SIMPOSIO INTERNACIONAL “GLICANOS EN EL ESTUDIO DE LA RESPUESTA INMUNE Y LAS ENFERMEDADES INFECCIOSAS”

COMITÉ ORGANIZADOR

Dr. Héctor Manuel Mora Montes
Dra. Leila Maria Lopes Bezerra
Dr. Iván Martínez Duncker Ramírez

LA REALIZACIÓN DE ESTE EVENTO FUE POSIBLE GRACIAS AL APOYO FINANCIERO RECIBIDO POR PARTE DEL CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA A TRAVÉS DE LA RED TEMÁTICA GLICOCIENCIA EN SALUD 270918.

CONACYT
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Centro de Investigación en Dinámica Celular

Universidad Autónoma del Estado de Morelos

Universidad de Guanajuato

GLICANOS EN EL ESTUDIO DE LA RESPUESTA INMUNE Y LAS ENFERMEDADES INFECCIOSAS

3 y 4 OCT 2016
10-16 h

EDIFICIO DE POSGRADO UNAM

October 3rd

Novel analytical tools for glycobiotechnology.

Enzymatic synthesis of glycans: developing new vaccines for diarrhea treatment.

Peptidorhamnomannan epitopes of Sporotrhix spp. as a diagnostic tool.

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

October 4th

Paracoccidioides spp as resilient genetic tractable organism: towards functional genomic studies.

Mechanisms of synthesis and hydrolysis of cell wall alpha-1,3-glucan in Paracoccidioides brasiliensis.

N and O-glycosylation are involved in cell wall organization, adhesion and virulence in the ubiquitous plant pathogen Botrytis cinerea.

The immunoglycobiology of Candida spp. and Sporothrix spp.

Schedule
Programa

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Advanced tools for structure-function studies of glycoconjugates

Sabine L Flitsch

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Carbohydrates are the most abundant biomolecules on Earth and have a multitude of function depending on biological context. They are important components of many biopharmaceuticals including therapeutic antibodies and vaccines and as such of great value to human health. Our understanding of glycans in terms of structure and function is limited due to the complexity of these biomolecules at many levels: heterogeneity of samples; structural complexity due to carbohydrate sequence and conformation; conjugation to diverse biomolecules; and polyvalency as part of clustering of ligand presentation. These intrinsic complex features are very difficult to define, but can dramatically affect biological function.

To address these challenges, our aim is to develop an integrated synthetic and analytical toolset that can be used by the scientific community. Defined carbohydrate and glycoconjugate probes can be prepared using in vitro biocatalysis, taking advantage of the abundance and exquisite selectivity of diverse glycoenzymes. For structural analysis we have developed tandem mass spectrometry methods incorporating ion mobility spectrometry for assignment of three dimensional shape. And finally, we have shown that glycan arrays are ideally suited for functional studies of interactions between carbohydrate ligands and proteins, cells or microorganisms.

Laurent et al. *Chemical Communications*, 4400-4412 (2008); Rannes et al. *Journal of the American Chemical Society* **133**, 8436-8439 (2011); Castangia et al. *Angewandte Chemie International Edition* **51**, 13016-13018 (2012); Noble et al. *Journal of the American Chemical Society* **134**, 13010-13017 (2012); Šardzík et al. *Journal of the American Chemical Society* **134**, 4521-4524 (2012); Martinez et al. *RSC* Advances **44**, 21335-21338 (2013); Both et al. *Nature Chemistry* **6**, 65-74 (2014); Noble et al. *Org. Biom. Chem.***12**, 9272-9278 (2014); Huang et al. *Carbohydrate Res.* **415**, 60-65 (2015); Formisano et al. *Biosensors and Bioelectronics* **85**,103-109 (2016).

Enzymatic synthesis of glycans: developing new vaccines for diarrhea treatment

Dra. Juana Elizabeth Reyes Martínez

Profesor Asociado "C", Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato.

Diarrhea is one of the biggest problems in Caribbean and Latin-American countries causing thousands of deaths each year, affecting mainly children and elderly people. Infections caused by *E. coli* are also one of the leading causes of diarrhea in travellers from Europe and US to developing countries.

Bacterial cell wall polysaccharides play an important role in the virulence of many pathogens; those are antigenic polysaccharides with potential use for vaccine development.

Complex glycans are used for basic science studies, in pharmaceutical, food and biotechnology industries. Developing new catalytic routes for complex glycans production is very important in glycobiology research. Our research is focused on developing new catalytic routes for the synthesis of glycans present on the cell wall of human pathogens as *E. coli* and *Klebsiella pneumoniae*, organisms causing diarrhea and lung infections respectively. Current glycan synthesis strategies are time consuming and produce large amounts of toxic waste therefore alternative synthesis strategies are needed. Glycosyltransferases show great specificity and hundreds of glycosyltransferases have been identified from genomic databases showing a great potential for glycan synthesis.

Peptido-rhamnomnnans epitopes of *Sporothrix* spp. as a diagnostic tool

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Several emerging species of the Fungi kingdom are important pathogens for human and/or animals. The pathogenic species of the *Sporothrix* genus, *S. schenckii s. str., S. brasiliensis* and *S. globosa*, are the etiological agents of human sporotrichosis, a subcutaneous mycosis that has also extracutaneous clinical forms. This disease also affects domestic cats and, cat-transmitted sporotrichosis is an emerging healthy problem mainly associated with *S. brasiliensis*. The fungal cell wall is composed by 80% of carbohydrates and, among the main cell wall components pathogenic fungi we highlight the structural polysaccharides, chitin and beta-glucans. These structural polysaccharides are already known to play a pivotal role in host recognition by innate immune cells. Our general hypothesis is that other polysaccharides or glycoproteins can also play an important role in the immune response. Besides these structural polysaccharides, *Sporothrix* spp. presents on the cell surface a peptido-rhamnomannan (PRM) that is recognized by antibodies present on patient's sera. Previous work of our group showed that PRM has O-linked oligosaccharides bearing the ConA binding sites and important epitopes of α-Rha*p* 1→4 α-GlcA*p* and α-Rha*p* 1→2 α-GlcA*p*. These epitope are present in an enriched cell wall PRM fraction isolated by affinity chromatography, the SsCBF antigenic fraction. This antigen was applied to develop and validate a serological diagnostic test (ELISA) that is useful not only for the diagnosis of all clinical forms of sporotrichosis but also, for the patients' therapeutic follow-up.

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

Jorge Humberto Ramírez Prado (Centro de Investigación Científica de Yucatán, A.C., México)

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 are the components of the *N*-linked glycan portion of the glycoprotein (Ruiz-Baca et al., 2009). A detailed bioinformatic analysis of the *S. schenkii* genome has allowed the identification of the 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 residues are shared with most members of the glycosyl-transferase superfamily (cl11394). This high similarity between a small fraction of their conserved residues prevents the use of traditional homology searches (e.g. BLAST) for identifying likely L-rhamnosyl-trasnferases within a genome. Currently, for the gamma-proteobacteria class, there are reported about 11,500 L-rhamnosyl-trasnferase sequences, of which 2581 are unique (NCBI, March 2016). These are distributed among multiple orders of gamma-proteobacteria. In Eukaryotes on the other hand, there are only about 50 sequences reported (mostly from plants). More flexible and sensitive homology search strategies are based on regular expressions (search for highly variable motifs) or "Hidden Markov Models" (HMM, from residue-probability matrices). Currently the standard for detecting L-rhamnosyl-transferases by HMM is the probability matrix hmm TIGR01556 (Interpro, TIGRFAMs). However, this matrix is too specific for gamma-proteobacteria so its use on fungi (*S. schenckii* specifically) has turned out negative results. To overcome this limitation we have generated more permissive HMM matrices based on the unique sequences reported at NCBI. The application of these matrices to the set of predicted proteins from the genome of *S. schenckii* has recovered the sequences for approximately 30 hypothetical proteins (E-values <6.0E-05), two of which were recovered by multiple matrices and present typical signatures from the glycosyl-transferases superfamily. It should be noted that fragments of the domains present in the L-ramnosiltransferasas can be found in other types of proteins and thus the high number of hypothetical proteins recovered.

Paracoccidioides spp as resilient genetic tractable organism: towards functional genomic studies

Fernando Rodrigues

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Paracoccidioides spp. cause paracoccidioidomycosis, one of the most prevalent systemic mycosis in Latin America. The conidia/mycelium transition to the pathogenic yeast form is critical for the establishment of disease. A better understanding of factors that mediate both transition to and yeastform virulence may reveal new targets for the design of rational preventive/therapeutic strategies.

Functional genomic studies will reveal such virulence mediators, but are largely hampered by the resilience of *Paracoccidioides spp.* to genetic manipulation. We have been developing molecular tools to genetically modify these fungi to identify putative virulence factors. Cdc42 is an inter kingdom conserved pivotal molecule for the establishment and maintenance of polarized growth. The analysis of clinical and environmental *Paracoccidioides spp.* isolates showed that CDC42 expression is a key determinant of the heterogeneity of cell shape and size, and possible of virulence in *Paracoccidioides spp*. By antisense technology we knocked-down CDC42's expression in *Paracoccidioides spp*. yeast cells, promoting a decrease in cell size and homogenous cell apical growth and altering the typical polymorphism of wild-type cells. Importantly, reduced expression levels of this gene resulted in an increased phagocytosis and decreased virulence in a mouse model of infection. The auxotrophic nature to organic sulfur of the yeast form of *Paracoccidioides spp.* led us to evaluate the relevance of the SCONC, a negative regulator of the inorganic sulfur assimilation pathway, in the dimorphism and virulence of this pathogen. SCONC down-regulated transformants started transition, but were unable to sustain yeast growth on inorganic sulfur compounds, correlating its metabolism with cellular energy, redox and oxidative stress imbalances. Down-regulation of SCONC had impact on *Paracoccidioides spp.* its virulence in vivo. The fact that yeast form of *Paracoccidioides spp*. is multinucleated implies that upon in vivo killing massive amounts of DNA are likely to be released. This led us to hypothesize that recognition of *Paracoccidioides spp*. by TLR9 would impact the physiopathology of the infection. In contrast to what one would expect, TLR9 plays a protective role early after intravenous infection with *Paracoccidioides spp*. by decreasing inflammation. Indeed, TLR9 deficient mice presented a neutrophil mediated tissue damage with increased expression of several cytokines, such as TNF-α and IL-6. Thus, we unraveled a protective role for TLR9 early upon infection with *Paracoccidioides spp*. In all, the development of a molecular tool-box for genetic manipulation of *Paracoccidioides spp.*, allowed us to perform functional genomic studies and to provide evidences on new virulence factors that account for its pathogenesis.

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Mechanisms of synthesis and hydrolysis of cell wall alpha-1,3-glucan in *Paracoccidioides brasiliensis*

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Paracoccidioides spp. is causative agent of paracoccidioidomycosis, a human systemic mycosis for which the portal of entry is via inhalation of airborne propagules into the respiratory tract. While in the mycelial (M) phase, the cell wall has -1,3-glucan as the sole neutral glucose polymer, in the yeastlike (Y) phase, it is reduced to a minimum (5%), and substituted by -1,3-glucan, which is the major neutral cell wall polysaccharide of the pathogenic yeastlike (Y). - 1,3-glucan has been proposed as virulence factor in this fungus, as well as in *Blastomyces dermatitidis* and *Histoplasma capsulatum*.

Our group have been researching different genes involved in the synthesis, and hydrolysis of cell wall -(1,3)-glucan in *P. brasiliensis*. A gene with high identity with *H. capsulatum* -1,4-amylase (AMY1), expressed preferentially in the pathogenic Y phase, was identified, and complemented an *H. capsulatum* amy1 mutant, a result that suggest an important role for its product in the synthesis of -1,3-glucan in *P. brasiliensis*. In silico amino acid analysis of its deduced protein led to the identification of all four conserved regions of the -amylase family, critical moieties for biological activity, and amino acids associated with specificity to hydrolyze glucosidic -1,4-linkages. Also, a single gene for an - 1,3-glucanase was identified and its product analysed, showing -(1,3)- glucanase activity.

In the present work, a summary of molecular and biochemical data on *P. brasiliensis* (1,3)-glucan synthesis, and hydrolysis of the cell wall -(1,3)- glucan of this medically important fungus is presented.

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The immunoglycobiology of *Candida spp.* and *Sporothrix spp*.

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The cell wall of the opportunistic pathogen *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 *Candida non-albicans*, and other fungal pathogens such as the causative agent of sporotrichosis, *Sporothrix schenckii*. Here, we studied the relevance of cell wall components of *S. schenckii*, *C. parapsilosis, C. tropicalis, C. guilliermondii* and *C. krusei* during cytokine stimulation and phagocytosis by human monocytes and monocyte-derived macrophages, respectively. Our results showed that these organisms have similar cell wall composition, but different degrees of cell wall porosity, and this physical parameter correlates with the ability to stimulate cytokine production. Removal of *O*- or *N*-glycans affected the stimulation of cytokines in a species specific-manner. As previously reported in *C. albicans*, *S. schenckii* stimulated cytokine production in a morphology-dependent way.

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