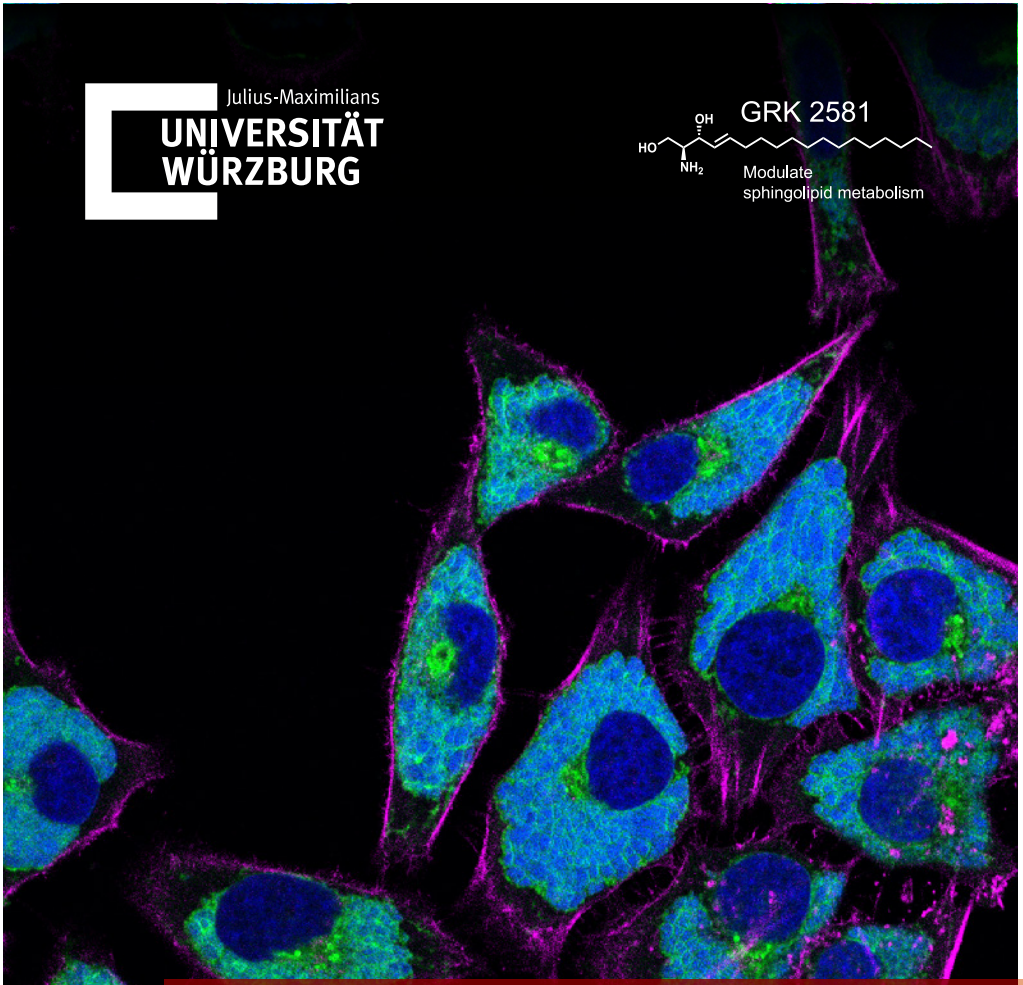
Julius-Maximilians
UNIVERSITÄT
WÜRZBURG

GRK 2581

Modulate
sphingolipid metabolism



International Symposium on

SPHINGOLIPIDS IN INFECTION 2022

Speaker Overview

20 – 22 September 2022 | Würzburg Germany

Tuesday, 20th September 2022

12:00 – 13:00	Registration & Standing Lunch	Gartenpavillon
13:00 – 13:20	Welcome & Introduction	Room „Würzburger Stein“
13:20 – 13:40	Introduction GRK2581	Room „Würzburger Stein“
13:45 – 14:05	*Young Investigator* **Agata Prell** Target and off-target effects of sphingosine kinase inhibitors on the sphingolipidome in different cell lines	Room „Würzburger Stein“
14:10 – 14:30	*Young Investigator* **Ingo Fohmann** Neisseria meningitidis induces SphK1 activation and production of S1P in brain endothelial cells	Room „Würzburger Stein“
14:30 – 14:50	Break	Gartenpavillon
14:55 – 15:25	*Key Note* **Prof. Frances Platt** The Niemann-Pick disease type C lysosomal pathway: an unexpected infectious disease hub	Room „Würzburger Stein“
15:30 – 16:00	*Key Note* **Prof. Tony Futerman** The sphingolipid anteome: implications for evolution of the sphingolipid metabolic pathway	Room „Würzburger Stein“
16:05 – 16:25	*Young Investigator* **Dr. Caroline Barisch** Role of sphingolipids in mycobacterial infections	Room „Würzburger Stein“
16:30 – 17:00	*Key Note* **Prof. Wiebke Herzog**	Room „Würzburger Stein“
17:05 – 17:35	Poster Session Intro	Room „Würzburger Stein“
17:40 – 17:55	Conclusion & Outlook	Room „Würzburger Stein“
18:00 – 21:00	Dinner with Poster Session (+ election best poster)	Gartenpavillon

Wednesday, 21st September 2022

09:00 – 09:15	Introduction	Room „Würzburger Stein“
09:15 – 09:45	*Key Note* **Prof. Josef Pfeilschifter** The role of sphingolipid kinases in renal fibrosis	Room „Würzburger Stein“
09:50 – 10:15	*Young Investigator* **Romana Scheffel** Deciphering the role of sphingosine-1 phosphate on blood-brain barrier function	Room „Würzburger Stein“
10:15 – 10:35	Break	Gartenpavillon
10:40 – 11:00	*Young Investigator* **Enrico Garbe** Systematic metabolic profiling identifies de novo sphingolipid synthesis as hypha-associated and essential for Candida albicans filamentation	Room „Würzburger Stein“
11:05 – 11:25	*Young Investigator* **Maria Batliner** The Quorum-Sensing Molecule Farnesol Inhibits Dihydroceramide Desatruase in Dendritic Cells	Room „Würzburger Stein“
11:30 – 11:50	*Young Investigator* **Marcel Rühling** Potential roles of sphingolipids during intracellular Staphylococcus aureus infection	Room „Würzburger Stein“
12:00 – 13:30	Lunch	Gartenpavillon
13:40 – 14:10	*Key Note* **Prof. Sarah Spiegel** My journey with Sphingosine-1-Phosphate: from insipid lipid to a key regulator	Room „Würzburger Stein“
15:00 – 16:30	*Key Note in honour of Prof. Sibylle Schneider-Schaulies* **Prof. Erich Gulbins** Sphingolipids in viral and bacterial infections	Toscanasaal
16:40 – 18:00	Guided tour of the UNESCO world heritage site Residence	Residence (meeting point at the museum entrance desk)
18:00 – 21:30	Residenz cellar with dinner and best abstract poster award	Residence cellar

Thursday, 22nd September 2022

09:00 – 09:30	*Key Note* **Dr. Fikadu Tafesse** The roles of lipids in bacterial and viral infection	Room „Würzburger Stein“
09:35 – 09:55	*Young Investigator* **Trushnal Waghmare** Role of Neutral Sphingomyelinase 2 and Sialophorin as virus effector in T cells	Room „Würzburger Stein“
10:00 – 10:20	Introduction Workshop Session	Room „Würzburger Stein“
10:25 – 11:40	Workshop Session and presentation outcome	Room „Würzburger Stein“ & „Iphöfer Julius-Echter-Berg“ & Gartenpavillon
11:45 – 12:05	*Young Investigator* **Jonas Weinrich** Role of Sphingolipids in Simkania negevensis Infection	Room „Würzburger Stein“
12:10 – 12:30	*Young Investigator* **Louise Kersting** Fluoxetine derivatives inhibit acid ceramidase activity and cause lysosomal trapping of SARS-CoV-2	Room „Würzburger Stein“
12:35 – 12:50	Conclusion	Room „Würzburger Stein“
13:00 – 14:00	End and Lunch Packs	Gartenpavillon

Juliusspital
WEINGUT. WÜRZBURG.

LAGEPLAN STIFTUNGSGELÄNDE

WER? WO? WAS?

WEINGUT: Das Büro für Großhandel, die Verwaltung und das Tagungszentrum finden Sie in der Zehntscheune, Klinikstraße 1.
PKW-Zufahrt nur über Koellikerstraße 4 (Navi) zum öffentlichen Parkhaus Juliusspital.

VINOTHEK: Die Vinothek befindet sich außerhalb des Geländes in der Koellikerstraße 1a.
Bitte nutzen Sie die öffentlichen Parkhäuser Juliusspital oder Parkhaus Pleich (Parkleitsystem).
Eine Ladezone zum kurzzeitigen Parken ist vorhanden.
Öffnungszeiten: Montag – Freitag von 9.30 bis 18.30 Uhr, Samstag 9.00 bis 16.00 Uhr

TREFFPUNKT WEINPROBEN UND FÜHRUNGEN: Der Treffpunkt sowohl für alle Führungen und Weinproben ist der Figurenbrunnen im Park des Juliusspitals. Dieser liegt zentral in der Mitte des Stiftungsgeländes. Der Zugang ist über Klinikstraße, Juliuspromenade und Koellikerstraße möglich.
Das ausführliche Angebot finden Sie auf der Homepage unter „Besuchen".

VERANSTALTUNGEN, TAGEN UND FEIERN: Zugang zum Gelände erhalten Sie über die Klinikstraße und die Juliuspromenade, Zufahrt jedoch nur über das Parkhaus in der Koellikerstraße.
Alle Veranstaltungen finden in der Regel in der Zehntscheune (1. Obergeschoss/ Eingang links) oder im Gartenpavillon statt.
Bitte achten Sie auch auf die ausgewiesenen Treffpunkte auf Ihren Einladungen oder Eintrittskarten etc.

RESTAURANT: Im gemütlichen Ambiente der Weinstuben Juliusspital erwartet Sie fränkische Gastlichkeit und regionale Kulinarik. Treten Sie ein und entdecken Sie die Vielfalt an frisch zubereiteten Gerichten in Verbindung mit unseren speziell ausgewählten Weinen von den Toplagen Frankens.
Telefonische Reservierung (empfehlenswert) über die Rufnummer 0931/54080.
Ihre Gastgeber, Familie Kulinna, freuen sich Ihnen abwechslungsreiche Gaumengenüsse zu servieren.
Unser Tipp: Die Fisch- und Wildspezialitäten sind mehrfach ausgezeichnet.

ANFAHRT

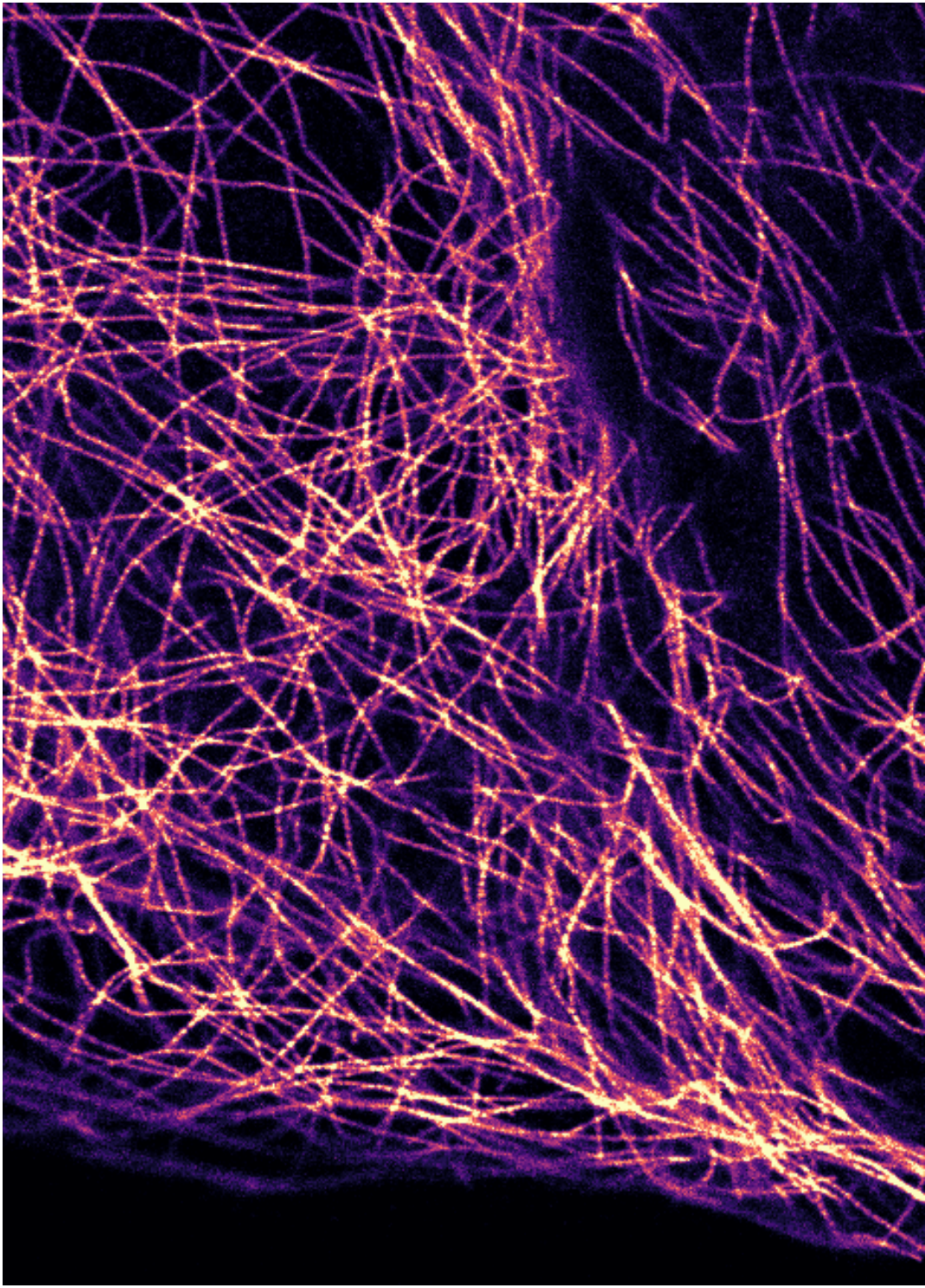
MIT DEM PKW: Zufahrt nur über Koellikerstraße 4 möglich. Bitte orientieren Sie sich an der Ausfahrt Stadtmitte Würzburg/Hauptbahnhof und geben Sie in das Navi unbedingt Koellikerstraße 4 ein. Über die Krankenhauseinfahrt gelangen Sie in das öffentliche Parkhaus Juliusspital (Parkleitsystem) bzw. zur Warenausgabe.

MIT DER BAHN: Ankunft Würzburg Hauptbahnhof (ICE-Bahnhof)
Richtung Innenstadt (Straßenbahnschienen folgen). Wegstrecke ca. 1 km.
Biegen Sie die erste schmale Straße nach rechts und nächste Gabelung links ab. Nach nur ca. 200 m erreichen Sie die Zehntscheune, Klinikstraße 1. Sie befinden sich mitten auf dem Gelände des Juliusspitals.

ZU FUSS: Das Juliusspital liegt im Zentrum von Würzburg. Viele öffentliche Verkehrsmittel bringen Sie zur Haltestelle – Juliuspromenade. Vom Zentrum kommend nutzen Sie den Haupteingang Juliuspromenade 19 und folgen Sie der Ausschilderung zur Zehntscheune / Gartenpavillon.

Juliusspital Weingut Würzburg
Klinikstraße 1, 97070 Würzburg
Telefon +49 (0) 931 / 3 93-1400, Fax +49 (0) 931 / 3 93-1414
weingut@juliusspital.de, www.juliusspital-weingut.de

19 10
VDP. DIE PRÄDIKATSWEINGÜTER
FRANKEN



KEY NOTE SPEAKER

PROF. FRANCES PLATT

University of Oxford,
Oxford, UK

Frances M. Platt is Professor of Biochemistry and Pharmacology and Head of the Department of Pharmacology, University of Oxford, UK.

She received her Ph.D. in Animal Physiology from the University of Bath, UK. After completing postdoctoral training at Washington University Medical School, St. Louis, she joined the faculty at the University of Oxford and was the recipient of a five-year Lister Institute Senior Research Fellowship. Her expertise relates to glycosphingolipids (GSL) and in particular glycosphingolipid (GSL) lysosomal storage diseases. She and her colleagues pioneered a novel approach to treat these inherited diseases that has led to the development of an approved drug (miglustat) for type 1 Gaucher disease and Niemann-Pick disease type C1 disease. She was awarded the Alan Gordon Memorial award from the UK Gaucher Association, the Horst-Bickel Award in recognition of her role in developing substrate reduction therapy for lysosomal disorders and the “Above and Beyond” award from National Tay-Sachs and Allied Diseases USA.

She has published extensively in this field over the past 20 years and co-edited a book titled Lysosomal Disorders of the Brain. She was an Editor for the Journal of Biological Chemistry (2009-2014) and serves on the advisory board of multiple lysosomal storage disease charities and organizations (UK and USA).

She was elected Fellow of the Academy of Medical Sciences in 2011 and Fellow of the Royal Society in 2021.

TUESDAY, SEPTEMBER 20, 2022 AT 14:55 HRS

THE NIEMANN-PICK DISEASE TYPE C LYSOSOMAL PATHWAY: AN UNEXPECTED INFECTIOUS DISEASE HUB

Mycobacterium tuberculosis (Mtb) survives and replicates within host macrophages (M) bit the underlying mechanism are incompletely understood. We previously found that lipids shed by persistent mycobacteria unexpectedly inhibit NPC1, the lysosomal membrane protein deficient in most cases of the rare neurodegenerative lysosomal storage disorder Niemann-Pick disease type C (NPC). Inhibition of NPC1 leads to a drop in lysosomal calcium levels, blocking phagosome-lysosome fusion leading to mycobacterial survival. The production of specific cell wall lipid(s) that inhibit NPC1 could therefore have been a key step in the evolution of intracellular persistence. We therefore analysed lipid extracts from clinical ***Mtb*** strains from multiple ***Mtb*** lineages, ***Mtb*** complex members and non-tubercular mycobacteria to determine if they all inhibit the NPC pathway. We found evidence of NPC1 inhibitory lipids in all clinical isolates from ***Mtb*** lineages 1, 2, 3 and 4, ***Mycobacterium bovis*** and the non-tubercular mycobacteria, ***Mycobacterium abscessus*** and ***Mycobacterium avium***. Interestingly, lipids from ***Mycobacterium canettii***, which resembles the common ancestor of the ***Mtb*** complex members, did not inhibit the NPC1 pathway. Therefore the evolution of NPC1 inhibitory mycobacterial cell wall lipids evolved early and post divergence from ***Mycobacterium canettii***-related mycobacteria and that this activity contributes significantly to intracellular persistence and the promotion of disease.

KEY NOTE SPEAKER

PROF. TONY FUTERMAN

Weizmann Institute of Science,
Rahovot, Israel

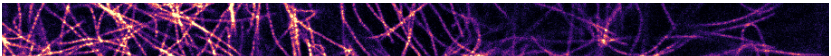
Prof. Tony Futerman received his BSc degree in Biochemistry from the University of Bath, England in 1981, and his PhD degree from the Weizmann Institute of Science (Rehovot, Israel) in 1986 where he discovered that acetylcholinesterase, a key enzyme in terminating neuronal transmission, is attached to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor.

From 1987 to 1990, he was a postdoctoral fellow at the Carnegie Institution (Baltimore, USA), where he analyzed the sites of sphingolipid synthesis.

In 1990, he joined the staff of the Weizmann Institute and he is currently the Joseph Meyerhoff Professor of Biochemistry in the Department of Biomolecular Sciences. His current research field is **“The regulation of sphingolipid metabolism”.**

Prof. Futerman runs a laboratory of approximately 20 scientists, postdoctoral fellows and students, and one of his main focuses is understanding how sphingolipid metabolism is regulated in both the biosynthetic and degradative pathways, with defects in the latter causing some human genetic diseases such as Gaucher disease.

More recently, he has become interested in studying the likelihood that generally accepted evolutionary pathways could lead to the development of the complexity seen in the sphingolipid metabolic pathway. He has published close to 300 manuscripts. He chaired the Gordon conference on Glycolipid and Sphingolipid Biology in 2006, the Lysosomal Diseases Gordon Conference in 2011, and chaired the Molecular Medicine of Sphingolipids meeting in 2012 and 2018. Between 1995-2000 he was a member of the editorial board of the Journal of Neurochemistry, and a member of the editorial board of the Journal of Biological Chemistry from 2000 to 2012. Recently, he started a new journal, BioCosmos: new perspectives on the origin and evolution of life.



TUESDAY, SEPTEMBER 20, 2022 AT 15:30 HRS

THE SPHINGOLIPID ANTEOME: IMPLICATIONS FOR EVOLUTION OF THE SPHINGOLIPID METABOLIC PATHWAY

Modern cell membranes contain a bewildering complexity of lipids, among them sphingolipids (SLs). Advances in mass spectrometry have led to the realization that the number and combinatorial complexity of lipids, including SLs, is much greater than previously appreciated. SLs are generated by four enzymes in the anabolic pathway, namely serine palmitoyltransferase, 3-ketodihydrosphingosine reductase, ceramide synthase and dihydroceramide D4-desaturase 1. Some of these enzymes depend on the availability of substrates and specific cofactors, which are themselves supplied by other complex metabolic pathways. The evolutionary pathway of these four enzymes is poorly understood, and likely depends on the co-evolution of the metabolic pathways that supply the other reaction components. We now introduce the concept of the 'anteome', from the Latin ante ('before') to describe the network of metabolic ('omic') pathways that must converge in order to allow these pathways to co-evolve to permit SL synthesis. We also suggest that current origin of life and evolutionary models lack appropriate experimental support to explain the appearance of this complex metabolic pathway and its anteome.

KEY NOTE SPEAKER

PROF. WIEBKE HERZOG

Friedrich-Alexander-Universität Erlangen, Germany

Wiebke Herzog studied Biology at the Free University in Berlin and one year at Exeter University, UK. She received her PhD for working at the Max Planck institute for immunobiology, Freiburg and trained as a PostDoc at the University of California, San Francisco. As the recipient of a NRW return fellowship and consecutively as a Heisenberg fellow, she joined the faculty at the University of Muenster from where she recently moved to the Friedrich-Alexander University Erlangen as a chair of developmental biology.

Her major research interest is the regulation of vascular development by different signaling pathways, mainly using zebrafish as a model system. When analysing the brain vasculature and the formation of the blood-brain barrier, her group found an interesting cross talk between Wnt- and Sphingosine-1 phosphate (S1P) signalling. Current research focusses on the S1P mediated regulation of blood-brain barrier tightness and on the role of Wnt and S1P in vessel regeneration.

TUESDAY, SEPTEMBER 20, 2022 AT 16:30 HRS

PROF. JOSEF PFEILSCHIFTER

Institute of General Pharmacology and Toxicology,
Goethe-University Frankfurt am Main, Germany

Prof. Josef Pfeilschifter studied Medicine at the University of Regensburg and the Technical University of Munich, Germany. He was a postdoctoral fellow at the Departments of Physiology at the University of Regensburg (Germany) and University of Zurich (Switzerland). In 1987 he joined the Pharmaceuticals Division of Ciba-Geigy Ltd. in Basel (Switzerland). In 1992 he was appointed Professor of Pharmacology at the Biocenter of the University of Basel (Switzerland). Since 1996 Prof. Pfeilschifter is Professor of Pharmacology and Toxicology and Chairman at Goethe-University Frankfurt am Main (Germany).

His major research interest is the signaling capacity of lipid mediators in general and particularly their role in inflammatory and fibrotic kidney diseases.
of the Royal Society in 2021.

WEDNESDAY, SEPTEMBER 21, 2022 AT 9:15 HRS

THE ROLE OF SPHINGOLIPID KINASES IN RENAL FIBROSIS

Over the last decade, various sphingolipid subspecies have gained increasing attention as important signaling molecules that regulate a multitude of physiological and pathophysiological processes including inflammation and tissue remodeling. These lipids have been shown to accumulate in various chronic kidney diseases that typically end in renal fibrosis and ultimately renal failure. I will summarize the effects and contributions of those enzymes that regulate the generation of these lipids, notably the sphingosine and ceramide kinases to renal fibrotic diseases. Strategies of manipulating these enzymes for therapeutic purposes and the impact of existing drugs on renal pathologies will be discussed.

KEY NOTE SPEAKER

PROF. SARAH SPIEGEL

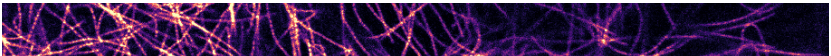
Professor and Chair of the Biochemistry and Molecular Biology Department, Virginia Commonwealth University School of Medicine, USA

Dr. Sarah Spiegel is a Professor and Chair of the Biochemistry and Molecular Biology Department at Virginia Commonwealth University School of Medicine. She received her PhD in Biochemistry in 1983 from the Weizmann Institute in Rehovot.

Her early research focused on the role of ganglioside GM1 in cell signaling. After joining the faculty of the Department of Biochemistry at Georgetown University Medical School, her focus shifted to the roles of the bioactive sphingolipid metabolite, sphingosine-1-phosphate (S1P), whose functions as a pleiotropic signaling lipid were discovered in her lab and opened a new area of research focused on this bioactive sphingolipid metabolite. As a result of her work, it is now recognized that S1P regulates numerous biological processes and is critical for health and diseases.

Her work paved the way for the discovery of FTY720/fingolimod, used for treatment of multiple sclerosis, and recently unraveled a unique aspect of its mechanism of action. In 2002, she became Chair of the Department of Biochemistry & Molecular Biology at the Virginia Commonwealth School of Medicine. In 2007, she assumed the Mann T. and Sara D. Lowry Chair in Oncology at the Massey Cancer Center, where she co-directed the Cancer Cell Signaling Program.

She has received many awards for her work, including VCU Distinguished Scholarship Award, the Women in Science, Dentistry, and Medicine (WISDM) Professional Achievement Award (2007), the Virginia Outstanding Scientist of the Year (2008), the Ernst and Berta Scharrer Medal from Goethe University (2008), the ASBMB Avanti Award in Lipids (2009), NIH Merit Award (2003), election as a Fellow of the American Association for the Advancement of Science (2009), Journal of Lipid Research Special Lectureship (2015), Distinguished Mentor Award (2018), Eicosanoid Research Foundation's Outstanding Achievement Award (2019), Selected as a Fellow of the American Society of Biochemistry and Molecular Biology (2021) and has been a keynote speaker at numerous international meetings.



WEDNESDAY, SEPTEMBER 21, 2022 AT 13:40 HRS

MY JOURNEY WITH SPHINGOSINE-1-PHOSPHATE: FROM INSIPID LIPID TO A KEY REGULATOR

In this lecture I will present my adventures with the bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) intertwined with those of my family life as a wife, mother, and grandmother. My lab has been involved in the study of sphingolipids and sphingolipid metabolites as signaling molecules from their origin. Our research was/is focused on the enigmatic lipid mediator, S1P whose role as a signaling lipid was discovered in my lab more than three decades ago. Our lab has discovered that many important physiological and pathophysiological processes important for inflammation and cancer are regulated by S1P. We were the first to clone and characterize sphingosine kinases, SphK1 and SphK2, and S1P phosphatases, the enzymes that regulate S1P levels, providing molecular tools for the field. Our identification of the S1P family of GPCRs set the stage for the elucidation of their important functions. We also showed that S1P is a critical factor that influences cells' fate and developed the concept of the sphingolipid rheostat, and the paradigm of inside-out signaling by S1P. The puzzle of how such a simple molecule as S1P can have such diverse roles has been resolved by our finding that S1P functions not only as a ligand for S1P receptors, but also has important intracellular actions. I will discuss what we used to know about S1P and what we know now, describe well-established concepts in S1P biology and actions. I will also highlight emerging and new concepts in S1P actions and describe evolving concepts from bench to clinic of targeting the SphK/S1P/S1PR axis that suggest that it is a useful therapeutic approach for several human diseases. It has been an exciting journey so far, with many surprises along the way, that still continues. Supported by NIH grant 2R01GM043880.

KEY NOTE SPEAKER

PROF. ERICH GULBINS

Department of Molecular Biology at the
University of Duisburg-Essen, Essen, Germany

Erich Gulbins studied Medicine in Heidelberg, Germany, London, UK, and Louisville, KY, USA. He then trained at the La Jolla Institute for Allergy and Immunology, San Diego, Ca, USA and the Institute of Physiology, University of Tuebingen, Germany, before becoming Associate Professor at the Dept. of Immunology, St Jude Children´s Research Hospital, Memphis, TN, USA, and Professor and Chair at the Institute of Molecular Biology, University Hospital Essen, University of Duisburg, Essen, Germany.

Research programs mainly deal with inflammation and infection, cystic fibrosis, tumor pathogenesis, major depression and their treatment. His group studies molecular mechanisms how sphingolipids contribute and control bacterial and viral infections, in particular pulmonary inflammation/infections. A focus of the group is transfer of these findings to the clinic. His group also works on the role of mitochondrial ion channels and mitochondrial sphingolipids in tumor biology as well as the function of ceramide and sphingosine for the interaction of malignant tumors with their host.

Finally, he is interested in the role of sphingolipids, in particular ceramide and the acid sphingomyelinase, in the pathogenesis of major depressive disorder and as targets for novel treatments of this disease.

WEDNESDAY, SEPTEMBER 21, 2022 AT 15:00 HRS

SPHINGOLIPIDS IN VIRAL AND BACTERIAL INFECTIONS

Sphingolipids have been shown to play a central role in many viral and bacterial infections. The acid sphingomyelinase and neutral sphingomyelinase generate ceramide, although in different cell compartments of the cell, i.e. lysosomes and the extracellular leaflet of the plasma membrane or intracellular membranes, respectively. Ceramide controls receptor aggregation and thereby receptor functions, but also plays a role in signal transduction contributing to immune cell activation or inactivation, depending on co-stimuli and the cellular context. Sphingosine, which is consumed from ceramide by activity of ceramidases, directly kills many pathogenic bacteria, but also has an impact on viral infections, most likely by binding to viruses and/or their receptors.

KEY NOTE SPEAKER

DR. FIKADU TAFESSE

Associate Professor of Molecular Microbiology & Immunology, Oregon Health & Science University, USA

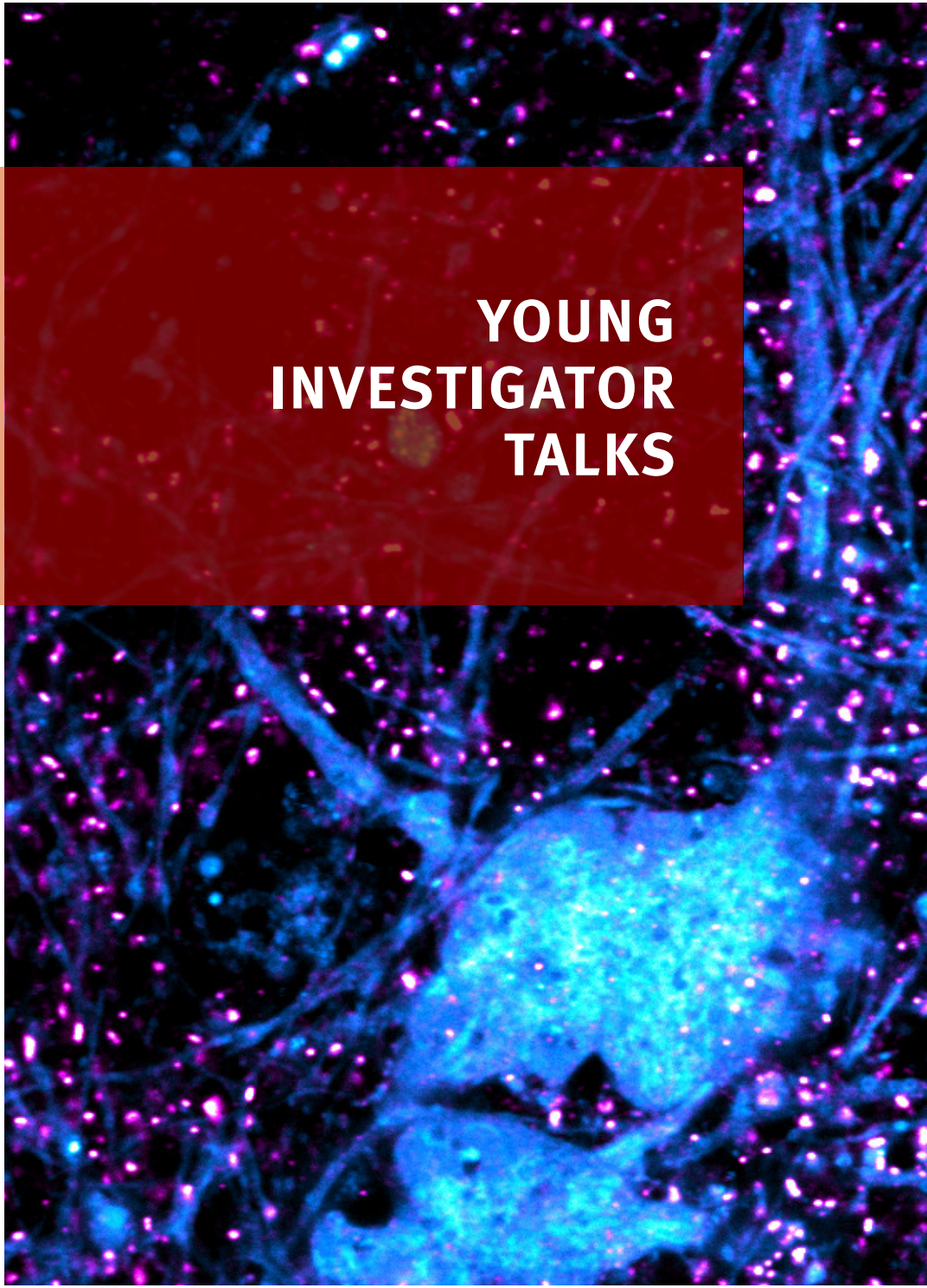
After his BSc studies in Plant Sciences at the Alemaya University in Ethiopia and his Master degree in Biotechnology from the University of Hannover, Germany, he received his PhD in Biochemistry at the Utrecht University in the Netherlands.

Despite the continuous effort to end the spread of infectious diseases, they remain the leading cause of death worldwide. The Tafesse Lab studies host-pathogen interactions of viruses (SARS-CoV-2, HIV and flaviviruses) and bacteria (M. tuberculosis). We focus on identifying and characterizing the host factors that are used by pathogens to secure invasion, persistence and propagation. We are especially interested in studying the role of cellular lipids in microbial pathogenesis and their significance on innate and adaptive immunity. We employ genome-wide genetic screens, various lipidomic analysis techniques and state-of-the-art microscopy tools to define the mechanisms of interactions at the host-pathogen interface.

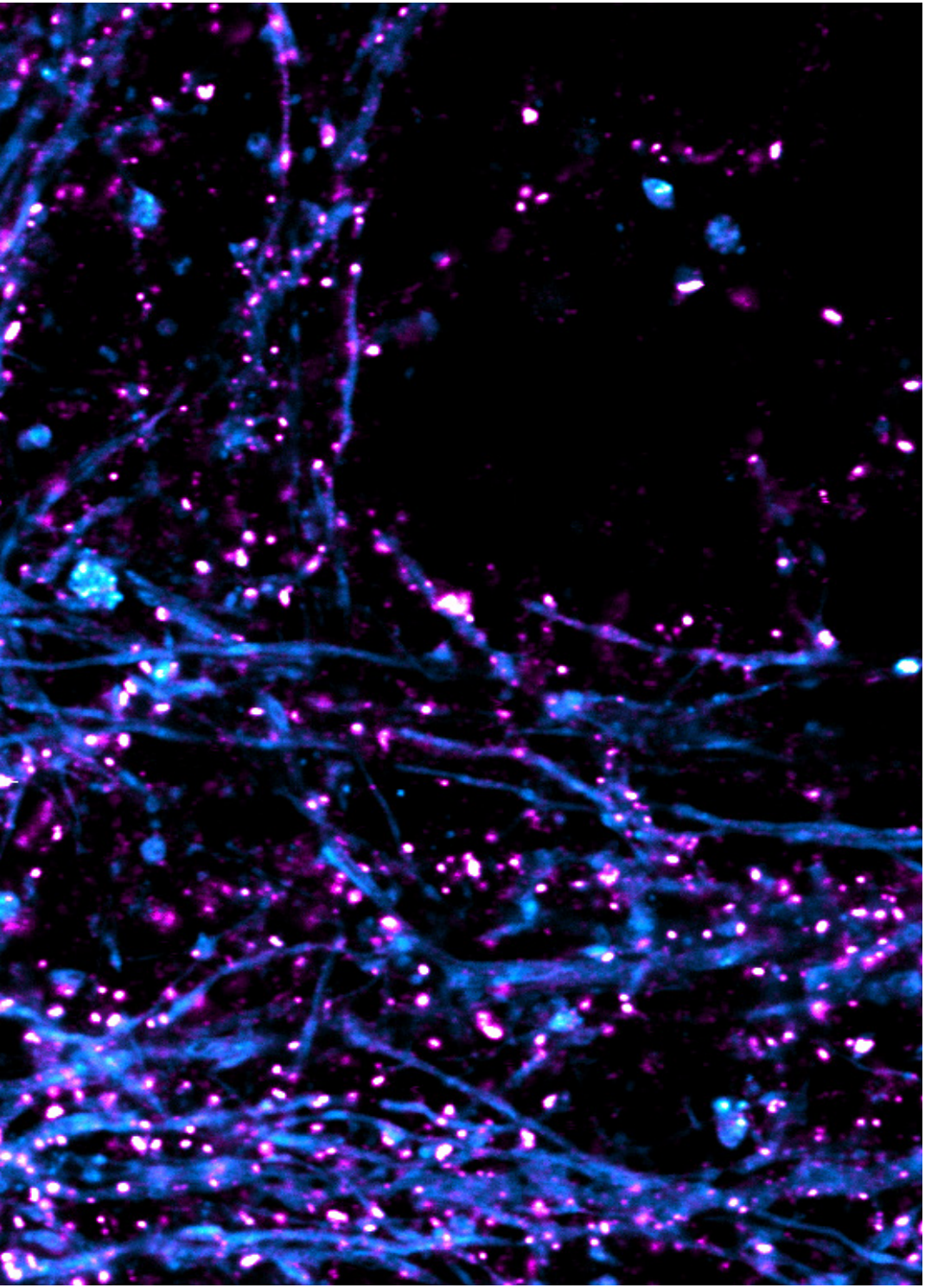
THURSDAY, SEPTEMBER 22, 2022 AT 9 HRS

THE ROLES OF LIPIDS IN BACTERIAL AND VIRAL INFECTION

Despite the continuous effort to end the spread of infectious diseases, they remain the leading cause of death worldwide. Our laboratory studies host-pathogen interactions of viruses (SARS-CoV-2, Zika virus and HIV) and bacteria (M. tuberculosis). We are especially interested in studying the role of cellular lipids in microbial pathogenesis and their significance on innate and adaptive immunity. In this seminar, I will discuss our finding that M. tuberculosis, the causative agent of tuberculosis, uses the sphingomyelin biosynthesis pathway to enter phagocytic cells to establish infection. I will also discuss our recent studies on how emerging and re-emerging pathogens remodel the host lipidome during infection. Using non-targeted lipidomics, we mapped alterations in host lipids following Zika virus and SARS-CoV-2 infections. ZIKV significantly alters host lipid composition, with the most striking changes seen within subclasses of sphingolipids. Ectopic expression of ZIKV NS4B protein results in similar changes, demonstrating a role for NS4B in modulating sphingolipid pathways. Remarkably, SARS-CoV-2 also rewires host lipid metabolism, significantly altering hundreds of lipid species during infection. We correlate these changes with viral protein activity by transfecting human cells with each SARS-CoV-2 protein and performing lipidomics. We find that lipid droplet plasticity is a key feature of infection and that small-molecule glycerolipid biosynthesis inhibitors can block SARS-CoV-2 propagation. This inhibition was effective against the different SARS-CoV-2 variants of concern, indicating that glycerolipid biosynthesis is a conserved host dependency factor supporting this evolving virus.



YOUNG INVESTIGATOR TALKS

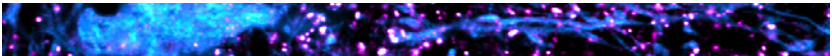


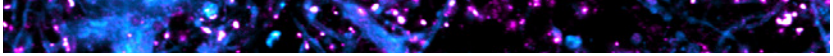
AGATA PRELL

Freie Universität Berlin, Germany

Agata Prell studied Chemistry at the Adam Mickiewicz University in Poznan and then Nutritional Science at the University of Potsdam. In 2021 she started her PhD at the Institute of Pharmacy at the Freie Universität Berlin in the group of Prof. Burkhard Kleuser.

The focus of her project is on analytical methods, especially mass spectrometry, for the identification and analysis of sphingolipids involved in infection. Currently she investigates the activity of sphingosine kinases, which regulate the balance of ceramides, sphingosine and sphingosine 1-phosphate. Abnormal sphingosine kinase expression and activity are found in various diseases, including viral and bacterial infections. Hence, modulation of their activity is a potential therapeutic target. Numerous inhibitors exhibiting a specificity against one or both of the two isoforms have been developed. However, off-target effects have been reported for a few of these compounds. The aim of the project is to investigate the effects of selected sphingosine kinase inhibitors on the entire sphingolipidome in different cell types in order to uncover further possible off-target effects.





TUESDAY, SEPTEMBER 20, 2022 AT 13:45 HRS

Target and off-target effects of sphingosine kinase inhibitors on the sphingolipidome in different cell lines

Agata Prell, Fabian Schumacher, Dominik Wigger, Burkhard Kleuser

Freie Universität Berlin, Institute of Pharmacy, Department of Pharmacology & Toxicology, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

E-Mail: aprell@zedat.fu-berlin.de

Objectives: Sphingosine kinases 1 and 2 (SphK1 and 2) catalyze the phosphorylation of sphingosine (Sph) to the bioactive lipid mediator sphingosine 1-phosphate (S1P). Together with other sphingolipid metabolizing enzymes, SphKs regulate the balance of ceramide, Sph and S1P. These bioactive metabolites participate in many cellular and physiological processes affecting cell homeostasis and function such as differentiation, immunity, and the determination of cell fate. Ceramide induces cell death, whereas S1P has the opposite effect, promoting cell survival. Abnormal SphK expression and activity are found in various diseases, including inflammatory and immune related-diseases, cancer, diabetes and viral and bacterial infections. Therefore, modulation of SphK activity is a potential therapeutic target. Numerous inhibitors exhibiting a specificity against one or both of the two SphK isoforms present in mammals have been developed. However, off-target effects have been reported for a few of these compounds. The aim of this project is to investigate the effects of selected SphK inhibitors on the entire sphingolipidome in order to uncover further possible off-target effects.

Methods: Liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used for the quantification of sphingolipid metabolites in Chang, HepG2 and Huvec cells treated with the SphK inhibitors 5c, K145, SKI-II, DMS, PF-543, SLM6031434 or ABC294640. Stable isotope-labeled palmitate, a precursor of sphingolipids, was added in order to distinguish between the *de novo* synthesized and intrinsic sphingolipid species. Inhibition of dihydroceramide desaturase (DES1) has already been described for SKI-II and ABC294640 as an off-target effect. Hence, intracellular conversion of non-physiological d_7-C13 dihydroceramide was assessed by LC-MS/MS in order to determine DES1 activity in the presence of the SphK inhibitors investigated.

Results: Inhibition of SphKs by SKI-II, PF543, SLM6031434 and DMS was confirmed in all studied cells lines. However, treatment of the cells with SphK1 inhibitor 5c did not significantly alter S1P levels and even led to Sph depletion. Surprisingly, both tested SphK2 inhibitors K145 and ABC294640 increased S1P and dihydro-S1P levels in Chang and HepG2 cells. The novel DES1 activity assay confirmed the previously reported inhibition of DES1 by SKI-II, a dual SphK inhibitor, and ABC294640, in all studied cell lines. In addition, 5c, K145, PF-543 and SLM6031434 also seem to have an inhibitory effect on DES1.

Conclusion: Comprehensive profiling of sphingolipids by LC-MS/MS provided an overall picture of the sphingolipidome in cells treated with different SphK inhibitors and revealed unexpected effects, some of which were even opposite to the desired effect. Therefore, we recommend that if SphK inhibitors are planned to be used, their effect on the cellular sphingolipidome should be investigated first.

Literature

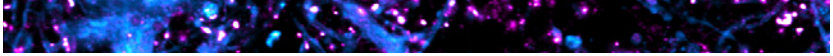
1. Cingolani F, Casasampere M, Sanllehí P, Casas J, Bujons J, Fabrias G. Inhibition of dihydroceramide desaturase activity by the sphingosine kinase inhibitor SKI II. J. Lipid Res. 2014; 55 (8): 1711-1720
2. Alsanafi M, Kelly S, Jubair K, McNaughton M, Tate R, Merrill A, Pyne S, Pyne N, Native and Polyubiquitinated Forms of Dihydroceramide Desaturase Are Differentially Linked to Human Embryonic Kidney Cell Survival. Mol Cell Biol. 2018; 38 (23): e00222-18
3. Arish M, Husein A, Kashif M, Saleem M, Akhter Y, Rub A, Sphingosine-1-phosphate signaling: unraveling its role as a drug target against infectious diseases. Drug Discov Today. 2016; 21 (1): 133-142
4. Solger F, Kunz T, Fink J, Paprotka K, Pfister P, Hagen F, Schumacher F, Kleuser B, Seibel J, Rudel T, A Role of Sphingosine in the Intracellular Survival of Neisseria gonorrhoeae. Front Cell Infect Microbiol. 2020; 10: 215

INGO FOHMANN

Julius-Maximilians-Universität Würzburg, Germany

Ingo Fohmann graduated in food chemistry at the University of Würzburg in 2018. Afterwards he obtained his state-certification as a food chemist at the Bavarian food safety authority, where he worked on the automation of ELISA assays.

In 2020, he joined Prof. Alexandra Schubert-Unkmeirs lab at the Institute for Hygiene and Microbiology. As a PhD student of RTG2581, he is studying the sphingolipid metabolism at the blood-brain-barrier during infection with N. meningitidis. His current studies focus on the manipulation of the Sphingosine kinase/Sphingosine 1-phosphate/Sphingosine 1-phosphate receptor axis and down-stream effectors in brain endothelial cells by N. meningitidis and the identification of responsible bacterial virulence factors.



TUESDAY, SEPTEMBER 20, 2022 AT 14:10 HRS

Neisseria meningitidis induces SphK1 activation and production of S1P in brain endothelial cells

Ingo Fohmann[1], Agata Prell[2], Maria Batliner[1], Burkhard Kleuser[2], Fabian Schumacher[2], Simon Peters[1], Alexandra Schubert-Unkmeir[1]

[1]Institute for Hygiene and Microbiology, Julius-Maximilians-Universität Würzburg, Würzburg, Germany.
[2]Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany.

Neisseria meningitidis (*Nm*) is an obligate human pathogen capable of crossing the meningeal blood cerebrospinal fluid barrier (mBCSFB) to eventually cause meningitis. Analyses of RNAseq data from infected BECs showed differential regulation of the sphingolipid salvage pathway enzymes, including upregulation of sphingosine kinase 1 (SphK1), which generates bioactive sphingosine 1-phosphate (S1P). S1P plays an important role in maintaining endothelial barrier properties via S1P receptors (S1PRs). We hypothesize that *Nm* manipulates the SphK/S1P/S1PR axis in order to enter BECs.

BEC cell line hCMEC/D3 was infected with *Nm* serogroup B strain MC58. LC-MS/MS was used for the sensitive, specific and simultaneous quantification of sphingolipid metabolites in BECs over 8 hour infection time. SphK enzymatic activity was measured in BECs either infected with wildtype strain MC58 or isogenic mutant strains (lacking capsule (*siaD),* outer membrane proteins (*opcA, opa*) or Type IV pili (Tfp) (*pilE*) expression). In addition, purified Tfp were applied. Changes in expression of SphK1/2 or S1PR1/2/3 were measured using qPCR. The roles of SphKs and S1PRs in *Nm* adherence and invasion were determined using gentamicin protection assays, and immunostaining in the presence of SphK inhibitors or S1PR antagonists and RNAi.

LC-MS/MS revealed a significant increase of S1P levels in BECs infected with *Nm* MC58. Infection of BECs with *Nm* MC58 led to an upregulation of SphK enzymatic activity. Infection of BECs with *Nm* MC58 and isogenic mutants or purified Tfp revealed, that the Tfp significantly contribute to SphK activation. In addition, SphK1 expression was transiently increased after 4h *Nm* infection, whereas SphK2 expression was not altered. Pharmacological inhibition of SphK with PF-543, K145 or SLM6031434 or RNAi of SphKs significantly reduced the number of viable intracellular *Nm* without affecting bacterial adherence. Moreover, application of S1PR1+3 antagonist FTY720 similarly reduced the numbers of viable intracellular *Nm.*

In summary, in this study, we demonstrate a comprehensive measurement of the sphingolipid profile in hCMEC/D3 infected with *N. meningitidis* and revealed a significant impact of the pathogen on the salvage pathway. In particular, our data show that *N. meningitidis* leads to activation of SphK1, resulting in a significant increase in S1P levels and interference with S1PR diminishes bacterial uptake suggesting a role of the SphK/S1P/S1PR axis in bacterial invasion.

1. Simonis, A., et al. PLoS Pathog, 2014. **10**(6).
2. Peters, S., et al. Infect Immun, 2019. **87**(8).

DR. CAROLINE BARISCH

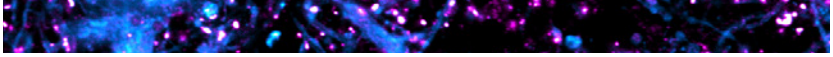
Universität Osnabrück, Germany

Caroline Barisch studied Biology at the University of Bayreuth and received her PhD from the University of Kassel in 2010. During her PhD in the lab of Prof. Markus Maniak she isolated lipid droplets from the model organism Dictyostelium discoideum and characterized the lipid droplet proteome.

In 2011, Caroline started her postdoc in the lab of Prof. Thierry Soldati at the University of Geneva, Switzerland. During the first years, she used the D. discoideum / M. marinum infection system to study lipid droplet dynamics during mycobacteria infection.

2016 she was promoted “maître assistante” and established several tools to monitor lipid flows from D. discoideum to intracellular mycobacteria. Besides lipid metabolism, Caroline also worked on zinc and ZnT transporters and their role in the bactericidal defense of D. discoideum and during mycobacteria infection.

In September 2019, Caroline started the junior research group Molecular Infection Biology at the University of Osnabrück, Germany. The main research interest of the group is to unravel the molecular mechanisms by which intracellular mycobacteria acquire host lipids. **www.barischlabuos.com**



TUESDAY, SEPTEMBER 20, 2022 AT 16:05 HRS

Role of sphingolipids in mycobacterial infections

Stevanus A. Listian[1], Matthijs Kol[2], Edwin Ufelmann[1], Stefan Walter[3], Florian Fröhlich[3,4], Joost CM Holthuis[2,3] and Caroline Barisch[1,3]

[1]Division of Molecular Infection Biology, University of Osnabrück, Germany
[2]Division of Molecular Cell Biology, University of Osnabrück, Germany
[3]Center of Cellular Nanoanalytic Osnabrück (CellNanOS), University of Osnabrück, Germany
[4]Division of Molecular Membrane Biology, University of Osnabrück, Germany

Tuberculosis (Tb), caused by *Mycobacterium tuberculosis* (*Mtb*), is responsible for millions of deaths every year. Although it is well established that *Mtb* relies on host lipids during infection, the molecular mechanisms of how this pathogen remodels the lipid metabolic network of its host to support its persistent lifestyle are so far poorly understood. Combining the application of functionalized lipid probes with mass spectrometry-based lipidomics and advanced microscopy techniques, we analyse metabolic lipid flows between mycobacteria and their host at the subcellular and ultrastructural level using the *Dictyostelium discoideum*/ *Mycobacterium marinum* infection system.

A recent search for genes whose transcript levels are up- or down-regulated during infection of *Dictyostelium* with *M. marinum* yielded numerous components of the host lipid metabolic and transport machinery. Profound changes in expression were observed for enzymes of the sphingolipid metabolic network. Strikingly, mycobacteria express an outer-membrane protein capable of hydrolysing host sphingomyelin (SM) that promotes their replication inside macrophages. While this clearly indicates that sphingolipid metabolism is intimately linked with mycobacterial infection, how individual sphingolipid species contribute to this process is largely unexplored. Consequently, this project aims to unravel the role of sphingolipids in mycobacteria infections.

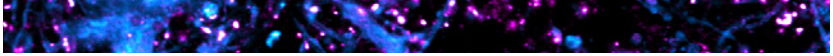
Since the lipid composition and complex sphingolipid profile of *Dictyostelium* are mostly unknown, we first determined all lipid classes synthesized by this organism using a mass spectrometry-based lipidomics approach. This revealed that *Dictyostelium* produces mainly inositol phosphorylceramide (IPC) and very likely not SM. We confirmed this result using a complementary biochemical approach, in which we monitored the turnover of NBD-ceramide to NBD-IPC by *Dictyostelium* lysates by lipid extraction and thin layer chromatography. Surprisingly, the IPC synthase of *Dictyostelium* has not been annotated so far. Using a systematic bioinformatics approach, we identified two potential complex sphingolipid synthases, CSS1 and CSS2. The functional analysis of both candidates using a cell-free assay in a defined lipid environment revealed that CSS2 is an IPC synthesizing enzyme. In cells overexpressing CSS2-GFP, we observed that this protein is located at the Golgi apparatus in *Dictyostelium*, similar to the yeast IPC synthase Aur1.

Finally, by targeting sphingolipid biosynthetic pathways (including CSS2) using genetics and drugs as well as clickable sphingolipid species, we are currently investigating the role of sphingolipids in mycobacteria infection and are determining host-to pathogen lipid flows. We believe that these efforts will help to uncover novel pathways by which intracellular mycobacteria exploit host (sphingo)lipids.

ROMANA SCHEFFEL

Friedrich-Alexander-Universität Erlangen, Germany

I did my Bachelor's and Master's degree in Molecular Biotechnology in Vienna. After that, I worked for about three years as a technician in R&D at the start-up Austrianni. The focus of Austrianni is the development of antibody-based therapies against tuberculosis. In 2020, I decided to do my PhD and changed fields again. In the laboratory of my professor Wiebke Herzog, we are investigating the development of blood vessels with the help of the model organism zebrafish. My project focuses on the development of capillaries in the brain and the formation of the blood-brain barrier and how/if a functional blood-brain barrier is formed again during the regeneration of capillaries after a stroke, traumatic brain injury, etc. I am also working on the development of the blood-brain barrier.



WEDNESDAY, SEPTEMBER 21, 2022 AT 09:50 HRS

Deciphering the role of sphingosine-1 phosphate on blood-brain barrier function

Scheffel, Romana[1,2]; Drexler, Hannes[3]; Herzog, Wiebke[2,3]

[1] Cells in Motion Interfaculty Centre, University of Muenster, Waldeyerstraße 15, 48149 Muenster, Germany
[2] University of Erlangen-Nuernberg, Developmental Biology, Staudtstraße 5, 91058 Erlangen, Germany
[3] Max-Planck Institute for Molecular Biomedicine, Roentgenstrasse 20, 48149 Muenster, Germany

A dysfunctional blood-brain barrier (BBB) is a common complication of a plethora of diseases, like Alzheimer's disease, epilepsy, infectious diseases, as well as ischemic incidents and traumatic brain injuries. Hence, it is crucial for modern therapies to understand the linkage between BBB state and cerebrovascular injury or regeneration.

Sphingosine-1 phosphate (S1p) signaling is essential for BBB maintenance. However, the detailed functions and the downstream mechanisms of S1p fine-tuning barrier tightness remain elusive. Interestingly, signaling through S1p receptor 1 or S1p receptor 2 can have opposing effects on barrier permeability. The basis of this opposition remains unknown. We are combining *in vitro* and *in vivo* techniques we seek to address the effects of S1p receptor signaling on BBB function.

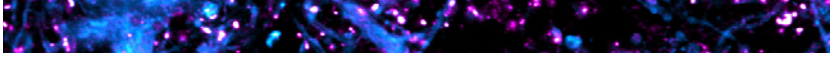
As an *in vitro* model we have established human cerebral microvascular endothelial cell (hCMEC/d3) cultures which mimic BBB properties and have used that model for whole proteome and phospho-proteomic analyses. Our data revealed a large set of differential protein phosphorylation patterns depending on S1p signaling stimulation and duration. Based on our findings, we are now deciphering the signaling cascades downstream of S1p receptor 1 activation and their impact on BBB maintenance and disruption. Ultimately, we aim to transfer our insights from *in vitro* experiments to animal models. Therefore, we are using transgenic zebrafish (*Danio rerio*) to investigate effects of S1p signaling on the BBB *in vivo*. Lastly, we make use of established cerebrovascular injury models in the zebrafish to address the regulation of BBB function or disruption in the context of the S1p signaling pathway.

ENRICO GARBE

Friedrich-Schiller-Universität Jena, Germany

Studied biochemistry in Jena and currently in the final stages of my PhD in the research group “Host Fungal Interfaces” at ZIK Septomics in Jena.

My main scientific interest is the polymorphic, human pathogenic fungus Candida albicans, which is part of the natural human microbiome. The main focus of my work is on metabolic adaptations of the fungus that occur during or enable the transition to the pathogenic stage. For example, the use of different nutrient sources in the host or the stage-specific regulation of biosynthetic pathways.



WEDNESDAY, SEPTEMBER 21, 2022 AT 10:40 HRS

Systematic metabolic profiling identifies *de novo* sphingolipid synthesis as hypha-associated and essential for *Candida albicans* filamentation

Enrico Garbe[1]*, Franziska Gerwien[1]*, Dominik Driesch[2], Tina Müller[3], Bettina Boettcher[1], Markus Gräler[3] and Slavena Vylkova[1]

[1] Septomics Research Center, Friedrich Schiller University and Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany

[2] BioControl Jena, Jena, Germany

[3] Department of Anesthesiology and Intensive Care Medicine, Center for Molecular Biomedicine (CMB) and Center for Sepsis Control and Care (CSCC), Jena University Hospital, 07740 Jena, Germany

*Equal contributions

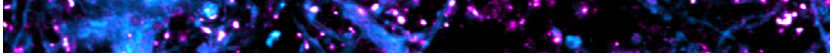
The reversible yeast-to-hypha transition of the opportunistic human fungal pathogen *Candida albicans* is viewed as the key virulence factor of this fungus. Consequently, the hypha-associated gene expression, as well as the underlying regulatory circuits and hypha-inducing stimuli have been subject of intense research. Yet, filamentation-associated metabolic changes and potential morphotype-specific metabolic signatures have been far less studied. Hence, we combined transcriptomics with global metabolic profiling for the SC5314 wild type and two-filamentation-affected mutants in three different hypha-inducing and two yeast-promoting conditions to systematically identify stimuli-specific and general metabolic adaptations during filamentation. A total of 638 unique metabolites were identified providing a state-of-the-art description of the fungal metabolome in both morphotypes. Analogous to hypha-associated gene expression, we observed a conserved group of 26 metabolites with either consistent enrichment (4) or depletion (22) during the hyphal switch. Notably, this group contained the initial three intermediates of *de novo* sphingolipid biosynthesis, indicating a specific hypha-associated induction of this pathway and connection to filamentous growth. Indeed, a pharmacological inhibition of this pathway via two different inhibitors resulted in strongly impaired filamentation in otherwise hypha-inducing conditions, which could be circumvented by the additional supplementation with downstream metabolites. Altogether, our results verified the critical role of *de novo* sphingolipid biosynthesis, in particular the fungal-specific synthesis of acidic glycosphingolipids, as a general prerequisite for the yeast-to-hypha transition in *C. albicans*. Furthermore, we identified a metabolic core signature of *C. albicans* hyphae and the morphotype-dependent induction of distinct metabolic pathways.

MARIA BATLINER

Julius-Maximilians-Universität Würzburg, Germany

Maria Batliner received her BSc in Biomedical Science from the University of Essex, UK in 2018, and her Master's degree in Biomedical Research from Imperial College London, UK in 2019. In 2020, she moved to Würzburg, Germany to do her PhD at the Institute for Hygiene and Microbiology in Prof. Oliver Kurzai´s department.

Maria's research focuses on the quorum-sensing molecule farnesol, an acyclic sesquiterpene alcohol produced by the opportunistic pathogenic fungus Candida albicans. She is investigating the effects of farnesol on sphingolipid metabolism in human monocyte-derived dendritic cells and the molecular mechanisms underlying these effects.



WEDNESDAY, SEPTEMBER 21, 2022 AT 11:05 HRS

The Quorum-Sensing Molecule Farnesol Inhibits Dihydroceramide Desatruase in Dendritic Cells

Maria Batliner[1], Fabian Schumacher[2], Dominik Wigger[2], Jan Dudek[3], Agata Prell[2], Ingo Fohmann[1], Simon Peters[1], Alexandra Schubert-Unkmeir[1], Burkhard Kleuser[2], Oliver Kurzai[1], and Natalie E. Nieuwenhuizen[1]

[1]Institute for Hygiene and Microbiology, Julius-Maximilians-Universität Würzburg, Würzburg, Germany.
[2]Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany.
[3]Deutsches Zentrum für Herzinsuffizienz Würzburg, Universitätsklinikum Würzburg, Würzburg, Germany

Sphingolipids play important roles in immune responses, including in the phagocytosis of the fungal pathogen *Candida albicans* by dendritic cells (DCs). *C. albicans* produces the quorum sensing molecule farnesol (FOH) to regulate filamentation and biofilm formation. Recently, we found similar effects on the differentiation of DCs from monocytes in the presence of FOH or sphingosine-1-phosphate (S1P). In this study, we aimed to determine the effects of FOH and S1P on sphingolipid metabolism in DCs and investigate their molecular mechanisms. Monocytes were isolated from leucocyte cones and differentiated into DCs using GM-CSF and IL-4, in the presence of FOH, S1P or solvent control. The phenotype of generated DCs was assessed by flow cytometry. Sphingolipid profiling of the DCs was conducted using LC-MS/MS. Subsequently, S1P- and FOH-treated cells were incubated with d_7-C13-dihydroceramide, in order to determine dihydroceramide desaturase (DEGS) activity. Reactive oxygen species (ROS) are known inhibitors of DEGS and were therefore measured in S1P- and FOH-treated cells using the 2',7'-Dichlorfluorescein-Diacetate assay. Both FOH and S1P downregulated CD1a expression and upregulated CD1d expression on DCs. This phenotypic effect is partly regulated by the nuclear receptor PPAR-γ. Interestingly, while S1P and FOH had similar effects on DC differentiation, the sphingolipid profiles that they induced were clearly distinguishable from one another. DCs differentiated in the presence of FOH, unlike S1P, showed the accumulation of sphingolipids involved in the *de novo* synthesis pathway, while S1P-treated DCs accumulated sphingolipids of the salvage pathway. Subsequently, we found that FOH treatment, but not S1P, inhibited DEGS. To investigate the molecular mechanisms through which FOH inhibits DEGS activity, we found FOH to induce ROS generation and increase oxidative stress in monocytes compared to the mock-treated cells. Additionally, FOH decreased mitochondrial oxygen consumption rate (OCR) in DCs measured using Seahorse XFe96 Analyzer. Nevertheless, the functional consequences of the differently altered sphingolipid metabolism upon FOH- and S1P-treatment in the context of *C. albicans* infection remain to be elucidated.

MARCEL RÜHLING

Julius-Maximilians-Universität Würzburg, Germany

Marcel Rühling studied Biochemistry at the Julius Maximilians University of Würzburg. During his Bachelor studies he worked in the group of Prof. Dr. Jürgen Seibel on enzyme engineering of levansucrase SacB. He completed his Master's degree in 2020 and conducted research for his Master thesis in the group of Martin Fraunholz at the Chair of Microbiology, where Marcel investigated the role of acid sphingomyelinase (ASM) in epithelial and endothelial cells during infection with *Staphylococcus aureus*. He continues to study the sphingolipid biology of this host-pathogen interaction in his PhD research.

In my research I investigated the role of ASM in plasma membrane repair of mammalian cells upon damage by the pore-forming staphylococcal α-toxin. Thereby, my colleagues and I observed that ASM was secreted by lysosomes of host cells in a Ca2+-dependent manner after treatment with α-toxin. This led to endocytosis of the damaged membrane areas and reestablishment of plasma membrane integrity. Interestingly, we also observed the internalization of S. aureus by its host cells to be dependent on Ca2+, ASM activity and plasma membrane sphingomyelin, whereas α-toxin was not required in this process. Thus, I am currently investigating the cell invasion mechanisms of S. aureus, its sphingolipid dependency and how the mode of pathogen internalization affects the outcome of intracellular S. aureus infection.

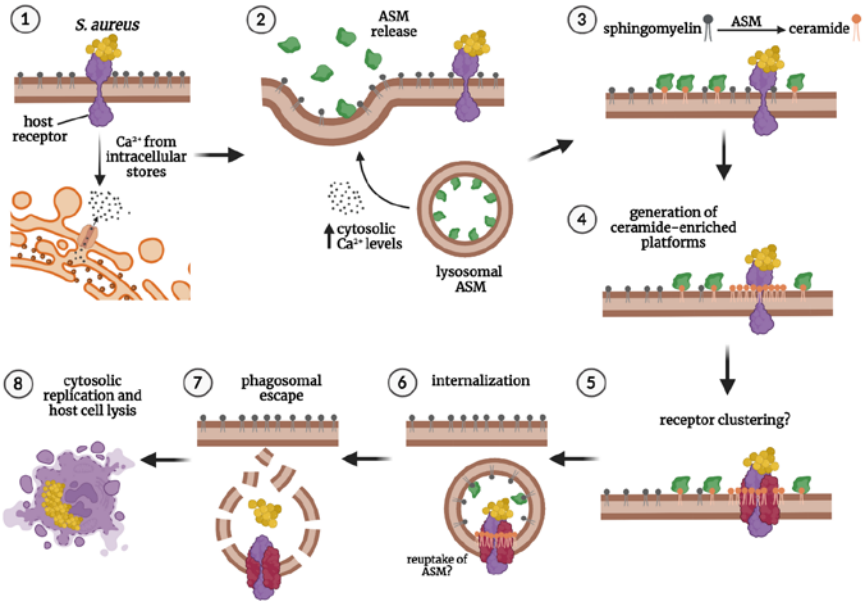
WEDNESDAY, SEPTEMBER 21, 2022 AT 11:30 HRS

Potential roles of sphingolipids during intracellular *Staphylococcus aureus* infection

Marcel Rühling[1], Christian Kappe[2], Andreas Iwanowitsch[1], Alicia Kempf[1], Kerstin Paprotka[1], Christoph Arenz[2], Martin Fraunholz[1]

[1]Chair of Microbiology, Julius Maximilians Universität Würzburg, Germany; [2]Institute of Chemistry, Humboldt University of Berlin, 12489 Berlin, Germany.

Staphylococcus aureus is an opportunistic pathogen, which can be internalized by phagocytes and non-professional phagocytes (NPPC) such as epithelial and endothelial cells. Here, we describe that internalization of *S. aureus* by NPPC is dependent on changes in intracellular Ca^{2+} levels (1) and sphingomyelin (SM) in the extracellular leaflet of host cell plasma membranes (PM), since pretreatment of host cells with the Ca^{2+}-permissive ionophore ionomycin, Ca^{2+} depletion with the Ca^{2+} chelator BAPTA-AM and the staphylococcal sphingomyelinase β-toxin decreased cellular uptake of *S. aureus.* During host cell invasion of diverse pathogens such as *Neisseria* or *Trypanosoma* the lysosomal acid sphingomyelinase (ASM) is required, whereby the lysosomes are recruited to host cell plasma membranes in a Ca^{2+}-dependent manner (2). We therefore tested for an effect of ASM on *S. aureus* internalization by direct enzyme inhibitors or functional inhibitors of ASM (FIASMA). We found that in both, epithelial and endothelial cells, bacterial uptake was diminished upon enzyme inhibition, suggesting that *S. aureus* internalization requires membrane remodeling by ASM (3-5). Furthermore, we observed processing of a visible range FRET probe for ASM activity shortly after uptake of *S. aureus* by host cells. Thus, we hypothesize that lipid composition and/or structure of *S. aureus*-containing phagosomes is determined during cell entry (6). In NPPC, *S. aureus* escapes from its phagosomes (7) to replicate within the cytosol (8). We observed that chemical inhibition of ASM as well pretreatment of cells with β-toxin increased phagosomal escape rates of *S. aureus*. Translocation of sphingomyelin from luminal to cytosolic leaflets of phagosomal membranes preceded phagosomal escape of *S. aureus.* We hypothesized that cytosolically exposed SM may serve as autophagy signal, however, we did not observe recruitment of a fluorescent autophagy reporter to *S. aureus*-containing phagosomes that stained positive for cytosolic SM. Further, removal of phagosomal sphingomyelin by β-toxin overexpression did not abrogate formation of autophagic vesicles. In summary, our data suggest that *S. aureus* invasion is dependent on host cell ASM and that this mode of uptake influences downstream events of intracellular infections such as phagosomal escape.

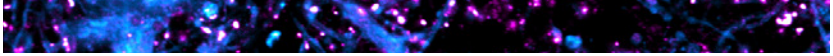


TRUSHNAL WAGHMARE

Julius-Maximilians-Universität Würzburg, Germany

After completing my master's degree in Virology from the National Institute of Virology, Pune, India, I started working as a PhD student at the Institute of Virology and Immunobiology, University of Würzburg, Germany.

My research project aims to identify early targets and receptors involved in the activation of Neutral Sphingomylinease 2, induced by the Measles virus in T cells.



Role of Neutral Sphingomyelinase 2 and Sialophorin as virus effector in T cells

Trushnal Waghmare[a], Franziska Rombach[a], Janna Eilts[b], Veronika Perschin[c], Lothar Jänsch[d],Josef Wissing[d] ,Florian Erhard[a], Elita Avota[a], Markus Sauer[b], Christian Stigloher[c],Niklas Beyersdorf [a], Sibylle Schneider-Schaulies[a], Lars Dölken[a]

[a]*Institute of Virology and Immunobiology, University of Würzburg,*
[b]*Institute of Biotechnology and Biophysics, University of Würzburg,*
[c]*Imaging Core Facility, Biocentre, University of Würzburg,*
[d]*Helmholtz centre for Infection Research, Braunschweig,Germany*

E-mail: trushnal.waghmare@uni-wuerzburg.de

Measles virus (MV) contact in T cells was found to activate the Neutral Sphingomyelinase2 (NSM2). Moreover, NSM2 activation fully accounted for virus-induced cytoskeletal paralysis and inhibition of cell expansion. However, the mechanism of receptor-mediated NSM2 activation in T cells targeting proliferative signaling pathways by MV is still unexplored.

We performed a study aiming to comprehensively map the phospho-proteomic changes that are induced early following MV (or mock) exposure of wild-type and NSM2 deficient Jurkat T cells and identified CD43 (Sialophorin) as a potential MV receptor candidate whose ablation, in turn, prevented MV-induced NSM2 activation and rendered Jurkat cells resistant to MV-mediated proliferation inhibition.

To further understand the molecular events following the putative binding of MV to CD43, we took into account that the surface of lymphocytes is densely covered by membrane protrusions, mainly microvilli, which play a role in the organization of receptors and sphingomyelin clustering proved to be important in their formation and stability.

Using the 4X-expansion microscopy technique, we were able to localize CD43 within the microvilli of T cells. Analyzing cells deficient for CD43 with even high resolution by scanning electron microscopy (SEM), we observed denser microvilli structures compared to wild-type cells and after reintroduction of CD43 expression in CD43-deficient cells. Interestingly, this particular phenotype was also visible in Jurkat cells deficient for CD45, which were included as controls for potential effects arising from changes in the glycocalyx upon CD43 depletion. These findings agree with observations made earlier by others in non-T cells, where a critical role of highly glycosylated proteins in regulating membrane architecture and receptor-mediated communication was established.

Current studies address whether ligation of CD43 by antibodies, Galectin-1 or MV specifically and differentially causes NSM2 activation in T cells to reveal the importance of CD43 in virus-induced physical T cell paralysis.

JONAS WEINRICH

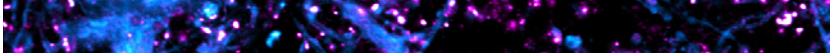
Julius-Maximilians-Universität Würzburg, Germany

Jonas D. Weinrich studied Biochemistry at the University of Würzburg. During his master's he spend a year in the group of André Hoelz at Caltech (USA), working on the membrane attachment of the Nuclear Pore Complex.

In 2020 he started his PhD in the group of Vera Kozjak-Pavlovic as a member of the DFG funded GRK 2581.

Here they are investigating the interplay of the host sphingolipid metabolism and the *Chlamydia*-related bacterium *Simkania negevensis*. *S. negevensis* is an environmental Chlamydiales member with a higher metabolic capacity than other *Chlamydiae*, which is associated with different respiratory diseases, like community acquired pneumoniae in children.

Jonas will present his ongoing research on the dependency of *S. negevensis* on the acidic sphingomyelinase and CERT-mediated ceramide transport. In contrast to other *Chlamydiae*, *S. negevensis* is not affected by the inhibition of the *de novo* synthesis pathway.



THURSDAY, SEPTEMBER 22, 2022 AT 11:45 HRS

Role of Sphingolipids in *Simkania negevensis* Infection

Jonas D. Weinrich[1], Sanisha Sunuwar[1], Fabian Schumacher[2], Dominik Wigger[2], Fabienne Wagner[1], Eva-Maria Hörner[1], Thomas Rudel[1], Burkhart Kleuser[2], and Vera Kozjak-Pavlovic[1]

[1]*Department of Microbiology, Biocenter, Julius-Maximilians-Universität Würzburg, Am Hubland, 97074 Würzburg, Germany.*
[2]*Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str, 2+4, 14195 Berlin, Germany.*

Simkania negevensis (Sne) is a gram-negative bacterium of the Chlamydiales order. It displays a characteristic, obligate intracellular, biphasic life cycle, with two distinct forms. So called Elementary Bodies (EBs) are infectious, small, and metabolically mainly inactive, while Reticulate Bodies (RB) are replicative, bigger, and metabolically active. EBs enter the cell, where they are redifferentiating into RBs and multiply within a tubular double membrane compartment, termed Simkania Containing Vacuole (SnCV), which spans the whole cell and is in close contact to the Endoplasmatic Reticulum (ER) and mitochondria.

To form the SnCV and for survival Sne depends on different host metabolites, e.g., lipids.
Sphingolipids are major membrane constituents and therefore highly abundant lipids in mammalian cells. A delicate equilibrium of different sphingolipids is crucial for survival of the cell. For example, ceramide (Cer), the central player of the sphingolipid metabolism, is among other functions, like membrane curvature and signaling, linked to apoptosis. An import of Cer into mitochondria leads to Cer induced apoptosis.

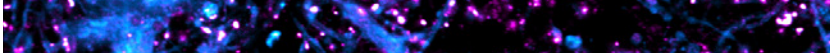
Our project focuses on a better understanding of the influence of changes in the sphingolipid metabolism during Sne infection.
To investigate this, we first performed an extensive screen of different sphingolipid metabolism inhibitors to identify key players for the survival of Sne. During this screen we found first indications that treatment with Desipramine or ARC39, both are Acidic Sphingomyelinase (ASM) inhibitors, leads to a significant and dose-dependent reduction in the number of cells with an established infection, characterized by a fully developed inclusion after three days. We could observe the same effect after using HPA-12, a Ceramide Transport Protein (CERT) inhibitor. CERT shuttles Cer from the ER, the location of the *de novo* Cer synthesis, to the Golgi apparatus, where Cer gets further modified. CERT inhibition was previously shown to impair normal development of *Chlamydia trachomatis* (Ctr) inclusions. In contrast to Ctr infection, after treatment with Myriocin, a potent Serine Palmitoyl Transferase (SPT) inhibitor, we could see no effect on the number of infected cells in our experiments. To further analyze the impact of Sne infection on sphingolipids, we performed lipidomics analysis. Here we could see a shift of the sphingomyeline to Cer ratio towards Cer, which points to an increased sphingomyelinase activity.

Those early findings lead to our hypothesis, that Sne infection is dependent on ASM, as well as on CERT mediated Cer transport, but in contrast to Ctr is not dependent on the *de novo* synthesis pathway.

LOUISE KERSTING

Julius-Maximilians-Universität Würzburg, Germany

I did my bachelor studies in chemistry at the universities of Mulhouse (France) and Freiburg and moved to Würzburg for my master studies. Now, I am a 3rd year PhD student in the research group of Prof. Jürgen Seibel and an associate to the RTG2581. My research focuses on acid sphingomyelinase and acid ceramidase activity in viral infections and the total synthesis of structures targeting these enzymes. I design functionalized inhibitors and sphingolipid analogs that can be stained with fluorescent dyes by bio-orthogonal reactions, aiming to study cellular distribution. My projects are carried out in interdisciplinary collaborations, combining chemistry, virology and microbiology as well as super-resolution microscopy. I believe that the chemical synthesis of functionalized sphingolipids and inhibitors is of great interest to the sphingolipid community and that the chemists' perspective is significant for investigating biological processes.



THURSDAY, SEPTEMBER 22, 2022 AT 12:10 HRS

Fluoxetine derivatives inhibit acid ceramidase activity and cause lysosomal trapping of SARS-CoV-2

Louise Kersting*, Nina Geiger, Jan Schlegel, Linda Stelz, Sofie Fähr, Viktoria Diesendorf, Valeria Roll, Marie Sostmann, Eva-Maria König, Sebastian Reinhard, Daniela Brenner, Sibylle Schneider-Schaulies, Markus Sauer, Jochen Bodem and Jürgen Seibel

*Institute of Organic Chemistry, Am Hubland, Julius-Maximilians-University Würzburg
Email: louise.kersting@uni-wuerzburg.de*

Abstract

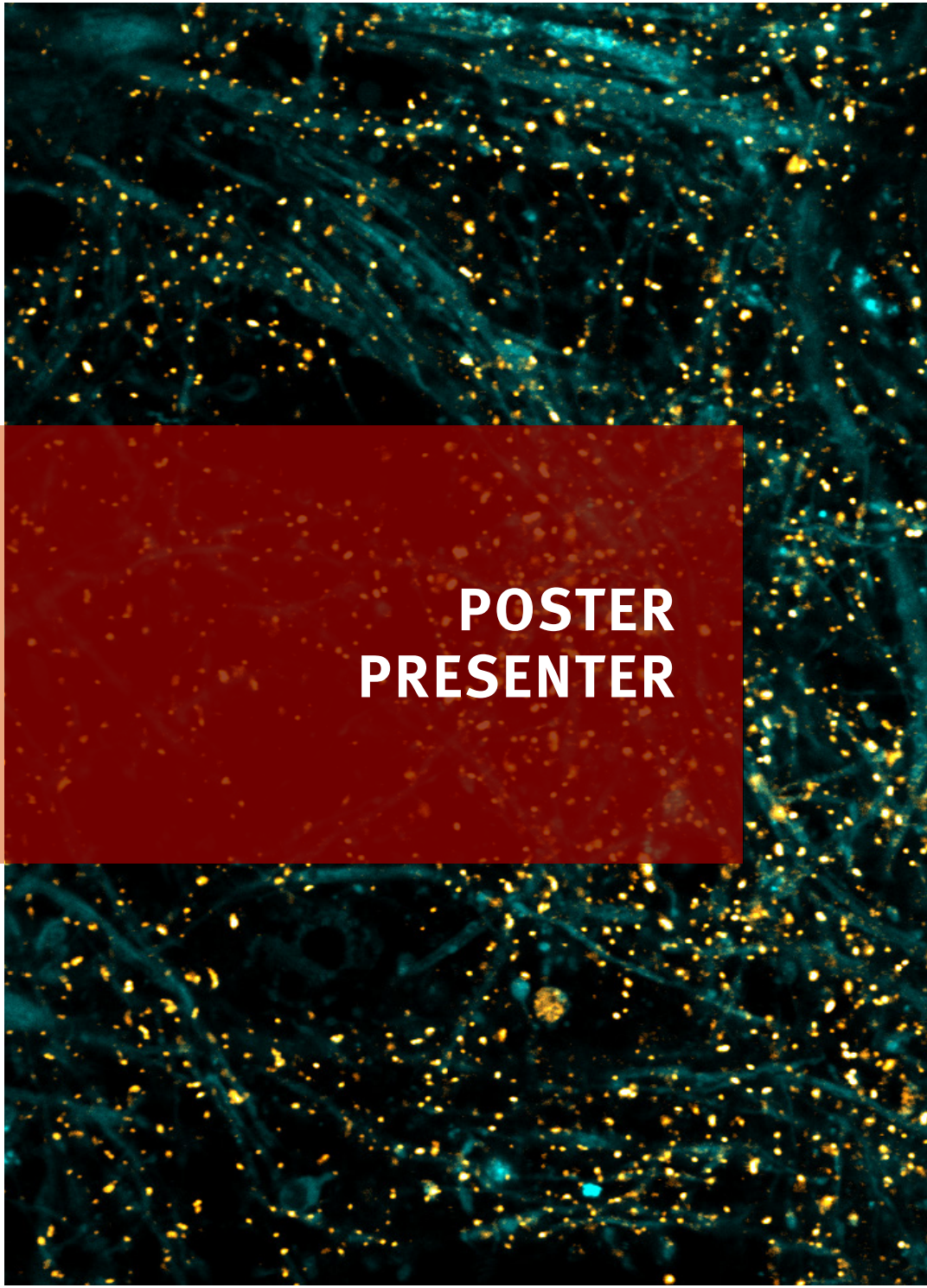
In the context of finding drugs with anti-SARS-CoV-2 activity, we and others reported the effectiveness of the serotonine reuptake inhibitor Fluoxetine in blocking viral infection.[1-3] This drug is also known to have an impact on the sphingolipid metabolism where it acts as a functional inhibitor of acid sphingomyelinase (FIASMA). This enzyme is typically found in lysosomes and catalyzes the degradation of sphingomyelin to phosphocholine and the hydrophobic molecule ceramide, which clusters in cellular membranes and thus enables receptor binding. It was demonstrated that acid sphingomyelinase (ASM) is activated upon infection and supports viral uptake at the entry stage. Hence, ASM inhibition by Fluoxetine and other pharmacological inhibitors prevented viral entry.[2] We wanted to investigate the role of lysosomal enzymes and lysosomal egress, rather than the entry steps, in the infection process and designed Fluoxetine analogues fulfilling two criteria: Firstly, these derivatives should exhibit antiviral activity but no ASM inhibition and secondly they should carry modifications allowing bio-orthogonal labeling with fluorescent dyes after incorporation into cells. The chemically synthesized derivative **AKS466** is a highly effective compound, which meets these requirements. We achieved this effect by replacing the amino group of Fluoxetine by an amide chain. After verifying the non-toxicity and antiviral activity of **AKS466**, we investigated its cellular distribution and observed its accumulation in lysosomes. We therefore assume that the inhibition mechanism of SARS-CoV-2 by **AKS466** occurs in this compartment. High-resolution imaging of dye-labeled **AKS466** and SARS-CoV-2-RNA fluorescence in situ hybridization (FISH) revealed that the intracellular production of RNA is not down-regulated – however, the viral RNA was enriched in lysosomes. Hence, we suggest that the inhibition can be attributed to lysosomal trapping of SARS-CoV-2. To analyze the implication of other lysosomal sphingolipid catabolizing enzymes, we performed acid ceramidase (AC) assays and confirmed the inhibition of this enzyme by **AKS466**. Furthermore, addition of exogenous C6-ceramide impacted viral replication as well. We therefore consider lysosomal ceramide concentrations and AC activity as potent antiviral targets for future research.

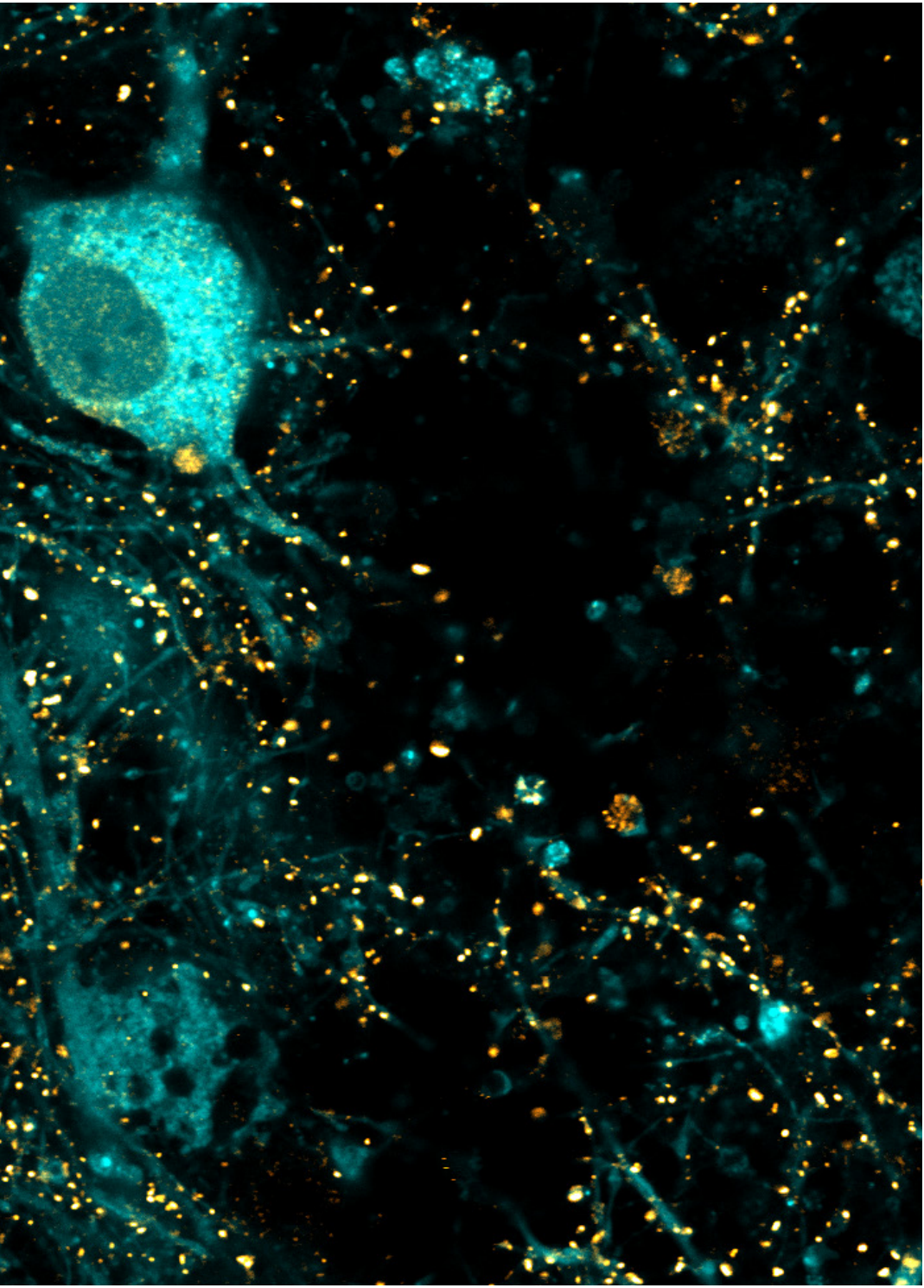
References:

[1] M. Zimniak, L. Kirschner, H. Hilpert, N. Geiger, O. Danov, H. Oberwinkler, M. Steinke, K. Sewald, J. Seibel, J. Bodem, *Sci. Rep.* **2021**, *11*, 5890.

[2] A. Carpinteiro, M. J. Edwards, M. Hoffmann, G. Kochs, B. Gripp, S. Weigang, C. Adams, E. Carpinteiro, A. Gulbins, S. Keitsch, C. Sehl, M. Soddemann, B. Wilker, M. Kamler, T. Bertsch, K. S. Lang, S. Patel, G. C. Wilson, S. Walter, H. Hengel, S. Pöhlmann, P. A. Lang, J. Kornhuber, K. A. Becker, S. A. Ahmad, K. Fassbender, E. Gulbins, *Cell Rep. Med.* **2020**, *1*, 100142.

[3] S. Schloer, L. Brunotte, J. Goretzko, A. Mecate-Zambrano, N. Korthals, V. Gerke, S. Ludwig, U. Rescher, *Emerg. Microbes Infect.* **2020**, *9*, 2245-2255.


POSTER
PRESENTER

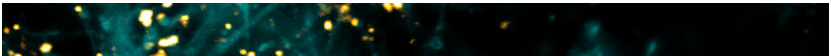


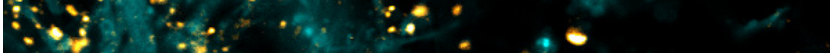
POSTER PRESENTER

DANIELA BRENNER

Julius-Maximilians-Universität Würzburg, Germany

Currently, I am in the third year of my PhD in organic chemistry in the research group of Prof. Dr. Seibel (University of Wuerzburg). My research topics focus on the role of sphingolipids in various biological questions, divided into the area of infectious diseases such as SARS-CoV-2 and chronic diseases like diabetes. Especially, I focus on the total synthesis of functionalized sphingolipid derivatives. These analogs can be coupled with fluorescent dyes in bioorthogonal reactions to investigate the cellular distribution as well as the metabolic pathway and associated enzymes. As part of the RTG2581 (research training group, funded by the German Research Foundation), I am involved in many collaborations with the departments of microbiology, virology, immunology, and pharmacy, working together on devising and analyzing the sphingolipid variants. With these insights, we, as a team, hope to reveal and examine the detailed role of these sphingolipids and their associated enzymes. With the symposium, I anticipate to gain more knowledge from other research fields and to offer the chemical view on the very broad, but highly interesting field of sphingolipids.





18-Azido-1-deoxysphinganine functions as a lipid probe for investigating the role of deoxysphingolipids in type 2 diabetes

Daniela Brenner, Dominik Wigger, Janna Eilts, Fabian Schumacher, Markus Sauer, Burkhard Kleuser, Jürgen Seibel

Type 2 diabetes (T2D) is a worldwide metabolic disorder that leads to chronically elevated blood glucose concentrations due to a deficiency of the hormone insulin and/or reduced insulin action. This leads to a number of long-term consequences such as neuropathy and impairment of the entire cardiovascular system, which is associated with a greatly increased lethality. One main cause for the onset of T2D is a compromised functionality of insulin-producing β-cells, yet the origin for this deterioration has still not been thoroughly explored.

Deoxysphingolipids, a class of non-canonical sphingolipids deficient of a hydroxyl group at C1, were recently discovered to be more concentrated in the blood plasma of diabetic patients and can therefore serve as a biomarker for early diagnosis. Additionally, as 1-deoxysphinganine proved to be cytotoxic to different cell lines, elevated plasma levels of deoxysphingolipids could be a cause to the impairment of insulin-producing β-cells. Previous studies in β-cells with exogenous 1-deoxysphinganine have shown that 1-deoxysphinganine-mediated toxicity is characterized by intracellular formation of 1-deoxydihydroceramides and triggers multiple signaling pathways, including cytoskeletal remodeling, senescence, necrosis, and apoptosis. Further *in vitro* assays of an alkyne-functionalized 1-deoxysphinganine revealed accumulation of deoxysphingolipids in mitochondria and ER, leading to swelling and fragmentation of the former and enlargement of the latter organelle. Yet, cellular transport of 1-deoxysphinganine as well as its effect on the organelles in β-cells have still to be identified.

Therefore, we synthesized 18-azido-1-deoxysphinganine, a novel lipid probe, in 13 steps. The azido ($-N_3$) group at the end of the sphingoid backbone was specifically chosen due to its three main advantages. While azido groups are relatively small functional groups with minimal influence on the size or the polarity of the lipid, this modification enables the observation of possible metabolites of this lipid by LC-MS(/MS) considering the difference in molecular weight. Additionally, azido groups can be readily utilized in a bioorthogonal reaction with an alkyne functionalized fluorescent dye and consequently visualized. Employing the copper free method of strain-promoted azide-alkyne cycloaddition (SPAAC) enables live-cell imaging and can therefore reveal the lipid pathway. By combining both methods of LC-MS(/MS) and fluorescent microscopy we expect to gain new insight on the effect of 1-deoxysphinanine on insulin-producing β-cells.

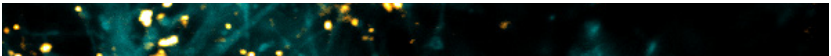
POSTER PRESENTER

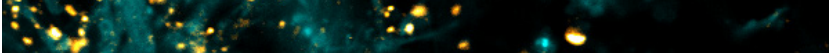
JANICE CHITHELEN

Julius-Maximilians-Universität Würzburg, Germany

Completed my Bachelor studies in microbiology and biochemistry in 2015 from Mumbai University in 2015 after which I further pursued my Masters studies in a niche field like virology at University of Pune (India). While doing my Masters thesis work and dissertation on sequencing and phylogenetic analysis of respiratory syncytial virus from clinical samples, I became interested in pursuing further research especially on Paramyxoviruses and other RNA viruses.

In 2018, I began as a graduate research fellow at University of Würzburg in the lab of Prof. Dr. Jürgen Schneider-Schaulies where we investigated compounds targeting viral and host factors for a therapy against measles virus infections. One of the aspects was to study the involvement of sphingolipid metabolism using acid ceramidase and sphingosine kinase inhibitors and their effect on potential downstream signaling pathways with and without measles virus infection in primary human lymphocytes.





Title

Sphingolipid Inhibitors Ceranib-2 and SKI-II Reduce Measles Virus Replication and Affect Cellular mTORC1 Downstream Signaling in Primary Lymphocytes

Authors :

Janice Chithelen, Hannah Franke, Nora Länder, Annika Grafen, Prof. Dr. Jürgen Schneider-Schaulies

Institute for Virology and Immunobiology, University of Würzburg, Würzburg

Abstract :

The Ceramide → Sphingosine → Sphingosine-1-phosphate rheostat catalyzed by acid ceramidase (aCDase) and sphingosine kinases (SphK) respectively is an important pathway within the sphingolipid metabolism. Previously, we reported that inhibitors Ceranib-2 and SKI-II inhibited measles virus replication (MV) in tumor BJAB cell line as well as primary human lymphocytes. We also observed deactivation of pp70 (immediate downstream substrate of cellular sensor mTORC1) with the inhibitor treatments in absence of measles infection (Grafen et al. 2019). Since viruses require host cellular machinery for the synthesis of their own viral proteins during their replication cycle, in the following study we sought to investigate the effect of both inhibitors on mTORC1 associated protein translation pathways namely the mTORC1→EIF4E and the S6 kinase→ribosomal protein (rpS6) axes along with expression of viral proteins. This was studied using the GFP expressing wild type MV IC323eGFP strain and its infection in primary human lymphocytes (from healthy blood donors) which are primary targets in wild type measles infection. We observed that in uninfected cells, the major effect of both the inhibitors was on EIF4E phosphorylation, while in MV infected cells, they predominantly dampened the infection effect by reducing the rpS6 expression and also viral protein GFP and hemagglutinin expression levels was affected.

References :

1) Chithelen J, Franke H, Länder N, Grafen A, and Schneider-Schaulies J, 2022. The sphingolipid inhibitors ceranib-2 and SKI-II reduce measles virus replication in primary human lymphocytes: effects on mTORC1 downstream signaling. Frontiers in Physiology, DOI: 10.3389/fphys.2022.856143).
2) Grafen A, Schumacher F, Chithelen J, Kleuser B, Beyersdorf N, Schneider-Schaulies J. Use of Acid Ceramidase and Sphingosine Kinase Inhibitors as Antiviral Compounds Against Measles Virus Infection of Lymphocytes *in vitro*. *Front Cell Dev Biol*. 2019;7:218. Published 2019 Oct 1. doi:10.3389/fcell.2019.00218

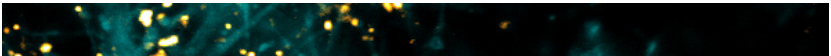
POSTER PRESENTER

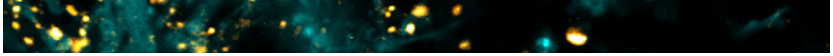
PAULINA CRUZ DE CASAS

Julius-Maximilians-Universität Würzburg, Germany

I studied Biochemical Engineering in the Autonomous University of Mexico and Biological Engineering in the Université de Technologie de Compiègne. This was a double Engineering diploma that allowed me to obtain the equivalent of a Masters degree (Engineering title). Afterwards, I move to Würzburg and joined Wolfgang Kasntenmüller´s lab and the GRK2581 for my PhD.

My PhD research project is focused on understanding what role are sphingolipids playing in T cell biology, for example migration, effector function or survival, during steady state and homeostatic conditions, but also upon infection. Understanding the role of sphingolipids or sphingolipid metabolizing enzymes in basic T cell biology might uncover new therapeutic approaches enhance the function of these cells during inflammation, disease or infection.





Role of Smpdl3b in CD8⁺ T cells

Paulina Cruz de Casas (PhD student)
Intitut für Systemsimmunologie, Julius-Maximilians-Universität Würzburg
Email: paulina.cruz-de-casas@uni-wuerzburg.de

CD8⁺ T cells can be divided into three types, naïve, effector and memory, which are different at a transcriptional level, and therefore differ in their phenotype (e.g. cytokine (CK) and chemokine receptors) and function. These cell types further differ in the amount and composition of lipid rafts that are found in their plasma membranes. Therefore, we hypothesized that the biophysical composition of the plasma membrane determines the functionality of CD8⁺ T cells and contributes to the differential responsiveness of naïve vs memory T cells. Lipid rafts can be defined as highly ordered membrane microdomaines with a particular lipid and protein composition, that reduce membrane fluidity and serve as receptor clustering platforms. Currently, however, it is unclear which enzymes modulate the plasma membrane composition, specifically in memory CD8⁺ T cells. To identify such enzymes, we interrogated our own and published data sets and identified the gene Smpdl3b as a potential candidate. Smpdl3b is a phosphatase that belongs to the family of sphyngomyelinases (SM) and that forms part of lipid rafts. Indeed, we found that the presence of this enzyme is required for memory T cell survival and function. Our preliminary data suggest that Smpdl3b could prioritize TCR- over cytokine-mediated signaling by altering lipid raft composition. Therefore, modulating biophysical parameters of the plasma membrane might be a promising novel angle to modulate T cell functionality for adoptive immunotherapy approaches.

Literature:

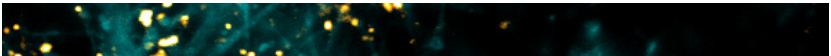
1) Gerlach, C., et al. (2016). The Chemokine Receptor CX3CR1 Defines Three Antigen-Experienced CD8 T Cell Subsets with Distinct Roles in Immune Surveillance and Homeostasis. Immunity. *https://doi.org/10.1016/j.immuni.2016.10.018*
2) Heinz, L., *et al.* (2015). The Lipid-Modifying Enzyme SMPDL3B Negatively Regulates Innate Immunity. Cell Reports. https://doi.org/10.1016/j.celrep.2015.05.006
3) Kaech, S., Cui, W. (2012). Transcriptional control of effector and memory CD8+ T cell differentiation. *Nature Immunology Reviews.* https://doi.org/10.1038/nri3307
4) Gerlach, C., *et al.* (2016). The Chemokine Receptor CX3CR1 Defines Three Antigen-Experienced CD8 T Cell Subsets with Distinct Roles in Immune Surveillance and Homeostasis. *Immunity. https://doi.org/10.1016/j.immuni.2016.10.018*
5) Kersh, E., *et al.* (2003). TCR Signal Transduction in Antigen-Specific Memory CD8 T Cells. *The Journal of Immunology.* https://doi.org/10.4049/jimmunol.170.11.5455
6) Kurd, N., *et al.* (2020). Early precursors and molecular determinants of tissue-resident memory CD8+ T lymphocytes revealed by single-cell RNA. 10.1126/sciimmunol.aaz6894
7) Prakaash, D., *et al.* (2021). Multi-scale simulations of the T cell receptor reveal its lipid interactions, dynamics and the arrangement of its cytoplasmic region. *PLOS Computational Biology. https://doi.org/10.1371/journal.pcbi.1009232*

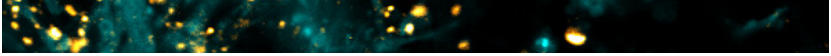
POSTER PRESENTER

DR. SHAGHAYEGH DERAKHSHANI

Standard BioTool GmbH, Munich, Germany

Shaghayegh Derakhshani received her PhD from Würzburg University for working on a project within GRK2157 “3D Tissue Models for Studying Microbial Infections by Human Pathogens” under supervision of Prof. Dr. Sibylle Schneider-Schaulies at the Institute for Virology and Immunology of Würzburg University. She then trained as a PostDoc at BioMed X Institute in Heidelberg at the Immunology department working on Intestinal Epithelial Barrier in Autoimmune Diseases. During her PostDoc she got fascinated by the power of CyTOF© (Cytometry by Time of Flight) technology in providing comprehensive information of the phenotype and function for every single cell. She works now as Application Scientist at Standard BioTools /former Fluidigm, the company which developed and owns CyTOF machines.





Imaging Mass Cytometry Identifies Structural and Cellular Composition of the Mouse Tissue Microenvironment

Shaghayegh Derakhshani, Kyle Driscoll, Qanber Raza, Michael Cohen, Smriti Kala, Liang Lim, Geneve Awong, Andrew Quong, Christina Loh
Standard BioTools Canada Inc., Markham, Ontario, Canada

Abstract

Understanding cellular and structural composition of tissues can be highly composition through quantitative evaluation of selected cellular and structural tissue markers facilitates prediction of disease progression. Specifically, in preclinical models, changes in immune cell infiltration, adhesion state of epithelial cells, and composition of extracellular matrix in response to drug treatments are regularly probed using conventional techniques, yet these techniques require an excessive investment of time and resources. Imaging Mass Cytometry™ (IMC™) is a vital state-of-the-art tool to deeply characterize the complexity and diversity of any tissue without disrupting spatial context. The Hyperion™ Imaging System utilizes IMC, based on CyTOF® technology, to simultaneously assess up to 40 individual structural and functional markers in tissues on a single slide, providing unprecedented insight into the organization and function of the tissue microenvironment. We and others have previously demonstrated the application of IMC in combination with Maxpar® panel kits to highlight cellular composition of human tissues. Here, we showcase the recently released Maxpar OnDemand™ Antibodies for IMC application on mouse tissue. We introduced 11 additional methodically curated biomarkers to our existing mouse antibody catalog, providing the basis for the use of high-multiplex imaging in preclinical investigations.

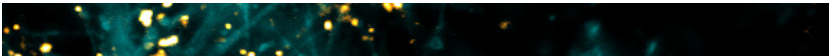
POSTER PRESENTER

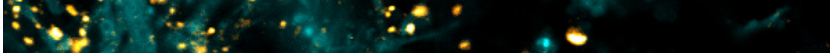
JANNA EILTS

Julius-Maximilians-Universität Würzburg, Germany

I studied Biology at the University of Würzburg and completed my Master's thesis at the Department of Biotechnology & Biophysics in Prof. Markus Sauer's lab.

As part of the RTG2581 I am currently continuing my studies in this lab as a PhD, focusing on super-resolution fluorescence imaging. I am especially interested in further developing the novel technique Expansion Microscopy and to make it applicable for the visualization and analysis of sphingolipids.





Title

Visualizing neuronal sphingolipid and protein organization using Expansion Microscopy

Authors

Janna Eilts[1], Christian Werner[1], Daniela Brenner[2], Jürgen Seibel[2], Markus Sauer[1]

[1]Department of Biotechnology and Biophysics (JMU Würzburg)
[2]Institute for Organic Chemistry (JMU Würzburg)

Abstract

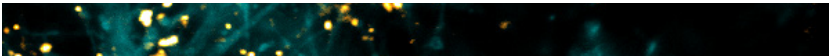
In recent years Expansion Microscopy (ExM) has been established as an efficient method for super-resolution fluorescence imaging with conventional microscopes. With the help of a swellable hydrogel, cross-linked structures in a biological sample are physically expanded, allowing imaging of cellular organization below the diffraction limit. In contrast to proteins, which can be easily incorporated into the polymer network via their amino groups, lipids lack these functional groups and are thus not fixable. To analyze sphingolipid distribution in the membranes of different cell types with ExM, we used a functionalized ceramide containing an amino group for cross-linking and an azido group for clicking with a functionalized fluorescent dye. We used this method to investigate the sphingolipid composition in synaptic regions of hippocampal mouse neurons, in particular at the presynapse. Here, the lipid composition of the dynamic membrane could influence the efficiency of vesicle release into the synaptic cleft through its biophysical properties or via the regulation of protein organization. Applying post-expansion labelling on 4-8-fold expanded samples (Tillberg et al. 2016, Damstra et al. 2022) enabled us to visualize ceramide distribution with a high signal-to-noise ratio. We observed an enrichment of clickable ceramides in several areas, including synaptic regions. To validate this, we applied co-immunostainings with synaptic markers. Our results demonstrate that post-expansion immunostaining achieves a higher labeling density due to improved epitope accessibility.

POSTER PRESENTER

DR. VLADIMIR GIRIK

University of Geneva, Switzerland

After finishing my undergraduate studies at the Kazakh National University in 2011 pursued my studies at the Technical University of Dresden obtaining a master's degree in Molecular Bioengineering in 2013. I continued my graduate education at the University of Geneva in the lab of Prof. Howard Riezman where my project focused on investigating sphingolipid metabolism in the yeast *Saccharomyces cerevisiae* using caged chemical probes. In 2020 I joined the lab of Prof. Paula Nunes-Hasler at the Department of Pathology and Immunology at the University of Geneva as a postdoctoral researcher to study the role of sphingolipids in phagocytosis and antigen cross-presentation with a focus on the ER-phagosome membrane contact sites.



Development and characterization of KSR1-based ceramide-specific sensors

Vladimir Girik, Maral Azam, Larissa van Ek, Prof. Anne-Claude Gavin, Prof. Paula Nunes-Hasler

Department of Pathology and Immunology, University of Geneva, Switzerland

E-mail : Vladimir.Girik@unige.ch, Paula.Nunes@unige.ch

Development of genetically encoded lipid probes provides an opportunity to investigate subcellular dynamics of lipid species[1]. We sought to develop ceramide-specific lipid biosensors for studying ceramide transport at the ER-phagosome contact sites using several candidate proteins previously shown to bind ceramides *in vitro* [2–4]. We found that while most probe candidates showed localization in the nucleus, C1 domain of the human pseudokinase KSR1 localized to the plasma membrane or in the endolysosomal compartments depending on the position of a fluorescent tag. C-terminally tagged KSR1 probe (C-KSR) responded to treatment with palmitate known to increase ceramide levels. Moreover, C-KSR relocalized to the plasma membrane in response to the treatment with Sphingomyelinase (SMase) or during phagocytosis. Furthermore, we characterized the binding of KSR1 to different lipids probe using liposome microarray-based assay (LiMa)[5]. No binding between KSR1 probe and liposomes of different lipid compositions has been observed. Interestingly, we detected ceramide binding by the tandem C1 domains of PKC[6] – an established sensor for a structurally similar lipid diacylglycerol (DAG). Further lipidomic characterization will be performed to provide an insight into ceramide sensing by the C1 domain of KSR1.

1. Wills, R. C., Goulden, B. D. & Hammond, G. R. V. Genetically encoded lipid biosensors. *Mol. Biol. Cell* **29**, 1526–1532 (2018).
2. Yin, X. *et al.* A Ceramide-binding C1 domain mediates kinase suppressor of Ras Membrane Translocation. *Cell. Physiol. Biochem.* **24**, 219–230 (2009).
3. Wang, G., Krishnamurthy, K., Umapathy, N. S., Verin, A. D. & Bieberich, E. The carboxyl-terminal domain of Atypical protein kinase Cζ binds to ceramide and regulates junction formation in Epithelial cells. *J. Biol. Chem.* **284**, 14469–14475 (2009).
4. Mukhopadhyay, A. *et al.* Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. *FASEB J.* **23**, 751–763 (2009).
5. Saliba, A. E. *et al.* A quantitative liposome microarray to systematically characterize protein-lipid interactions. *Nat. Methods* **11**, 47–50 (2014).
6. Codazzi, F., Teruel, M. N. & Meyer, T. Control of astrocyte Ca2+ oscillations and waves by oscillating translocation and activation of protein kinase C. *Curr. Biol.* **11**, 1089–1097 (2001).

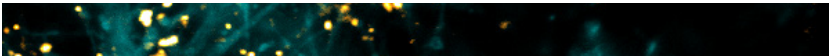
POSTER PRESENTER

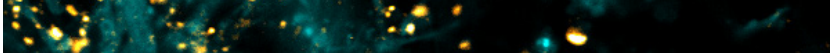
DR. PIA HEMPELMANN

Ruprecht-Karls-Universität Heidelberg, Germany

I am Pia Hempelmann, a Postdoc in the laboratory of Dr. Doris Höglinger at the Biochemistry Center Heidelberg, Germany.

I started my scientific carreer in Hanover, Germany, where I performed my B.Sc. in Biology. Afterward, I moved to Heidelberg for my M.Sc. in Biochemistry. In my Master's thesis, I already worked on cellular sphingolipid metabolism, particularly on the impact of the glucosylceramide synthase inhibitor PDMP on subcellular sphingolipid distribution. We published this story in 2021 in the International Journal of Molecular Sciences. Following this, I started my PhD in 2019 in the group of Dr. Doris Höglinger at the Biochemistry Center in Heidelberg and finished it recently, in July 2022. During my PhD, I worked on lysosomal sphingolipid export and the role of the lysosomal protein STARD3. I found STARD3 as the first lysosomal sphingosine exporter potentially involved in sphingolipid recycling at lysosome-ER contact sites. I started my Postdoc in August 2022 to further investigate the role of STARD3 in sphingolipid transfer at lysosome-ER contact sites.





STARD3: A Sphingolipid Transporter at Lysosome-ER contact sites

Dr. Pia Hempelmann

Biochemie Zentrum, Heidelberg University

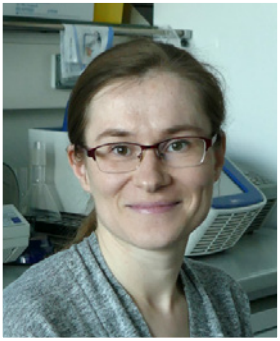
E-mail: pia.hempelmann@bzh.uni-heidelberg.de

Sphingolipid transport between organelles has been increasingly studied during the last years and a large body of evidence points to protein-mediated sphingolipid transfer at organelle contact sites. This is well characterized for the transfer of ceramide, glucosylceramide and glycolipids along the sphingolipid biosynthetic pathway from the endoplasmic reticulum (ER) towards the plasma membrane. However, the catabolic pathway is far less well studied. Here, a crucial, but unexplored step is the recycling of sphingosine backbone upon lysosomal sphingolipid degradation and its re-integration into the biosynthetic pathway at the ER. In this study, we propose that the lysosomal cholesterol transporter STARD3 acts as a sphingosine transfer protein.

STARD3 has been previously described to tether lysosomes to the ER via its FFAT motif. At this contact, its START-domain transfers cholesterol from the ER to lysosomes to support endosome maturation. We employed functionalized, photocrosslinkable sphingosine in intact cells to show that STARD3 also binds to sphingosine. In addition, in vitro studies confirmed sphingosine binding and molecular dynamic simulations identified the cholesterol-binding pocket in the START-domain to bind to sphingosine as well. Functionally, we could show that overexpression of STARD3 drives the sphingosine metabolism towards biogenesis of higher sphingolipid species such as sphingomyelin, while depleting cellular Stard3 levels results in a delayed sphingosine metabolism, which indicates STARD3 as a lysosomal sphingosine exporter. This transfer is dependent on a functional FFAT motif, consistent with sphingosine transfer taking place at the lysosome-ER contact site.

Overall, we hypothesize that STARD3 transfers sphingosine from the lysosome towards the ER while shuttling cholesterol in the opposing direction in analogy to several other bi-directional lipid transfer proteins acting at organelle contact sites.

POSTER PRESENTER

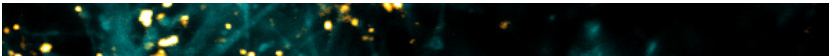


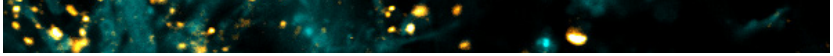
DR. DENISA JAMECNA

Ruprecht-Karls-Universität Heidelberg, Germany

I did my Biology studies at Masaryk University in Brno and at University College London. For my PhD, I joined the group of Dr Bruno Antonny at the Université Côte d'Azur in Nice. There, I studied how intrinsically disordered N-terminal regions in OSBP and related proteins affect membrane tethering geometry and dynamics of membrane contact sites.

Currently, I work as a postdoc in the lab of Dr Doris Höglinger at the Biochemie Zentrum Heidelberg. My research focuses on the mechanism of sphingosine transport at the lysosome - ER interface. My work combines biochemistry, chemical biology and structural biology. In particular, I use photoactivatable and clickable lipid analogs incorporated into liposomes to investigate sphingosine binding by isolated lipid transfer proteins or lipid binding domains. I also aim to characterize how various factors such as point mutations or auxiliary lipids in liposomal membrane affect protein interaction with sphingosine.





Characterization of sphingosine binding by STARD3 START domain - *in vitro* studies

Denisa Jamecna, Pia Hempelman, Fabio Lolicato, Walter Nickel, Doris Höglinger

Abstract

Sphingolipids make up approximately 10 mol% of eukaryotic lipids and are defined by a common sphingoid backbone. They are particularly enriched at the plasma membrane and as such they undergo constitutive turnover along the endocytic pathway. The bulk of sphingolipid recycling takes place in late endosomes and lysosomes. Here, catabolism of complex sphingolipids produces sphingosine and long chain sphingoid bases, which are then exported to the ER and the Golgi for synthesis of new sphingolipids. Despite its importance for cellular lipid homeostasis, the mechanism of sphingosine export from the acidic compartments into the ER is completely unknown. In our work, we propose that lysosomal sterol transporter STARD3 also acts as a sphingosine transporter at the lysosome-ER interface.

STARD3 consists of a transmembrane domain anchored at the lysosome, an FFAT motif mediating interaction with ER-resident proteins and a lipid transport domain called START. To investigate sphingosine binding by START domain, we utilize an *in vitro* approach involving photocrosslinkable and clickable (pac) lipid analogs incorporated into liposomes. We show that purified START domain crosslinks with both pac-cholesterol and pac-sphingosine. Moreover, competition crosslinking experiments suggest a higher affinity for sphingosine. Molecular dynamic (MD) simulations confirm that sphingosine can accommodate into the sterol binding cavity of START. Combining MD simulations and mutagenesis studies, we identify and examine the role of arginine 351 in pac-sphingosine binding. In addition, our cell biology experiments show that overexpression of STARD3 supports the metabolic conversion of lysosomal sphingosine into higher sphingolipids, whereas STARD3 depletion results in delayed sphingosine metabolism. This corresponds well with the hypothesis that STARD3 mediates export of lysosomal sphingosine during sphingolipid recycling.

In conclusion, we identified STARD3 as a putative sphingosine transporter operating at at the lysosome – ER contact sites. Our work paves the way for further research on protein mediated sphingosine transport as well as on mechanisms that regulate the recycling of cellular sphingolipids.

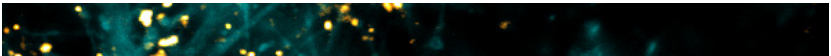
POSTER PRESENTER

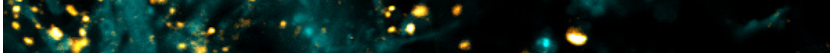
PUTRI MANDASARI

Julius-Maximilians-Universität Würzburg, Germany

Putri Mandasari did her Master's degree in Food Science in Halle (Saale). It was only during her Master thesis, she developed great interest in immunology.

She then started PhD program at PD Niklas Beyersdorf's Lab, which allows her to deepen her knowledge of immunology. Within project 3 in GRK 2581, her project focuses on investigating the role of neutral sphingomyelinase 2 (Nsm2) and acid ceramidase (Ac) in immune responses. To approach this, inducible knock-out mouse model is used *in vitro* and *in vivo* to study the consequences of Nsm2- and Ac-deficiency in immune cells, especially CD4+ T cells, in steady-state condition. Furthermore, measles virus will be also used *in vivo* as chronic viral infection model to understand better how Nsm2 and Ac contribute to reach immunity.





Acid ceramidase is a key regulator of cytokine secretion by mouse CD4$^+$ T helper cells

Putri Mandasari, Claudia Hollmann, Niklas Beyersdorf

Institute for Virology and Immunobiology, University of Würzburg, Versbacher Str. 7, 97078 Würzburg, Germany

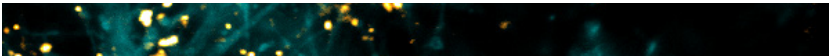
Background:
Acid ceramidase (Ac) – a sphingolipid-metabolizing enzyme that hydrolyses proapoptotic ceramide – was reported to be aberrantly expressed in several human tumors. Many Ac inhibitors have, thus, been investigated as potential therapeutics for cancer. Yet, the importance of Ac in immune responses has not been fully elucidated.
Methods:
To study the role of Ac in different CD4$^+$ T cells subsets, we generated tamoxifen-inducible knock-out mouse models for Ac in a) all cells (iAc-KO^{ubi}) and b) in the Treg compartment only (iAc-KO^{Treg}). After incubation with 4-OH-Tamoxifen *in vitro*, purified CD4$^+$ T cells from iAc-KO and wild-type (wt) littermates were cultured for 10 days to allow for loss of Ac protein expression. The cells were then (re-)stimulated with Dynabeads® mouse T-Activator CD3/CD28 at optimal and sub-optimal bead to cell ratios for 24 and 48 h. Intracellular cytokine expression (FACS staining) as well as secretion (multiplex assay) were detected.
Results:
Our results using CD4$^+$ T cells from iAc-KO^{ubi} mice showed that the frequency of CD4$^+$ Foxp3$^+$ regulatory T cells (Treg) among CD4$^+$ T cells was reduced in iAc-KO compared to wt T cell cultures. In line with this finding, we observed less IL-2 secretion and lower CD25 expression by CD4$^+$ T cells which might explain the reduced Treg survival in iAc-KO compared to wt cultures. IFNγ and IL-10 secretion into culture supernatants, but not expression of these cytokines, were greatly increased after Ac knock-out. Ac deletion in Treg only, in contrast, led to a different phenotype compared to Ac deletion in all (CD4$^+$ T) cells as indicated by our preliminary results (activation, proliferation and cytokine secretion).
Conclusions:
Our *in vitro* findings suggest that genetic deletion of Ac primarily affects cytokine secretion by CD4$^+$ Foxp3$^-$ conventional T helper cells (Tconv). Reduced IL-2 secretion by Tconv appears to secondarily deplete Treg in cultures of iAc-KO^{ubi} cells. Increased IFNγ and IL-10 secretion after iAc knock-out might enhance type 1 immunity *in vivo*, which could be beneficial in clearing chronic viral infections. We will, thus, follow this up in a mouse model of chronic measles virus infection of the brain.
This study was funded by the DFG (GRK 2581-P3).

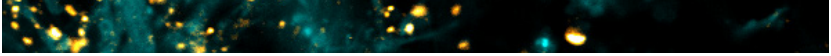
POSTER PRESENTER

HENRIK NURMI

Åbo Akademi Turku, Finland

I'm a 4th year biochemistry PhD student at Åbo Akademi University in Turku, Finland; currently working in the lipid transfer protein group at the department of biochemistry under Dr Peter Mattjus. My main research focus is the glycolipid transfer protein (GLTP), a transporter of complex glycosphingolipids at the ER-Golgi interface. Currently, we are working on elucidating the non-transportatory roles and effects of GLTP in the cell, such as its interactions at the ER-Golgi membrane contact sites, its influence on cell metabolism, and its effect on vesicular trafficking pathways in the cell





In vivo effects of glycolipid transfer protein (GLTP) knockout in HeLa cells

The glycolipid transfer protein (GLTP) has recently been linked to multiple cellular processes and functions aside from its best-known function as a lipid transport protein. For instance, GLTP has been proposed to act as a sensor and regulator of glycosphingolipid homeostasis in the cell. GLTP may also be involved in facilitating or regulating vesicular transport, through its previously determined interaction with the endoplasmic reticulum membrane protein VAP-A (vesicle-associated membrane protein associated protein A). In this study, we have characterized the phenotype of HeLa cells in which GLTP has been knocked out via CRISPR/Cas9, in comparison to wild-type HeLa cells. We show that GLTP knockout affects multiple cellular functions and processes, such as motility, three-dimensional growth and cohesion, lipidome composition and even cellular metabolism. Notably, we also find evidence which suggests that GLTP, through its interaction with VAP-A, indeed does affect vesicular trafficking in the cell. Taken together, we show direct effects of GLTP on multiple cellular processes, most significant of which are its evident effects on cellular metabolism and vesicular trafficking.

POSTER PRESENTER

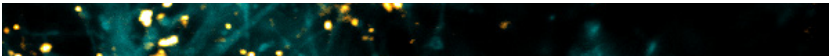
VERONIKA PERSCHIN

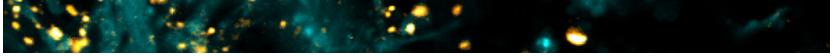
Julius-Maximilians-Universität Würzburg, Germany

Veronika Perschin studied Biology in Marburg, Townsville and Würzburg. Throughout her studies, parasitology and infections are a common theme.

Her bachelor's thesis aimed at understanding New Permeability Pathways that are established by Plasmodium falciparum upon infection of human erythrocytes – these are essential nutrient uptake and waste removal routes. During her Master's thesis, she studied Plasmodium berghei in mouse liver. This is the symptom free, but massive proliferation of the parasite and thus a crucial bottleneck for the establishment of systemic infection.

In her doctoral project, she is using C. elegans as a model organism to study the influence of acid sphingomyelinase (ASM) on Staphylococcus aureus infection. The worms have a different sphingoid base (iso-branched) and they have three ASM genes compared to one in humans. Their innate immune system is highly conserved and tissue repair is limited, which allows to study the infection on a cellular or ultrastructural resolution in an animal model.





Acid sphingomyelinase deletion mutants show increased resistance to *Staphylococcus aureus* infections, formation of electron dense multilamellar bodies and accumulation of various sphingolipid species

Veronika Perschin[1], Marcel Rühling[2], Agata Prell[3], Fabian Schumacher[3], Martin Fraunholz[2], Burkhard Kleuser[3], Gholamreza Fazeli[1], Christian Stigloher[1]

[1]Imaging Core Facility, University of Würzburg, Würzburg, Germany

[2]Chair of Microbiology, University of Würzburg, Würzburg, Germany

[3]Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

Staphylococcus aureus exerts its pathogenicity partly by the secreted pore forming α-toxin. The formation of pores in the plasma membrane of the host cell leads to lysosome exocytosis and release of lysosomal acid sphingomyelinases (ASM) to the outer leaflet of the plasma membrane. There, ASM catalyses the conversion of sphingomyelin to ceramide, which leads to internalization of the damaged membrane in small vesicles. Whether α-toxin or even the whole pathogen is engulfed during this process varies among the infection models and it is unclear whether this effort to repair the membrane contributes to the cell toxicity of *S. aureus in vivo*. *Caenorhabditis elegans* is susceptible to *S. aureus* and thus a well-established model to study its pathogenicity. *C. elegans* has three ASM isoforms: ASM-1, ASM-2 and ASM-3 that are all homologous with the human ASM. Here, we used deletion alleles of the three *asm* genes to investigate the role of sphingomyelinases in *S. aureus* infection.

We fed control worms as well as single, double and triple *asm* mutants with *S. aureus* and quantified their survival. None of the control worms, but almost half of the *asm-1* and *asm-3* single or double mutants survived after 72 hours of feeding on *S. aureus*. *asm-2* mutation did not protect the worms against *S. aureus* infection, and even abrogated the protective effect of *asm-1* and *asm-3* in double or triple mutants, implying divergent roles of ASM-2 vs. ASM-1 and ASM-3 in the pathogenicity of *S. aureus*.

Ultrastructural analysis of high pressure frozen specimen revealed excessive lipid accumulation in *asm-1,* and *asm-3* mutants probably in lysosomes, which resemble multilamellar bodies described in human Niemann-Pick patients. *asm-1/asm-3* double mutants showed the highest count of such organelles, whereas these were rarely observed in controls and *asm-2,* confirming the different mode of action of ASM-2 compared to either ASM-1 or ASM-3.

To test if this phenotype is due to a disturbed sphingolipid metabolism, we analysed the sphingolipidome of *C. elegans* using liquid chromatography tandem-mass spectrometry. We found that only *asm-1* increased total levels of dihydrosphingomyelin, sphingomyelin and 2-OH-sphingomyelin, but interestingly also dihydroceramides compared to controls. These results indicate the significance of ASM-1 on regulating the levels of multiple sphingolipid species.

Our findings show that host ASM activities shape the outcome of experimental infection of *C. elegans* with *S. aureus*. Thereby *asm-1* demonstrates the strongest protective effect in the survival assays as well as the strongest influence on the sphingolipid levels. Next, we aim to reveal the mechanisms of increased resistance of the *asm* mutants challenged with *S. aureus*. We will test whether *asm* mutants fail to engage membrane repair response, which eventually renders them less susceptible to intracellular toxicity or if the innate immune response is generally upregulated in *asm* mutants.

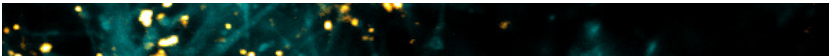
POSTER PRESENTER

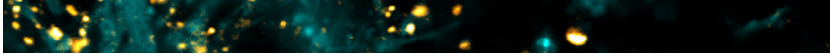
DR. SIMON PETERS

Julius-Maximilians-Universität Würzburg, Germany

I did my bachelor studies in biology at the University of Cologne and moved to Würzburg for my master studies. In 2017, I started my PhD in the group of Prof. Alexandra Schubert-Unkmeir where I have been a RTG2581 associate postdoc for 2 years now.

My research focuses on the interaction between the human exclusive pathogen *Neisseria meningitidis (Nm)* and the host sphingolipid metabolism. In my current work, I investigate the role of glycosphingolipids (GSL) in the meningococcal traversal of the nasopharyngeal epithelial barrier. There is evidence that a specific subset of GSL play an important role for *Nm* to transmigrate from its colonization site in the nasopharynx into the bloodstream. Therefore, I aim to identify the interaction partner on the bacterial and host side.





***Neisseria meningitidis*: Importance of host glycosphingolipids at the nasopharyngeal barrier**

Introduction:

Neisseria meningitidis (*Nm*) is a major cause of bacterial meningitis and sepsis. A critical step in the pathogenesis of *Nm* is the passage through the epithelial barrier of the nasopharynx, a step that is still poorly understood. Recent published data on human brain microvascular endothelial cells have shown that *Nm* interacts strongly with GM1, a glycosphingolipid (GLS) strongly abundant on the epithelium (1). This interaction seems to be essential for the ability of *Nm* to invade the cells.

The aim of this study was to investigate the role of GLS on the ability of *N. meningitidis* to overcome the epithelial barrier in the nasopharynx. We hypothesize that interaction between *Nm* and GLS (e.g. GM1) is an important perquisite for bacteria to enter and overcome the epithelial barrier without destroying it.

Methods:

Human lung epithelial cells (Calu-3) were grown on permeable cell culture inserts as liquid – liquid (LLI), or air – liquid interface (ALI) model and further characterized by immunofluorescence imaging, Transepithelial electric resistance (TEER) and permeability (NaF and Fitc-dextran) measurements. The cells were exposed to different *Nm* strains and their effect on the barrier integrity was evaluated by TEER measurement and qPCR analysis of tight junction protein expression. The ability of the bacteria to cross the barrier was determine by transmigration experiments in the presence or absence of cholera toxin b subunit (CtxB), blocking the interaction between *Nm* and a specific GLS subset. GM1 distribution on the cells and accumulation at the site of bacterial interaction with the cell were visualized by structured illumination and direct stochastic superresolution microscopy (SIM / *d*STORM).

Results:

Here we demonstrate that only the Calu-3 air – liquid interface model exhibits distinct epithelial barrier properties, such as its multilayer structure and mucus production. Whereas the barrier integrity was generally not weakened during infection, the distribution of GM1 (or closely related GSL) has shifted from a uniformly distributed pattern to clustering and strong accumulation around the bacteria. The ability of *Nm* to overcome the epithelial barrier was found to be depended on the strain used. In addition, the ability of *Nm* to transmigrate trough the barrier was affected by the pretreatment of the cells with CtxB, which resulted in a decrease in bacterial transmigration through the epithelial barrier.

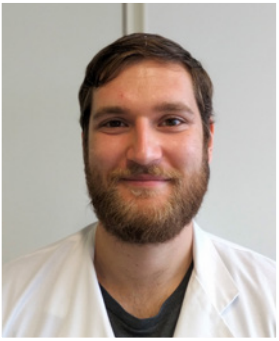
Discussion:

The results demonstrate that the ALI model offers various advantages over the classical LLI model. In addition, the ability of *Nm* to initially cross the epithelium of the nasopharynx is not based on the disruption of the barrier, but strongly relies on the interaction with host GSL.

Reference:

1. Schlegel J, Peters S, Doose S, Schubert-Unkmeir A, Sauer M. 2019. Super-Resolution Microscopy Reveals Local Accumulation of Plasma Membrane Gangliosides at Neisseria meningitidis Invasion Sites. Frontiers in Cell and Developmental Biology 7.

POSTER PRESENTER

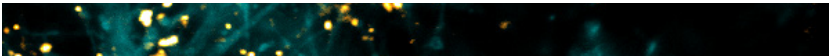


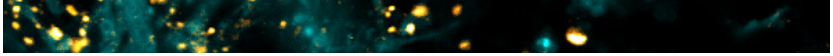
FLORIAN SALISCH

Justus-Liebig-Universität Gießen, Germany

I received my Bachelor´s degree from the Humboldt University in Berlin and continued with my Master´s degree at the Hannover medical school. Currently, I am a PhD student in the group of Prof. John Ziebuhr at the University of Gießen and part of the RTG2581.

The main topic of my research is how sphingolipids influence the replication of coronaviruses. Of particular interest is the role of sphingomyelinases during viral entry and formation of replicative organelles.





In vitro screening of a compound library targeting the sphingolipid metabolism reveals potential inhibitors of coronaviral replication

Florian Salisch[1], Jürgen Seibel[2], Jochen Bodem[3], Elita Avota[3], Sibylle Schneider-Schaulies[3], Christin Müller[1], John Ziebuhr[1]

[1] Institute of Medical Virology, Justus Liebig University Giessen; [2] Institute of Organic Chemistry, Julius Maximilians University Würzburg; [3] Institute of Virology and Immunology, Julius Maximilians University Würzburg

E-Mail: florian.salisch@viro.med.uni-giessen.de

In general, viruses are known to manipulate the host cell metabolism including cellular lipid homeostasis to facilitate their replication. For example, sphingolipids (SLs) as key structural components of cellular membranes are supposed to be involved in viral uptake (e.g. SARS-CoV-2) and/or the formation of virus-induced replicative organelles in the cytoplasm of infected host cells (e.g. West Nile virus). Therefore, SLs and related factors could serve as potential cellular drug targets against newly emerging viruses such as SARS-CoV-2.

In this study, we screened a compound library of 400 small molecule inhibitors targeting the SL metabolism for their antiviral activity against coronaviruses. In subsequent studies, the three identified hit compounds were evaluated regarding cytotoxicity and antiviral activity against (i) three different coronaviruses (SARS-CoV-2, HCoV-229E and MERS-CoV) in (ii) permanent as well as primary cell cultures. In detail, we demonstrated for the small molecule inhibitor AKS-294 a pan-coronaviral antiviral activity with effective concentration (EC_{50}) in the nanomolar rage and no detectable cytotoxicity up to 100 µM. This compound was further analyzed using primary airway epithelial cells differentiated under air-liquid-interface conditions to validate the antiviral potency in a relevant *ex vivo* system. Here 10 and 1µM significantly reduced viral replication.

Taken together, our findings suggest that targeting SL metabolism reduces coronaviral replication and could thereby provide new strategies for broad-spectrum antiviral drug therapy.

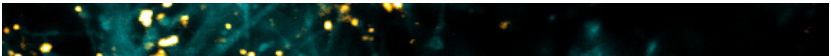
POSTER PRESENTER

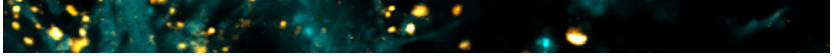
REBEKKA SCHEMPP

Julius-Maximilians-Universität Würzburg, Germany

Rebekka Schempp studied Biochemistry at Ulm University, Germany. In 2020 she started her PhD in in the lab of Dr. Elita Avota in the Institute of Virology and Immunology, University of Würzburg, Germany.

Her project is part of the GRK2581 -“Sphingolipids in Infection” and focuses on the role of the neutral sphingomyelinase 2 (NSM2) in T cells. She is specifically interested in the sphingomyelinase-mediated lipid and protein topology changes at the plasma membrane of T cells.





Neutral sphingomyelinase 2 (NSM2) mediated regulation of plasma membrane lipids in T cells

Rebekka Schempp[1], Janna Eilts[2], Fabian Schumacher[3], Burkhard Kleuser[3], Markus Sauer[2], Elita Avota[1]

[1] Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany

[2] Department of Biotechnology and Biophysics, University of Würzburg, Würzburg, Germany

[3] Institute of Pharmacy, Free University of Berlin, Berlin, Germany

NSM2 is a neutral sphingomyelinase associated with the plasma membrane (PM) inner leaflet. There, the activity of the enzyme has the potential to regulate both lipid composition and protein topology, and thereby, membrane proximal signaling. Although NSM2 activity associated sphingolipid changes at the whole cell level are documented, knowledge about local PM-associated modulations of lipid and associated spatial organization of proteins is scarce. Previous work of the lab showed that Measles Virus (MV) inhibits TCR or mitogen stimulated T cell proliferation in contact dependent manner in vitro, which is associated with activation of acid (ASM) and NSM2 sphingomyelinases followed by ceramide (Cer) release at the PM.

To analyse the influence of NSM2 activity on other lipids, wild type Jurkat cells were contacted with MV and Mass Spec analysis of the PM fractions showed an increase in Diacylglycerol (DAG) content. To further analyze the regulatory function of NSM2 in DAG production Jurkat T cells overexpressing wild type NSM2 or the enzyme dead mutant H639A were generated and characterized. Then we focused on the reverse reaction by Sphingomyelinsynthase (SMS) 1/2. SMS1 and 2 are both generating SM and DAG using Cer and Phosphatidylcholine (PC) as substrate. SMS1 is localized in the Golgi whereas SMS2 is predominantly localized at the PM. We could show that overexpression of NSM2 leads to an impaired SMS activity *in vitro* as well as *in situ* measured by TLC.

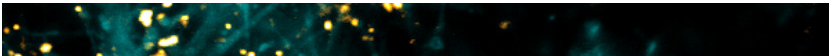
POSTER PRESENTER

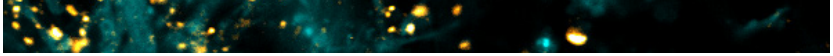
MARIE SCHÖL

Julius-Maximilians-Universität Würzburg, Germany

Marie Schöl studied Biology at the Albert-Ludwigs-Universität in Freiburg and at the ETH in Zürich. In 2020, she moved to Würzburg to start her PhD at the Institute of Virology and Immunology under the supervision of Prof. Dr. Sibylle Schneider-Schaulies and Prof. Dr. Lars Dölken.

Marie`s project is part of the GRK2581 -"Sphingolipids in Infection" and focusses on the role of the neutral sphingomyelinase 2 (NSM2) in T cells. She is specifically interested in the interactome of NSM2 and its changes upon activation. To resolve the interactome she uses proximity-dependent biotin labelling with the engineered peroxidase APEX2 in combination with mass spectrometry.





Stable expression of APEX-tagged neutral sphingomyelinase 2 (NSM2) in T cells to identify effector proteins

Marie Schöl[1], Burkhard Kleuser[2], Thomas Hennig[1], Francisco Zapatero[3], Gisa Gerold[3], Marco van Ham[4], Lothar Jänsch[4], Elita Avota[1], Florian Erhard[1], Sibylle Schneider-Schaulies[1] and Lars Dölken[1]

[1]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany
[2] Institute of Pharmacy, Free University of Berlin, Berlin, Germany
[3] Department of Biochemistry & Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Hannover, Germany
[4] Department of Cellular Proteomics, Helmholtz Centre for Infection Research, Braunschweig, Germany

E-Mail: marie.schoel@uni-wuerzburg.de

Abstract

Neutral sphingomyelinase 2 (NSM2) is a key enzyme of sphingolipid metabolism that catalyses the conversion of sphingomyelin into ceramide at the cytosolic leaflet of the plasma membrane. In T cells, NSM2 activity essentially regulates cytoskeletal dynamics, cell polarization and T Cell Receptor (TCR) signalling. On spatiotemporally dysregulated activation (as by measles virus (MV) interaction with the T cell surface), NSM2 is centrally involved in T cell paralysis. To shed light on the role of NSM2 in T cells we perform a comparative analysis of the NSM2 interactome under physiological conditions and in response to activation. Proximity-dependent biotin labelling using engineered peroxidase APEX2 combined with mass spectrometry is a powerful tool to investigate protein-protein interactions in living cells. We expressed NSM2-APEX2 in Jurkat T-cells to identify NSM2 interacting proteins under physiological conditions and further monitor changes of those interactors upon activation. Under physiological conditions, the NSM2 interactome contains proteins associated with pathways already known to be NSM2 dependent like PM-Golgi trafficking, lipid metabolism or exosome secretion. The dataset provides also evidence for as yet novel NSM2 interacting proteins of which the role in NSM2 activation or downstream signalling needs to be established.

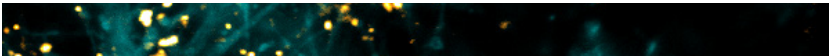
POSTER PRESENTER

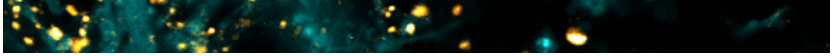
CHRISTINE STERNSTEIN

Julius-Maximilians-Universität Würzburg, Germany

Christine Sternstein studied chemistry at the University of Würzburg and finished her master studies in 2018 in the area of bioorganic chemistry with her thesis regarding the synthesis of functionalised sphingolipids.

In 2019, she continued her research as a PhD student in the group of Prof. Dr. Jürgen Seibel with the investigation of lipids and their metabolism. As an organic chemist, she contributes to this interdisciplinary project by the design and total synthesis of tailor-made sphingolipids and complex cholesterol conjugates. These derivatives of natural substances find their application in biological experiments. The functionalised lipids enable the tracking of the lipid metabolism as well as their localisation within a biological system.





Development of an effective functionalised lipid anchor for membranes (FLAME) as temporary marker for mesenchymal stromal cells

Christine Sternstein*, Stephan Altmann, Theresa-Maria Böhm, Cenk Gurdap, Julian Fink, Jessica Meyer, Erdinc Sezgin, Regina Ebert, Jürgen Seibel.

**Institute of Organic Chemistry, Julius-Maximilians-University of Wuerzburg*

E-Mail: christine.sternstein@uni-wuerzburg.de

The glycosylation of cellular membranes – the glycocalyx – is crucial for the survival and communication of cells. Associated with cancer and other diseases, the investigation of this carbohydrate coating is of high scientific interest.[1] As our target is the engineering of the glycocalyx, we designed a functionalised lipid anchor for the introduction in cellular membranes called FLAME. Since cholesterol introduces very effectively into membranes,[2, 3] we developed a twice cholesterol-substituted anchor in a total synthesis by applying orthogonal protecting group chemistry. We labelled the compound with a fluorescent dye, which qualifies the compound for visualising cells. FLAME was successfully incorporated in the membranes of mesenchymal stromal cells (MSCs) as a temporary marker.

The availability of an azido function - a bioorthogonal[4] reacting group within the compound - enables the convenient coupling of alkyne-functionalised molecules allowing the final step without protecting groups. Furthermore, toxicity assays with living mesenchymal stromal cells were performed, revealing no influence on proliferation and apoptosis. These properties combined render FLAME useful for the addition of molecules to the membranes of MSCs.

Cholesterol anchors tend to insert into the lipid-disordered phase of giant unilamellar vesicles due to poor interaction between the cholesterol moieties and sphingomyelin.[2] The influence of FLAME on the sphingolipid distribution in membranes will be investigated in future experiments.

Literature:

[1] L. Möckl, K. Pedram, A. R. Roy, V. Krishnan, A.-K. Gustavsson, O. Dorigo, C. R. Bertozzi, W. E. Moerner, *Dev. Cell* **2019**, *50*, 57-72.e56.

[2] N. Stuhr-Hansen, J. Madl, S. Villringer, U. Aili, W. Römer, O. Blixt, *ChemBioChem* **2016**, *17*, 1403-1406.

[3] B. R. Peterson, *Org. Biomol. Chem.* **2005**, *3*, 3607-3612.

[4] C. Besanceney-Webler, H. Jiang, T. Zheng, L. Feng, D. Soriano del Amo, W. Wang, L. M. Klivansky, F. L. Marlow, Y. Liu, P. Wu, *Angew. Chem.* **2011**, *123*, 8201-8206.

POSTER PRESENTER

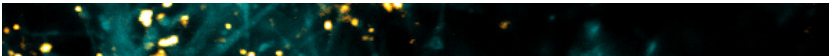
FABIENNE WAGNER

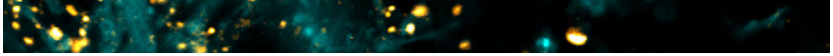
Julius-Maximilians-Universität Würzburg, Germany

I did my Bachelor's and Master's degree in Biochemistry at the Julius-Maximilians Universität of Würzburg.

After obtaining my Master's degree, I left academia and moved to Karlsruhe to work for L'Oréal Produktion Deutschland GmbH u. Co. KG. There I was working as a Junior Quality Manager and gathered insights in quality management and quality assurance processes.

In 2020, I decided to go back to academia and back to Würzburg to do my PhD in the laboratory of Prof. Thomas Rudel at the chair of Microbiology. I am a member of the GRK2581 and I am in my 3rd year of my PhD program. My research project focuses on the role of sphingolipids during *Chlamydia trachomatis* infection of phagocytic cells more precisely neutrophils and M2-like macrophages.





Sphingolipids interfere with infection and replication of *C. trachomatis* inside phagocytic cells

Fabienne Wagner, Adriana Moldovan, +Fabian Schumacher,
+Burkhard Kleuser and Thomas Rudel
Chair of Microbiology, Julius-Maximilians-Universität of Würzburg, Germany
+Institute of Pharmacy, Freie Universität Berlin, Germany
E-Mail: fabienne.wagner@uni-wuerzburg.de

Chlamydia trachomatis (*C.tr.*) is the leading cause of sexually transmitted diseases and infectious blindness. Even though attempts for its development started more than 100 years ago, no vaccine against *C.tr.* is available so far. As we get closer to the post-antibiotic era alternative antimicrobial therapeutics need to be developed. Sphingolipids, a class of membrane lipids and bioactive compounds, have been shown to play a major role in various infectious diseases and therefore are of interest as potential treatment strategy. In case of *C.tr.* infection, it was shown that neutrophils, the first effector cells at the site of infection, are paralyzed by *C.tr.*. Consequently, their activation and apoptosis are inhibited. Furthermore, *C.tr.* can survive within these phagocytic cells and might use them as Trojan horse to infect deeper tissues. We investigated the role of sphingolipids during neutrophil response to *C.tr.* infection by performing sphingolipid profiling. First results indicate that sphingosine is involved in survival pathways and potentially operates during host cell defense mechanisms. The concrete antimicrobial activity now remains to be investigated. Macrophages are another type of phagocytic cells that are involved in clearing of infections. While M1 macrophages increase inflammation and stimulate the immune system, M2 macrophages on the other hand decrease inflammation and support wound healing processes. Recent findings show that *C.tr.* can infect M2-like macrophages and form infectious progeny. Still, it is not known whether *C.tr.* development inside these phagocytic cells depends on the same sphingolipid metabolites as in Hela cells, in which the ceramide and sphingomyelin dependency is well characterized. To gain first insights, a study with established inhibitors was performed and the ceramide dependency could be confirmed. There seems to be no tissue tropism regarding the need for ceramide. The exact transport routes for ceramide acquisition need to be elucidated and will be investigated further.

Literature:

1. de la Maza LM, Zhong G, Brunham RC. Update on Chlamydia trachomatis Vaccinology. Clin Vaccine Immunol. 2017 Apr 5;24(4):e00543-16. doi: 10.1128/CVI.00543-16. PMID: 28228394; PMCID: PMC5382834.
2. Zucca M, Savoia D. The post-antibiotic era: promising developments in the therapy of infectious diseases. Int J Biomed Sci. 2010 Jun;6(2):77-86. PMID: 23675180; PMCID: PMC3614743.
3. Kunz TC, Kozjak-Pavlovic V. Diverse Facets of Sphingolipid Involvement in Bacterial Infections. Front Cell Dev Biol. 2019 Sep 19;7:203. doi: 10.3389/fcell.2019.00203. PMID: 31608278; PMCID: PMC6761390.
4. Rajeeve K, Das S, Prusty BK, Rudel T. Chlamydia trachomatis paralyses neutrophils to evade the host innate immune response. Nat Microbiol. 2018 Jul;3(7):824-835. doi: 10.1038/s41564-018-0182-y. Epub 2018 Jun 25. PMID: 29946164.
5. Tietzel I, Quayle AJ, Carabeo RA. Alternatively Activated Macrophages Are Host Cells for Chlamydia trachomatis and Reverse Anti-chlamydial Classically Activated Macrophages. Front Microbiol. 2019 May 7;10:919. doi: 10.3389/fmicb.2019.00919. PMID: 31134002; PMCID: PMC6524708.

ORGANIZER

Research Training Group 2581
University of Würzburg
Speaker: Prof. Juergen Seibel

https://www.uni-wuerzburg.de/grk2581

SYMPOSIUM LOCATION

Juliusspital Zehntscheune
Klinikstraße 1
97070 Würzburg