Satellite Session 1: Glycobiology

Lectures

L22.1

Adventures in RBC biology: inspired by Elwira Lisowska and Jerzy Koscielak

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As a resident in the 1970's, I became interested in transfusion medicine and was fascinated by the biochemistry of human blood group antigens. Because of a clinical issue at the time about whether antibodies to Lewis blood group antigens were clinically significant, I read the work of SI Hakomori and others about glycosphingolipid structures, and, thus, came across the papers of J Koscielak. Because of a hemolytic transfusion reaction caused by an anti-M antibody, I read the seminal studies of V Marchesi, O Blumenfeld, and E Lisowska regarding the glycophorin A sequence and the MN antigen polymorphisms. These antigens encode glycopeptide epitopes; the role of oligosaccharides only increased my fascination.
Following clinical training, my interest in glycobiology led me to pursue a postdoctoral research training with V Ginsburg at the NIH. These years with Vic were transformative. Because of his generosity, his postdocs had the opportunity to have private meetings with eminent individuals who came to visit. As such, I made the acquaintance of Prof. Lisowska and Koscielak, in particular. You may imagine the thrill I had, as a young trainee, to meet the people whose papers initially inspired me.
As a faculty member, I pursued studies of glycosphingolipid and glycoprotein structure and function, often using blood group antigens as models. In 1991, Fogarty Foundation sponsored a scientific visit to Poland. As such, my family and I were hosted by Prof. Koscielak in Warsaw and Prof. Lisowska in Wroclaw. This trip was important professionally, in enhancing friendships and collaborations, and personally, given my Polish heritage.
Our Laboratory's research now focuses on (patho)physiological issues related to blood transfusion, particularly related to red cells. The availability of powerful mouse models allows for investigations of the mechanisms underlying hemolytic transfusion reactions, the "red cell storage lesion," and the consequences of transfusing older, stored red cells. We currently use cell culture methods, animal models, human volunteer studies, and studies in human patient populations.
The guidance and friendship of many individuals, particularly those from my "Polish connection," are incredibly important in any success that we have had, and always function as a lodestar in terms of what it means to try to be a scientist.

L22.2

Genetic alterations of the glycosylation pathway leading to diminished infectivity of '*Candida albicans*'

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It is generally accepted that mannoproteins, the important structural elements of *C. albicans* cell wall, act as the major adhesins, antigens and immunomodulatory molecules of the cell. Moreover, they play an important role in *C. albicans* morphology.
The morphological switch i.e. the yeast to hyphae transition is thought to be required for *C. albicans* virulence.
The present talk will focus on the impact of dolichol biosynthetic and protein glycosylation pathways on the cell wall integrity, adhesion, biofilm formation and morphological transition, in the pathogenic yeast *C. albicans*. In particular, we concentrate on the early events in protein glycosylation, localized in the endoplasmic reticulum (ER). The subject relies on our earlier observation indicating inter relation between protein glycosylation and sensitivity towards external agents, including antifungal drugs.
To this end we have constructed the set of mutants perturbed in the dolichol biosynthetic pathway (rer2; cwh8), protein N- (alg7,13,14) and O glyco/mannosylation (dpm1, 2, 3). The results led us to the following conclusions:
– *ORF19.4028* (*RER2*) encodes the main *cis*-prenyltransferase in *C. albicans*. Rer2p activity as well as its functional interdependence with dolichol diphosphate phosphatase (Cwh8p) determine dolichol level and protein N-glycosylation.
– dolichol N-acetyl glucosamine ($DolPPGlcNAc_2$)- and dolichol phosphate mannose synthases (DPMS) constitute the complex of three subunits: Alg7p,Alg13p, Alg14p and Dpm1p, Dpm2p and Dpm3p.
– the diminished activity of *cis*-prenyltransferase either $DolPPGlcNAc_2$ or DPMS leads to severe defects in protein glycosylation, affects cell wall composition, integrity and morphology, as well as increases susceptibility to some antifungals and chemical agents
– the proper level of the mentioned proteins in *C. albicans* is required for hyphae formation, adherence and chlamydospore production
– transcriptional changes in response to the decreased expression of *RER2* in *C. albicans* include, among others, the activation of unfolded protein response (a common cellular respond to ER stress) and, surprisingly, some adjustment of central metabolism and the cell cycle
In conclusion: The defect in the ER located glycosylation might significantly alter *C.albicans* biology and fungus/host interaction and facilitate the search for the new antifungal drugs.

Acknowledgements:

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L22.3

Do nucleotide sugar transporters work as independent proteins to deliver substrates for glycosylation into Golgi lumen?

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The glycan moiety is synthesized and modified by glycosyltransferases located in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus (GA). The substrates required for glycosyltransferases are sugars, activated by the addition of a nucleoside mono- or diphosphate (UDP, GDP, or CMP) and transported into the ER and/or GA by nucleotide sugar transporters (NSTs). Although biosynthesis of nucleotide sugars is well understood, the mechanisms of their transport and distribution remain still unclear. For a long time, it was accepted that SLC35A2 (UGT) and SLC35A3 (NGT) are characterized by single, defined (UGT for UDP-galactose and NGT for UDP-N-acetylglucosamine) specificity. However, our data suggest that the role played by NGT and UGT in galactosylation might be coupled. Moreover, involvement in glycosylation of other NSTs from SLC35 family, such as SLC35A4 and SLC35A5, is not known. We showed that transporters are not independent in their action and may form complexes with other transporters and also glycosyltransferases. Given the diversity of glycan structures and the competitive nature of many glycosyltransferases it is remarkable that the cell can synthesize several different oligosaccharides with high precision. We hypothesize that cells may achieve this specificity by cooperative activity of respective NSTs and the spatial or temporal organization of a discrete subset of proteins into specific compartments and/or assemble them into functionally relevant protein complexes. Therefore, we propose that interaction between NSTs, as well as between NSTs and glycosyltransferases might constitute a general mechanism utilized in the glycosylation process. We also assume that other proteins, not directly connected with glycosylation process, might be involved in such complexes. This hypothesis I now being tested with cell lines with non-functional SLC35A3, SLC35A4 and SLC35A5 transporters, recently generated in our laboratory with the CRISPR-Cas technique.

L22.4

Endothelial proteoglycans

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Single mesoderm derived endothelial cells layer: coate and originate all blood and lymphatic vessels, express different cell surface proteins that control creation and action of vascular network in answer to hypoxia, organize the development and growth of the connective tissue as well as smooth muscle surrounding the vessel walls, interact with one another to suit local requirements of the normal and pathological cells e.g cancerous cells. Non-cytosolic sides of the endothelial cells are covered with hydrophilic coat or glycocalyx. Apparent thickness of the glycocalyx is ~ 0.5 μm. Glycocalyx is composed mainly by transmembrane and absorbed proteins that are mostly glycosylated. The glycocalyx glycoproteins consist of: proteoglycans, glycosaminoglycans (GAGs) and oligosaccharide chains of glycoproteins (some containing sialic acid residues). The amount of glycocalyx sulfate proteoglycan (SPG)-molecules vary between 10^5–10^6/cell. SPG are composed of: heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS) attached to a core protein. Fine structure of GAGs is tissue and cell type specific, dependent on aging and pathology. All GAGs form unbranched chains 30–150 disaccharide units, with exception of hyaluronic acid (HA) forming much longer chains. The vascular endothelium GAGs are attached mostly to syndecans-transmembrane proteoglycans and glypicans anchored to the cell membrane by glycosylphosphatidylinoinositol (GPI) linkage. Glypicans (59–65 kDa proteins carrying 2–5 HS chains) are involved in the suppression/modulation of growth and the activity of growth factors.

L22.5

Glycosylation in melanoma

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Malignant melanoma is one of the most aggressive human tumors. It originates from melanocytes - the pigment-containing cells. Changes in the repertoire of cell-surface glycosylation are an inherent hallmark of malignant transformation of melanocytes, affecting cell-cell and cell- ECM interactions; they lead to transition from an epithelial to a mesenchymal phenotype and acquisition of migratory, invasive and finally metastatic capabilities by melanoma cells. Unlike melanocytes and cells of early melanoma *in situ*, cells of florid melanoma *in situ* express β1,6-branched *N*-linked oligosaccharides. A marked and progressive increase of these oligosaccharides is frequently observed during the development of malignancy and promotes lung metastases and larger colonies formation. The β1,6-branched *N*-glycans, extended with poly-*N*-acetyllactosamine chains - another tumor specific structures, facilitate lung specific metastasis of melanoma cells *via* galectin-3 interaction. Normal human melanocytes do not express sLe^x and poorly express sLe^a, but melanoma cells from tumor biopsies and culture overexpress both isomers; these oncofetal structures are known as neoplastic differentiation antigens of melanoma. Although normal melanocytes express a high amount of Neu5Ac, an additional increase in α2,6-sialylation is specific for outer N-acetyllactosamine units during tumor progression. Both, $sLe^{x/a}$ expression and altered sialylation facilitate the association of malignant melanoma cells, respectively, with selectins and siglecs, causing interactions of circulating tumor cells with platelets, leukocytes, turning off immune response and supporting docking of tumor cells on the endothelium, promoting metastasis. Normal melanocytes do not express acetylated forms of sialic acids; however, modification by 9-*O*-acetylation has been recognized as a human melanoma specific marker; it makes cells much less susceptible to degradation, extends their lifetime *in vivo* protecting cells from apoptosis; it could be also related to the high malignancy and rapid spread of melanoma. Melanoma progression and liver metastases formation are accompanied by abnormal truncated structures, i.e., core fucosylated and non-fucosylated paucimannose *N*-glycans, and GlcNAc-terminated structures. Invasive melanoma displays higher expression of truncated mucin type *O*-glycan i.e. T and Tn antigens, which are positively-correlated with metastasis of melanoma. These oncofetal antigens are not expressed in benign nevus cells, thus they are predictors of poor prognosis.

L22.6

Chemical structure and biological role of the lipopolysaccharide from the bacteria of *Proteus* and *Providencia* genera

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The genus *Proteus* belongs to the *Enterobacteriaceae* family, where it is placed in the tribe *Proteeae,* together with the genera *Morganella* and *Providencia*. Currently, the genus *Proteus* consists of five species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri* and *P. myxofaciens*, as well as three unnamed *Proteus* genomospecies. Recently, two species *P. terrae* and *P. cibarius* are proposed, as a novel species in the genus *Proteus*. Itis known as a bacterial opportunistic pathogen, which causes complicated UTIs with a higher frequency, compared to other uropathogens. The bacteria of the genus *Providencia* are opportunistic pathogens causing acute enteric and urinary tract diseases, most often in little children and patients whose immune system has been compromised by surgery or burns. It contains 8 genus including *P. alcalifaciens*, *P. stuartii* and *P. rustigianii,* causing infection with the higher frequency. Lipopolysaccharide is composed of three genetically and structurally distinct regions: O-specific chain (O-antigen), the core oligosaccharide and lipid A, All three regions of *Proteus* LPS have been studied. The differences in the structure of O-antigens serve as a basis for the serological classification of *Proteus* strains. The serological classification scheme currently consists of 80 serogroups. *Proteus* O-antigens have a characteristic composition of monosaccharide and non-sugar components. All polysaccharides include amino sugars and, with one exception (*P. vulgaris* O53), all contain either d-glucosamine (GlcN) or d-galactosamine (GalN). Rather common in *Proteus* OPSs are also d-glucuronic acid (GlcA), and d-galacturonic acid (GalA). These hexuronic acids either have a free carboxyl group or, less often, are amidated with the a-amino group of amino acids, including L-alanine, L-serine, L-threonine, and L-lysine. The chemiacl structeres of O-antigens representing all *Proteus* serogroups were studied in details, some of them will be presented in the lecture, togheter with the results of study of the core and lipid A regions of these bacteria, as well with the data concerning of the LPS antigenic specificity and its biological role. The existing combined serological classification scheme of three species,*P. alcalifaciens, P. stuartii* and *P. rustigianii,* is based on the specificity of O-antigens (O-polysaccharides) and comprises 63 O-serogroups. Results of immunochemical studies of these antigens will be also presented.

L22.7

Endotoxin-ficolin-3 interactions – specificity and cross-talk within the complement system

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The complement system is a part of innate immunity and represents the first line of defence against infections. There are three pathways of complement activation: classical, alternative, and lectin-based. Ficolin-3, together with mannose-binding lectin (MBL), collectin-10 (CL-10), CL-11, ficolin-1, -2 and -3 is a key activator of the lectin pathway. Ficolin-3 in complex with MBL-associated serine proteases (MASP), specifically binds to pathogen-associated molecular patterns and initiates the inflammatory response followed by opsonisation, phagocytosis or direct lysis of pathogenic cell. Contrary to other lectins and ficolins, ficolin-3 specificity is poorly characterised. There are only few natural ligands identified that are recognised by human ficolin-3: the exopolysaccharide of *Aerococcus viridans* 86965 and O-specific polysaccharides (O-PS) of *Hafnia alvei* PCM (Polish Collection of Microorganisms) 1200, 1203, 1205 and 23, *Proteus vulgaris* O4 and *P. pennerii* O58 lipopolysaccharides (LPS, endotoxins). All ligands were demonstrated to activate lectin pathway *in vitro* and/or *in vivo* [1].
Herein the specificity of human ficolin-3 was studied using polysaccharides, oligosaccharides and monosaccharides characteristic for or isolated form *H. alvei* PCM 1200 and *Proteus* spp. LPS. LPS 1200-derived oligosaccharides were isolated by zwitterionic-type hydrophilic interaction liquid chromatography coupled on-line with electrospray ionisation mass spectrometry [2]. Both recombinant and plasma-derived proteins were analysed. Plasma-derived ficolin-3 was isolated by a protocol based on an exclusive ligand: the O-PS of LPS 1200 LPS [3]. Interactions were characterised using surface plasmon resonance (SPR) and the saturation transfer difference (STD) NMR. We have shown that the most dominant interactions were related to N-acetyl group and protons H2-H6 of Qui*p*4NAc and protons H1-H2 of glycerol of LPS 1200 O-PS. Additionally, optimisation of ficolin-3 purification protocol demonstrated that ficolin-3/MASP complexes interacts with immunoglobulins, what was in in agreement with recent findings suggesting cross-talk between IgG and ficolin-3 [4].

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L22.8

Neoglycoconjugates as components of antibacterial vaccines. Anti-pertussis vaccines revisited

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Pertussis (whooping cough) is a highly contagious respiratory tract disease caused by the Gram-negative bacterium *Bordetella pertussis*. Widespread vaccination has drastically reduced the prevalence of pertussis in many countries over the last decades. However in recent years the pertussis incidence has been on the rise and outbreaks still occur. The whole-cell pertussis vaccine has been very effective for years but at the same time it was reactogenic. Concerns related to the adverse effects following vaccination led to the development of subunit vaccines containing only a selected set of the isolated and inactivated antigens of *B. pertussis*. There are reports, that the immunity induced by the acellular pertussis vaccine wanes in the vaccinated populations. The observation indicates that these vaccines are devoid of important protective *B. pertussis* antigens. The vaccine containing an inactivated pertussis toxin induces the production of toxin-neutralizing antibodies, but it does not lead to destruction of bacteria. The pathogenesis of pertussis involves many virulence factors and the direct bactericidal activity is essential in anti-pertussis immunity. One of these disregarded antigens of *B. pertussis* is lipooligosaccharide (LOS). LOS is the most abundant surface molecule and a virulence factor of *B. pertussis*. It is a target for bactericidal antibodies during natural infection, but the endotoxic activity of LOS precludes its use as a component of acellular vaccines against *B. pertussis*. We have devised neoglycoconjugate comprising the non-toxic moiety of the *B. pertussis* LOS-derived oligosaccharide coupled to a carrier protein. Immunization of animals with this neoglycoconjugates yields the *B. pertussis* LOS-specific antibodies, but more importantly, these antibodies are bactericidal in complement-mediated *in vitro* assay. The neoglycoconjugate could be used as a new component of the pertussis vaccine.

Oral presentations

O22.1

The use of lectin microarray for assessing protein glycosylation

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Glycans or carbohydrates attached to glycoproteins can directly affect functions of such proteins, and therefore must be adequately analyzed and controlled throughout reaearch and development. Despite advances in glycoanalysis by mass spectrometric methods, the complexity of protein glycosylation is an analytical challenge. In this study, we evaluated the utility of a lectin microarray for assessing protein glycans. Using commercial lectin chips, which contain 45 lectins toward distinct glycan structures, we determined the lectin binding patterns of a variety of glycoproteins, including monoclonal antibodies. Lectin binding signals were analyzed to generate glycan profiles that were generally consistent with the known glycan patterns for these glycoproteins as determined by MS-based techniques. Conclusions:
(1) This is not a method to identify the complete glycan structures, but is very powerful in comparative glycan analyses among samples with very high sensitivity and high throughput. Actually, this technology has more than 100times higher sensitivity than Mass Spectroscopy,and the standard protocol for sample preparation is just Cy3 labeling onto glycoprotein. There is no need to detach glycan from glycoprotein.
(2) This technology is the best match for discovery and screening phases of biomarker development pipeline.
(3) This technology is also very promising in characterization and qualification of bio-similar and bio-better drugs such as IgG and EPO. The current Lectin Microarray is stronger than any other type of analysis tools from a view point of identifying isomers such as Sia(alpha)2-3, Sia(alpha)2-6, (beta)-Gal, and (alpha)-Gal, and also changes of epitopes.

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O22.2

Tricomponent, self-adjuvanting glycoconjugate vaccine against *C. albicans*

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Glycoconjugate vaccines have been proved to be a successful tool in fighting life-threatening infectious diseases. Despite significant progress in the vaccine development, no vaccine against *C.albicans* has been currently available on the market. This opportunistic fungus is responsible for severe nosocomial infections, especially in immunocompromised patients, and associated with high morbidity rate. In our initial attempt, a glycoconjugate vaccine based on protective β-mannan epitope and tetanus toxoid as a carrier has been proposed and tested in animals. While the conjugate was effective in rabbits, it showed poor immunogenicity in mice. In the search for the design of more immunogenic vaccine, we proposed to include an additional functionality in the conjugate; that ensures vaccine targeting to dendritic cells and their activation via interaction with cell surface receptors. Dendritic cells play a pivotal role in response to foreign antigens and are capable of tuning the immune response according to various stimuli. They express C-type lectin receptors on the surface that can be engaged for vaccine delivery with specific ligands. The receptor of this type – Dectin-1, recognize and binds β-glucans initiating a signalling cascade resulting in cytokines release and modulation/enhancement of the immune response. Laminarin, a short β-glucan from *Laminaria digitata* was chosen as targeting ligand and Dectin-1 activator.
To facilitate attachment of two different carbohydrate ligands, it was necessary to develop an orthogonal conjugation strategy. Ligands were sequentially added to the carrier molecule and finally labelling with a fluorescence dye, permitting observation of the vaccine uptake by dendritic cells, was also performed. The conjugation strategy is based on the transformation of protein amino groups into azides, carbodiimide driven conjugation of a first ligand to carboxyls on a protein surface, a coupling of a second ligand via "click chemistry" conjugation, restoration of amino groups by reduction of azides and final conjugation of a fluorescent dye with regenerated amines. The developed technique permitted the high density of ligand conjugation that reached 21 β-mannan trisaccharide units and three molecules of laminarin per molecule of tetanus toxoid carrier. Upon experiments on mice model, the tricomponent vaccine showed significantly improved performance when compared to the simple tetanus toxoid/ β-mannan conjugate.

Posters

P22.1

Camel glycophorins: purification and biochemical characterization

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Glycophorins represent a class of heavily glycosylated proteins present on the surface of erythrocytes. While human glycophorins have been thoroughly studied, glycophorins from other species remain largely unexplored. In particular, no attempt has been made to investigate glycophorins from camels, whose utility in research has been on the rise for their ability to produce single-chain antibodies. Using phenol extraction from ghosts of *Camelus dromadarius* erythrocytes and size-exclusion high-performance chromatography we found that camel erythrocytes contain three glycophorins, which we named X, Y and Z. Glycophorin X is visible in SDS-PAGE as monomer (29 kDa), dimer (49 kDa) and trimer (86 kDa), while glycophorin Y is visible as monomer (34 kDa) and dimer (62 kDa). Glycophorin Z is single band of 108 kDa. Using lectinoblotting, we found that *Arachis hypogea* (peanut) lectin binds to glycophorins X and Z after desialylation. The same glycophorins are bound by *Vicia villosa* and *Salvia sclarea*. In contrast, lectins from *Phaeseolus vulgaris* (E), *Aleuria aurantia* and *Griffonia simplicifolia* (II) bind to glycophorin Y. PNGase F treatment of glycophorins caused a complete loss of *Phaeseolus vulgaris* (E) lectin binding to glycophorin Y. The binding of lectins to glycophorins X and Z was unaffected by PNGase F treatment. Thus, it may be concluded that glycophorins X and Z contain O-linked oligosaccharide chains, while glycophorin Y contains N-linked oligosaccharide chains.

P22.2

Glycophorin D is the receptor for *Plasmodium reichenowi* EBA-140 merozoite ligand on chimpanzee erythrocytes

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Plasmodium reichenowi, the malaria parasite which infects chimpanzees, is morphologically identical and genetically similar to *P. falciparum*, the human parasite responsible for malignant malaria. Genomes comparison of *P. falciparum* and *P. reichenowi* revealed major differences in genes coding for proteins engaged in erythrocytes invasion [1].
Erythrocyte binding ligand 140 (EBA-140) is a member of the *P. falciparum* erythrocyte binding antigens (EBA) family [2]. The amino acid sequence of *P. reichenowi* EBA-140 ligand is 81% identical with *P. falciparum* erythrocyte binding region (Region II) [3]. Until now the receptor for *P. reichenowi* EBA-140 ligand on chimpanzee erythrocytes was not identified.
In order to evaluate the molecular basis of theerythrocyte recognition by *P. reichenowi* EBA-140 ligand, its binding region (Region II) was expressed in HighFive insect cells, similar as homologous *P. falciparum* Region II[4]. The protein secreted into the medium was purified in one step NiNTA affinity chromatography. Binding of EBA-140 Region II to erythrocytes was examined by flow cytometry, surface plasmon resonance and immunoblotting using native, neuraminidase-, trypsin- or chymotrypsin- treated chimpanzee red blood cells. It was shown that the recombinant baculovirus-expressed *P.r.* EBA-140 Region II binds to chimpanzee erythrocytes but does not bind to human erythrocytes. Its binding to erythrocytes treated with neuraminidase or chymotrypsin was significantly decreased. These results indicate that the interaction of Region II with chimpanzee erythrocytes is specific and sialic acid-dependent. Moreover, the observed enzymatic profile of Region II binding pointed at glycophorin D homolog, as a putative receptor for *P. reichenowi* EBA-140 ligand on chimpanzee erythrocytes.
We have obtained, for the first time, the soluble, functional recombinant *P. reichenowi* EBA-140 binding region and identified the putative receptor – glycophorin D on chimpanzee erythrocytes. Further ligand-binding studies will be performed to characterize the receptor site on glycophorin D, especially with respect to sialic acid residues on its oligosaccharide chains.

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P22.3

Analysis of the N-glycan profile of human seminal plasma by means of MALDI-TOF Mass Spectrometry

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The human seminal plasma N-glycan profile was analyzed in pooled samples by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and compared in fertile and infertile men. Infertile patients were divided into the following groups: normozoospermic (N), asthenozoospermic (A), oligozoospermic (O), and oligoasthenozoospermic (OA). Samples from healthy fertile volunteers were collected as a control group (C). The N-glycan structures were confirmed by MALDI-TOF/TOF tandem mass spectrometry. Eighty-six oligosaccharide structures were identified in all the analyzed samples. The presence of following oligosaccharide types were detected: high mannose and hybrid type, biantennary, triantenary and tetraantenary terminated with Lewis^x and Lewis^y. Only minor number of oligosaccharides contained terminal sialic acid. In all the samples biantennary glycans were dominant, reaching 65–75% of the total carbohydrate moieties. High mannose and hybrid type glycans comprised 15–28% of the whole N-glycome. Three- and tetraantennary glycans, comprising about 3% of the total carbohydrate moiety in the control, in the normozoospermic infertile sample reached over 9%, so their relative content was 3-fold elevated. Increased content of highly branched glycans was also observed in O, A and OA groups. The differences among the studied groups were observed when we focused on the content of highly sialylated and highly fucosylated oligosaccharides. Glycans with at least two residues of antennary fucose were increased in the infertile subjects except of the normozoospermic group. Sialylated glycans were observed to be increased in the normozoospermic infertile group and 2.5-fold decreased in the asthenozoospermic group when compared to the control sample originating from fertile men. The difference was even more spectacular when highly sialylated glycans were considered. The increase of these glycans in the N *vs.* the C sample was almost 2-fold higher. These oligosaccharides were completely absent in group A and decreased by about one third in groups O and OA. Seminal plasma is suggested to hold a reservoir of glyco-biomarkers that may be helpful in prediction of fertility potential of male patients.

P22.4

The role of UDP-galactose:ceramide galactosyltransferase (UGT8) and galactosylceramide (GalCer) in cellular stress response and multidrug-resistance of breast cancer cells

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Based on our previous studies on UGT8, enzyme that is responsible for synthesis of galactosylceramide (GalCer), we proposed that the presence of GalCer protects breast cancer cells from cellular stresses induced by tumor microenvironment and anticancer agents, and probably this cytoprotective effect is associated with increased resistance to stress-induced apoptosis. Therefore, the goals of this study were: (1) identification of cellular stressors associated with increased resistance of breast cancer cells to apoptosis, (2) elucidation of the role played by GalCer in multidrug-resistance of breast cancer cells, and (3) identification of signaling pathways responsible for increased apoptosis-resistance of GalCer-rich breast cancer cells. The following human breast cancer cells were used in this study: (MDA/LUC-shUGT8) with highly decreased expression of UGT8 and (T47D/PURO-UGT8) with overexpression of UGT8. Breast cancer cells were subjected to microenvironment stressors: hypoxia, metabolic acidosis, and free radicals or treated with chemotherapeutic agents: doxorubicin, paclitaxel, etoposide. It was found, based on flow cytometry using Caspase-3-GLOW™ assay, that expression of UGT8 and accumulation of GalCer in human breast cancer cells increased their resistance to apoptosis induced by hypoxia and free radicals as well as doxorubicin and paclitaxel. This was confirmed further by WST-1 assay as the viability of breast cancer cells correlated with GalCer level. The question remains as to the exact mechanism by which GalCer mediates cytoprotective effects during stress- and drug-induced apoptosis. To answer this question we analysed the intracellular concentration of ceramide, known as pro-apoptotic molecule, by HILIC-ESI-MS/MS. However, no difference in ceramide concentrations was found between cells synthesizing high and low amounts of GalCer. Therefore, expression of genes involved in apoptosis-related pathways was analyzed using RT^2 Profiler™ PCR Array Human Apoptosis assay and Real-Time PCR using self designed primers. The level of expression of selected genes was also analysed on the level of protein using Western blotting. Gene expression profiling revealed statistically significant down-regulation of TNFRSF1B, TNFRSF9 and up-regulation of BCL2 in breast cancer cell lines with high expression of UGT8 and GalCer. These data suggest that GalCer can affect the expression of apoptosis-related genes on the level of transcription.

P22.5

Bordetella pertussis oligosaccharide-pertussis toxin conjugate-specific antibodies. Identification of *Bordetella pertussis* LOS-derived oligosaccharide epitopes

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Bactericidal antibody response is essential for protection against *Bordetella pertussis* infection [1]. The whole-cell pertussis vaccine has been very effective but highly reactogenic. The contemporary subunit vaccines contain only a few selected antigens isolated from the bacterium. Recently, a waning of the acellular vaccine-induced immunity has been observed among the vaccinated people. It indicates, that these vaccines are lacking some important protective *B. pertussis* antigens.

We have demonstrated that immunization of rabbits with *B. pertussis* 186 lipooligosaccharide (LOS)-derived oligosaccharide conjugated with pertussis toxin (PT) yielded the *B. pertussis* LOS-specific antibodies [2]. More importantly, these anti-OS-PT antibodies are bactericidal in complement-mediated *in vitro* assay.

The reactivities of these antibodies with *B. pertussis* LOS were demonstrated by ELISA. Additionally the surface plasmon resonance (SPR) experiment indicated that the antibodies reacted with complete OS (dodecasaccharide) and its fragments (the nonasaccharide devoid of the terminal trisaccharide and the pentasaccharide comprising the trisaccharide). We have investigated the binding of these antibodies to *B. pertussis* oligosaccharide by the saturation transfer difference (STD)-NMR spectroscopy. Both 1D and 2D STD NMR spectra were collected and analyzed. The STD-TOCSY-NMR experiment provided more detailed information about the OS epitope structures. The interaction studies of *B. pertussis* OS with antibodies specific for OS-PT suggest that the immunodominant epitopes are located predominantly in its distal trisaccharide and other terminal residues of its core sugars. The residues are recognized and bind to the anti-*B. pertussis* OS-PT antibodies, therefore the oligosaccharide fragments, comprising these epitopes, coupled to a protein carrier could be an important component of the effective pertussis vaccine.

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Acknowledgements:

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P22.6

Application of lectin microarrays for the analysis of seminal plasma glycome

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Lectin microarray is an emerging, sensitive and high-throughput technique for glycomic profiling. The method exploits simultaneous reaction of the sample with multiple lectins of different sugar specificities, immobilized on the single glass slide or microplate well. Up to 50 commercially available lectins of plant, invertebrate, or fungal origin are usually applied. Such a matrix is submitted for a direct interaction with a biological sample, labeled with a fluorescent dye. Resulting array of signals for each sample enables multifactor bioinformatics analysis. Subtle differences in lectin specificity enable detection of even minor differences in oligosaccharide structures among the samples. Application of the lectin microarrays enables fast gaining of remarkable amount of data, comparing to conventional lectin-ELISA or blotting, not burdened with laborious isolation procedures necessary for mass spectrometry. We made an attempt to introduce this method for the analysis of seminal plasma glycome, in search of potential glyco-biomarkers of male infertility. Current WHO data estimate that problems with conception concern about 15–20% of couples in the industrial countries, moreover, specialists warn about its increasing tendency. Although problem affect both sexes equally, evaluation of male infertility remains limited so far. Andrologists underline currently the urgent need for a search of new biomarkers, helpful in prediction of male fertility potential. Recent years brought interesting data, suggesting that glycosylation within male reproductive tract may be involved in fertilization outcomes. For this study we have chosen eighteen lectins with binding preferences covering different types of glycans, gathered in groups specific for mannose, sialic acid, fucose, N-acetyl glucosamine and galactose/N-acetyl-galactosamine. The set of glycoproteins and neoglycoconjugates based on BSA linked mono- and oligosaccharides recognizable by the lectins was used for optimization of the method, thus lectin printing, ligand concentration and experiment conditions. Finally, we have analyzed the samples of seminal plasma of fertile and infertile men, comparing their lectin reactivity. Further study will be aimed on the analysis of the glycosylation profile of prostate specific antigen as well as the other seminal plasma glycoproteins, indicated as potential carriers of altered glycoepitopes in our former research.

P22.7

Analysis of IgG-derived, free N-glycans using LC-MS technique

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Human serum IgG has been recognized as a biomarker in several rheumatic and other diseases. Briefly, it was shown that IgG carbohydrate moiety is affected under these pathological conditions by lacking, in different degree, the terminal galactose residues in conservative N-glycans; the diminished number of Gal residues is proportional to the severity of a disease. Therefore, it is interesting to determine the status of galactosylation of these IgG N-glycans 1° in order to determine the current clinical status of the disease and 2° to know the efficacy of the clinical treatment applied. IgG N-glycans can be analyzed in three different ways: 1° in a native immunoglobulin molecule using chosen lectins (two methods are mostly used: ELISA test or biosensor BIAcore with SPR technique as a detection system), 2° as the glycopeptides (obtained after protease treatment of IgG) or 3° as free oligosaccharides, released from the native IgG molecules by enzymatic or chemical method. In this investigation we have applied the chemical method to release N-glycans. IgG was isolated from human serum using affinity chromatography on a Sepharose – protein A/G column; the purity of IgG preparations was controlled using SDS-PAGE. The purified IgG samples were subjected to strong alkaline degradation (1 M $NaBH_4$ in 1 M NaOH, 100°C, 5 hrs) and the released N-glycans, in a reduced form, were isolated from the reaction mixture and re-N-acetylated using the Carbograph minicolumns (GL Sciences, Tokyo). The samples of N-glycans were desalted using BioGel P-4 column, eluted with Milli Q water and then were subjected to LC-MS analysis. The HPLC system (Dionex), containing AXH-1 GlycanPac column (Thermo Scientific), was equipped with a mass spectrometer MicrOTOF-Q II (Bruker) and electrospray ionization (ESI) source as a detector. Based on identified molecular m/z ions 12 different structures of the N-glycans could be established. These were biantennary chains, partially sialylated, galactosylated and fucosylated, with or without bisecting GlcNAc residue. Performed experiments revealed that it is possible to determine the N-glycosylation profile of serum IgG using LC-MS technique – performed on free, reduced N-glycans, obtained from the native IgG molecules by a chemical method (strong alkaline degradation). The method is versatile and can be used in the case of any glycoprotein.

P22.8

Cancer cell lines as model for studying glycosylation changes upon re-differentiation

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One of the most interesting phenomenon 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 in case of pathologies such as cancer progression and metastasis. The question, that in many cases remains still open is if these specific changes are only an additional effect of the processes or are crucial to their proper regulation and going on.

One of the research models of this work consists of Caco-2 adenocarcinoma cells, which spontaneously differentiate into cells of enterocyte-like morphology after several days growing in confluence. We have studied the N-glycome of membrane and secreted proteins (HILIC-HPLC), together with the expression of selected glycosyltransferases (RT-PCR) before and after differentiation. Finally, we checked the impact of glycosylation inhibitors (swainsonine and kifunensine) on the differentiation process. The second group of experimets involves SH-SY5Y neuroblastoma cell line which is a useful *in vitro* model of neuronal differentiation after stimulation with all-*trans* retinoic acid. The cells were analysed in terms of N-glycosylation changes before and after induction of differentiation.

In the case of "enterocytic model" (Caco-2) the main modification of N-glycans seen after differentiation was trimming of high-mannose species. The RT-PCR analysis showed the up-regulation of MAN1A1 and MAN1C1 alpha 1,2 mannosidases. As this phenomenon can suggest a switch of the glycosylation machinery towards formation of complex glycans, we checked the expression level of selected Golgi glycosyltrasferases and observed the up-regulation of MGAT3, MGAT5 and ST6GAL1. After Golgi alpha 1,2 mannosidases inhibition by kifunensine, we observed the down-regulation of the activity of intestinal alkaline phosphatase *in vivo* but not *in vitro* during differentiation, which can suggest that complex N-glycans are important for brush border formation and/or transport of brush-boarder enzymes towards the apical membrane.

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P22.9

Lectin-based analysis of fucosylated glycovariants of human milk immunoglobulin G

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Human milk is a source of bioactive glycoconjugates such as free oligosaccharides, glycolipids and glycoproteins. The oligosaccharide chains present in human milk support the immature immune system of breastfed infants and are involved in protection against infectious pathogens. The sialylated and/or fucosylated glycotopes of IgG take part in a variety of biological events, such as recognition of bacterial adhesins, immunoregulation, inflammation progression, complement activation, opsonization, and mediation of antibody-dependent cellular cytotoxicity.

The aim of the present work was to study the IgG concentration and fucosylation pattern of IgG glycovariants in milk samples in relation to milk maturation stages, namely colostrum, transitional and mature milk. The relative amounts of fucosylated glycotopes on IgG from milk of mothers delivered at term were analyzed by modified lectin-IgG-ELISA using α1,2-, α1,3-, and α1,6-fucose specific biotinylated *Ulex europaeus* (UEA), *Lotus tetragonolobus* (LTA), and *Lens culinaris* (LCA) lectins, respectively.

IgG concentration was almost unchanged during human milk maturation. Milk IgG elicited qualitative and quantitative differences in the fucosylation pattern in comparison to plasma IgG of lactating mothers. The relative amount of α1,6-fucosylated glycotopes on milk IgG versus plasma IgG was significantly higher. In contrast to plasma IgG, the expression of α1,2- and α1,3-fucosylated glycotopes on milk IgG was observed. Moreover, the fucosylation pattern of milk IgG is lactation-stage related. The expression of UEA- and LTA-reactive glycotopes on milk IgG showed a weak negative and positive correlation, respectively, with the day of normal lactation from the 1st to the 55th, but no correlation was found with expression of the LCA-reactive glycotope.

The observed differences and increase in fucosylation level of milk IgG in contrast to maternal IgG may be related to local synthesis of milk IgG in the mammary gland. Similar to secretory IgA glycans, highly fucosylated glycotopes of milk IgG may be considered as an additional element of the innate immune system transferred to newborns during breastfeeding. Moreover, fucosylated glycans provide milk IgG with further bacteria-binding sites, and therefore IgG has the potential to serve as soluble decoys for fucose-dependent pathogens, preventing adhesion to epithelial cells. However, further structural and functional investigations are needed.

P22.10

Mass spectrometry analysis of immunogenic form of enterobacterial common antigen (ECA_{LPS}) isolated from rough strains of *E. coli*

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Enterobacterial common antigen (ECA) is a surface antigen expressed by Gram-negative bacteria belonging to the *Enterobacteriaceae* family, including emerging drug-resistant pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* spp. ECA is a heteropolysaccharide built of the trisaccharide repeating unit, →3)-α-d-Fuc*p*4NAc-(1→4)-β-d-Man*p*NAcA-(1→4)-α-d-Glc*p*NAc-(1→ [1] and occurs as a cyclic form (ECA_{CYC}), a phosphatidylglycerol-linked form (ECA_{PG}), and a lipopolysaccharide (LPS)-associated form (ECA_{LPS}) [2]. LPS is the main component of the outer membrane of the cell envelope of Gram-negative bacteria, that is also a main virulence factor. The smooth type LPS is builtof an O-specific polysaccharide and a core oligosaccharide linked to lipid A. Recent studies have identified the covalent linkage between LPS and ECA of *S. sonnei* phase II [3]. ECA_{LPS} is the only immunogenic form of ECA capable of eliciting cross-reactive anti-ECA antibodies.

Here we present results of MALDI-TOF mass spectrometry (MS) and ESI MS^n analysis of ECA_{LPS} isolated from *E. coli* R1, R2, and R4. Structural analysis excluded its presence in *E. coli* R3. The composition of the isolated fractions elucidated by MS indicated the presence of ECA_{LPS} fragments consisted of the core oligosaccharides of LPS substituted with different number of ECA repeating units. The results presented herein might be useful in further screening and analyses of ECA_{LPS} among other *Enterobacteriaceae*, especially synthetizing smooth forms of LPS.

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P22.11

UDP-galactose transporter deficiency affects morphology of numerous subcellular structures

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UDP-galactose transporter (UGT) is responsible for UDP-galactose transport across the Golgi and ER membranes into the lumen of these organelles, where this nucleotide sugar is subsequently utilized as a substrate for a series of galactosylation reactions. Both MDCK-RCA^r and CHO-Lec8 cell lines bear inactivating mutations within their UGT-encoding genes. Glycoconjugates synthesized by these cells are significantly enriched in terminal *N*-acetylglucosamine and *N*-acetylgalactosamine residues due to impaired galactosylation. This feature allows for easy discrimination between mutant and wild type cells with GlcNAc- or GalNAc-specific lectins. Although mutant cells generally resemble their wild type counterparts in terms of morphology, viability and growth rate, a detailed comparative analysis of their subcellular structures has never been conducted.

UGT-deficient cells were mixed with the wild type cells and several organelles were subsequently visualized using indirect immunofluorescence approach. Surprisingly, mutant cells displayed alterations in numerous subcellular structures including cytoskeleton, ER, cis/medial Golgi compartment, trans Golgi network, endosomes, lysosomes and mitochondria. In case of some subcellular structures their amount was decreased (e.g. vimentin intermediate filaments) or elevated (e.g. microfilaments). In contrast, for other organelles a substantial difference in subcellular distribution (e.g. endosomes, lysosomes, trans Golgi network) or overall structure (e.g. cis/medial Golgi compartment) could be observed.

In order to confirm that these alterations result entirely from a mutation within the UGT-encoding gene and not from other mutations that might have accidently arisen upon generation of MDCK-RCA^r and CHO-Lec8 cell lines the UGT-encoding gene was inactivated in HepG2 cells via CRISPR-Cas9 approach. UGT-deficient HepG2 clones displayed several features typical of MDCK-RCA^r or CHO-Lec8 cells (e.g. decrease in vimentin content) which confirms that the observed phenotypes were indeed UGT-related. Importantly, some of these changes cannot be explained simply by undergalactosylation of Golgi-synthesized macromolecules. We therefore suggest that the biological role of UGT is much more complex than previously thought. This is the first finding showing that a depletion of a nucleotide sugar transporter not only impairs glycosylation but also affects overall morphology of the cell.

P22.12

Lipidomic profiling of *Nocardia* by MALDI-TOF MS

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Gram-positive bacteria from *Nocardia* genus are ubiquitous soil bacteria. Some of them are non-pathogenic while other approximately 30 species are considered to be of medical importance. *Nocardia* are etiologic agents of rare disease nocardiosis that affects predominantly immunocompromised patients.

Laboratory diagnostics of *Nocardia* is difficult and laborious. The MALDI (matrix-assisted laser desorption/ionization) Biotyper system for bacterial identification has already been utilized in clinical microbiology laboratories as a successful clinical application of proteomics. However, in cases of *Nocardia*, identification is not always reliable on species level due to small representation of clinical strains of this genus in reference databases [1]. Therefore other alternative techniques leading to proper identification of etiological agent of infection are needed. One of them could be bacterial lipid profiling, of structural components of cell wall envelope.

The aim of these studies was identification by MALDI-TOF MS method of lipids components of *Nocardia* cells using Bligh-Dyer extract from dry cells or from colonies grown on solid medium.

Eight *Nocardia* species from Polish Collection of Microorganism were used in the studies. Lipid compounds present in Bligh-Dyer extracts were analyzed by TLC and MALDI-TOF mass spectrometry. TLC analysis of lipid extracts from *Nocardia* spp. have shown the presence of phospholipids (phosphatidylglycerol – PG, phosphatidylethanolamine – PE and phosphatidylinositol – PI), phosphatidylinositol mannosides – PIM and glycolipids. The same lipid classes were next identified in MALDI-TOF mass spectra. Lipid profile of *Nocardia* spp. on MALDI-TOF mass spectra in negative ion mode shows common phospholipid profile – mainly PE and DPG with different fatty acyl chain length and diacyl trehalose. Very similar mass spectra were obtained for extracts of dry cell mass and live cells, respectively. Lipid profile of *Nocardia* on MALDI-TOF MS spectra in positive ion mode were more diverse and more lipid classes were identified. Besides phospholipids, di- and triacylated glycerol were identified. Additionally PIM class were mono and diacylated and contained from one to 4 mannose residue. Almost all species possess characteristic lipid compound which was not present in others. The results are promising for future use of whole cells lipid profile for *Nocardia* identification.

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P22.13

The role of Alg13 *N*-acetylglucosaminyl transferase in *Candida albicans* pathogenicity

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Background: The pathogenic potential of *Candida albicans* depends on adhesion to the host cells mediated by highly glycosylated adhesins, hyphae formation and biofilm growth. These factors require effective *N*-glycosylation of proteins. Here, we present consequences of up- and down-regulation of the newly identified *ALG13* gene encoding *N*-acetylglucosaminyl transferase, a member of the Alg7p/Alg13p/Alg14p complex catalyzing the first two initial reactions in the *N*-glycosylation process.
Methods: We constructed *C. albicans* strain *alg13Δ::hisG/TR*p-*ALG13* with one allele of *ALG13* disrupted and the other under the control of a regulatable promoter, TRp. Gene expression and enzyme activity were measured using RTqPCR and radioactive substrate. Cell wall composition was estimated by HPLC DIONEX. Protein glycosylation status was analyzed by electrophoresis of hexaminidase, a model *N*-glycosylated protein in *C. albicans*.
Results*:* Both decreased and elevated expression of *ALG13* changed expression of all members of the complex and resulted in a decreased activity of Alg7p and Alg13p and under-glycosylation of hexaminidase. The *alg13* strain was also defective in hyphae formation and biofilm growth. These defects could result from altered expression of genes encoding adhesins and from changes in the carbohydrate content of the cell wall of the mutant.

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P22.14

Application of lectin array technology for Biobetter characterization: correlation with FcγRIII binding and ADCC

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Lectin microarray technology was applied to compare the glycosylation pattern of the monoclonal antibody MB311 expressed in SP2.0 cells and a glyco-optimized variant (MB314). MB314 was generated by a plant expression system that uses genetically modified moss protoplasts (Physcomitrella patens) to generate a de-fucosylated version of MB311. In contrast to MB311, no or very low interaction of MB314 with lectins AOL, PSA, LCA and AAL were observed. These lectins are specific for mono / biantennary N-glycans containing a core fucose residue. Importantly, this fucose indicative lectin binding pattern correlated with increased MB314 binding to FcγRIII (which affinity is mediated through core fucosylation) and stronger antibody-dependent cellular cytotoxic (ADCC) effector function demonstrating that lectin microarrays are useful orthogonal methods for antibody development and characterization.

P22.15

Reactivity of two monoclonal antibodies mAbs 12 and 39 against *Actinomyces odontolyticus* polysaccharide

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Actinomycosis is a rare subacute or chronic bacterial infection that causes both suppurative and granulomatous inflammation. Characteristics include localized swelling with suppuration, abscess formation, fibrosis and sinus drainage of pus containing sulphur granules. Actinomycosis is defined as a hard mass-type lesion with a specific histopathological structure [1]. The goal of the study was to determine the chemical composition of polysaccharide antigens extracted from *A. odontolyticus* and to generate monoclonal antibodies reactive with the polysaccharide, collectively aimed to understand the role of exopolysaccharides in pathogenicity.

Polysaccharides of *Actinomyces* spp. were extracted from dry bacterial cell mass by using trichloroacetic acid and enzymes (DNase, RNase and protease). The extracts were further purified by ion-exchange chromatography (DEAE Sephadex A25) and gel filtration (TOYOPEARL HW 55 S). Composition and structure of polysaccharide was determined by gas-liquid chromatography – mass spectrometry (GLC-MS). Monoclonal antibodies were generated by the hybridoma technique [2]. The ELISA method was carried out for evaluation the specificity of monoclonal antibodies to the polysaccharide antigen. Then quantitative immunoprecipitation test has been performed with polysaccharides from *Actinomyces* spp. and *Tsukamurella pulmonis*. Actinomycetal cells were grown on thioglycolate medium for 7 days at anaerobic atmosphere. Scanning electron microscopy showed slightly-curved rods of *A. odontolyticus* with clubbed cells. Polysaccharide isolated from *A. odontolyticus* consists of ribose, mannose, glucose and mannosamine. Two hybridomas, clones 12 and 39, producing mAbs against polysaccharide antigen were selected. Both mAb were of IgM class. Quantitative microimmunoprecipitation test showed that monoclonal antibody anti *Tsukamurella pulmonis* polysaccharide precipitated polysaccharide antigen of *A. odontolyticus*, *A. israelii* and *T. pulmonis*. Titration of antibodies 12 and 39 have shown that the epitope detected by them is present on other *Actionomyces* polysaccharides: *Actinomyces naeslundii*, *A. israelii* and *Tsukamurella pulmonis.*

Conclusion: These initial results show that *Actinomyces odontolyticus* share with other studied strains common structural motives recognized by monoclonal antibodies mAbs 12 and 39.

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P22.16

An insight into the orphan nucleotide sugar transporter – SLC35A5

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SLC35A5 (A5) has been classified in the SLC35A subfamily based on amino acid sequence homology. Most of the proteins belonging to the SLC35 family act as transporters of nucleotide sugars. In this study, the subcellular localization of endogenous SLC35A5 protein was determined via immunofluorescence staining. A5 occurs predominantly in the endoplasmic reticulum but is absent from the Golgi apparatus, unlike recombinant A5, which is found exclusively in the latter organelle. Moreover we observed that endogenous SLC35A5 is often found in large (i.e. up to 1 μm in diameter) cytoplasmic vesicles, with more peripheral distribution when compared with the perinuclear area occupied by the Golgi apparatus and endolysosomal system. Based on these findings, one may assume that a subset of endogenously expressed A5 resides in a vesicular subcellular compartment non-corresponding to the staining patterns obtained with the most commonly used organelle markers. It has been described in literature, that nucleotide sugar transporters form homooligomers. Moreover, it has been reported that SLC35A2 and SLC35A3 are in close proximity to each other. These findings encouraged us to explore putative interactions of SLC35A5 protein. *In vivo* analysis using the FLIM-FRET approach revealed that A5 forms homomers and interacts with all other members of the SLC35A subfamily.

To determine potential role of SLC35A5 protein in glycosylation the corresponding gene was knocked out in HepG2 cells using CRISPR-Cas approach. Independent clones were generated and their glycans were analyzed. We observed a significant increase of chondroitin 4-sulfate in A5-deficient cells. Further analysis has shown that generated mutants synthetize higher amount of mature tri- and tetraantennary N-glycans. A significant increase of high-mannose structures was also observed.

This is the first the first comprehensive report on putative nucleotide sugar transporter - SLC35A5. We determined its subcellular localization and analyzed its interactions with other members of SLC35A subfamily. Additionally, we confirmed that A5 is involved in the glycosylation process.

P22.17

D-galactan-III identification and occurence among lipopolysaccharides from *Klebsiella pneumoniae* serotype O1 and O2

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Klebsiella pneumoniae is a type of Gram-negative bacterium that can cause pneumonia, bacteremia and urinary tract infections mainly in individuals with impaired immune system. Carbapenem resistant *K. pneumoniae* is a serious threat to global public health due to limited treatment options [1]. One of the main virulence factors of *Klebsiella* is lipopolysaccharide (LPS, endotoxin). LPS is characteristic component of the outer membrane of Gram-negative bacteria and it is composed of lipid A, core oligosaccharide and O-specific polysaccharide, also known as an O-antigen. The heterogeneity of O-antigens determines O-serotypes. There are nine main O-antigens found in *K. pneumoniae,* of which O1 and O2 serotypes are responsible for over 50% of all *Klebsiella* infections [2].

O-antigens of both O1 and O2 serotype are made of homopolymers of galactose, described as galactans. The O2 serotype express →3)-α-D-Galp-(1→3)-β-D-Galf-(1→ repeating units described as D-galactan-I (gal-I). O1 antigen consists of gal-I subunits capped by →3)-β-D-Galp-(1→3)-α-D-Galp-(1→ repeating units, termed as D-galactan-II (gal-II). Structural analyses reveal the occurrence of gal-I backbone decorated by the terminal D-α-Gal*p*, described as D-galactan-III (gal-III) within both O1 and O2 serogroup. Conversion of gal-I to gal-III is encoded by the putative glycosyltransferase genes (*gmlABC*) identified as a distinct operon adjacent to the *rfb* (*wb*) locus encoding gal-I synthesis [3]. The nature of modification encoded by *gmlABC* in O1 and O2 O-specific polysaccharides was determined by ^{1}H and ^{13}C NMR spectroscopy. Analyses include O-antigens from O1 and O2 clinical isolates (*gml*+ and *gml*-) and trans-complemented mutants.

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P22.18

The role of sulfatides in breast cancer progression

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It was proposed that sulfatides present on the surface of cancer cells are ligands for P-selectin expressed on the surface of activated endothelial cells, and similarly to P-selectin expressing platelets and sulfatide-expressing leukocytes such interaction facilitate formation of aggregates, which in turn increases their metastatic potential (Tsuruo *et al*., 2008). More recently, sulfatide was detected as P-selectin ligand on murine colon carcinoma cells (Garcia *et al*., 2007). These studies revealed that adhesion *in vitro* of activated platelets expressing P-selectin to cancer cells is mediated solely trough sulfatides present on the latter. Such interactions were also observed *in vivo*, and cancer cell-platelet aggregates were found in lungs. Very little is known about the presence of sulfatides in breast cancer cells and their involvement in breast cancer progression. To reveal the role of sulfatide in breast cancer progression, human breast cancer MDA-MB-231 cells were transfected with 3-O-sulfotransferase cDNA to obtain cellular model representing gain-of-function phenotype, overexpressing sulfatide. It was found that sulfatide is involved in aggregation of cancer cells with platelets as a ligand for P-selectin. Importantly, sulfatide expressed by cancer cells increased P-selectin expression on the platelet surface causing additional platelet activation. On the other hand, it was found that presence of sulfatide in MDA-MB-231 cells decreased their resistance to apoptosis induced by doxorubicin or hypoxia, and *in vivo* study showed that sulfatide decreased the *tumorigenic potential* of *breast cancer* cells in nude mice model. Our data suggest that sulfatide present on the surface of breast cancer cells is a physiological counter-receptor for P-selectin present on the surface of platelets, what facilitates formation of aggregates by metastatic cells, and therefore their survival in the hostile environment of bloodstream. In contrast, increased expression of sulfatide in primary tumor contributes to the increased sensitivity of breast cancer cells to apoptosis,what resulted in the reduced survival of cancer cells in a hostile tumor microenvironment as we have shown by *in vivo* experiment. To explain these conflicting results, we propose that initially sulfatide is a limiting factor for breast cancer malignancy, but when cancer cells with expression of sulfatide are released from the primary lesion and enter the bloodstream, the presence of this glycolipid increases their metastatic potential.

P22.19

How glycosylation may influence glycosylation: N-glycans of the human alpha1,4-galactosyltransferase (Gb3/CD77 synthase) are crucial for its activity

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The human alpha1,4-galactosyltransferase (A4GALT, Gb3/CD77 synthase, P^k synthase) transfers galactose residue from UDP-Gal to glycosphingolipid acceptors, synthesizing 2 or 3 carbohydrate antigens of the P1PK blood group system, depending on position 211 in the polypeptide chain. We found that a single nucleotide substitution c.631C>G in the open reading frame of A4GALT, resulting in replacement of Gln by Glu at postion 211, broadens the acceptor specificity, enabling the enzyme to attach Gal not only to another Gal, but also to GalNAc. As the result, the consensus enzyme synthesizes P1 and P^k (Gb3, CD77) antigens, while its p.Q211E variant additionally synthesizes the NOR antigen. P^k is a common antigen present in nearly all human population, while P1 is found in approximately 75% of Caucasians and 20% of Asians. Presence or absence of P1 antigen determines the P_1 or P_2 blood group, respectively. The rare NOR antigen terminates with unusual Gal(alpha1-4)GalNAc disaccharide, never before found in mammals. We obtained a recombinant catalytic domain of A4GALT in High Five insect cells and we show that the enzyme is glycosylated itself. There are two potential N-glycosylation sites within the catalytic domain of the enzyme. Analysis of the oligosaccharides released from the protein after PNGase F treatment revealed alpha1,6-fucosylated paucimannose structures, typical for insect glycoproteins, which shows that at least one of the potential N-glycosylation sites is occupied. In addition, we observed that removal of these glycans causes complete loss of A4GALT activity. To the best of our knowledge, this is the first case wherein N-glycans of a glycosyltransferase are crucial for its activity. We also demonstrate that P_2 blood group erythrocytes may be turned into P_1 blood group cells by enzymatic treatment with the soluble recombinant enzyme.

P22.20

Structural masquerade of *Plesiomonas shigelloides* CNCTC 78/89 – de-O-acetylation of O-polysaccharide reveals the galactan I of *Klebsiella pneumoniae*

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Plesiomonas shigelloides is a facultative anaerobic Gram-negative flagellated, rod-shaped bacterium belonging the *Enterobacteriaceae* family. It is widely distributed in nature, but predominantly isolated from aquatic environments and animals. Human infections with *P. shigelloides* are generally related to visiting countries with low sanitary standards, drinking unpurified water and eating uncooked shellfish. These bacteria induce i.a. an invasive shigellosis-like disease, gastroenteritis and diarrheal disease. The pathogenicity of *P. shigelloides* is not entirely understood but lipopolysaccharide (LPS, endotoxin) is considered as the main virulence factor.

The high-resolution magic angle spinning nuclear magnetic resonance spectroscopy (HR-MAS NMR) analysis of bacteria and LPS revealed the characteristic structural patterns of the O-acetylated polysaccharide in the ^{1}H NMR spectrum.

The O-specific polysaccharide (O-PS) of *P. shigelloides* 78/89 was isolated from LPS and then investigated by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and chemical methods. The analyses suggested that the *P. shigelloides 78/89*O-PS is composed of the disaccharide →3)-β-D-Gal*f*2OAc-(1→3)-α-D-Gal*p*-(1→repeating units. The O-acetylation is not complete and leads to the O-acetylation-related heterogeneity. The ^{1}H NMR spectrum of a non-O-acetylated glycoform of *P. shigelloides* PS showed the profile identical to this identified previously in HR-MAS NMR spectrum acquired using the whole-bacteria of *K. pneumoniae*strain Kp20, expressing the pure form of D-galactan I as a constituent of lipopolysaccharide (LPS). The observation confirmed that *P. shigelloides* 78/89 strain shares O-antigenic epitopes with these *K. pneumoniae* strain Kp20. This "identity-in-disguise" is an example of interspecies structural similarity of bacterial O-antigen epitopes that can lead to serological cross-reactivities. The type-specific reactions among remotely related bacteria are of special interest, particularlly in case of the antibiotic-resistant pathogens such as *Klebsiella*.

P22.21

N-glycosylation of serum proteins in Hashimoto thyroiditis

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Introduction: Hashimoto's thyroiditis (HT) is one of the autoimmune thyroid disease (AITD), the group of disorders associated with loss of immune tolerance to thyroid autoantigens, which leads to destruction of thyroid tissue by infiltrated cytotoxic T cells. Our studies were designed to answer the question whether glycosylation of serum proteins is disturbed in HT pathology.
Material and methods: The research material was serum proteins from HT patients and healthy individuals. Glycoproteins were depleted in IgG and digested using N-glycosidase F to release N-glycans. N-glycans was fluorescently labeled and analyzed using high performance liquid chromatography (HPLC). Obtained chromatograms were manually separated into 31 peaks. The percentage of total integrated area (% area) of each peak corresponds to the amount of glycan.
Results: The chromatographic analysis demonstrated repeated pattern of chromatograms for case and control samples. Statistical analysis for 19 HT and 20 C samples showed significant (p0.05) decrease of peak 23 intensity in HT group. Eight possible oligosaccharides were identified for this peak based on GlycoBase3.2. Among the assigned structures are mainly fucosylated (core or terminal) and sialylated di- and triantennary complex type N-glycans. One of the identified N-glycans is high-mannose structure.
We observed also changes in the intensity of peaks No. 20 and 25. for some HT and control samples, but not statistically significant between the groups. Two sialylated diantennary complex type N-glycans with bisecting GlcNAc were assigned to peak No. 20, which intensity increased in HT. Twelve structures were identified for peak No. 25 with decreased intensity in HT, most of them were diantennary sialylated complex-type N-glycans with bisecting GlcNAc. Six of the assigned glycans may have a core fucose.
Conclusion: Altered N-glycosylation of serum proteins may be important in autoimmune reactions against thyroid gland.

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P22.22

Determination of lysosomal exoglycosidases in hemolyzed blood

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Objective: Our goal was to provide information about possibility and conditions of the determination of α-fucosidase (FUC), β-galactosidase (GAL) and β-glucuronidase (GLU) in serum of hemolysed blood.Introduction: Determination of lysosomal exoglycosidases based on quantification at 410 nm released 4-nitrophenol from appropriate artificial substrates is seriously impaired by hemoglobin released during hemolysis. Hemoglobin released from erythrocytes absorbs light at 415, 540 and 570 nm. In recently published paper [1] we described method of determination of N-acetyl-β-hexosaminidase, the most active of exoglycosidases, in human serum of hemolysed blood.Methods: Serums without and with hemolysis were incubated with 4-nitrophenol derivatives of appropriate sugars as substrates. Released 4-nitrophenol was determined colorimetrically. After the incubation of serums from hemolyzed blood with substrates, hemoglobin was precipitated with trichloroacetic acid (TCA) before 4-nitrophenol determination.Results: Maximum activity in hemolyzed & non-hemolysed blood was observed at pH 4.3 in a citrate-phosphate buffer for FUC & GAL and at pH 4.5 in an acetate buffer for GLU. Km and incubation time for FUC, GAL and GLU was also very similar in hemolysed and non hemolysed blood.Conclusion: The modified method is appropriate for FUC, GAL and GLU determination in serum from hemolyzed blood.

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