INVITATION to hybrid - virtual and on-site meeting :

Advances in biology and chemistry of bacterial surface polysaccharide

Date - **April 28 2022**,

Agenda (times are in CET)

9.00 - 9.15, Welcome – Representative of Jan Kochanowski University, Kielce Poland.

Chair – Antoni Rozalski, University of Lodz, Lodz, Poland

Speakers

Keynote Lecture: 9.15 – 10.00

The sweet language of cells - a chemical perspective

Till Opatz , University of Mainz, Mainz, Germany

10.00 -10.45

Mycobacterial envelope fortifications: structure and function.

Jakub Pawelczyk, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

10.45 – 11.30

The melibiose-derived glycation product mimics a unique epitope present in human and animal tissues.

Andrzej Gamian, Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland

11.30 -12.15

Multifaceted *Proteus* polysaccharides – serology, structure, and biological role.

Dominika Drzewiecka, University of Lodz, Lodz, Poland

12.15 – 13.15 Coffee break

13.15 – 14.00

Interaction of factors specific for complement lectin pathway with microbial surface structures.

Maciej Cedzynski, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland .

14.00 – 14.45

Past and future endotoxin studies of *Proteus mirabilis* (03) 1959 strain.

Wieslaw Kaca, Jan Kochanowski University, Kielce , Poland

14.45 – 15.00 - Concluding Remarks – Wieslaw Kaca,

==========

Registration for on-line meeting only on ZOOM platform closes April 25, 2022 and is free. Contact – Dr Paulina Zarnowiec, e-mail: paulina.zarnowiec@ujk.edu.pl, phone: +48 41 349 6120

Venue: Jan Kochanowski University , Uniwersytecka 7, aula CB4, Pl 25-406 Kielce, Poland.

ABSTRACTS

The Sweet Language of Cells – A Chemical Perspective

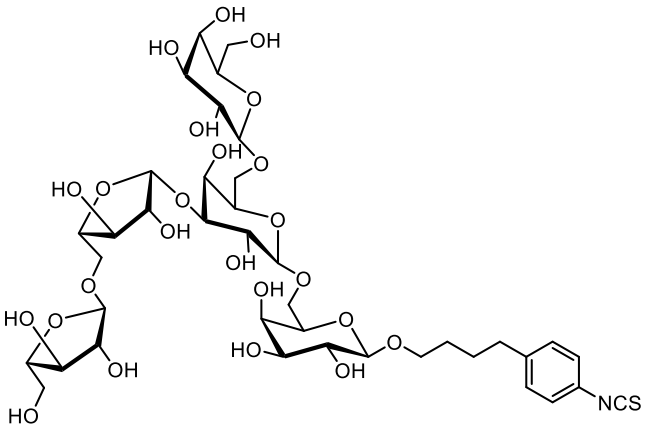
Till Opatz*, Matthias Krumb, Jens Langhanki, Robert Forster

Department of Chemistry, Johannes Gutenberg University, Mainz, Germany

Keywords: carbohydrates, cellular communication, neoglycoconjugates

Carbohydrates are key components of intercellular communication and not only play an important role in the immune system but also allow the selective targeting of certain cell types.

Examples for structural carbohydrates, the synthesis of a neoglycoconjugate vaccine for allergy prevention[1] and for the cell targeting of therapeutic nanocapsules and liposomes[2,3] using carbohydrates will be demonstrated and discussed.



Acknowledgements. Studies were supported by the Deutsche Forschungsgemeinschaft and the German Federal Ministry of Education and Research.

1. M. Krumb, M. Jäger, A. Voss, L. Immig, K. Peters, D. Kowalczyk, A. Bufe, T. Opatz, O. Holst, C. Vogel, M. Peters**,** *Chem. Eur. J.*, **2021**, *27*, 928. https://chemistry-europe.onlinelibrary.wiley.com/doi/abs/10.1002/chem.202002287
2. K. Wagener, M. Bros, M. Krumb, J. Langhanki, S. Pektor, M. Worm, M. Schinnerer, E. Montermann, M. Miederer, H. Frey, T. Opatz, F. Rösch, *Adv. Therap.*, **2020**, 1900185. https://onlinelibrary.wiley.com/doi/10.1002/adtp.201900185
3. M. Krumb, M. L. Frey, J. Langhanki, R. Forster, D. Kowalczyk, V. Mailänder, K. Landfester, T. Opatz, *Cells*, **2020**, 2087. https://www.mdpi.com/2073-4409/9/9/2087

Mycobacterial envelope fortifications: structure and function.

Laeticia Alibaud[1], **Jakub Pawełczyk[2],** Laila Gannoun-Zaki[1], Vipul K. Singh[1], Yoann Rombouts[3], Michel Drancourt[4], Izabela Szulc-Kielbik[2], Michal Kielbik[2], Yann Guérardel[3], Laurent Kremer[1], Magdalena Klink[2], and Jaroslaw Dziadek[2]

[1]Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, Université de Montpellier, France, [2]Institute for Medical Biology, Polish Academy of Sciences, Lodz, Poland, [3]Université de Lille 1, Unité de Glycobiologie Structurale et Fonctionnelle, France, UMR 8576, 59650 Villeneuve d'Ascq, France, [4]Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UMR CNRS 7278, IRD 198, INSERM 1095, Faculté de Médecine, Marseille, France

Keywords: *Mycobacterium tuberculosis*, *Mycobacterium marinum*, Lipooligosaccharides, THP-1-derived macrophages, TLR2, Infection

The mycobacterial cell envelope is essential for *Mycobacterium tuberculosis* growth and survival. It is a highly impermeable barrier thereby providing protection from many antibiotics, and also allows the pathogen to proliferate within macrophages and persist for extended periods in the infected host. It comprises mainly two types of lipids: (i) the very long-chain fatty acids, mycolic acids, covalently attached to the arabinogalactan/peptidoglycan backbone, and (ii) a vast panoply of structurally diverse, extractable compounds among which glycolipids are the first-line molecules involved in host-pathogen interactions. Lipooligosaccharides (LOSs) are cell surface-exposed glycolipids capable of modulating the host immune system, suggesting that they may be involved in the early interactions of tubercle bacilli with macrophages. We addressed whether LOS composition affects the uptake of *M. marinum* - a *surrogate* model for tuberculosis-like infection by professional phagocytes. Mutants with various truncated LOS variants were generated, leading to the identification of several previously uncharacterized biosynthetic genes (*wbbL2*, *MMAR_2321*, and *MMAR_2331*). Biochemical and structural approaches allowed resolving the structures of LOS precursors accumulating in this set of mutants. These strains with structurally defined LOS profiles were then used to infect both macrophages and *Acanthamoebae*. We provide the first evidence that LOSs inhibit the interaction between mycobacterial cell wall ligands and appropriate macrophage pattern recognition receptors, affecting uptake and elimination of the bacteria by host phagocytes.

The melibiose-derived glycation product mimics a unique epitope present in human and animal tissues

Andrzej Gamian

Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences

Glycation is a non-enzymatic process of binding of carbohydrates and other aldehydes with amino groups of proteins. Recently, we have reported on the synthetic melibiose-derived glycation product (MAGE) that mimics a unique epitope present in human and animal tissues [1]. In contrast to known classical advanced glycation end-products, the MAGEs are synthesized in anhydrous conditions. The generated mouse anti-MAGE monoclonal antibody recognized the native analogous product in living organisms of novel and unique structure, called AGE10. The MAGE cross-reactive autoantibodies were detected in patients with diabetes [1]. Analysis of serum of patients with Alzheimer's disease revealed that the level of AGE10 is lower than in healthy people, while immune complexes are in higher levels. Immunohistochemical pattern of muscle of diabetic patient contains more AGE10 epitope than muscle of healthy person at physiological level. Our other study revealed that tissue damaged with dioxin is recognized with MoAb anti-MAGE indicating that AGE10 is a marker of damaged tissue. There is no correlation of AGE10 with glucose and HbA1c levels, but there is positive correlation with glomerular filtration coefficient (eGFR) as revealed in diabetic patients with microangiopathy. In diabetic patients AGE10 was significantly higher in patients with microangiopathy, in whom was lower in patients treated with aspirin, thus AGE10 might be considered as a marker of metabolic diseases complications (obesity). In patients with allergic rhinitis and chronic Epstein-Barr virus infection at different stages of virus persistence (active and latent phase) AGE10 is lower while complexes are at higher levels. Possible participation of intestinal microbiome in formation of AGE10 will be discussed.

1. Staniszewska, M.; Bronowicka-Szydełko, A.; Gostomska-Pampuch, K.; Szkudlarek, J.; Bartyś, A.; Bieg, T.; Gamian, E.; Kochman, A.; Picur, B.; Pietkiewicz, J.; Kuropka, P.; Szeja, W.; Wiśniewski, J.; Ziółkowski, P.; Gamian, A. The melibiose-derived glycation product mimics a unique epitope present in human and animal tissues. Sci. Rep. 2021 doi:10.1038/s41598-021-82585-7.

Multifaceted *Proteus* polysaccharides – serology, structure, and biological role

Dominika Drzewiecka, Magdalena Moryl, Agata Palusiak, Małgorzata Siwińska, Agnieszka Torzewska, Agnieszka Zabłotni, Antoni Różalski

Department of Biology of Bacteria, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Poland

Keywords: biofilm, lipopolysaccharide, O serogroup, *Proteus*, urinary stones, vaccine

The lecture presents a progress in the studies carried in the Department of Biology of Bacteria, University of Lodz, Poland, on the polysaccharides produced by the bacteria belonging to the genus *Proteus*. These bacilli are widespread among animals and found in natural environments, but are also known as being human opportunistic pathogens. Lipopolysaccharide (LPS) and other surface polysaccharides are important virulence factors of the bacteria. On the basis of LPS serological studies, we have classified hundreds of clinical *Proteus* strains isolated from patients in central Poland to proper O serogroups. Moreover, we have indicated the serogroups prevalent among Polish patients and recognized several new O serogroups or subgroups, extending the previously existing serological classification scheme. Molecular bases of the serological differentiation of *Proteus* strains were also determined. Some serological similarities between *Proteus* and *Klebsiella* O antigens have been found, thus giving the possibility to employ them in future vaccines which are needed, since both genera play an important role in urinary tract pathogenesis. The influence of *Proteus* lipopolysaccharides in crystal formation and their aggregation in the urinary stones development was indicated, which is depending on the LPS concentration and chemical structure. Furthermore, it was demonstrated that planktonic or biofilm lifestyle may affect *Proteus* LPS length and structure. The studies on *Proteus* biofilm allowed to identify some factors significantly increasing the production of exopolysaccharides composing a biofilm matrix.

Interaction of factors specific for complement lectin pathway with microbial surface structures

Maciej Cedzyński[1], Jolanta Łukasiewicz[2], Katarzyna Kasperkiewicz[3], Gabriela Gajek[1], Aleksandra Man-Kupisińska[2], Anna Brzostek[4], Anna Maciejewska[2], Agnieszka Szala-Poździej[1], Czesław Ługowski[2], Jarosław Dziadek[4], Anna S. Świerzko[1]

[1]Laboratory of Immunobiology of Infections, Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland; [2]Department of Immunochemistry, L. Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, Poland; [3]Institute of Biology, Biotechnology and Environmental Protection, University of Silesia, Katowice, Poland; [4]Laboratory of Mycobacterium Genetics and Physiology, Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland

Keywords: complement, lectin pathway, collectin, ficolin, lipopolysaccharide, lipoarabinomannan

The lectin pathway of complement system (LP) is considered important branch of the immune response. The LP-specific pattern-recognising molecules (PRM) are some collectins (mannose-binding lectin, collectin-10, collectin-11) and ficolins (ficolin-1, ficolin-2, ficolin-3), forming complexes with proteins of the MASP family (MASP-1, MASP-2, MASP-3, MAp19, MAp44). As the lectins, they recognise variety of glycoconjugates exposed on the surface of bacteria, fungi, viruses, parasites or altered (apoptotic/necrotic/cancer) host's cells. Their ligands are pathogen-associated molecular patterns (PAMP) or danger-associated molecular patterns (DAMP). Recognition results in opsonisation what facilitates phagocytosis and initiation of complement activation by MASP, leading to further opsonisation with activated factors and/or direct lysis of target cells.

We present a review of our data concerning recognition of microbial antigens by collectins and ficolins, its molecular basis and biological consequences. Interactions of the mentioned PRM with bacterial cells, lipopolysaccharide (LPS) core oligosaccharides (*Proteus vulgaris*, *Hafnia alvei*, and *Yersinia enterocolitica*) and O-specific polysaccharides (*Hafnia alvei*), exopolysaccharide (*Escherichia coli*) as well as mycobacterial lipoarabinomannan (ManLAM) and antigen 85 (Ag85) complex will be discussed. Furthermore, we will demonstrate reactivity of collectins and ficolins with some clinical strains, isolated from patients suffering from hospital infections.

Acknowledgements. Studies were partially supported by National Science Center (Poland), grants 2015/17/B/NZ6/04250 and 2018/31/B/NZ6/03514

Past and future endotoxin studies of *Proteus mirabilis* (03) 1959 strain

Wieslaw Kaca, Dawid Gmiter, Katarzyna Durlik-Popinska Paulina Zarnowiec and Grzegorz Czerwonka

Department of Microbiology and Parasitology, Institute Biology of Jan Kochanowski University, Uniwersytecka 7, Pl 25-406 Kielce, Poland.

Smooth *Proteus mirabilis* and its rough Ra (strain 110) and Re type (strain R45) are one of most often studied *Proteus sp*. representatives. *P. mirabilis* (O3) S959 cells produce strain-specific biofilm preventing action of urease inhibitor. Investigations of synthetic flap fragments of urease indicate on its role on molecular mimicry on rheumatoid arthritis (RA) diseases. Based on phenotypic and bioinformatic analysis (O3) S1959 strain belongs to group A Dines type, with reduced domination features – DFI -0.55.

Composition of LPS is typical for *Proteus sp*. (O3)1959 O-specific O-polysaccharide of lipopolysaccharide is build up from Lysine linked to GalA and GlcNAc residues. Serological specificity of (3) S1959 LPS were presented by immnochemical methods, including eliposometry and AFM. Series of experiments biological activities of P. mirabilis (O3) S959 LPS of were performed. It was shown that (O3) S1959 LPS enhance toxic hemoglobin activities by detergent -like action. Complement activation were induced by different part of LPS (O3) 1959. Phage "otto' depolymerases were assed and role of free amino group of Lysine were confirmed. Molecular mimicry to collagens that may induce of inflammatory reaction were presented by anti-(O3) LPS 1959 antibodies from RA patients sera. Perspective of future studies of *P.mirabilis* (O3) S959 strain and its LPS will be presented.

Studies were partially supported by grand UJK 607 (WK), Preludium 17 NCN (DG), Preludium 18 NCN (K D-P).