with longer sonication times.

Green synthesis of glyconanovectors using lactosylated albumin for antiproliferative compounds transport.

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The use of nanostructures modified with carbohydrates provides the advantage of specifically interact with cell receptors. Recently, we reported the potential action of the lactosylated albumin nanoparticles (NPs) as drug nanovehicles with selective targeting toward an *in vitro* model of hepatocellular carcinoma. The present study is focused on obtaining glycated albumin under green synthesis conditions to prevent generation of toxic residues and to compare it with conventional chemical synthesis. Synthesis included lactose or lactobionic acid, $NaBH_3CN$ and bovine serum albumin (BSA). Recognition of exposed carbohydrates was done by *Ricinus Comunis* lectin (RCA) and characterization of modified BSA by SDS-PAGE and HPLC-MS. Both BSA-Lac Δ (green synthesis) and BSA-Lac $NaBH_3CN$ (conventional chemical synthesis) showed similar biorecognition by RCA and increase in BSA molecular mass. BSA-Lac Δ generated less toxic residues, thus was selected to formulate NPs to encapsulate antiproliferative agents such as Oregano Essential Oil (OEO) and carvacrol. NPs of BSA-Lac/AEO were 206.9 ±2.581nm in size and Z potential of -31.5 ± 0.65; NPs of BSA-Lac/carvacrol were 244.4 ± 5.15nm with Z potential of -30.3 ± 0.87. Both NPs showed lectin biorecognition.

RMN DETECTION OF G_{D3} AND G_{D2} GANGLIOSIDES IN T CELLS

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Introduction: Gangliosides are glycosphingolipids that contain one or more units of sialic acid that are ubiquitously expressed in vertebrate tissues, cells and body fluids, being particularly enriched in lipid rafts of cell membranes. Recently, our research group, using monoclonal antibodies and flow cytometry, reported that activated human CD4+ T lymphocytes overexpress G_{D3} ganglioside and neoexpress G_{D2} ganglioside. Nonetheless, a direct detection of these gangliosides remains to be performed. **Objective:** The aim of this work was to detect the presence of G_{D2} and G_{D3} through TLC (thin layer chromatography) and NMR (nuclear magnetic resonance), using MOLT-4 cells as a model of CD4+ T- lymphocytes. **Methodology:** MOLT-4 cells were cultivated in T-25, T-75 and T-125 flasks using RPMI medium at 37 °C in a humidified atmosphere containing 5 % CO_2. The ganglioside profile was monitored every 48 h during 12 days of culture by flow cytometry, TLC and RMN^{1}H. **Results:** The IMF and cell percentage was higher for G_{D3} than G_{D2} at 4 and 6 days of culture, interestingly, the viability and proliferation also increased at this time. We also identified characteristic spots of gangliosides using resorcinol-H_2SO_4 reactive in TLC and NMR^{1}H signals in dimethvl sulfoxide-d_6 (DMSO) solvent, allowing the assignment of carbohydrate residues compared to the shift of ganglioside standards. **Conclusion:** It was possible to detect and characterize the dymanics of G_{D3} and G_{D2} expression in MOLT-4 cells.

Autoclaving improves the techno-functional properties of common bean (*Phaseolus vulgaris* L.) carbohydrates.

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Common beans (*Phaseolus vulgaris* L.) are an important source of proteins, resistant starch (RS), dietary fiber (DF) and other bioactive compounds that could reduce the risk of multiple non-communicable diseases. Autoclaving with subsequent cooling steps could increase the amounts of RS, reduce the content of α-galactooligosaccharides (α-GOS) and improve the techno-functional properties. Dehulled black beans and distilled water were placed in food pouches and autoclaved at 100°C or 121°C during 15 or 30 min and then cooled at 4°C for 24 h. Processed beans were blended with distilled water, pH was adjusted to 11.5 and stirred at 60°C for one hour, and then centrifuged at 4,000 rpm for 30 min. The protein-containing supernatant was discarded, and the pellet was solubilized in distilled water to dry using a spray dryer. RS, DF and α-GOS content were determined using assay kits from Megazyme. The water holding capacity (WHC) and solubility were also analyzed. Unprocessed black bean carbohydrates and wheat flours were used as controls. Processing reduced (p<0.05) the RS content up to 27% with two times autoclaving-cooling cycle treatment. Autoclaved beans had similar DF concentrations as whole wheat flour. The α-GOS content of bean flours was as low as (p<0.05) the wheat flours. All treatments increased (p<0.05) the WHC. The solubility of autoclaved bean flours was lower (p<0.05) than wheat flours. Autoclaved bean flour could be considered as pregelatinized starch which can be incorporated in dry mixes, such as a thickening agent for puddings, sauces, creams, or dairy products.

Molecular diagnosis of a patient with congenital disorder of glycosylation type I.

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Congenital disorders of glycosylation are multisystemic diseases caused by defects or aberrations in the glycosylation process, these defects occur in the activation, presentation or transport of carbohydrates precursors; glycosidases, glycosyltransferases and proteins involved in the maintenance of Golgi homeostasis. N-glycosylation defects are detected by isoelectric focusing (IEF), the study of isoforms of serum transferrin (N-glycosylated protein) defined by its isoelectric point. The migration profile in the IEF is determined by the glycans associated with transferrin, particularly sialic acid, which allows to establish the presence of an alteration of N-glycosylation. When these isoforms are separated by IEF, it is possible to observe the sialylation profile of the individual; In a healthy individual, the main isoform is the tetrasialilated, while in people who have a CDG-I there is a decrease in the tetrasialilated isoform, an increase in the disialilated isoform, and the presence of the asialilated isoform. In an individual with a CDG-II there is a presence of the monosialilated isoform in addition to the asialiated and disiallylated, although the decrease in tetrasialilated isoform is also evident. In the laboratory of human glycobiology and molecular diagnosis, we received a serum sample obtained from a male patient who presented the following clinical characteristics: minor dysmorphies, microcephaly, brain disorders, epilepsy, psychomotor regression, intellectual disability, muscular atrophy, strabismus, nystagmus, blindness cortical, among others. After the analysis of the clinical picture, an IEF of the patient's serum transferrin was performed, resulting in a hypoglycosylation profile corresponding to a CDG-I, based on the patient's symptoms, two platforms designed for the identification and comparison of diseases with the patients were used. due to the type I profile, as well as the suggestion of the platform about the potentially affected genes, it was decided that the candidate genes to be analyzed by sequencing are: DPM1, ALG6 and PMM2 since they are the only ones thrown by the platform that they cause CDG and that are in the synthesis pathway of the glycan precursor of the N-glycosylation pathway. This work aims to identify the mutation responsible for causing type I CDG in the patient by amplifying the genes cDNA: DPM1, ALG6 and PMM2 by PCR followed by Sanger sequencing for the identification of the mutation in any of the candidate genes.

Genetic variants identified by exomic analysis in congenital disorders of glycosylation.

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Introduction: Congenital Disorders of Glycosylation (CDG) are human genetic diseases due to 130 different defects in the pathway of glycoconjugates. These pathologies encompass defects of nucleotide-sugar biosynthesis or transporters, glycosyltransferases, vesicular transport, as well as in lipid and glycosyl-phosphatidylinositol anchor glycosylation. The clinical features range from a severe multisystem to mild phenotype and often associated with neurological impairments (hypotonia, psychomotor disability, strabismus, ie). Analysis of serum transferrin isofocusing (Tf-IEF) combined with Next-Generation Sequencing (NGS) plays a significant role in CDG diagnostic. **Objective:** Report improvements in CDG diagnosis in Argentinean patients. **Methodology:** patients were referred by pediatricians from Argentinean medical centers. Ethical permissions and informed consents were obtained. Transferrin analysis were performed according to standard methods. Genomic DNA from patients with altered Tf-IEF by the exome sequencing of genes associated with human diseases OMIM database (clinical exome sequencing, CES). **Results:** A neurological multisystem phenotype was observed in four patients with Tf-IEF CDG-type I (di- and asialo-Tf increased). We identified a homozygous missense variant (c.G753T; p.Arg251Leu) in exon 2 of ALG-2 gene OMIM #607906). In silico analysis showed a moderately pathogenic variant Functional tests are underway. The fourth patient had non CDG gene variants and abnormal Tf-IEF as part of the syndrome, deserves further study.
Conclusion: It must to keep in mind a CDG in any unexplained syndrome, in particular when there is neurological involvement. Our results highlight the usefulness of NGS to CDG diagnosis. Together it provides insights to guide research towards new therapies (precision medicine). CONICET-UCC-UNC.

Genetic analysis of multiple osteochondromatosis in a cohort of argentinean patients.

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Introduction: Mutations in two tumors suppressor genes, *EXT1* and *EXT2*, have been identified in multiple osteochondromatosis disease (MO), an autosomal dominant O-glycosylation disorder (EXT1/EXT2-CDG). The *EXT1* (8q24.11,MIM 133700) and *EXT2* (11p12,MIM #133701) genes, encode glycosyltransferases involved in heparan sulfate elongation in chondrocytes. The risk of malignant chondrosarcoma is 2%-5%. **Aim:** To enhance the power in genetic testing and to improve to our knowledge of the molecular bases of OM in Argentina. **Methods:** A longitudinal study including 35 unrelated MO patients (20% (n: 7) families) with history of OM). Clinical phenotyping from all 55 patients (35 index and 20 family affected members) were obtained. *EXT1* and *EXT2* were analyzed using a skeletal dysplasia panel (SkeletelSeqV5, n=368 genes, SeqCap EZ (Roche Nimblegen) and run on a NextSeq (Illumina) and MLPA. Bioinformatic analysis was performed. **Results:** The index patients had an average age: 13.56 (2.21-55.3 years-old); male 63.6% and 36.4% female. Main clinical features were: chronic pain and fatigue 74.5% (41/55); spinal tumors 10.9% (6/55); 18.1% short stature (10/55); limb length discrepancy 27.9% (12/43) and intellectual disability 10.9% (6/55). Clinical severity was classified as class I 34%, as class II 23% and as class III 43%. The life quality (PEDsQL 4.0) evaluated in children 7-18years-old, was 72.6% (DE 14.21) and adults (SF36) 32.86% (DE 9.77). A total of 20 different pathogenic variants in 35 probands, 13 (65%) in *EXT1* and 7 (35%) in *EXT2* were identified 13 (47%) frameshifts, 9 (31%) nonsense, 2 (6%) splicing and 5 (17%) missense. No *EXT1* or *EXT2* mutation in 5 (12%) patients whilst one patient presented *FLNB* variant (Larsen syndrome, MIM150250). Three patients with pathogenic variants showed mental disability (low CI), normal EEG, brain TAC and karyotype. **Discussion:** mutations were identified in 29/35 (83%) patients with OM. Interestingly, in our cohort (80%) were sporadic, in comparison to data where 90% have an affected parent. Phenotype cannot be predicted based on mutation type or gene. Recent studies (Inubushi et al, 2019) showed data supporting Palovarotene (a retinoic acid receptor) is a potential therapeutic agent for MO. In this sense, the genetic advances in this field will be necessary to include patients into future biologically based therapeutics. CONICET, FONCyT, UCC.

Study of expression of specific glycans on platelet membrane in congenital disorders of glycosylation patients.

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Introduction: Congenital Disorders of Glycosylation (CDG) are human genetic diseases due to defects in the pathway of glycoconjugates. Most of these patients present thrombo-hemorragic events. **Objective:** To contribute to the knowledge of the physiopathological bases associated with thrombo-hemorrhagic alterations in CDG patients. **Methodology:** The expression of total glycans in platelet membrane we studied in a platelet population (PRP) of normal controls (6) and PRP from a PMM2-CDG. The platelet population was identified by flow cytometry labeling using the anti CD-41-PE antibody. A panel of 10 biotinylated lectins labeled with Streptavidin and conjugated with an Alexa-647 antibody was tested to detect glycans. We compared the value of MFI observed in the control group for each lectin studied in relation to the values obtained in the patient PMM2-CDG, by means of a Student's T test considering a significant difference the lower values $p<0.05$. **Results:** The MFI values showed no significant differences between the controls and the patient with PMM2-CDG in the following readings: AAL, CON-A, UEA-I, RCA-I, MAA-I and WGA**.** In addition, we were able to corroborate that expression of PNA, GSL-I and GSL-II lectins was not observed, indicating very low levels of *O*-glycans in the platelet membrane proteins. However, this study showed significant differences ($p <0.05$) in the expression of the SNA lectin between the controls and the patient. **Conclusion:** For the first time, we have characterized the profile of glycans present in platelets of a PMM2-CDG patient, observing a decrease in the expression of SNA lectin ($p <0.05$), consequently, a lower sialic acid content. The decrease in sialic acid profile could increase the platelet clearence, (thrombocytopenia) and as a result, a potential risk of hemorrhage in these patients. We observed that the platelet proteins are mainly *N*-glycosylated. CONICET, FONCyT, UCC.

Clinical utility of congenital disorder of glycosylation gene panel in patient with refractory epilepsy.

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Introduction: Congenital Disorders of Glycosylation (CDG) are a growing group of multisystemic diseases caused by defects in the formation or processing of glycoproteins and/or glycolipids. Most types of CDGs present in early infancy and the clinical manifestations may include failure to thrive, hepatopathy, hypoglycemia, protein-losing enteropathy, developmental delay, hipotonia, neurologic abnormalities, eye abnormalities, immunologic, skin and skeletal findings. Type I CDG includes a group of disorders where there are defects in the biosynthesis of dolichol-linked oligosaccharides in the cytosol or endoplasmic reticulum (ER), as well as defects involving the transfer of oligosaccharides onto nascent glycoproteins. CDG-IK is a type I CDG caused by homozygous or compound heterozygous mutation in *ALG1* gene (OMIM605907), encoding beta-1,4-mannosyltransferase on chromosome 16p13. This type of CDG is characterized by predominant neurologic involvement.
Case Report: We present a patient referred at one year for refractory seizures, lack of eye contact, failure to thrive, developmental delay and acquired microcephaly. She had mild hyperammonemia, abnormal coagulation studies, her LFTs were elevated and cholesterol was low. Her brain MRI showed cortical and central atrophy, visual evoked potentials showed axonal neuropathy and electroretinography has a normal response. **Methodology and Results:** Transferrin isoelectrofocusing revealed a typical CDG type I pattern. No other pathological biochemical result was found. A gene panel sequence analysis (test of 102 genes of Congenital Disorders of Glycosylation) was made. Two heterozygous likely pathogenic variants in trans configuration were identified in *ALG1*, c.826C>T (p.Arg276Trp) and c.863-2A>G.
Conclusion: Family was counseled and ketogenic diet was started. She improves her refractory epilepsy and repetitive epileptic status and she is free of seizures.
CDGs should be suspect in patients with microcephaly, neurologic involvement and liver compromise. Molecular panels are a rapid and accurate tool for diagnosis and should be consider early in the diagnostic workup. CONICET, FONCyT, UCC.

Evaluation of galectin-4 in serum and tissue of patients with premalignant lesions and cervical cáncer.

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Introduction. Galectin-4 is a protein involved in several cellular functions, by recognizing to β-galactosides of glicoconjugates. In differents cancer types the seric concentration and tissue expression of patients has been evaluated. In patients serum galectina-4 increased and has been associated with tumor progression. On the other hand, in tissue patients a expression change was associated to the stage of progression, early recurrence, metastasis, poor prognosis and lower survival. Our working group showed a lower expression of the gene; that codes for this protein, in cervix malignant tumors. Nevertheless, the expression of galectin-4 in serum and tissue of patients of cervical cancer was not assessed. To evaluate the galectina-4 expression in serum and tissue of patients whit cervical cancer and to determinen if there is an association. **Metodology.** Serum and tisue samples was obtained from woman: healthy, with premalignant lesions and cervical cancer. The patients serum were evaluated in an ELISA and the detection in tissue was performed by histochemistry with antibody specific for galectin-4. **Results.** We observed an increased of galectin-4 concentration in serum from patients with premalignant lesions and cervical cancer. On the other hand, the galectin-4 expression in patients tissue has a market increase respect to progression grade. **Conclusions.** The galectin-4 increment in serum and greater expression of tissue patients with cervical cancer can be asociated with the patology progression. Changes in the pattern galectin-4 expression have been proposed as a posible biomarker for the follow-up, prognosis or predictor of cancer development.

Determination of epigenetic modifications in *LGALS9* promoter and the relationship with its expression in cervical cancer.

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Introduction. Cervical cancer is the second cause of mortality in women in developing countries. Alterations in epigenetic mechanisms as DNA hypermethylation and deregulation of histone acetylation in some gene promoters have been described in the last years, and this have been related with tumor progression. Galectin-9 (Gal-9), coded by the *LGALS9* gene, is a protein with antitumoral functions and its low expression has been associated with poor prognosis in cervical cancer. To date, little is not known about the factors that modify Gal-9 expression. **Metodology**. To determine epigenetic modification associated to Gal-9 expression, we used cell lines and biopsies of cervical cancer. Chromatin Immunoprecipitation (ChIP) was performed to verify histone acetylation H3K9ac and H3K14ac adjacent to *LGALS9* promoter. Expression of Gal-9 was performed by immunocytochemestry and immunohistochemistry. **Results.** In tumoral cell lines, we detected a loss of H3K9ac, that was present in non-tumoral cell line HaCaT; H3K14ac was present in the tumoral cell lines suggesting that is not related with Gal-9 expression. In HaCaT cells, Gal-9 expression was higher than in tumor cell lines and we observed a different expression pattern between the different cell lines. In cervical cancer tissues, we observed a low expression of Gal-9 and in advanced stages of cancer it was null. **Conclusion**. The loss of H3K9ac is related to lower expression of Gal-9 in cervical cancer cell lines. Low expression of Gal-9 in cervical cancer tissue could be related to loss of acetylation of H3K9.

Evaluation of anticancer and antioxidant capacity of: *Nephelium lappaceum, Melicoccus bijugatus, Manilkara zapota* via the modulation of intracellular sumoylation processes.

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Cancer, the second leading cause of death worldwide, caused 8.8 million deaths in 2015. Exotic fruits have important quantities of phenolics acid, flavonoids, tannins and others compounds that possesses antioxidant and/or anticancer properties. In this work, we evaluated the antioxidant/anticancer properties of different extracts from *Melicoccus bijugatus, Nephelium lappaceum, and Manilkara zapota*, exotic fruits endemic in the south of México. For that, the skin, pulp and seeds of each fruit were collected separately. The seeds have been previously dried. The skin and pulp were macerated and extracted with methanol using phytochemical techniques. The solvent was removal and after lyophilized, its cytotoxicity was measured on three cancer cell lines: colic (HCT116), prostatic (DU145) and breast (MCF7) by Incucyte® cell live assays and MTT cell viability assays. *Manilkara zapota* seeds show the best antiproliferative properties. In a second part of this work, we investigated the cellular mechanisms by which these compounds could affect cancer cells proliferation and viability. We focused on SUMOYlation processes, a post-translational modification of proteins consisting in the covalent linkage of one or several residues of SUMO-1 or SUMO-2 (Small Ubiquitin Like Modifier) onto lysines of the targeted proteins.We hypothesized that phenolic acids could modulate cancer cells SUMOYlation levels. We evaluated the effect of our different fruit extracts on SUMO-1 and SUMO-2 levels by Western Blot using specific antibodies. The total extracts containing phenolic acid impact the sumoylation level in cancer cell lines. *Manilkara zapota* seeds extract, showed a low level in SUMO 2/3 and SUMO1 on HCT116 cell lines.

Characterization and functional assay of the PIGX promoter: a clue of its regulation.

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PIGX encodes for an essential component of glycosylphosphatidylinositol-mannosyltransferase I, that participates in the synthesis of glycosylphosphatidylinositol. Data from *The Human Protein Atlas* suggest that the gene could be useful as a prognostic biomarker in breast and liver cancer. Also, evidence from our research group suggests that *PIGX* could function as a prognostic gene in cervical cancer. The overexpression is associated with a good prognosis, whereas its downregulation with poor survival. Thus far, there is no evidence about the signals that regulate the gene expression neither the regulatory elements of the *PIGX* gene. In this study we identified, characterized, cloned and evaluate the activity of the putative promoter. Preliminary results indicate the promoter contains core elements such as GC, TATA and CCAAT boxes and Inr sites; also we identified binding sites for the transcription factors LEF1 and TCF, CBF, c-Myc, c-Myb, and, FOXM1, and for retinoic acid receptors. These results may be useful to elucidate the signals that promote the *PIGX* expression, that in turns is associated with good prognosis in cervical cancer.

Impact of *O*-GlcNAcylation over the PI3-Kinase/Akt pathway in oral squamous cell cancer.

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Oral squamous cell carcinoma (OSCC) is a public health problem with an increased incidence in young people. In the OSCC is expected that alterations in glycosylation could modify signaling pathways that promote tumor progression. *O*-GlcNAcylation is a reversible posttranslational modification that involves the addition of GlcNAc to Ser/Thr residues of cytosolic, nuclear and mitochondrial proteins, regulated by the enzymes OGT and OGA. In several tumors enhanced *O*-GlcNAcylation triggers the growth, invasion and cell resistance to death. These events are currently unknown in OSCC and how this modification affects the regulation of the PI3-kinase/ Akt pathway remains unclear. The purpose of this work was to determine the level of the *O*-GlcNAcylation in OSCC tumors and healthy tissues, as well as the impact of this modification in the activation of this pathway in the SCC-152 cell line.

Our results showed that OSCC tumors overexpressed the *O*-GlcNAc modification both in the cytoplasm and the nucleus, such as the SCC-152 cells. On the contrary, healthy tissues exhibited lower expression of the *O*-GlcNAcylation that is localized mainly in the cytoplasm. In this cell line, treatments that stimulated this modification, activated the PI3-Kinase/Akt pathway with an increase in pAkt, however, significant changes were not observed in proliferation, it did not affect the morphology and structure integrity of the colonies. In conclusion, this indicates that *O*-GlcNAcylation stimulates the PI3K/Akt pathway by Akt phosphorylation, and impacts the integrity of the colonies which could be related to the tumor growth and survival.

Key words: Oral squamous cell carcinoma, *O*-GlcNAcylation, PI3-K/Akt pathway.

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Analysis of galectin-9 expression in cervical cancer.

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Introduction. Galectin-9 is a protein that recognizes and binds to glycan structures and plays an important role in chemotaxis, differentiation, aggregation, adhesion and apoptosis. Its function varies depending of the cell type and its expression level. In different cancer types it has been reported altered expression of galectin-9. In cervical cancer it has found that a low expression is associated with the cancer progression. **Methodology.** To evaluate the galectin-9 expression in cervical cancer cell lines we have performed real time PCR, western blot, and cytochemical assays, additionally we have evaluated the galectin-9 expression in cervical cancer tissues by immunohistochemical assays. **Results.** The levels of mRNA and protein in the four cervical cell lines were significantly lower with respect to the control cell line. The cytochemical analysis showed that the localization of galectin-9 was more diffuse in the cytoplasm of normal cell line with respect the tumor cell lines. In cervical tissues the galectin-9 signal is low or absent while in normal tissues is always expressed. **Conclusions.** Expression of Galectin-9 is lower in cervical cancer cells and tissue. The decrease of expression during cell transformation could be associated with malignant progression due to the lack of antitumor function.

Detection of the cancer-associated t antigen using an *Arachis hypogaea* agglutinin biosensor.

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The T, Tn and STn antigens are immature *O*-glycans that are expressed in tumor cells, due to incomplete glycosylation, and are known as pan-carcinoma glycobiomarkers. In the case of the T or Thomsen-Friedenreich (TF) antigen, the increase in its expression is related to the processes of cancer cell proliferation and metastasis, hence its detection can favor the medical diagnosis (in early stages) and prognosis.

An impedimetric biosensor was developed for the detection of the T antigen, using the *Arachis hypogaea* lectin (PNA) as biorecognition agent. The formation of the complex between PNA and glycoproteins containing the T antigen (asialofetuin) was monitored by electrochemical impedance spectroscopy, measuring the increase in the biosensor impedance. The response of the biosensor was directly related to the amount of glycoprotein applied on the sensor surface.

With the PNA biosensor, T-antigen expression was evaluated in blood serum samples and it was able to discriminate between control samples (individuals without cancer) and case samples of patients with different carcinomas (skin, colon, breast, prostate, stomach, kidney, lung, liver and rectum). The same samples were analyzed with a *Vicia villosa* agglutinin biosensor (developed by our group) that has specificity for the Tn antigen, to compare the expression of both antigens in the different carcinomas. The results were distinct for both biosensors, confirming that the use of different lectins allows to monitor the expression of diverse cancer-associated antigens.

Slowly digestible starch of beans (*Phaseolus vulgaris L.*) improves the efficiency of glucose metabolism and oxidative stress in rats with induced diabetes.

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The consumption of carbohydrates that have a postprandial response of prolonged absorption, sustained and gradual energy release, is an emerging area of study to control and improve the complications of diseases related to metabolic syndrome (MetS) and diabetes. Methodology. Bean starch was treated by pullulanase debranching and retrogradation to produce slowly digestible starch (SDS). *In vitro* digestibility was evaluated and kinetics were fitted into the Logarithm of Slope model (LOS) to obtain first-order rate coefficients and extent of digestion. The effect of SDS consumption was evaluated in male Wistar diabetic rats (220 g), with isocaloric diet adding 30% SDS for 4 weeks. Metabolic biomarkers, nutritional parameters and biomarkers of oxidative stress were evaluated. To evaluate the postprandial response and degree of bioaccessibility *in vivo*, a biokinetic monocompartmental model was used to describe. Statistical analyses of all the data obtained were evaluated using one-way ANOVA. Results. The content of SDS in the product was 65.13%. The degree of hydrolysis of SDS was 51.5%. The kinetic profile of amylolysis in vitro was characterized by a linear phase. The rate constant (k) was lower for SDS and higher for native starch (NS). The effect of SDS in diabetic animals improved the nutritional parameters, decreased the insulin resistance index (TyG) and lipoperoxidation, and increased antioxidant enzyme activity. The postprandial response in vivo showed a prolonged absorption of glucose and gradual energy release, generating a reduction in hyperglycemia. Conclusion. The daily consumption of SDS in the diet can improve the efficiency of carbohydrate metabolism and the oxidative stress condition.

Study of the potential role of the human papillomavirus E6 protein in the *PIGX* expression.

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The glycogene *PIGX* is a potential biomarker for cervical cancer. The gene downregulation in patients is associated with poor survival. Since there is no evidence about the signals that regulate the gene expression, we had identified the putative gene promoter and some of the potential regulatory sites. The infection and persistence of some specific genotypes of human papillomavirus (HPV), such as type 16, is the main risk factor for the cervical cancer development. The oncogenic effect of HPV is mainly due to three viral proteins. The HPV16-E6 protein displays several functions that can promote cell transformation. Among others, E6 can modify the host gene expression by several mechanisms. For instance, E6, through the interaction of transcription factors and regulatory elements can modify the gene expression. Here, we expressed ectopically the HPV16-E6 protein in a keratinocyte cell line and quantified the *PIGX* mRNA. Our results suggest that the presence of the E6 HPV-16 protein decreases the glycogene expression. Moreover, the results of *in silico* analysis of the gene promoter suggests that E6 could be decreasing the gene expression through the retinoic acid receptor system.

Serum galectin-9 as a potential biomarker for cervical cancer.

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Cervical cancer is the second cause of death by cancer in Mexican woman. Nevertheless, there are no biomarkers for treatment response to cervical cancer. Galectin expression has been related to tumor growth, apoptosis, invasion, metastasis and cell transformation. The changes in galectins expression between normal and cancerous tissues indicate that these proteins may be used as prognostic biomarkers and to determine treatment response. Galectin-9 expression has been shown to be altered in different cancer types. In cervical cancer galectin-9 expression is decreased in tumors, while concentration in serum is showed to be increased. We evaluated the concentration of Galectin-9 in serum of patients with normal cytology, premalignant lesions and cervical cancer. A blood sample was taken and then the serum Galectin-9 concentration determined using R&D Systems Human Galectin-9 Quantikine ELISA Kit. Results showed increased expression in patients with cervical cancer. Concentration of Galectin-9 was also related to cancer progression, the serum of patients in stage IV (metastatic cancer) had the higher concentration. Therefore, differential concentration of Galectin-9 might serve as a potential biomarker for cervical cancer.

Streptozotocin (STZ)-induced type 2 DM, increases inflammatory cytokines in serum.

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Introduction. Streptozotocin (STZ)-induced type 2 DM, STZ-DM2, in animals, is used as a model of human type 2 DM. STZ-DM2 is characterized by hyperglycemia, insulin resistance and process inflammatory, were increased plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). All it provides by adipose tissue dysregulation in consume excessive saturated fat and carbohydrates simples. The objective of the study is to determinate if other proinflammatory cytokines rise (gamma interferon, INF-γ and monocyte chemotactic protein 1, MCP–1) and if anti-inflammatory cytokine (IL-2) decreases in serum Wistar rats with STZ-DM2, after 6 weeks with hypercaloric diet, HD. Procedure: We used an enzyme-linked immunosorbent assay (ELISA) multiplex method to determine the cytokines in serum of healthy, HD (fructose 20% and saturated animal fat 20%) and HD + STZ-DM2 (1 dose, 40 mg/kg intraperitoneal) groups. Results and discussion: Leptin satiety modulating hormone, as well as the inflammation state in obesity and diabetes, significantly increased 7.2 times in HD and 2.48 times in STZ-DM2. All the cytokines were increased in pathologic states. IFN-γ increased in 1.73 times in HD and 1.28 times in STZ-DM2. MCP-1 increased 1.34 times in STZ-DM2 and had not changes with HD. IL-6 (1.58 HD vs healthy and 1.67 in STZ-DM2) and TNF-α increased 1.56-1.67 times in HD and STZ-DM2, respectively. All results confirm the inflammatory state. Even though IL-2 had not significant changes in the conditions. Conclusion: In STZ-DM2 the inflammatory cytokines increased. Supporting that inflammatory state of STZ-DM2 is similar to human type 2 DM.

Antitumor effect of glycosylated steroidal saponins derived from diosgenin *in vitro*.

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Introduction. Diosgenin and its derivative glycosylate Diosgenin 3-Glu have diverse biological activities. However, their complete role on cancer cells is not currently established. In this study the antiproliferative, apoptotic, selective and antitumoral activities of Diosgenin, Diosgenin 3-Glu, MF10, and MF-11 were probed. **Methodology**. The antiproliferative effect on tumor cells was determined by means of violet crystal staining, while in lymphocytes was by carboxyfluorescein labeling. Necrotic cell death was evaluated by the activity of the cytoplasmic enzyme lactate dehydrogenase (LDH) in supernatants from cell cultures. Apoptosis was morphologically evaluated as well as by immunodetection of the active caspases (-3, -8, and -9) under an optical microscope. **Results.** Diosgenin and its glycosylated derivatives evidenced antiproliferative activity on tumor cells, with IC_{50} values ranging from 3 to 17 µg/mL. Meanwhile in lymphocytic cells the proliferative activity was slightly affected. In addition, these compounds did not induce necrosis in tumor cells neither in lymphocyte cells. Our results evidenced that Diosgenin and its glycosylated derivatives induce programmed cell death apoptosis in tumor cells. **Conclusions.** Diosgenin and its derivatives have antiproliferative activity and induce to apoptotic death in tumor cells, without significantly affecting non-tumor cells, showing that they have a selective action and they can be antitumor agents with therapeutic potential.

Tn tumoral carbohydrate antigen promotes the growth of vascularized tumors.

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The Tn antigen (GalNAc-O-Ser/Thr) is a tumor-associated carbohydrate antigen expressed in 90% of adenocarcinomas. It constitutes both a diagnostic tool and a target for immunotherapies. Tn originates from a blockage in the O-glycosylation pathway, which prevents Tn to be elongated to core 1, by the core-1-synthase, and its associated chaperone, COSMC. Previous studies show that the presence of Tn is associated with malignancy and is related to the metastasis potential of the tumoral cell. Furthermore, it is recognized by the C-type receptor, MGL, present on dendritic cells and macrophages, and may modulate the immune response. In this study, we developed a tumoral model to study the immunomodulatory role of Tn during tumor growth. We generated variants of the lung cancer murine cell line, LL2 that express different levels of Tn by mutations in the COSMC chaperone gene. We confirmed the expression of Tn with specific lectins, such as VVL and HPA, using an antibody against Tn, evaluating the T-synthase activity and confirming the recognition by the MGL2 receptor. Interestingly, when inoculated in syngeneic mice, Tn+ cells produced tumors that grew faster and presented higher levels of vascularization. In addition, Tn+ cell tumors presented more CD11c+ F4/80+ cells that expressed higher level of MGL2, together with an increased expression of FoxP3 and regulatory cytokines, such as IL10 and IL4, in infiltrating CD4+ T cells. These results indicate that Tn has a relevant role in the tumor development and suggest it may be responsible for immunomodulary processes triggered by the tumor cells.

The Tn antigen promotes tumor growth and metastasis.

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Altered glycosylation is considered a universal cancer hallmark. One of the most frequently observed aberrant glycophenotype is the expression of the truncated O-glycan structures such as the Tn antigen (GalNAc-Ser/Thr). Tn expression is associated with decreased overall survival and increased risks of recurrence. However, little is known about the role of the Tn antigen in cancer progression and metastasis *in vivo.* Thus, in this work we generated an experimental metastatic cancer model to study cancer growth and metastasis and the immune response. A Tn expressing murine breast cancer cell line derived from the highly metastatic 4T1 cell line was generated (4T1-Tn+), together with a Tn-negative cell clone (4T1-Tn-). The glycophenotype of the generated cells indicates higher levels of GalNAc-containing membrane glycans in 4T1-Tn+ as compared to 4T1-Tn- and the parental cell line. *In vitro* proliferation and colony forming assays did not show any differences between cell lines. Remarkably, when injected in mice, the 4T1-Tn+ cells produced significant bigger tumors and promoted more lung micrometastasis. Furthermore, when using a metastatic preclinical model, with resection of primary breast tumor, the expression of the Tn antigen was associated with a decreased mice survival and the presence of higher number of lung metastasis. Immunological studies revealed lower leukocyte infiltrate in the tumor microenvironment, with presence of $CD11c^{+}F4/80^{+}$ cells expressing the Tn-recognizing lectin receptor MGL2 in Tn+ tumors. Our results indicate that the Tn antigen plays a central role in the malignant properties of cancer cells, favoring metastasis and immunomodulation.

Identification, characterization and evaluation of the *LGALS9* gene promoter.

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Introduction: Galectin-9 encoded by the *LGASL9* gene located on chromosome 17 (17q11.2) consist of 11 exons. It's a tandem repeat galectin for its two DRCs, distributed in liver, small intestine, thymus, kidney, spleen, lung, cardiac and skeletal muscle, brain, pancreas, prostate. TNF, IL-1β and IFN-γ induce their expression in primary cultures of astrocytes. To date the promoter of the *LGALS9* gene has not been described in humans, so identifying and characterizing it will provide information on its regulation.

Methodology: *in silico* analysis using the program Eukaryotic Promoter Database identified two possible promoter regions upstream of the start site of transcription of the *LGALS9* gene in humans, different fragments of the promoter (2089, 1479, 727 and 222 bp) were amplified by PCR and cloned in the pGL4.12 reporter vector, the activity of the different fragments was measured by luciferase assays, using the Dual Luciferase Assay System kit.

Results: the activity of the fragment of 727 bp that contains the possible promoter region (600 bp) is high with respect to the other fragments. In this fragment, TATA boxes, binding sites to TFIIB an Inr, were identified. In addition to other sites of response to transcription factors such Sp1, GATA-1, AP1, AP2.

Conclusions: a fragment of 727 bp, which contains the start site of the transcription of the *LGALS9* gene, shows promoter activity. This region contains characteristic sites of a classical promoter, such a TATA boxes and Inr sites, so we can conclude that this region is the promoter of the *LGALS9* gene.

Identification and partial characterization of a lectin from *Moringa oleifera* seeds.

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The *Moringa oleifera* tree is broadly distributed in the tropical region of Mexico coast, from many parts of the tree has been attributed different medical properties; from the seeds we isolated a lectin by affinity chromatography using rabbit's erythrocyte stroma. It is a 10-kDa protein and pI from 3 to 4 as determined by 2D electrophoresis. The highest agglutinating activity was observed in presence of rabbit´s erythrocytes; although erythrocytes from several animal species included human ABO were agglutinated in less proportion. Purification processes increased 12 times the specific activity of the lectin. Neither simple sugars nor di or three saccharides tested were able of inhibit the agglutinating activity; however, glycoproteins like lactoferrin (14.4 mM) fetuin (6.25 mM) or thyroglobulin (1.8 mM) inhibited the agglutinating activity of 4 Hemagglutination units in presence of rabbit's erythrocytes. Our results indicate that *M. oleifera* lectin possesses a broad CDR that recognizes oligosaccharide sequences.

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Functional characterization of the C.303 G> C mutation in the isoform of del177 of the CMP-sialic acid transporter.

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CMP sialic acid transporter (SLC35A1) located in Golgi is proposed as a 10 transmembrane domains (TM1-TM10) protein with both residues -NH2 and -COOH oriented to cytosolic side of the Golgi membrane. TM1, TM3, TM7 (NIQM motif), as well as loops 3 (Golgi), 4 (Golgi), 7 (Golgi), 8 (cytosol) and the VGII residues of the carboxyl terminal end, have been reported as indispensable for the operation of SLC35A1. Mutations in SLC35A1 lead to an erroneous sialylation process, which triggers the presence of congenital glycosylation disorders, such as SLC35A1-CDG. Mohamed et al. reported that the point mutation c.303 present in a patient reduces 50% the function of SLC35A1. Salinas Marín et al. reported that the human isoform DEL177 is functional. Objective of this project is elucidate whether the c.303 change does not affect the function of the isoform, since it could affect as a therapeutic alternative for patients with SLC35A1-CDG. Methodology: We obtained Lec2 cells transfected with the plasmid of177 with the point mutation c.303 C> T. From these transfected cells, molecular and metabolic analyze were performed to establish the behavior of this modification in the Lec2-CHO complementation model. Results: The c.303 G>C mutation generates a reduction of the function in the DEL177 isoform, in the Lec2-CHO complementation system. Conclusions: Here, we report that the point mutation c.303 G> C in del177 decreases the transport of CMP-Sialic acid, suggesting the importance of Gln 101 both in the three-dimensional structure and in the function of the isoform.

Expression dynamics of the *O*-glycosylated proteins recognized by *Amaranthus leucocarpus* lectin in T cells and their relationship with Moesin as an alternative mechanism of cell activation.

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Glycoproteins are crucial for T cell activation and function. We have previously shown that *Amaranthus leucocarpus* lectin (ALL, specific for Ser/Thr-O-GalNAc) induces a costimulatory function similar to that generated via CD28. Also, our data indicate that ALL-recognized glycoproteins are inducible in $CD4^+$ lymphocytes after in vitro activation with anti-CD3/CD28 but constitutive in $CD8^+$. Other experiments suggest that in $CD4^+$ lymphocytes the main protein recognized by ALL is moesin, a 70KDa protein classically associated with cytoskeleton; however, the identity of the other proteins recognized by this lectin is unknown and whether they are expressed in $CD8^+$ lymphocytes or not. In this work, we evaluated and compared by Lectin blot the glycoprotein profiles recognized by ALL in $CD4^+$ and $CD8^+$ lymphocytes and determined by mass spectrometry the identity of immunoprecipitated proteins with ALL obtained from each subpopulation.

Our data shows that after activation with anti-CD3/CD28 the number of bands recognized by ALL increased from 4 to 12 and from 7 to 13 (0h-48h) in $CD4^+$ and $CD8^+$ cells respectively. Furthermore, analysis of the ALL immunoprecipitated glycoproteins showed a 70kDa protein in $CD8^+$ cells identified as moesin, which coincides with previous reports in the $CD4^+$ subset.

These data show that moesin is the main protein recognized by ALL in both $CD4^+$ and $CD8^+$ lymphocytes, although there are also other proteins that are differentially expressed in each subpopulation. We think that the O-glycosylation in moesin modulates its location towards the cell membrane in order to provide a previously undescribed secondary activation signal on T cells.

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Redistribution of L-fucose residues in pig spermatozoa exposed to the perfluorated compounds PFOS and PFOA *in vitro.*

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Spermatozoa require different stages of maturation to be able to fertilize the ovule: epididymal maturation, capacitation and acrosomal reaction (AR), in which there are the removal and redistribution of glycoconjugates in the plasma membrane. The perfluorooctane-sulfonate (PFOS) and the perfluorooctanoic-acid (PFOA) are perfluorated compounds (PFCs) considered persistent toxics. The aim of this work was to evaluate if there is an alteration in the distribution of L-fucose residues mediated by these PFCs. The Ulex europaeus lectin (UEA) conjugated with FITC, a specific lectin for L-fucose residues was used. Semen samples of normospermic pigs were analyzed by fluorescence microscopy under 3 experimental conditions: fresh spermatozoa washed with PBS (F), after 4 hours of capacitation induction (CI) and after 20 minutes of progesterone AR induction (ARI), in the presence of both PFCs. In control F spermatozoa, we observed that L-fucose was concentrated mainly on the apical zone of the head; in control CI the fluorescence labeled homogeneously on the head and the tail; in control ARI fluorescence was concentrated on the apical zone or homogeneously on the sperm. PFCs modify the control fluorescence patterns in several ways, possibly by altering the integrity of the membrane, the changes in the distribution of L-fucose residues are the consequence of the toxicity of PFCs and may affect the ability of the spermatozoa to recognize molecules present in the oviductal epithelium and the zona pellucida of the ovule.

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Characterization of the ganglioside expression profile in human CD8+ T lymphocytes.

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Gangliosides (GGs) are sialic acid (Sia) containing glycosphingolipids. An important role of GGs is to participate in the formation of lipid rafts and microdomains, which help form signaling cascades, involved in different cellular functions, such as proliferation, differentiation and survival.

It has been reported that anti-CD3/CD28 mediated activation of human naive CD4+ T lymphocytes induces expression changes in b-series GGs, particularity GD3 overexpression and GD2 neoexpression, the latter colocalizing with the TCR during activation-induced clustering.

The objective to this work was to determine the expression profile of b-series GGs (GD3, GD2, GD1b and GT1b) in human CD8+ T lymphocytes.

The peripheral blood mononuclear cells (PBMCs) were obtained by a sucrose gradient and subsequently purified by a negative gradient purification kit isolate human total CD8 + T lymphocytes. Afterwards, the cells were activated and ganglioside expression was measured by flow cytometry at (12, 24, 48 and 72h.). The neo-expression of GD2 ganglioside was observed from 12h post-activaton, there was also a decrease in the GD3 ganglioside after 12h. (non-significant data) and an increase in the GT1b ganglioside after 48h post-activation (significant data).

Real time PCR was performed to determine is GD2 neoexpression was induced by an increased expression of the GD2 synthase. The GD2 synthase was found at baseline in resting cells and increased it expression since 12h post-activation.

Subsequently, to analyze the location of the GD2 ganglioside, confocal microscopy was performed, observing that at 3h. post-activation the GD2 ganglioside is scattered throughout the cell, however, since 6h. It begins clustering along with the TCR.

This observation suggests that the GD2 ganglioside could be important for TCR clustering and thus and efficient activation as occurs with CD4+ T cells.

Characterization of the polysylation of activated human CD4 + T lymphocyte glycoproteins.

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Introduction. Human naïve CD4+ T cells are the subset of T lymphocytes responsible for modulating the adaptive immune response through cytokine secretion. We have previously reported that the activation of these cells is accompanied by increased *de novo* sialylation, that allowed us to identify the overexpression of the genes coding for polysialyltransferases ST8Sia II and ST8Sia IV and the resulting ability of these cells to synthesize polysialic acid. In unpublished data, we evaluated the overexpression of PSA in activated CD4+ T lymphocytes by use of anti-PSA NCAM 12E3 monoclonal antibody and we identify the expression of at least eight glycoproteins carrying this modification. The identity of these proteins as well as the PSA attachment sites to *N*- or *O*-glycans has not been described. The caracterizarion of polysialylation contributes to elucidation of relevance and function of these glycoproteins in CD4+ T lymphocyte biology. **Objective**. Identify the proteins carrying PSA, and the type of the glycan to which PSA is attached. **Methods.** Through mass spectrometry we determine the identity of the polysilated proteins of the CD4 + T lymphocyte, the proteins analyzed were previously isolated through immunoprecipitation with the anti-PSA monoclonal antibody NCAM 12E3. *N*-glycan association was evaluated by PNGase F digestion of whole lysates from activated CD4+ T lymphocytes and analyzed by western blotting. **Results.** The susceptibility of the proteins to digestion with PNGase F demonstrates that in at least three of the eight polysiallylated glycoproteins, the polysialic acid is associated with *N*-glycosylation. Mass spectrometry revealed several proteins with direct involvement in T cell function.

Polysialic acid is involved in human CD4+ T lymphocyte activation.

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CD4 + T lymphocytes orchestrate the immune response after activation by the antigen-presenting cells. During activation, rearrangements of the cytoskeleton, gene expression and molecules associated with activation occur, as well as an increase in proliferation due to the production of the cytokine IL-2. Additionally, it has been reported that the activation of CD4+ T lymphocytes leads to an asialofenotype in the surface glycans as a consequence of the reduction of sialic acid (Sia) in α2,3 and α2,6 linkages. However, although the reduction of Siaα2,3 and Siaα2,6 is well characterized, the presence of the Siaα2,8 link has been little studied in these cells. In the present work we evaluate the total incorporation of Sia on the surface of human naïve CD4+ T lymphocytes, both at rest and activated by anti-CD3/anti-CD28 antibodies. We observed that the activation induces an increase in the total Sia of the cell surface, independently of the reduction reported in Siaα2,3 and Siaα2,6. This led us to postulate that the activation of human naive CD4+ T lymphocytes presents an increase in total Sia at the expense of Siaα2,8 structures. The analysis in the expression of the α2,8 sialyltransferases allowed us to identify that the activation increases the expression of the coding genes for ST8Sia I, ST8Sia II and ST8Sia IV, indicating the possible synthesis of gangliosides and polysialylated proteins. Based on the increase in the polysialtransferases ST8Sia II and ST8Sia IV, the possible presence of polysialic acid (PSA) in the human naive CD4+ T lymphocyte, a type of glycosylation that had not been described in these cells, was suggested. Through the use of the monoclonal antibody anti-PSA clone 12E3 we confirmed in this work, that CD4+ T lymphocytes synthesize PSA through the expression of both polysialiltransferasas. Additionally, by silencing the ST8SIAII and ST8SIAIV it was identified that the PSA is associated to the modulation of the activation of the immune response mediated by IL-2, IL-2R and IFN-γ.

The 13 KDa allergenic protein from *Fraxinus uhdei* pollen is *O*-GlcNAcylated

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The pollen from *Fraxinus uhdei* is the most abundant in Mexico City, which cause allergic reactions and enhance health risk. I information of proteins involved in human diseases remains poorly understood. Our main objective is to characterize the structural modifications of pollen's allergenic proteins as well as their role in human diseases. We have determined that pollen proteins showed post-translational modifications and are specific glycosylated for *N*-acetyl-glucosamine, chitobiose, and have been involved in cross-reactivity with several allergens. For the characterization of allergenic proteins were determined the reactivities of sera from allergic to pollen and grass pollen-sensitized patients, they were screened by 1D and 2D immunoblot against pollen extract. In 1D analysis of sera showed binding to variability proteins between 10-200 kDa; whereas, WB revealed with serum from allergic patients indicated the relevance of a 13 kDa protein. *F. uhdei* saline extract showed several proteins which seems to be glycosylated, as determined by lectin-blot; immunodetection was positive for WGA and in lesser extent by ConA in 13 kDa protein, suggesting presence of O-glcnacylated post-translational modification. Further ongoing proteomic analysis of diseased patient samples will allow us to determine the immunogens and their putative reaction mechanisms. Outcoming results will help to understand molecular processes of proteins involved in immune response for allergic respiratory diseases.

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