Paths of Glycobiology

Jerzy Kościelak Memorial Conference

ABSTRACT BOOK

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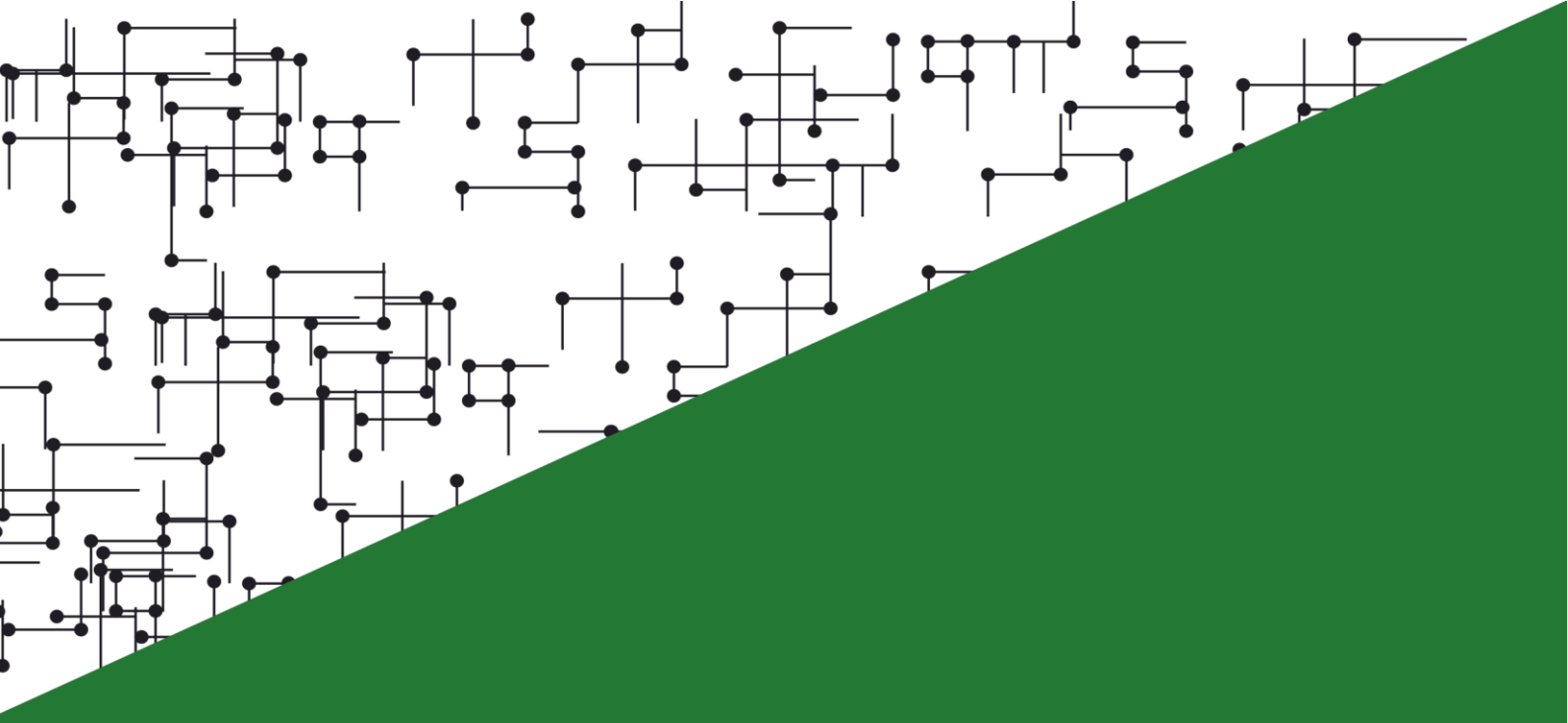
POSTER SESSION. PROKARYOTIC GLYCOCONJUGATES



PLENARY LECTURES

PL1

High-performance computing in glycoscience: Rebuilding glycans to understand their functions, from viruses to humans

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The 3D structure information deposited in public repositories, such as the RCSB Protein Data Bank (www.rcsb.org) and the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/), often covers only the protein component of complex biomolecular systems, while features of essential and common (co)post-translational modifications are completely or partially missing, or incorporated with glaring structural errors. In this talk I will introduce how we use GlycoShape, our MD-generated library of 3D glycan structures soon to be released, to restore this key missing information and thus to rebuild the glycoproteins of interest back to their functional state. I will show how this approach is unique in its potential to capture the diverse degrees of flexibility characteristic of the structure of complex carbohydrates, where 3D ensembles are not random distributions of structures, but strictly regulated by sequence and branching. Within this framework, I will present how this approach can capture not only the nature, but also the biological function of different glycosylation motifs in viral infection and protein folding.

PL2

Exploring the glycolipid landscape: glycosphingolipids as regulators of cancer cell plasticity

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Cancer cell plasticity is a highly heterogeneous programme and has recently been included as a hallmark of cancer. The process usually enables cancer cells to dynamically respond to selective pressure in the primary and metastatic tumor microenvironment, leading to the acquisition of characteristics associated with different cell types. Consequently, cancer cells overcome selective constraints, leading to an increased metastatic potential and acquired drug resistance in cancer patients. Interestingly, there is a growing body of evidence suggesting that glycosylation promotes the reversible transition of epithelial and mesenchymal (EMT) cancer cells. Our biomarker discovery pipelines in ovarian cancer patients allowed us to identify glycans that are naturally linked to neutral glycosphingolipids. Further characterization of cancer cell lines and spatial glycolipidomics in patient-derived samples led us to hypothesize that the presence of neutral glycosphingolipids is associated with epithelial cancer cells, while the presence of sialic acid-terminated glycosphingolipids is associated with mesenchymal cancer cells. Therefore, we analyzed large transcriptomic data sets and found genes encoding glycosyltransferases coinciding with the expression of classic EMT markers (e.g. CDH1 and VIM). We further validated these findings using our paired sgRNA CRISPR-Cas9-mediated genomic deletion of key glycosyltransferases, resulting in the acquisition of mesenchymal traits as evidenced by acquired drug resistance, in vitro experiments assessing cell motility and proliferation as well as in vivo zebrafish embryo cluster formation and cancer cell dissemination. We provide a comprehensive data set and shed new light onto an underestimated class of biomolecules during cancer cell plasticity in ovarian cancer. Understanding the interaction between cancer cell plasticity and glycosylation has potentially important implications for the development of new cancer therapies considering the function of specific glycoproteins and glycolipids that are involved in cell signaling, cell adhesion, and immune recognition.

PL3

N-glycan diversity in nematodes

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Nematodes represent a large phylum of invertebrate animals occupying a range of ecological niches; they include free-living species as well as parasites of plants and animals. Over the past quarter century various glycomic studies have been performed, our own being based on an off-line HPLC/MALDI-TOF-MS approach complemented by use of chemical and enzymatic treatments to investigate the fine structure of nematode N-glycans. In recent years, we have completed major surveys of the N-glycomes of *Caenorhabditis elegans*, *Dirofilaria immitis* and *Trichuris suis*, whereby each species has its own glycostructural characteristics. While C. *elegans* has N-glycans with highly decorated cores, but also some degree of complex glycosylation, *D. immitis* presents rather extended N-glycan antennae, partly modified with glucuronic acid. The N-glycome of *T. suis* is again different, as the core regions are rather simple, but the LacdiNAc-based antennae carry fucose, HexNAc-substituted fucose and phosphorylcholine modifications. While glycomutants have yielded much information regarding glycobiosynthetic pathways in C. *elegans*, the use of natural glycan arrays enables investigation of which parasite glycan modifications may interact with the lectins, pentraxins and antibodies of mammalian hosts. Whether as a 'smoke-screen' or as immunomodulators, there is every indication that glycans are important for host-parasite interactions, while there is still much to be learnt regarding how these organisms elaborate their highly heterogeneous and varied glycomes.

PL4

Supply of glycosylation substrates to the mammalian Golgi: a sharp snapshot getting blurred

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Glycosylation is a process of enzymatic attachment of sugar residues to macromolecules. This process occurs due to the sequential action of glycosyltransferases. N-glycosylation and O-glycosylation take place mainly inside the endoplasmic reticulum (ER) and the Golgi apparatus of eukaryotic cells and nucleotide sugars (NS) serve as substrates for glycosylation reactions. These compounds are synthesized in the cytoplasm. Therefore, translocation of NS across the organelle membranes is essential. This process is thought to be mediated by a group of multi-transmembrane proteins from the SLC35 family, i.e., nucleotide sugar transporters (NSTs). Despite many years of research, some uncertainties/inconsistencies related with the mechanisms of NS transport and the substrate specificities of NSTs remain.

A historical view of the experimental approaches used to study NS transport and evaluate the most important achievements will be discussed. Also various aspects of knowledge concerning NSTs, ranging from subcellular localization up to the pathologies related with their defective function will be summarized. Additionally, the outcomes of our research performed using mammalian cell-based models and its relevance in relation to the general context will be presented.

PL5

O-antigen variability among clinical isolates of *Klebsiella pneumoniae* relevant for passive and active immunisation

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Klebsiella pneumoniae is a nosocomial pathogen, pointed out by the WHO as "critical" regarding the highly limited options for the treatment of infections.

Lipopolysaccharide (LPS, O-antigen) and capsular polysaccharide (K-antigen) are its virulence factors and surface antigens, determining O- and K-serotypes and encoded by O- or K-loci. They are promising targets for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics. To make such immunotherapy effective, knowledge about O/K-antigen structures and distribution among clinical isolates is necessary.

Research carried out over the last decade in the Laboratory of Microbial Immunochemistry and Vaccines at the Hirszfeld Institute has identified novel *K. pneumoniae* O-antigens. A new galactan was identified for O1 and O2 serotypes [1,2]. Discrepancies for O2 serotyping between Kaptive-based genetic predictions (O2 variant 2) and the phenotype (O2 variant 1) were explained by the presence of insertion sequences in O-loci [3]. Three subtypes O3, O3a, O3b were identified within O3 serotype, previously considered to have uniform O-antigen structure. The presence of the O3, O3a, O3b is dependent on diversity within genes encoding O-antigen (*WbdA and WbdD*) [4]. Next new O-antigen have been identified for isolates Kp175, Kp231, Kp254 and 3936/19. Finally monoclonal antibodies were developed for diagnosis and treatment of infections caused by *K. pneumoniae* [1,5]. Additionally O-antigen variability of NDM-producing *K. pneumoniae* responsible for a countrywide outbreaks in Poland (2012-2018) is discussed. This study was partially supported by the National Science Centre, Poland (grant no. 2018/31/B/NZ7/04002).

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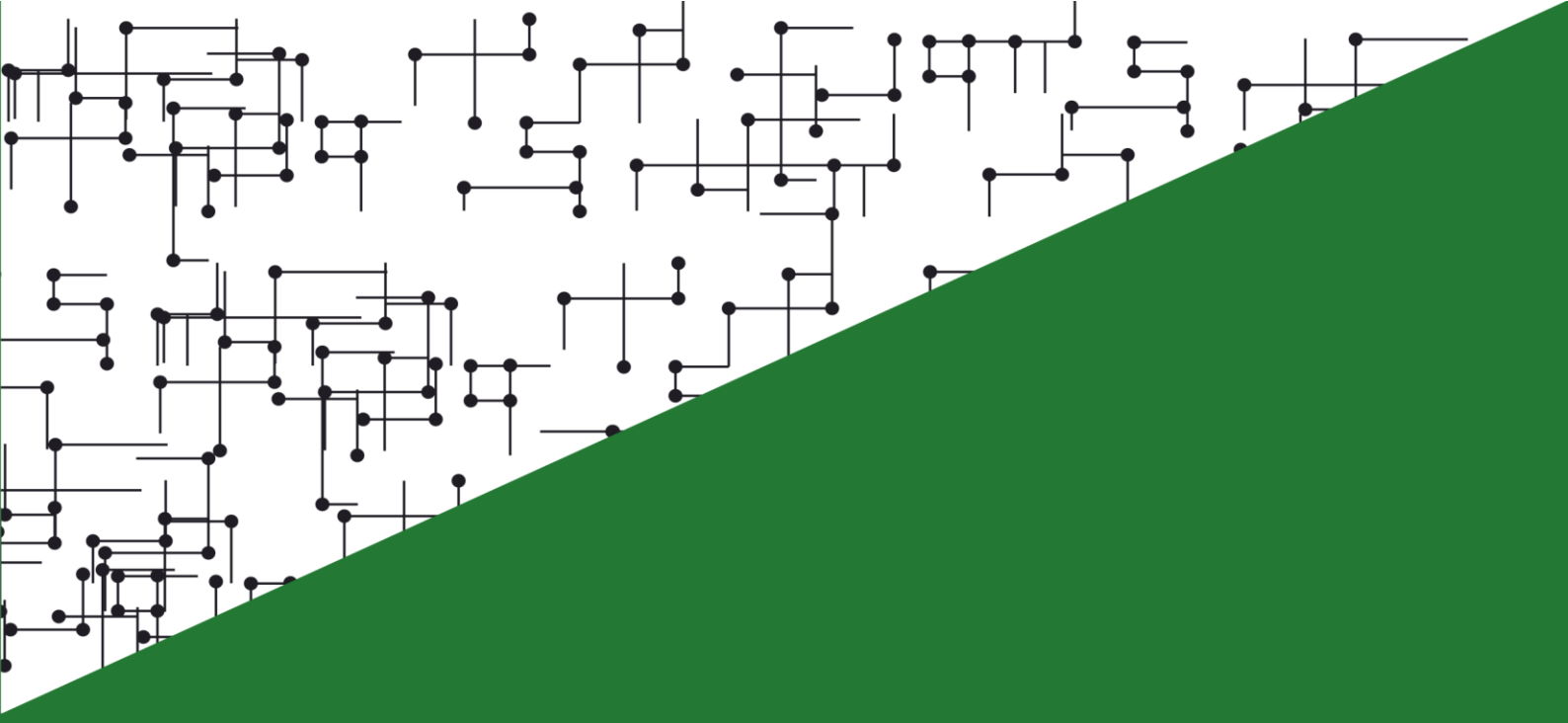

SESSION 1.
GLYCANS IN HUMAN DISEASES

S1.01

The link between serum cytokines levels and N-glycosylation of CD4+ T cells in Graves' disease

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Background: Graves' disease (GD) is the most common cause of hyperthyroidism and an archetypal example of antibody-dependent autoimmunity. GD development depends on Th2 humoral response, which contributes to B cell overactivation, increased IgG secretion and cytokine production by Th2 cells. Most of T cell receptors undergo N-glycosylation. The structures of N-oligosaccharides can be modified by proinflammatory cytokines, as shown mainly in vitro and in animal models. Our study aimed to assess a correlation between the proinflammatory cytokines and T cell N-glycan structures in GD and healthy donors.

Methods: Blood and serum samples from GD patients before (GD) and after TSH normalization as a result of methimazole treatment (GD/T), and from healthy volunteers (CTR) were used in the study. CD4+ T cells were isolated from PBMCs using an automated magnetic sorter. N-glycans were released from cellular proteins by N-glycosidase F and analyzed by MALDI-TOF mass spectrometry. Serum cytokines concentrations were measured by flow cytometry. Spearman's rank correlation was used to evaluate the link between cytokines levels and N-glycan species identified in CD4+ T cells.

Results: Among a set of the analysed proinflammatory cytokines, TGFβ, IL-4, and IL-6 serum levels correlated with the amount of CD4+ T cell N-glycans. IL-6 seems to mostly correlate with the content of oligomannose structures in CD4+ T cells from GD/L patients. The strongest correlations in the analysed groups were determined for IL-4 and the content of both oligomannose and galactosylated complex-type structures, some of them fucosylated.

Conclusions: The correlation between proinflammatory cytokine levels and N-glycosylation of CD4+ T cells may be relevant to T cell-dependent autoimmunity in GD. Further analysis is needed to confirm that N-glycosylation is modified by proinflammatory cytokines in thyroid autoimmunity.

The study was supported by the National Science Centre, Poland (grant no. UMO-2015/18/E/NZ6/00602).

S1.02

Galactosylceramide down-regulates the expression of pro-apoptotic *TNFRSF1b* and *TNFRSF9* genes and up-regulates the expression of anti-apoptotic *BCL-2*

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It was shown that galactosylceramide (GalCer) increases the resistance of breast cancer (BC) cells to apoptosis induced by doxorubicin [1]. Recent studies using a BC cellular model: MDA-MB-231 cells with suppressed synthesis of galactosylceramide (GalCer; MDA.Δ.UGT8.4) and MCF7 and T47D cells that over-produced GalCer (MCF7.UGT8 and T47D.UGT8) revealed that GalCer accumulation correlated with decreased expression of pro-apoptotic TNFRSF1b and TNFRSF9 and increased expression of anti-apoptotic Bcl-2. This data suggested that GalCer may affect the expression of certain apoptotic genes on transcriptional level. Consistent with this hypothesis, MDA-MB-231 and MCF7.UGT8 BC cells with high GalCer content showed lower activity of *TNFRSF1B* and *TNFRSF9* promoters than cells lacking GalCer. In contrast, the activity of the *BCL2* promoter in MCF7.UGT8 was higher than in MCF7 cells without GalCer. However, no difference in *BCL2* promoter activity was observed between MDA-MB-231 cells with high levels of GalCer and GalCer-negative MDA.Δ.UGT8.4 cells. Instead, we found that GalCer increased the stability of Bcl-2 mRNA. As a candidate protein that simultaneously increases the expression of *TNFRSF1B* and *TNFRSF9* genes and decreases the expression of *BCL2* gene and stability of Bcl-2 transcripts is p53. Therefore, its expression was analysed in BC cells containing various amounts of GalCer. In agreement with this hypothesis, BC cells with high GalCer content were characterized by significantly lower expression of p53, and inhibition of p53 using siRNA resulted in decreased expression of *TNFRS1B* and *TNFRS9* genes and increased expression of *BCL2* gene.

In conclusion, these studies validated hypothesis that GalCer regulates the expression of specific apoptotic genes (*TNFRSF1b*, *TNFRSF9* and *BCL-2*) in BC cells on the transcriptional level by the activation of specific signaling pathways most probably involving p53.

Grant support for this work was provided by the National Science Centre, Poland (grant no. 2019/35/B/NZ5/01392).

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S1.03

Yersinia eneterolitica outer membrane vesicles as a target for human complement system

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Gram-negative bacteria constantly release outer membrane vesicle (structures 20-250 nm diameter in size) to their environment. They secrete these cellular components as a way of promoting pathogenesis, surviving stress condition and regulating microbial interactions with bacterial communication. OMVs contain proteins, nucleic acids, other cellular materials and they have a similar bilayer outer membrane as they parent cell, with lipopolysaccharide (LPS) in its outer leaflet. Bacterial LPS plays an important role in complement system activation what can leads to Systemic inflammatory response syndrome development (SIRS). Many functions of bacterial OMVs and their interaction with immune system are not known, including complement system activation. OMVs as unique complex structures, with possible outer membrane structure modification (in this Lipid A toxicity reduction) can also stimulate human organism to produce specific antibodies and serve as a vaccine. Better understanding and their thorough characterization is crucial. High purity OMVs of *Yersinia enterocolitica* O:3 (YeO:3) wild-type depleted of virulence plasmid (pYV-) and its LPS-dependent chemotypes (Ra mutant with complete outer and inner core of LPS (YeO3-c-R1) and Re deep rough LPS mutant with truncated inner core (YeO3-R1-M205) cultivated in three different temperatures (4, 22 and 37 °C) were obtained. We characterized YeO3 OMVs by using physicochemical methods: dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), their zeta potential and were depicted by transmission electron microscopy (TEM). LPS from OMVs was isolated and O-specific polysaccharide, core oligosaccharide and Lipid A structures were determined by MALDI-TOF mass spectrometry and compared with bacterial LPS. We also proved binding of human Mannose Binding Lectin (MBL) with high molecular mass region of OMVs in western blot method what is the evidence, that OMVs effectively activate lectin pathway of complement system. Despite that, activation of classic pathway was also observed. Flow cytometry analysis of OMVs with specific surface antigens antibodies are ongoing.

S1.04

Lectin-based study reveals the presence of disease-relevant glycoepitopes in bladder cancer cells and ectosomes

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Background: Numerous studies have shown that during neoplastic transformation there are rapid changes in the biosynthesis of glycans followed by the appearance of tumor-associated carbohydrate antigens on the cell surface. Those specific glycoepitopes can also be transferred between cells via the extracellular vesicles (EVs). The aim of this study was to compare the glycosylation profiles of T-24 urothelial bladder cancer cells, HCV-29 normal ureter epithelial cells, and ectosomes, one of EV subpopulation, released by both cell lines.

Methodology: For lectin blotting, material obtained from T-24 and HCV-29 cells (*i.e.*, the whole cell extracts, membrane protein fractions, and ectosome samples) containing an equal amount of protein) was separated by SDS-PAGE. After western blot, the membranes were probed with biotinylated PHA-E, PHA-L, MAA, SNA, AAA, and GNA lectins. Surface glycosylation of untreated or treated with 1-deoxymannojirymycin (DMJ) T-24 and HCV-29 cells as well as of ectosomes derived from those cells was analyzed by flow cytometry with the use of the same panel of lectins.

Results: On-blot and flow cytometry stainings revealed the abundance or enrichment of β-1,6-branched complex type N-glycans (reaction with PHA-L) and α-2,3- (reaction with MAA) and α-2,6-linked (reaction with SNA) sialic acids and in ectosomes derived from HCV-29 and T-24 cells. This enrichment was still present on-blot for DMJ-treated ectosome samples, but in flow cytometry percentage of PHA-L-, SNA- and MAA-positive ectosomes and RFI decreased. Therefore, preferential sorting of β-1,6-branched complex type N-glycans into ectosomes may not concern surface proteins. Intensities of PHA-E, GNA and AAA-positive bands from HCV-29- and T-24-derived ectosomes were lower than those of the corresponding bands from the parental membrane fractions and kept similar values after DMJ-treatment. Moreover, in flow cytometry analysis, no changes for those stainings were observed. It suggests that bisecting GlcNAc, high mannose and fucosylated glycans are neither crucial nor required for protein sorting into ectosomes.

S1.05

Variability of the profile and degree of serum immunoglobulin G N-glycosylation in SARS-CoV-2

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COVID-19 is an infectious disease caused by the SARS-CoV-2 virus (Severe Acute Respiratory Syndrome Coronavirus 2). Immunoglobulins' glycosylation affects effector functions and is essential in many steps of the inflammatory cascade, therefore it is an important parameter for assessing the immune response against COVID-19 [1]. It is also well known that immunoglobulin G (IgG) effector functions are controlled by N-glycosylation.

The study aimed to examine the profile and degree of N-glycosylation of blood serum IgG in the context of SARS-CoV-2 infection. The blood sera were divided into COVID-19, convalescents, and healthy subjects (control). The profile and degree of IgG N-glycosylation was determined using a modified lectin-ELISA test with biotinylated lectins specifically reacting with sialic acid (SA) (MAA-detecting α2,3-linked SA, and SNA-specific to SA linked α2,6), galactose (GSL-II), GlcNAc (RCA-I), core fucose (AAL, LCA), fucose of Lewis^x oligosaccharide structure (LTA), fucose of Lewis^y oligosaccharide structure (UEA), and highly-branched N-glycans (PHA-L).

Afucosylated IgG is produced in response to various viral infections [2-4], which all target surface-exposed, membrane-embedded proteins. COVID-19 patients with severe symptoms have increased levels of afucosylated IgG anti-severe SARS-CoV-2 in comparison to patients with mild disease [5]. The reduction of IgG fucose content in patients with COVID-19 was associated with an increased abundance of non-neutralizing, afucosylated immunoglobulin G that was not present in patients with the milder disease [5-7].

Our study found significantly the lowest reactivity with core fucose-specific lectins in the convalescent group. We also showed significantly higher reactivities with LTAs specific to Lewis^x structures in the group of COVID-19 patients. Antennary fucose as part of Lewis^x oligosaccharide structures activates leukocytes, contributing to the development of inflammation. The lowest reactivities obtained with PHA-L in the COVID-19 group indicate that the development of the disease is accompanied by the presence of typical IgG biantennary N-glycans.

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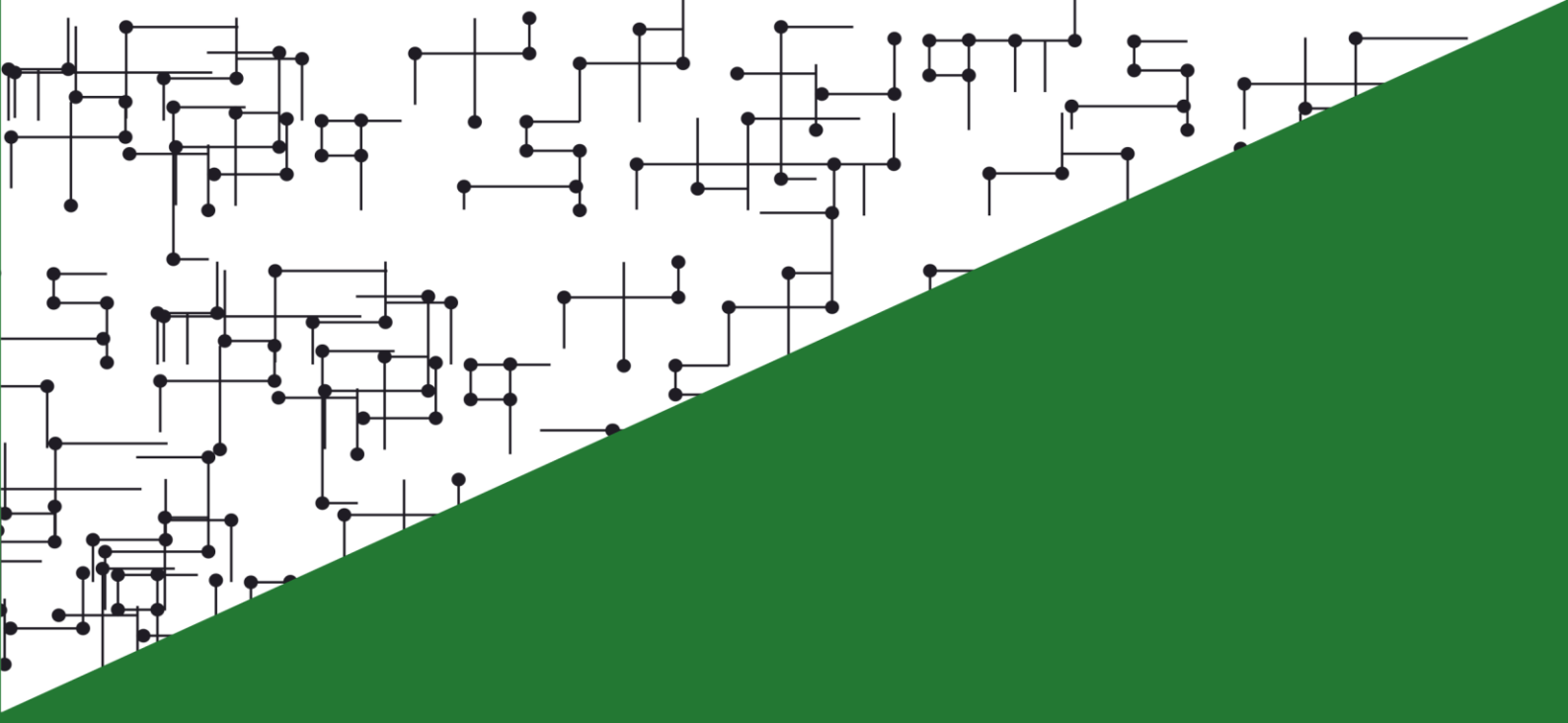

SESSION 2.
CELLULAR BIOLOGY OF GLYCOCONJUGATES

S2.01

N-Glycosylation dynamics in differentiating intestinal epithelial cells

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The intestinal epithelium is a huge organ of key importance in controlling the homeostasis of the human body. But not only the absorption of nutrients is important here. The epithelium forms a very complex physical and biological barrier (known as an intestinal epithelial barrier) in which every cell has its unique tasks in maintaining the delicate balance between digestion, absorption, immunity and tolerance, interactions with microbiota and metabolism. Each of these tasks is, at least to some extent, mediated by glycosylation. One of the most interesting phenomena of glycosylation is its dynamics. Unlike other biomolecules, the shape of glycans attached to proteins or lipids can be changed dramatically during several cellular and physiological processes such as organogenesis and differentiation, but also accompany pathologies such as cancer progression and metastasis.

Our research model are CaCo-2 adenocarcinoma cells, which spontaneously differentiate into enterocyte-like cells in vitro. Our initial studies clearly showed that the process of differentiation of these cells is accompanied by a characteristic dynamic "switch" of protein N-glycosylation, including the increased trimming of oligomannose structures by type I alpha-mannosidases. We also demonstrated that chemical inhibition of all alpha-mannosidase I impair to some extent the process of differentiation into fully functional enterocytes. Our last study concerns the impact of metformin, a broadly used anti-hyperglycemic agent, on the N-glycosylation of differentiating enterocytes. We show, that metformin causes dramatic remodeling of the cellular N-glycome by influencing the fucosylation and sialylation of several glycan species. For the in-depth glycomics, we used modern mass spectrometry methods thanks to the development of the glycomics core facility at the Jagiellonian University Medical College.

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S2.02

N-glycans and galectins as multilevel master regulators of FGF/FGFR signaling and cell fate

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Fibroblast growth factors (FGFs) and their receptors (FGFRs) constitute plasma-membrane localized signaling hubs that transmit signals from the extracellular environment to the cell interior, governing pivotal cellular processes like motility, metabolism, differentiation, division and death. FGF/FGFR signaling is critical for human body development and homeostasis; dysregulation of FGF/FGFR units is observed in numerous developmental diseases and in about 10% of human cancers. A vast majority of FGFs (15 out of 22 members) and all FGFRs are N-glycosylated, but the significance of these modifications was unknown. Besides cell surface localization of FGF/FGFR, FGFRs were detected in nuclei, but the mechanism behind receptor trafficking to this compartment is elusive.

Here we have demonstrated that N-glycans of FGFR1 serve as a cell surface targeting signals and inhibitors of receptor autoactivation. Glycosylation-deficient variant of FGFR1 is unable to follow the secretory pathway and accumulates in the ER and in the nuclear envelope. ER/nuclear FGFR1 interacts with a discrete set of ER/nuclear proteins and displays high level of activation that is FGF-independent, implicating presence of a novel intra-nuclear FGFR1 signaling cascade.

We have shown that N-glycans of the D3 domain of cell surface-localized FGFR1 serve as a binding site for the specific set of multivalent galectins (galectin-1, -3, -7 and -8). We have demonstrated that galectins cause differential receptor clustering, resulting in FGFR1 activation and initiation of downstream signaling cascades. Using protein engineering approaches, we have deciphered the mechanism of FGFR1 activation by galectins. We have altered the valency of galectins and demonstrated that multivalent interaction between galectins and FGFR1 resulting in receptor crosslinking and activation. Importantly, galectin/FGFR signaling triggers different cellular processes than FGF1/FGFR1 signaling, which is likely attributed to the fact that FGF1 and galectins activate distinct N-glycosylation variants of FGFR1. Combined treatment of cells with FGF1 and galectins results in enhanced amplitude of FGFR1 transmitted signals and altered cell response. Using high content imaging we have also shown that galectins differentially adjust FGFR1 endocytosis (acting as inducers and inhibitors), implicating their critical role in determining kinetics of signals transmitted by FGFR1.

Among 22 FGFs 15 are secreted in a conventional manner and contain putative N-glycosylation sites of unknown function. We have demonstrated that in the extracellular space, N-glycans of FGFs are recognized by a set of galectins, which anchor growth factors to the components of the extracellular matrix, affecting flow of FGFs to FGFRs and thus modulating signals transduction by FGF/FGFRs. We have demonstrated that multivalency of galectins is critical for modulation

of FGFs activity. Interestingly, our data implicate that inside cells galectins interact with intracellular FGF subfamily members (FGF11-FGF14).

Summarizing, our data demonstrate for the first time that galectins are master regulators of receptor tyrosine kinase signaling and cellular trafficking, where they simultaneously act at the level of ligand (FGFs) and receptors (FGFRs). For their cell surface activity, galectins strictly require multivalency and specific N-glycans of FGFs and FGFRs. On the other hand, lack of N-glycans spatially restricts FGFR1 from galectin binding and re-directs receptor to the nucleus, where it transmits signals in the ligand-independent manner.

S2.03

Human Gb3/CD77 synthase: a paradigm-shifting glycosyltransferase

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Originally, human Gb3/CD77 synthase (α1,4-galactosyltransferase, encoded by *A4GALT*), was considered quite an ordinary glycosyltransferase that synthesizes the terminal Galα1→4Gal disaccharide of globotriaosylceramide (Gb3, CD77, the P^k antigen). This view changed upon the discovery that Gb3/CD77 synthase can also produce terminal Galα1→4Gal of another glycosphingolipid blood group antigen called P1. The enzyme rose to prominence when it was discovered that a single point mutation c.631C>G (p.Q211E) broadens the specificity, making the enzyme able to synthesize Galα1→4GalNAc structure in addition to Galα1→4Gal. It was the first case of a glycosyltransferase changing the acceptor specificity due to a single point mutation. The Galα1→4GalNAc-terminated product called NOR, was found to be associated with the NOR polyagglutination syndrome before formally becoming the third blood group antigen of the P1PK system. But the specificity of Gb3/CD77 synthase proved to be even more complex. Our recent study showed that it can synthesize Galα1→4Gal structures also on N-glycan chains of glycoproteins. Surface proteins with such glycotopes may serve as functional receptors for Shiga toxin 1 produced by enterohemorrhagic *Escherichia coli* and *Shigella dysenteriae* serotype 1. This functional relevance came as a surprise because Gb3 glycosphingolipid was considered to be the only true receptor for Shiga toxins. We also showed that human Gb3/CD77 synthase carries two N-glycans, one of which is indispensable for enzyme activity and can allosterically modulate enzymatic activity. Moreover, we found that the genome of pigeon (*Columba livia*) contains 5-7 homologs of human Gb3/CD77 synthase, which differ in glycoprotein/glycosphingolipid specificity. This abundance of active genes in birds that are refractory to Shiga-producing bacteria strains represents yet another conundrum of Gb3/CD77 synthase activity, which is currently the subject of a multipronged investigation.

S2.04

SLC35A2-deficient MDCK cells display elevated levels of selected glycolytic enzymes

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SLC35A2 is the only supplier of UDP-galactose to the mammalian Golgi identified to date. Consequently, glycoconjugates synthesized by the cells deficient in SLC35A2 activity lack galactose and sialic acid. Importantly, mutations in the human SLC35A2 gene cause a subtype of a congenital disorder of glycosylation (CDG), namely SLC35A2-CDG. We have recently developed a Madin-Darby canine kidney (MDCK) cell line deficient in SLC35A2 activity using a CRISPR/Cas9 strategy. Interestingly, the resulting knockout had lost the epithelial characteristics typical of the parental cell line and displayed several hallmarks of the epithelial-to-mesenchymal transition (EMT). Glycolysis is a universal catabolic pathway in which glucose is converted to pyruvate in a series of ten enzyme-catalyzed reactions. Since the activation of EMT is thought to be an essential step for cancer metastasis and cancer cells are known to have increased rates of glycolysis (the Warburg effect), we decided to compare the levels of selected glycolytic enzymes between control and SLC35A2-deficient MDCK cells using immunoblotting. We found that the knockout cells displayed statistically significant increases in the levels of rate-limiting glycolytic enzymes such as pyruvate kinase M2 (PKM2) and two isoforms of phosphofructokinase: platelet (PFKP) and muscle (PFKM). We also observed an increased level of lactate dehydrogenase A (LDHA) in the SLC35A2 knockout. Our findings suggest that the loss of SLC35A2 activity may trigger some cancer-like traits in the otherwise non-malignant cells and provide a novel link between the delivery of UDP-galactose into the Golgi and the catabolism of glucose.

SESSION 3.
BACTERIAL GLYCOCONJUGATES

S3.01

Impact of the dephosphorylation on the function of Ba218.3 polysaccharide isolated from *Bifidobacterium animalis* ssp. *animalis* CCDM 218

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"Probiotics" is a well-known term, widely studied in the context of human health. However, the newest publications are pointing out that health-promoting properties are a feature of not only live and proliferating bacteria but also smaller bacterial components. In 2021, those molecules were defined by the International Scientific Association of Probiotics and Prebiotics as "postbiotics". Their advantage over probiotics is based on easy-to-define, less complex structure and function, which further allows the studies of structure-function relationships. Moreover, introduction of the structural modifications may allow the change or even improvement of properties exhibited by the analyzed components.

In our studies, we decided to investigate the role of Ba218.3 polysaccharide isolated and purified from *Bifidobacterium animalis* ssp. *animalis* CCDM 218. With the use of chemical methods and NMR spectroscopy, we defined its structure and confirmed the phosphorylation of the molecule. In the next step, we used 48% hydroxide fluoride to remove phosphorus from the structure and with the use of cells isolated from mice together with human cell lines we analyzed changes in polysaccharide properties. The obtained results indicated the presence of 9 sugar moieties and glycerol phosphate substitution in the Ba218.3 PS unit. The molecular mass of the molecule was determined to be approximately 1.24×10^4 Da. Investigation of the cytokine production by studied mouse cells and human cell lines induced by both variants of Ba218.3 PS molecule indicated that the presence of phosphorus impacts the immunomodulatory properties of the polysaccharide.

Finally, our results showed that dephosphorylation of Ba218.3 PS influences the function of the tested molecule, however further studies in mouse models of diseases might be necessary to confirm the presented changes.

S3.02

Morphology and carbohydrate surface antigens of *Flavonifractor plautii* PCM 3108

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The human digestive tract is one of the most complex microbial ecosystems. Microbiota has a great influence on the maintenance of homeostasis or the development of several diseases, such as inflammatory bowel diseases (IBD). IBD include Crohn's disease (CD) and ulcerative colitis (UC), which pathology is characterized by chronic inflammation of the digestive tract. It is known that patients with IBD have a greater amount of certain specific species of bacteria compared to healthy individuals.

Flavonifractor plautii is a strictly anaerobic, Gram-variable bacterium belonging to the Clostridiales. It is a component of the human gut microbiome and is well known for its ability to metabolize a wide range of flavonoids, however, that is the only one clearly defined property of this species. *F. plautii* is characterized by slow and minimal growth, thus its phenotypic identification is a challenge for microbiologists. Until now, three cases of infections have been described. The enrichment of the *F. plautii* has been indicated in patients with colorectal carcinoma, IBD and other gastrointestinal tract disorders.

The purpose of this study was to further analyze the biological properties of clinical isolate *F. plautii* PCM 3108. The complete structure of the exopolysaccharide was determined using NMR spectroscopy as the repeating unit of →2)-Rhaf-(1→3)-Rhaf-(1→. Bacterial cultures were also analyzed by transmission electron microscopy showing the bacterial cells morphology and structure of the cell wall. The ability to produce membrane vesicles was also demonstrated.

S3.03

Gut microbiome and bacterial markers assessed in mice with MC38 colorectal cancer

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An intake of dietary fiber results in a production of short-chain fatty acids (SCFA), contributing to the anti-inflammatory reaction by participation in maintaining a balance between the suppression of inflammatory mediators (TNFα, IL6) and the induction of anti-inflammatory cytokine (IL10).

The SCFA levels were determined in stool of mice with colorectal cancer and a control group to assess microbiota homeostasis disorders related to a cancer progression, as well as an influence of high-fiber diet on the concentration of SCFA in feces and cancer progression. Analytical methods (HPLC with UV/Vis) and NGS sequencing were applied.

A diet enriched with cellulose had a positive effect on an intestinal abundance of *Akkermansia muciniphila* in cancer-affected animals. Lactobacillus abundance decreased when diet enriched by 20% cellulose was used. Animals consuming cellulose-rich diet reveal a lower *Firmicutes:Bacteroidetes* ratio comparing to group on standard diet.

A diet with potato starch caused an increase of *Bifidobacterium* and *Faecalibaculum*, and a decline in abundance of *Blautia, Peptocdoccus, Ruminococcus* UCG-010 and *Anaeroplasma*. The diet was the main modulator of the gut microbiome, moreover, a diet rich in potato starch significantly changed the SCFAs profile and increased their fecal concentration.

S3.04

Antigenic and structural properties of the lipopolysaccharide of uropathogenic *Proteus mirabilis* Dm55 strain isolated from a patient in the central Poland

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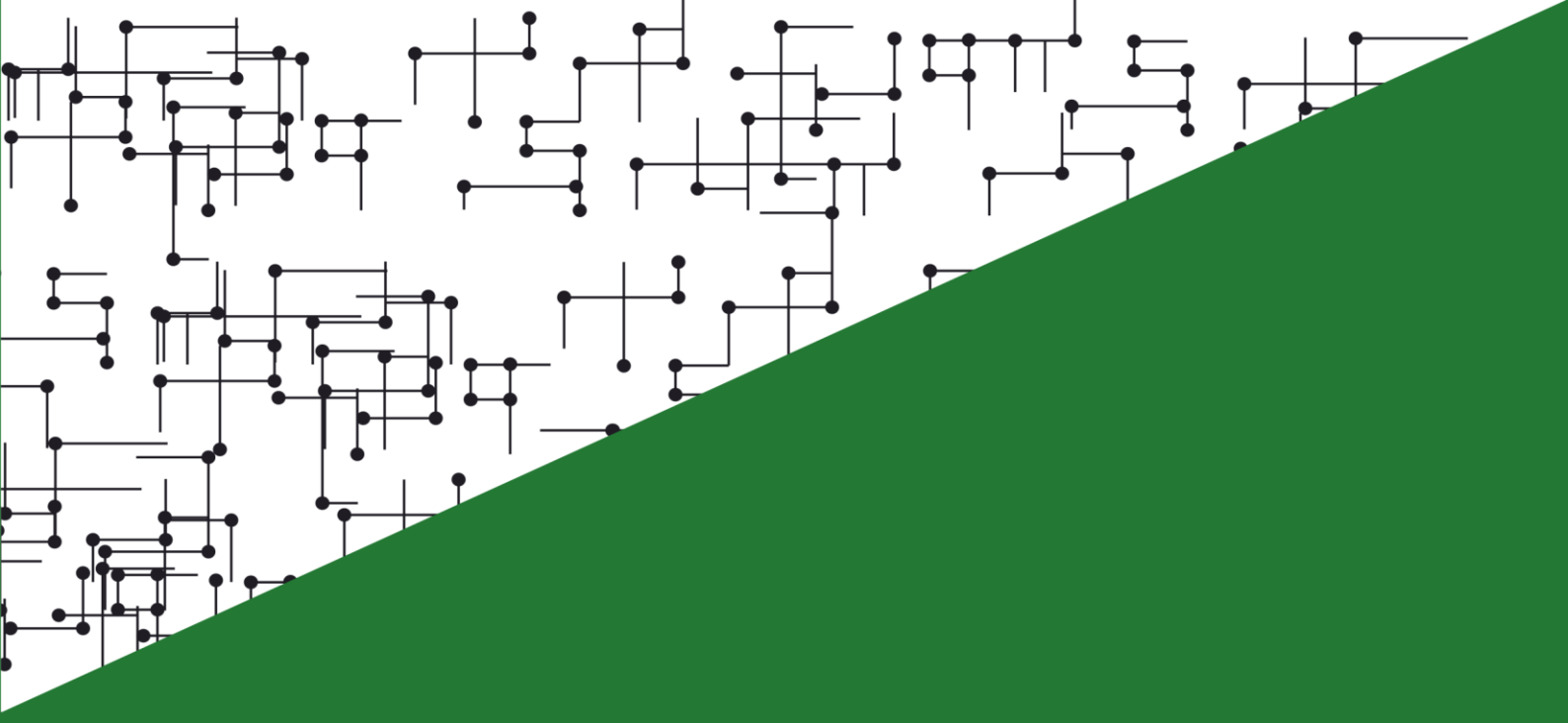
Proteus mirabilis clinical strains infecting patients in the central Poland have shown a big serological variety of their O antigens. The strain Dm55 comes from the urine of a patient from Lodz and the aim of the study was the characterization of its O antigen. The LPS of P. mirabilis Dm55 was extracted by the phenol-water method and tested in ELISA with the sera specific to each of the 84 Proteus O serotypes, recognized so far. The only cross reaction was observed with the O54 antiserum, but it was much weaker than in the homologous system. The reactivity of the obtained polyclonal rabbit serum against *P. mirabilis* Dm55 was checked in ELISA with the homologous LPS and with the LPSs representing all the described Proteus O serotypes. The antiserum reacted with a few LPSs (including O54 one), but the titers were lower than in the homologous system. The observed reactions were visualized using Western blotting. To confirm the uniqueness of the studied O antigen, the LPS of P. mirabilis Dm55 was subjected to mild acid hydrolysis. The high-molecular-weight O polysaccharide, isolated by fractionation of the supernatant by gel-permeation-chromatography on Sephadex G50 fine, was chemically studied using sugar and methylation analyses, mass spectrometry, and 1H and 13C NMR spectroscopy, including 1H,1H NOESY, and 1H,13C HMBC experiments.

The O-repeating unit appeared to be a branched three-saccharide containing one neutral and two N-acetamido sugars, the composition of which is structurally different from the other *Proteus* O polysaccharides, studied so far. Three-component units rarely occur in Proteus O antigens. The found common fragments in the cross-reacting OPSs may play a role of the antigenic epitopes. We propose to classify *P. mirabilis* Dm55 strain to a next, new O serogroup in the genus *Proteus*.



SESSION 4.
INVERTEBRATE GLYCOCONJUGATES

S4.01

Evolutionary glycomics of holozoans and early branching animals

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Glycans may be attached to other biological molecules and serve as tags, differentiating tissues, developmental stages and species. The totality of glycosylated molecules present on the cell surface is called a glycocalyx. In addition, intracellular N-glycans form tags that help recognize the folding state of proteins. The evolutionary origins and initial importance of these structures are largely unknown. Here we show, for the first time, life stage-specific N-glycomic profiles of a filasterean *Capsaspora owczarzaki*, a choanoflagellate *Salpingoeca rosetta* and of selected, early branching animals. A unique modification of the *Capsaspora owczarzaki* N-glycans is uncovered. In addition, bioinformatic analyses supplement the work, pointing to specific glyco-evolutionary changes at the onset of animals. We hope this analysis will increase the understanding of the currently enigmatic biology of the last common ancestor of all animals.

S4.02

Sialic acids specificity of *Plasmodium* parasites

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Plasmodium parasites which infect humans and great apes are largely host-specific. In the invasion of erythrocytes multiple proteins, expressed by *Plasmodium* merozoites, are involved. Erythrocyte recognition is dependent mostly on two multigene families: erythrocyte-binding ligands (EBL) and reticulocyte-binding ligands (RBL). *P. falciparum* has four members of EBL family, including erythrocyte binding antigen 140 (EBA-140), which enable the merozoite to interact with independent human RBCs receptors. The EBA-140 merozoite ligand was shown to bind glycophorin C (GPC) a minor erythrocyte sialoglycoprotein. Pf.EBA-140 ligand binds to human erythrocytes in a sialic acid dependent manner and N-acetylneuraminic acid is crucial for its binding.

The most common mammalian sialic acids (SA) are N-glycolylneuraminic acid (Neu5Gc) and N-acetytneuraminic acid (Neu5Ac), which is the metabolic precursor of Neu5Gc. Humans cannot produce Neu5Gc because of a mutation in the hydroxylase (CMAH) gene. Thus, human erythrocytes express only Neu5Ac, while most other primate erythrocytes carry a mixture of both SA, with Neu5Gc being dominant. Homologs of the EBL merozoite ligands, including EBA-140 protein, were identified in ape parasites including *P. reichenowi* and *P. praefalciparum*.

We aimed in comparative characteristics of the SA binding specificity of three recombinant *Plasmodium* EBA-140 ligands, from *P. falciparum, P. praefalciparum* and *P. reichenovi*, towards NeuAc and NeuGc acids. We have shown that there is a clear preference for the Neu5Gc binding in three studied species, including humans. Moreover, all ligands show the specificity towards both Neu5Gc and Neu5Ac, as well. It suggests that human *P. falciparum* parasite can recognize both sialic acids with a clear preference for Neu5Gc, as in case of ape ligands. Thus, the SA specificity seems unlikely to be the sole determinant of *Laverania* host recognition.

POSTER SESSION

EUKARYOTIC GLYCOCONJUGATES

P1.01

Immunoglobulin G O-glycosylation changes in the course of COVID-19

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Coronavirus disease 2019 (COVID-19), still remains a global health challenge. Immunoglobulin G (IgG) can promote disease sequelae in some infections. Many studies prove that IgG may play a role in the inflammatory pathogenesis of this severe disease. IgG O-linked glycosylation is a poorly understood process, little information is available about it so far. Estimated that about 10% of IgG3 polyclonal antibodies and about 13% of IgG3 monoclonal antibodies contain O-glycans in blood serum. Sołkiewicz et al. confirmed the presence of O-glycans in the serum IgG of women with advanced endometriosis and women with other gynecological diseases. The study aimed to examine the profile and degree of O-glycosylation of blood serum IgG in the context of SARS-CoV-2 infection.

The blood sera were divided into three groups: patients with COVID-19, convalescents, and healthy subjects (control). The profile and degree of IgG O-glycosylation were determined using a modified lectin-ELISA method with biotinylated lectins specific to O-glycans: MPL-specific to complete O-glycans (T antigen), VVL-reacting with truncated O-glycans (Tn antigen), Jacalin-specific to antigen T, Tn and sialo-T. The lack of significance in the reactivity with VVL between the tested groups and the significantly lower reactivity with MPL in the group of convalescents compared to the group of patients with COVID-19 and the group of healthy subjects indicates a decrease in the content of the T antigen. In addition, significantly higher reactivities observed in the group of patients with COVID-19 and in the group of convalescents compared to the control group most probably is caused by increased expression of core 3 types of O-glycans reacting with Jacalin. Increased reactivity of O-glycans is associated with the development of inflammation with COVID-19, which is confirmed by our previous results in patients with advanced endometriosis.

P1.02

Fourier-Transform InfraRed (FT-IR) spectroscopy shows alterations in the molecular composition of EV derived from malignant melanoma cells treated with glycosylation inhibitors

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Background: Intercellular transport of proteins through extracellular vesicles (EVs) is one of the factors promoting carcinogenesis. The aim of this study was to evaluate whether manipulating the glycosylation status of melanoma cells affects their molecular composition as well as the molecular composition of the EVs they release.

Methodology: WM266-4 melanoma cells were treated with 1-deoxymannijirymycin (DMJ-1) and tunicamycin for 24, 48, or 72 hours. Cells were cultured in a serum-free medium for 24 hours before harvesting the cells and conditioned media. EVs were isolated from the conditioned media by differential centrifugation combined with low-vacuum filtration. The quality and purity of EVs samples were verified by nanoparticle tracking analysis, transmission electron microscopy, and Western blotting. Flow cytometry with a panel of six lectins was used to analyze changes in glycosylation profiles of melanoma cells and EVs. Fourier Transform Infrared Spectroscopy (FTIR) was used to compare the infrared absorption spectra for different cell subpopulations and EVs.

Results: Flow cytometry showed changes in the glycosylation profile of cells and EVs. 24 and 48 hours of treatment with tunicamycin reduced expression of selected EV glycoepitopes. Interestingly, after 72 hours expression of all epitopes increased. The FTIR results showed that EV populations derived from melanoma cells treated with DMJ-1 or tunicamycin differ in the content of protein and lipid components. In addition, the secondary structure of proteins in EVs showed significantly greater compositional changes compared to proteins present in cells. Both the use of DMJ-1 and tunicamycin resulted in an increase in the occurrence of the α-helix compared to EV derived from untreated cells. Tunicamycin treatment led to much greater fluctuations in the presence of side chains in EV proteins. Thus, it can be concluded that 72 h treatment with DMJ and tunicamycin significantly affects the folding and secondary structure of proteins present in EVs.

P1.03

Functional effect of HCV-29- and T-24-derived ectosomes on recipient cells viability and migratory properties

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Background: Bladder cancer is a malignancy that still remains a therapeutic challenge. Extracellular vesicles (EVs) promote angiogenesis, migration, and metastasis, and inhibit apoptosis in bladder cancer. This effect may depend on their glycosylation status. Thus, the aim of this study was to compare how changes in glycosylation profiles of ectosomes derived from T-24 urothelial bladder cancer cells, HCV-29 normal ureter epithelial cells affect viability and migration properties of recipient cells.

Methodology: For Alamar Blue assay, T-24, HCV-29, HUVEC, and Hs27 cells were seeded onto 96-well plates. The next day, cells were incubated with T-24- or HCV-29-derived ectosomes for 18 h. Fluorescence intensity was measured at 560/595 nm. For wound healing assay T-24, HCV-29, HUVEC, and Hs27 cells were grown to confluence on 6-well plates. Then, the medium was removed, and cell-coated surfaces were scraped. Next, T-24- and HCV29-derived ectosomes were added to each well and the wounded areas were allowed to heal for 18 h.

Results: Approximately 24% of wound width closure was observed for normal HCV-29 cells in control conditions, while T-24 cells displayed a slightly higher rate of ~30%. When added to recipient cells, T-24-derived ectosomes manifested stronger pro-proliferative and promigratory activity in comparison to ectosomes from HCV-29 cells. When ectosomes were isolated from DMJ-treated cells, the aforementioned effects were diminished. It suggests that glycans carried by ectosomes were involved in the modulation of recipient cell function. Also, two-fold increase in cell viability after the addition of T-24-derived ectosomes, but not HCV-29-derived ectosomes for both normal and cancerous bladder cell lines as recipient cells. HCV-29- and T-24-derived ectosomes also increased the viability and motility of HUVEC cells and Hs27 fibroblasts. This supports the hypothesis that ectosomes can modulate the function of various cells present in the tumor microenvironment.

P1.04

Inactivation of the gene encoding TSTA3 protein causes aberrant production of GDP-fucose upon fucose supplementation

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In mammalian cells exist two GDP-fucose biosynthetic pathways, “de novo” and “salvage”. The first utilizes mannose or glucose as a biosynthetic substrate, making up to 90% of the total pool of GDP-fucose. Two enzymes, TSTA3 and GMDS, take part in this process. The second pathway, the so-called “salvage”, uses free fucose originating from lysosomal and proteasomal degradation of glycans and/or the environment and produces up to 10% of the general pool of GDP-fucose in cells.

Recent studies in our laboratory have suggested that protein SLC35C1, a transporter of mentioned nucleotide sugar, could discriminate between different GDP-fucose pools, the “de novo”-derived over the “salvage”-derived. To prove that hypothesis, we developed a cell line with TSTA3 knockout. A lack of TSTA3 protein led to almost completely abolishing fucosylation and its recovery upon fucose supplementation similar to wild-type cell line. Although the production of GDP-fucose was dramatically increased compared to the wild-type cell line and cell line lacking with SLC35C1 transporter. To study that effect, if it depends on removing the last enzyme or blocking the action of the entire de novo biosynthesis pathway, we generated GMDS knockout. Upon fucose supplementation, knockouts differed from each other in terms of GDP-fucose synthesis as well as the production of fucosylated structures. Moreover, we observed various effects on fucose uptake by cells and the level of enzymes involved in the GDP-fucose synthesis through the salvage pathway.

To date, nucleotide sugars biosynthetic pathways were considered to work independently. Our data suggest that the action of one biosynthetic pathway could be regulated by another. In addition, this study gives new insights into treating Congenital Disorders of Glycosylation.

P1.05

A comparative approach to decoding key glycosylation patterns in neurotransmitter receptors

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Glycans cover the surfaces of most membrane and secreted proteins. Modifications in glycan composition in the neurotransmitter receptors like GABA (γ-Aminobutyric acid) have been found to be involved in impairing subunit assembly, protein mobility, cell surface expression, protein stability, and ligand binding. In addition to its effect on neural transmission in many neuropsychological and neurodevelopmental diseases, the efficacy of medical interventions is also affected. To fully address these effects, rational drug design requires understanding of how glycans alter receptor function on a molecular level. The comprehensive predictions of glycoprotein dynamics obtained from Molecular Dynamics (MD) simulations are still computationally expensive. Here, we introduce GlycoSHIELD, a reductionist technique that can be utilized to quickly construct completely glycosylated protein structures on desktop computers. We use GlycoSHIELD, with a focus on the GABA(A) Receptor (GABA(A)R), to predict span and shielding effects of its glycoforms associated with diseases like schizophrenia and to explain how glycans affect shielding both with and without incorporating modifications. Our Solvent Accessible Surface Area (SASA) calculations of chosen glycoforms indicate significant impact on the structural environment of GABA(A)R subunits. Our plans include utilization of the obtained data to understand the connection between shielding patterns and types of glycan modifications on binding sites of therapeutic value.

P1.06

Transcriptional up-regulation of *UGT8* gene expression in breast cancer cells

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Expression of UGT8, enzyme involved in synthesis of galactosylceramide (GalCer), is increased in breast cancer (BC) tumors with highly malignant phenotype [1]. As a result, BC cells accumulate GalCer, which acting as anti-apoptotic molecule increases their tumorigenic and metastatic properties [2]. For these reasons, the understanding of molecular mechanisms involved in up-regulation of *UGT8* gene expression is of great importance not only in gaining insight into biology of BC, but also in developing new therapeutic strategies.

The *UGT8* promoter and deletion mutants were obtained by PCR and inserted into pGL3 Basic Vector. Their activities were analyzed using the Dual-Luciferase Reporter Assay System (Promega). The short promoter fragments were amplified by PCR and electrophoretic mobility shift assay (EMSA) was performed with nuclear fractions from BC cells. JASPAR database was used to identify transcription factors (TFs), which bind to DNA fragments. The expression of TFs in BC cells was analyzed by qPCR and Western blotting. Functional analysis of TFs was performed using cellular models with silenced expression (*loss-of-function*) or overexpression (*gain-of-function*) of TFs.

UGT8 gene promoter is highly active in invasive MDA-MB-231 cells, representing mesenchymal-like" phenotype, but not in weakly invasive and "luminal epithelial-like" T47D cells, revealing that *UGT8* gene expression is regulated on the transcriptional level. It was found that strong enhancer elements are present in the region located within ~1132 to ~1665 bp from the transcription start site. Based on the results obtained by EMSA with promoter fragments and nuclear fractions from BC cells with high (MDA-MB-231) and low (T47D) expression level of UGT8, supported by in silico analysis, 5 TFs were chosen and validated by qPCR and Western blotting. Based on these results functional analysis of LHX6, GR and SOX4 in MDa-Mb-231 cells was performed.

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P1.07

The impact of generalized hypoglycosylation - what can we learn from patients with Congenital Disorders of Glycosylation?

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Background: Glycosylation plays a crucial role in human physiology and pathophysiology. Congenital Disorders of Glycosylation (CDG) form a large group of inherited diseases resulting from variants in genes responsible for the glycosylation process.

Aim: The aim of this work is to present and analyze the clinical effects of impaired glycosylation, based on the data of CDG patients.

Methods: A retrospective analysis of 33 patients with CDGs, who were hospitalized in Children's Memorial Health Institute in Warsaw, Poland in years 1995 – 2023, together with the literature review, were performed.

Results: We identified 33 patients with different CDGs, mostly with N-glycosylation or mixed glycosylation disorders. More than a half of patients presented in the perinatal period, in patients with stable course of the disease the diagnosis was delayed. The most frequent symptoms were: neurological involvement (hypotonia, developmental delay, cerebellar hypoplasia, seizures), dysmorphia, malformations, gastrointestinal symptoms (feeding difficulties, regurgitations, diarrhea), ocular symptoms (nystagmus, strabismus), proteinuria, hypertransaminasemia and hypothyroidism. The most severe symptoms were third space effusions, generalized oedemas, hematological disturbances – cytopenias non-responsive to treatment or hyperleukocytosis, thrombocytosis, thrombosis, coagulopathy with bleeding tendency, recurrent severe infections or sepsis-like episodes of unknown origin, sometimes with multiorgan failure. Severe symptoms worsened during episodes of fever, infection or surgical procedures. In one patient anti-inflammatory treatment with corticosteroids and aspirin resulted in the resolution of chronic severe pericardial effusion. Patients with MPI-CDG were treated with oral mannose with good effect.

Conclusions: Generalized hypoglycosylation most often leads to multisystem disease. The pathogenesis of symptoms is unknown, they may result from deficiency of glycoproteins, but also from dysregulation of different systems (like immune and hematological system) resulting from impaired glycosylation; unfolded protein response is also taken into account. The further research on the pathogenesis of CDG symptoms may provide clues for development of personalized therapy for these diseases.

P1.08

How to study the localization of glycosyltransferases: human alpha1,4-galactosyltransferase as a model in cryo-EM and immunofluorescence techniques

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Human alpha1,4-galactosyltransferase (Gb3/CD77 synthase), encoded by the *A4GALT* gene, synthesizes three glycosphingolipid antigens of the P1PK blood group system: Gb3, P1 and NOR. The presence or absence of the P1 antigen determines P_1 and P_2 phenotypes, respectively, while the presence of all three antigens determines the P_1NOR-positive phenotype. The presence of rare c.631C>G mutation (rs397514502) in *A4GALT* causes p.Q211E substitution in Gb3/CD77 synthase, alters the enzyme specificity, rendering it able to synthesize all the three P1PK system antigens. Gb3 antigen is the main receptor for Shiga toxins, causing hemorrhagic colitis, hemolytic-uremic syndrome (HUS), and uropathogenic *E. coli* responsible for human pyelonephritis. Human Gb3/CD77 synthase expressed in CHO-Lec2 cells also produces Galα1→4Galβ1→4GlcNAcβ1→R (P1 glycotope) on N-glycoproteins, which serve as functionalize receptors for Shiga toxin type 1.

Studies about glycosyltransferase subcellular localization using cell lines may be challenging due to their occurrence in membrane organelles and tendency to form heterodimers. Here, we evaluated the human Gb3/CD77 synthase localization in CHO-Lec2 and HEK293T cells by two techniques: (1) immunogold reaction on Lowicryl Resin for low-temperature embedding visualized using TEM (JEOL F-200) and (2) immunofluorescence observed using confocal microscopy (LEICA SP8). The cryo-EM method showed that HEK293T cells are the preferable cell model to identify the membrane organelles. The obtained image contrast and quality of electrograms provided more valuable data about cell organelles topology than those obtained for CHO-Lec2 cells. Using immunofluorescence, we confirmed trans-Golgi localization of human Gb3/CD77 synthase in CHO-Lec2 and HEK293T cells and showed topology differences of the Golgi apparatus between these two lines. The Golgi in CHO-Lec2 cells exhibits perinuclear position, in contrast to HEK293T cells, which reveal more compactness and localization further away from the nucleus, simplifying the observations. Summarizing, using HEK293T cells facilitates the evaluation of protein localization in the Golgi apparatus compartment.

P1.09

Sweet *Plasmodium* invasion

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Discovering and analyzing interplay between malaria parasites and human cells is important for understanding inner-workings of *Plasmodium*. It is also a way to improve or design malaria drug/ vaccine. The important invasion interaction is one between merozoite PfRH5 and erythrocyte basigin.

Basigin is heavy glycosylated surface receptor, composed with N-glycans of varied structures. Throughout this and other ligand-receptor reactions merozoite can get inside the erythrocyte where it can multiply and cause lysis. This leads to symptoms of malaria – fever, nausea and fatigue.

We examed the binding between recombinant RH5 and recombinant BSG1 and BSG2 isoforms obtained in HEK293 cells. Using SPR and BLI methods we have shown an interaction between parasite protein – RH5 and human BSG. This interaction will be studied in details, especially in the context of glycosylated mutants of BSG2, in order to evaluate a role of basigin glycans in the RH5 binding and parasite invasion.

P1.10

Glycosylation-dependent clustering modulate FGFR signaling and cell fate by a network of extracellular galectins

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The transmission of signals from the extracellular environment to the cell is mediated by cell surface receptors. Fibroblast growth factor receptors (FGFRs) are integral membrane proteins that belong to the receptor tyrosine kinase (RTK) superfamily, activated by fibroblast growth factors (FGFs). FGFRs are N-glycosylated at several positions and these modifications regulate FGFRs signaling, which is critical for human body development and homeostasis. Galectins are family of lectins, which are capable of reading glycol-code in form of N-glycosylation of cell surface proteins, leading to contribute multiple cellular functions, including endocytosis, signaling, apoptosis, division and immune surveillance. We demonstrated that a precise set of extracellular multivalent galectins -1, -3, -7 and -8 directly bind to the N-glycan chains in D3 domain of FGFR1, triggering differential clustering of FGFR1. The galectin activation of the receptor and initiation of downstream signaling cascades, revealed different consequences for the cell physiology, affecting on cell viability and metabolic activity. In summary, our findings might be relevant for the development new therapeutic strategies, identifying a novel regulatory module in FGFR signaling and cell fate through distinct members of galectin family.

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P1.11

FGFR1 trafficking between the plasma membrane and the nuclear envelope is regulated by receptor N-glycosylation

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Fibroblast growth factors (FGFs) and fibroblast growth factor receptors (FGFRs) comprise a system transmitting signals through the plasma membrane and thereby controlling fundamental cellular processes. The balanced FGF/FGFR1 signalling is crucial for the development and homeostasis of the human body and abnormal FGFR1 is frequently observed in various cancers. Fibroblast growth factor receptor 1 (FGFR1) is a heavily N-glycosylated receptor tyrosine kinase, which in addition to predominant localization to the plasma membrane, was also found inside the cells, mainly in the nuclear lumen. However, the exact mechanism of FGFR1 nuclear transport still remains unclear.

Here we assembled a glycosylation-free (GF) mutant of FGFR1, FGFR1.GF, and demonstrated its primary localization to the nuclear envelope. We showed that FGFR1.GF crosses the nuclear pore complexes as it is present in the inner layer of the nuclear membrane. Furthermore, we showed that inefficient secretion, rather than FGFR1 destabilization by lack of N-glycans, is responsible for FGFR1 accumulation in the nuclear envelope. We also constructed single and multiple N-glycosylation mutants of FGFR1 and demonstrated that only the coexistence of N-glycans of the immunoglobulin-like domains D2 and D3 of FGFR1 allows the potent transport of FGFR1 to the plasma membrane. Moreover, we observed a high level of intracellular FGFR1.GF autoactivation, which implies the possibility of nuclear FGF-independent FGFR1 signalling. Using mass spectrometry and proximity ligation assay we identified novel binding partners of the FGFR1.GF mutant, providing insights into its cellular functions. These data indicate that N-glycosylation of FGFR1 has an crucial role in the FGFR1 kinase activity regulation and trafficking between the nuclear envelope and plasma membrane, which has an influence on the cellular function of the receptor.

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P1.12

Fibroblast growth factors N-attached-glycans serves as new layer of information used by extracellular galectins to modulate cell signaling and physiology

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Fibroblast growth factors (FGFs) employ their receptors (FGFRs) and regulate cellular processes pivotal for development and homeostasis, such as cell division, metabolism, differentiation, migration, and apoptosis. FGFs are organized into seven subfamilies: FGF1, FGF4, FGF7, FGF8, FGF9, FGF11 and FGF19. Among them, only members of FGF1 and FGF11 subfamilies are devoid of classical signal peptides. FGFs belonging to all other subfamilies (16 proteins) possess putative N-glycosylation sites and are released by cells via classical secretory pathway, where their N-glycosylation may occur. However, the presence of N-glycans on secreted FGFs and the functional significance of these modifications are largely unknown. We have recently confirmed N-glycosylation of FGFs representing all five secreted groups. We have also identified a specific set of extracellular lectins, galectins − 1, -3, -7 and − 8, as novel FGFs interacting partners. Galectins are β-galactose-binding lectins, engaged in many cellular processes, such as endocytosis, autophagy, cell division or apoptosis and are strongly implicated in cancer. Our data provide evidence for a direct, and N-glycosylation-dependent interaction between model FGFs and identified galectins. Particular galectins differentially modulate FGF4 cell binding and internalization, thus altering amplitude of FGF4 signaling and fine-tuning cellular processes. Moreover, galectins multivalency is strictly required for adjustment of FGF4 activity. Galectins bind FGF4 N-attached glycans and capture FGF in the extracellular matrix, regulating growth factor release and relocation to FGFR. Summarizing, our data reveal a novel module of FGF/FGFR signaling regulation, in which FGFs N-glycans are additional layer of information differentially read by multivalent galectins and used to modulate signal transduction and cell physiology.

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P1.13

Immunohistochemical analysis of AGE10 epitope in prostate cancer

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Advanced glycation end products (AGEs) are a heterogeneous group of compounds formed in a non-enzymatic reaction between reducing sugars and amino groups of proteins. Elevated levels of AGEs have been observed in various inflammatory diseases and cancers, including prostate cancer (PCa). The unique AGE epitope (AGE10) has recently been identified in human serum using synthetic melibiose-derived AGE (MAGE) [1,2]. AGE10 is identified with its synthetic analogue derived from a disaccharide which exhibits pro-apoptotic, genotoxic, and immunogenic properties [3,4]. However, the role of AGE10 in cancer and tissue damage remains unclear. Prostate cancer is the second most common cancer diagnosed in men. Screening tests typically measure the concentration of prostate-specific antigen (PSA). However, PSA testing has low specificity for PCa, as elevated PSA levels can also occur in benign prostatic hyperplasia and prostatitis. The introduction of PSA screening has had minimal impact on mortality rates in men, emphasizing the need for new methods of early diagnosis. In this study, our objective was to perform immunohistochemical analysis to examine the presence of AGE10 in prostate cancer. We analyzed tissue slides containing prostate cancer and prostate intraepithelial neoplasia (PIN). We observed the presence of AGE10 in both types of lesions. The reactivity is localized in cell cytoplasm and not in nucleus. Results, compared to P63 antigen, indicate some diagnostic value of AGE10 epitope.

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POSTER SESSION
PROKARYOTIC GLYCOCONJUGATES

P2.01

Structural diversity among *Edwardsiella tarda* core oligosaccharides

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Edwardsiella tarda is a Gram-negative bacterium and a pathogen of farmed fish. It is the etiological agent of a systematic disease called edwardsiellosis, which has been reported to affect a wide range of freshwater and marine fish [1]. In addition to fish, *E. tarda* is also an occasional human pathogen known to cause both gastroenteritis and extraintestinal infections in humans [2].

Lipopolysaccharide (LPS) is the main virulence factor of these bacteria. It is a powerful activator of innate immune responses. This molecule present in smooth bacteria is composed of three distinct regions – hydrophobic lipid A (center of endotoxicity), a core oligosaccharide, and the O-antigen. The structural analysis of endotoxins is important, since the physiological and pathophysiological effects strongly depend on their chemical structure.

The first structure and genomics of the core oligosaccharide of pathogenic *E. tarda* strain EIB 202 has been studied before [3]. Herein, the other 13 *E. tarda* core oligosaccharides were isolated from bacterial strains and analyzed by MALDI-TOF mass spectrometry and 1H, 13C NMR spectroscopy. The differences responsible for identification of other *E. tarda* oligosaccharide core types were attributed to additional substitution by one or two O-acetyl group/s and lack of glycine in comparison with core oligosaccharide presented in EIB 202 strain. By 2D NMR spectroscopy the core substitution by O-acetyl group/s has been confirmed. These results suggest the significant conservatism among of *E. tarda* core oligosaccharides.

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P2.02

Structural studies of *Plesiomonas shigelloides* O5 lipopolysaccharide

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Plesiomonas shigelloides is a Gram-negative rod belonging to the Enterobacteriaceae family. *P. shigelloides* is associated with episodes of intestinal infections and outbreaks of diarrhoea in humans. It was ranked third among etiological agents in outbreaks of travellers' diarrhoea in Japan and China [1]. The extraintestinal infections caused by this bacterium, e.g. meningitidis, bacteremia and septicemia, usually have gastrointestinal origin and serious course. Lipopolysaccharide (LPS, endotoxin), the main component of the outer membrane of the cell envelope of Gram-negative bacteria, is built of an O-specific polysaccharide and core oligosaccharide covalently linked to lipid A.

Except for the detailed serotyping schemes of *P. shigelloides* proposed by Shimada, Sakazaki and Aldova, LPS, the main surface antigen and a virulence factor of these bacteria has not been extensively investigated. The structure of *P. shigelloides* O5 LPS was determined by chemical analysis, mass spectrometry and NMR spectroscopy. The O-specific polysaccharide of *P. shigelloides* O5 has the following structure: →4)-β-D-ManpNAc-(1→4)-α-d-GlcpNAc-(1→. The same structure was identified before in the O-specific polysaccharide of *Hafnia alvei* strain 38 [1], *Serratia marcescens* O2 and O3 [2]. The core oligosaccharide and lipid A are identical with these of the *P. shigelloides* serotype O33 [3] and O74 [4], respectively.

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P2.03

Unusual structural elements of O-antigen from *Plesiomonas shigelloides* O68 (strain CNCTC 138/92) investigated by HR-MAS NMR spectroscopy

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Plesiomonas shigelloides is a potential human and animal pathogen that has been implicated in outbreaks of food poisoning with acute gastroenteritis. With the gradual increase in antibiotic-resistant strains of *P. shigelloides* development of vaccine therapy is of urgent interest.

The carbohydrate antigens expressed on the surfaces of *P. shigelloides* bacterial cells have been classified to 102 O-serotypes. To date, structures of the O-specific polysaccharide (O-PS) component of the lipopolysaccharide (LPS) have been determined only for the serotypes O1, O12, O17, O22, O24, O33, O36, O37, O51, O54, O74 [1]. The presence of unique sugar residues and rare substituents were reported.

We have investigated the structures of O-antigens from a set of *P. shigelloides* strains by high-resolution magic angle spinning (HR-MAS) NMR spectroscopy directly on bacterial cells. The studies revealed apparent similarities between the 1H HR-MAS NMR profile of *P. shigelloides* O74 (strain CNCTC 144/92) [2] and that of O68, suggesting the common structural elements in their O-PS.

In-depth studies by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and chemical methods indicated that the *P. shigelloides* O68 O-PS is composed of a trisaccharide repeating unit with the →4)-α-D-Glcp6OAc-(1→4)-β-D-GlcpNAcyl3NAc-(1→3)-β-FucpNAc4N-(1→ structure, in which 3-acetamido-2,3-dideoxy-glucosamine is acylated with D-3-hydroxybutyric acid. The presence of the substituent and acetyls make this O-PS more hydrophobic than typical O-antigen. The structural elements of similar nature to these present in *P. shigelloides* O74 may influence the biological and physicochemical properties of their LPS. The unique structure indicates a need for studies of biological activities that is essential for development a vaccine against the *P. shigelloides*.

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P2.04

Structure of the core oligosaccharide of *Plesiomonas shigelloides* serotype O68. Can we identify the "minimal common structure" among *Plesiomonas* cores?

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Bacteria expose on the cell surface a variety of complex carbohydrate molecules that are essential for the structural integrity and interactions with hosts. Gram-negative bacteria produce lipopolysaccharides (LPS), which are the main components of the outer membrane of bacterial envelopes and play a major role in the pathogen interactions with the immune system of the host and manifest endotoxic activities similar to those of enteric bacteria. *Plesiomonas shigelloides* has been reported as the most common etiological agent in outbreaks of travellers' diarrhea. 102 serotypes have been identified for *P. shigelloides*, but only for 15 strains complete or partial LPS structures have been elucidated. Despite the rising knowledge of *P. shigelloides* LPS structures over the past three decades, this virulence factor is still poorly characterized. The core oligosaccharides were described for strains assigned to serotypes O1 [1,2], O12 [3], O13 [4], O17 [5,6] and O36 [7], O22 [8], O24 [9], O33 [10], O37 [11], O54 [12], O74 [13]. We now report on structural studies of the core oligosaccharides isolated from *P. shigelloides* strain CNCTC 138/92 lipopolysaccharide [serotype O68]. NMR spectroscopy and mass spectrometry were the principal methods used. It was concluded that the main core oligosaccharide of the strain is composed of eleven monosaccharide residues having the following structure: The core is not substituted by phosphate, but instead by glycine, both being rather unusual features. This structure represents a novel core oligosaccharide among *P. shigelloides* O-antigens. Additionally, we have now also compared the structures of all *P. shigelloides* described to date in an attempt to identify the common conserved structural elements of the core OS and indicate the variability of this segment of *P. shigelloides* LPS.

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P2.05

Chemical characteristics of glycolipids present in *Cutibacterium acnes* cells and secreted extracellular vesicles

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Cutibacterium acnes is one of the most common species found on the surface of human skin. These Gram-positive lipophilic bacteria are facultative anaerobes inhabiting areas of the skin where sebaceous glands are abundant. Two or four glycolipids were found in the membrane lipid profile of C. *acnes* strains. Due to the presence of sugar components, bacterial glycolipids are an interesting research object, especially in the context of their potential immunogenic and antigenic properties.

Extracellular vesicles (EVs) are spherical nanostructures produced by all living organisms. EVs are purposely secreted by bacteria to aid in communication and contribute to numerous functions.

In our studies, we identified, isolated and purified cellular glycolipids present in 4 *Cutibacterium acnes* phylotypes. Glycolipids were identified not only in whole-cell extracts but also in EVs secreted by these bacteria. Depending on the TLC retention factors glycolipids were designated A, B, C and D. Glycolipids were analyzed by TLC, GC/MS and MALDI-TOF MS.

Of the whole-cell extracts, only one phylotype of C. *acnes* (DSM 16379) contained all 4 glycolipids. The others (DSM 1897, PCM 2334 and NCTC 13655) contained glycolipids A and C. In lipid extracts obtained from EVs, the presence of glycolipid A (all phylotypes) and glycolipid C (DSM 1897 and NCTC 13655) were also found. Based on the GC MS and MALDI-TOF analysis data, glycolipid A - contains 2 fatty acid chains and 2 glucose residues attached to a glycerol backbone. Glycolipid C contains the same sugar-glycerol backbone and three fatty acyl residues.

The chemical characteristics of C. *acnes* glycolipids are the starting point for further research on the antigenic properties of these compounds in the context of searching for new vaccine components and diagnostic tools. Studies in the function of glycolipids in EVs are also very promising research goals.

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