17th

Mucins in Health and Disease

17-20 June 2024 • Gothenburg • Sweden

A Marcus Wallenberg symposium

Program

www.mucinconference.com

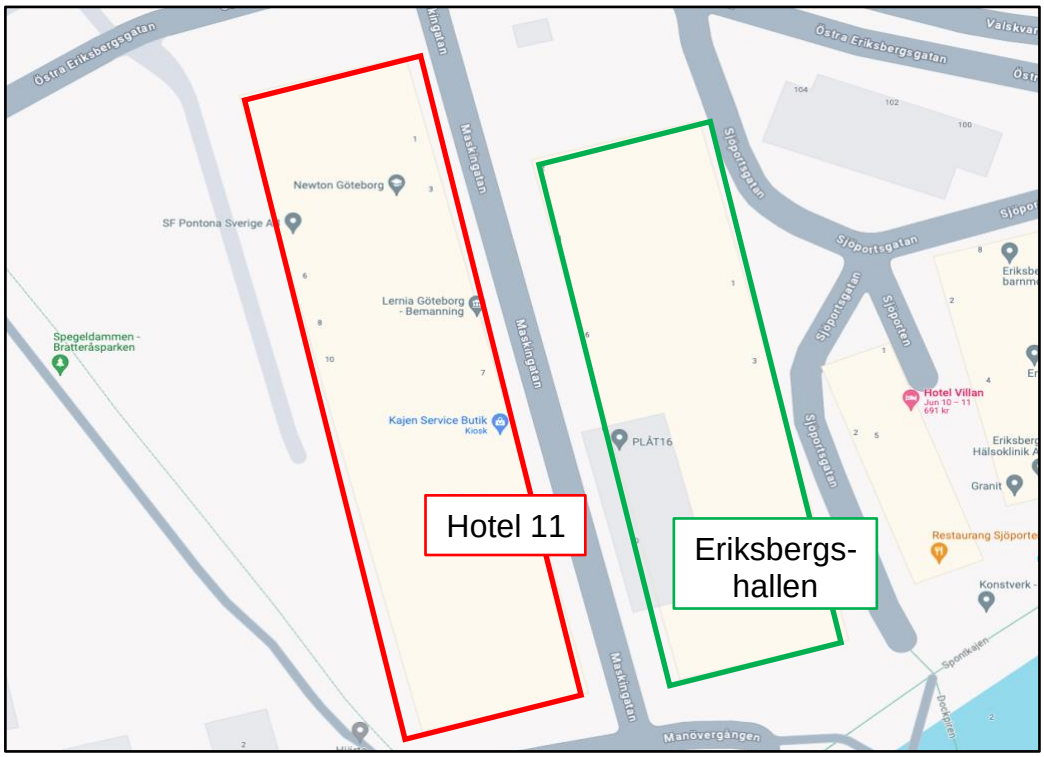
twitter.com/mucinmeeting

#mucin2024

CONTENT

Practical information

Venue
Hotel 11
Maskingatan 11
417 64 Gothenburg
Telephone: +46 31 779 11 11
Fax: +46 31 779 11 10
Email: q.11@strawberry.se



Opening hours Hotel 11 restaurants and bar

The Social Bar & Bistro
Monday – Saturday: 5 pm – 11.30 pm

Eriksbergshallen (Dinners)
Monday – Friday 7 pm – 11.00 pm

Dinner recommendation for Sunday June 16th.
Hotel 11 restaurants are closed Sunday June 16th. We recommend the following restaurants:

Restaurang Faro: 1 pm – 11 pm (3 min walk from Hotel 11)
Italian restaurant

Feskarbrittas kro: 12 pm – 8 pm (3 min walk from Hotel 11)
Seafood restaurant

Atlantica: 3 pm – 09.30 pm (5 min walk from Hotel 11)
American restaurant

Krog Axet: 11 am – 11 pm (7 min walk from Hotel 11)
Modern Beer Hall

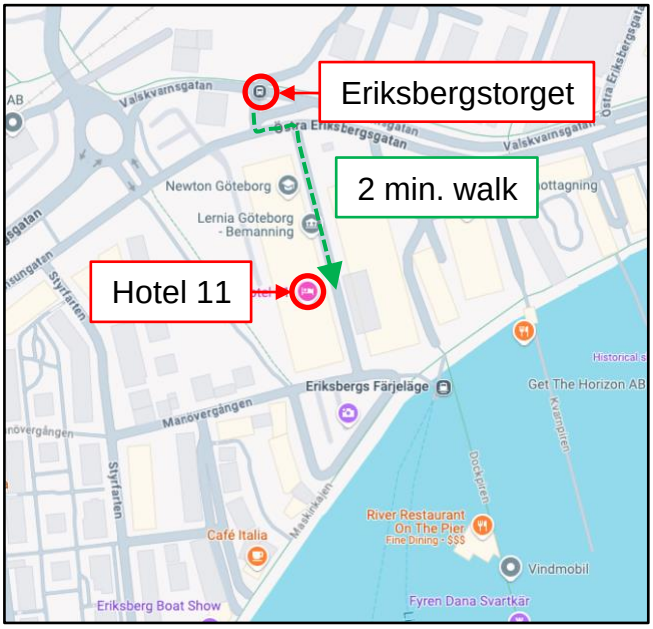
Travel directions by public transport

From Landvetter Airport (GOT)

• Flygbussarna (LINK)
Direct shuttle bus to Eriksbergstorget departs every 40 minutes.
Travel time 45 min.139.00 SEK pp.

From Gothenburg Central station

• Västtrafik (LINK)
Bus 21 to Eriksbergstorget departs every 11 minutes.
Travel time 15 min. 36.00 SEK pp.
Pay with debit/credit card on board.



How to get there by car

From Copenhagen/Malmö (3.5-4 hours)
Take E20 toward Malmö. From Malmö, take E6 toward Gothenburg. At Åbro-motet, take Söderleden mot Västra Frölunda. From Västra Frölunda, take Västerleden to Älvsborgsbron. After Älvsborgsbron, take exit toward Västra Eriksbergsgatan. Turn left onto Västra Eriksbergsgatan. At roundabout, take 1st exit onto Säterigatan. Säterigatan turns left and becomes Östra Eriksbergsgatan. Turn right into Propellergången for parking.
Google maps route (LINK)

From Stockholm (5-6 hours)
Take E4 toward Jönköping. After Jönköping, take road 40 toward Gothenburg. In Gothenburg, take Kungsbackaleden and take exit 75a to merge onto E45 toward Centrum. Continue onto Oscarsleden. Slight right onto the ramp to Oslo/Hisingen. Merge onto Älvsborgsbron. After Älvsborgsbron, take exit toward Västra Eriksbergsgatan. Turn left onto Västra Eriksbergsgatan. At roundabout, take 1st exit onto Säterigatan. Säterigatan turns left and becomes Östra Eriksbergsgatan. Turn right into Propellergången for parking.
Google maps route (LINK)

Taxi services

Costs approximately SEK 550 to/from Landvetter Airport (GOT). Ask for a fixed price.

The driver should have a taxi ID card clearly displayed in the vehicle. Service is included in the taximeter price. Avoid unlicensed taxis.

We recommend:
Taxi Göteborg: +46 (0) 31 650 00
VIP Taxi: +46(0) 31 27 16 11
Taxi Kurir: +46 (0) 31 27 27 27

Sightseeing in Gothenburg

Visit Gothenburg's official tourist service for attractions and sightseeing (LINK)
Also visit the conference website for recommendations (LINK)

Checking in at the Conference

Early check-in: Sunday 16th June, 18.00-20.00 pm at Hotel 11.

Late check-in: Monday 27th June, 08.00-09:00 am at Hotel 11.

For presenters

Please see session chair 30 minutes prior to the start of your session.
Bring your presentation on a memory stick.

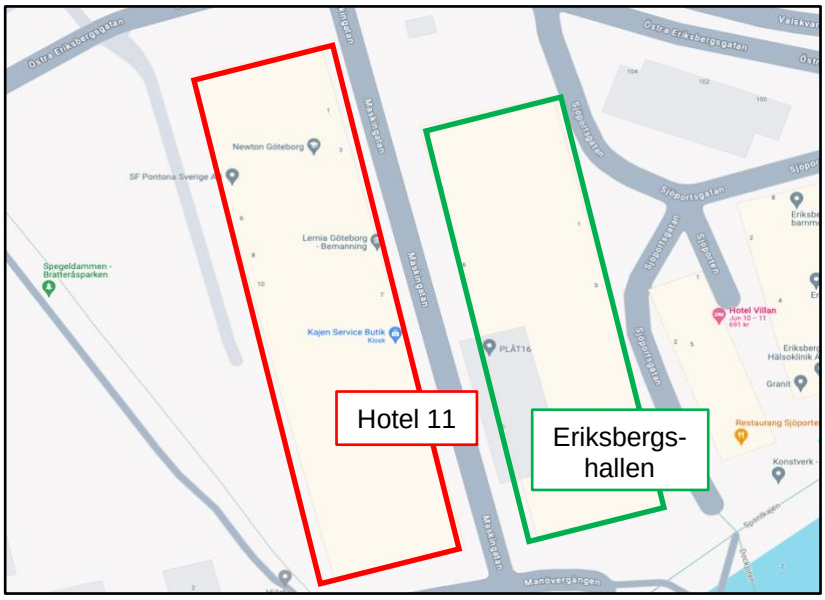
Invited speakers
Oral presentations are 20 minutes long, followed by 5-7 min of questions from the audience.

Selected oral presentation
Oral presentations are 10 minutes long, followed by 5 min of questions from the audience.

Poster presentation
Prepare your poster in portrait format (90 cm x 120 cm or 35.4" x 47.2"). The width must not exceed 90 cm.
Poster should be put up in poster area(s) on Monday 17th June, 13.00-13.30.
Poster should be removed by Thursday 20th June, 13.30.

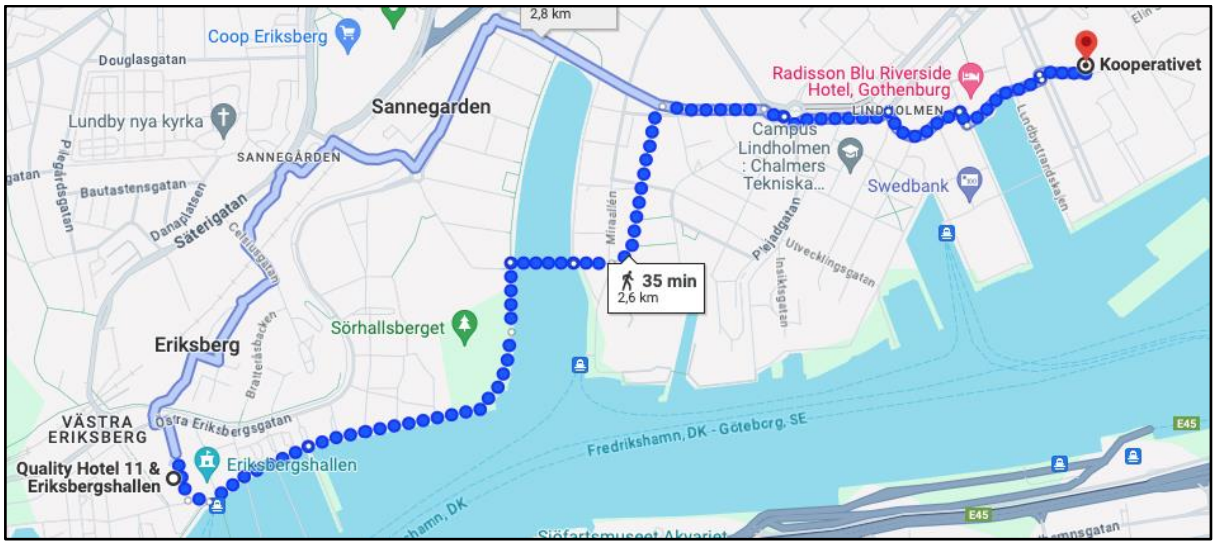
Social program

Monday 17th June
19.00-21.00 Welcome reception and dinner
Location: Hotel 11 (Eriksbergshallen)
Join the official reception sponsored by the City of Gothenburg and Region Västra Götaland.



Tuesday 18th June
15.30-17.30 Guided boat tour in Gothenburg harbor
Departure from Hotel 11 at 15.30 (don't be late!)
Enjoy a guided boat tour in Gothenburg harbor.

Wednesday 19th June
19.00-00.30 Conference dinner and party
Location: Kooperativet Lindholmen, Anders Carlssons gata 2 (LINK). 35 min. walk.
Join us for a night of celebration at Kooperativet Lindholmen, a vibrant venue housed in a historic shipyard building in the heart of Gothenburg. Dinner kicks off at 7:00 PM (19:00), featuring live jazz entertainment throughout the dinner. As the night unfolds, a DJ will take over, turning up the energy for an after-dinner dance party! Don't miss this chance to connect with colleagues and unwind after a productive conference.



GOLD SPONSORS

MARCUS WALLENBERG FOUNDATION FOR INTERNATIONAL SCIENTIFIC COLLABORATION

Wenner-Gren Stiftelserna
Wenner-Gren Foundations

City of Gothenburg

REGION VÄSTRA GÖTALAND

SILVER SPONSORS

MUCOSAL IMMUNOLOGY
Official Journal of the Society for Mucosal Immunology

GENOVIS

BRONZE SPONSORS

Cirqle

Appendix 1

17th Mucins in Health and Disease

17-20 June 2024 • Gothenburg • Sweden

CONFERENCE PROGRAM AT A GLANCE

Conference day
Session
Breaks, Dining, Activities

Sunday 16th June		
18:00 – 20:00	**Pre-registration (Hotel 11)**	
20:00 – 22:00	**Informal gathering: *Get to know each other!***	
Monday 17th June		
08:00 – 10.25	***Session 1: Keynote***	
8:00 – 9:00	**Registration**	
9:00 – 9:20	**Welcome by the City of Gothenburg and Region Västra Götaland: Lord Mayor Aslan Akbas**	
9:20 – 10.30	**Keynote**	**Carolyn R. Bertozzi**
10:20 – 11:00	***Coffee break***	
11:00 – 12:00	***Session 2: Mucins Past Present Future***	***CHAIRS: Gunnar C. Hansson and Burton Dickey***
11:00 – 11:30	Dave J. Thornton	Secreted mucins – The frontline of host defence
11:30 – 12:00	Sandra Gendler	A Mucin Odyssey – Tales of MUC1 and other Transmembrane Mucins
12:00 – 13:00	***Lunch***	
13:00 – 13:30	***Poster hanging + Mingle***	
13:30 – 15:30	***Session 3: Adaptation of Mucins and Mucous Cells***	***CHAIRS: Kris Chadee and Shai Bel***
13:30 – 14:00	Elisabeth Plender	Structural and genetic diversity in the secreted mucins, MUC5AC and MUC5B
14:00 – 14:30	Petar Pajic	A mechanism of gene evolution generating mucin function
14:30 – 14:45	***Short break***	
14:45 – 15:00	Sophie Janssens	AGR2 as a novel regulator of IRE1b in goblet cell quality control
15:00 – 15:15	Ana M. Jaramillo	MIA3 is a specialized trafficking protein that is required for MUC5AC production
15:15 – 15:30	John D. Dickinson	Autophagy regulates lysosome-mediated degradation of mucin granules
15:30 – 16:00	***Coffee break***	

16.00 – 18.00	***Poster session (Odd poster numbers)***	
19:00	***Welcome reception by City of Gothenburg and Region Västra Götaland***	
	Dinner at Eriksbergshallen	
Tuesday 18th June		
9:00 – 10:30	***Session 4: Models in Mucin Research***	***CHAIRS: Sara K. Linden and Maitrayee Chatterjee***
9:00 – 9:30	Douglas Fudge	Fiber-reinforced slime in hagfishes
9:30 – 10:00	Kelly Ten Hagen	A novel Cysteine-rich adaptor protein is required for mucin packaging and secretory granule stability in vivo
10:00 – 10:15	Thaher Pelaseyed	MUC17 is an essential small intestinal glycocalyx component that is disrupted in Crohn's disease
10:15 – 10:30	Brendan Dolan	The role of mucus and secreted mucins in the clearance of the normal airways
10:30 – 11:00	***Coffee break***	
11:00 – 12:45	***Session 5: Mucin Biosynthesis and Processing***	***CHAIRS: Michael Tuvim and Marcelo Guerin***
11:00 – 11:30	Bart Lambrecht	New mechanisms in type 2 inflammation
11:30 – 12:00	Deborah Fass	Evolutionary Mechanisms for Generating Functional Diversity in Gel-Forming Mucins
12:45 – 12:10	Ori Avinoam	The Unique Exocytosis Mechanism of Large Vesicles in Exocrine Tissues
12:15 – 12:30	Christian V. Recktenwald	The generation of immunogenic gluten peptides occurs during their Goblet cell-mediated transport - Implications for Celiac Disease
12:30 – 13:30	***Lunch***	
14:00 – 15:00	***Poster session + Coffee (Even poster numbers)***	
15:30– 17:30	***Guided boat tour***	
18:00 – 19:00	***Interactive session (Eriksbergshallen)***	
18:00 – 19:00	*Meet the expert*	
19:00 – 20:30	***Dinner at Eriksbergshallen***	
Wednesday 19th June		
9:00 – 10:30	***Session 6: Mucin Biophysics***	***CHAIRS: Manfred Frick and Scott Danielsen***
9:00 – 9:30	Katharina Ribbeck	Mucin Glycans and Microbial Virulence: Exploring Therapeutic Potential in Host-Derived Molecules
9:30 – 10:00	Gregg Duncan	Mucus gels and innate lung defense
10:00 – 10:15	Maude A. Liegeois	Peroxidase-mediated mucin cross-linking drives pathologic mucus gel formation in IL-13-stimulated airway epithelial cells

10:15 – 10:30	Thomas Crouzier	Topical reinforcement of the cervical mucus barrier to sperm
10:30 – 11:00	***Coffee break***	
11:00 – 12:30	***Session 7: O-glycosylation of Mucins***	***CHAIRS: Filipa Marcelo and Ana Luis***
11:00 – 11:30	Yoshiki Narimatsu	Cell-based Mucin Array for Discovery and Characterization of Mucinases and Carbohydrate Binding Modules
11:30 – 12:00	Stacy Malaker	Recent developments in the MS-based analysis of mucin-domain glycoproteins
12:00 – 12:15	Carol de Ram	Mucin O-glycan degradation patterns of Akkermansia muciniphila, Ruminoccocus torques, and a synthetic gut-associated microbial community
12:15 – 12:30	Geraldine Nouailles	Unraveling the role of α-(1,2)-fucosylation in bacterial pneumonia
12:30 – 13:30	***Lunch at Hotel 11***	
14:00 – 15:45	***Session 8: Mucin-Microbe Interactions***	***CHAIRS: Raphael Valdivia and George Birchenough***
14:00 – 14:30	Nathalie Juge	Mucin glycan foragers in the human gut: the case of Ruminococcus gnavus
14:30 – 15:00	Maher Abou Hachem	The adaptation hallmarks of Akkermansia muciniphila enzymes to human O-glycans
15:30 – 16:00	***Coffee break***	
16:00 – 16:15	Mikael E. Sellin	Maturation of Human Intestinal Epithelia involves Fortification of the Apical Surface against Salmonella Attack
16:15 – 16:30	Elena Fekete	Characterization of the founding member of the first CBM family known to target sulfated glycans
16:30 – 16:45	Jinyi Su	Salmonella enterocyte invasion through MUC1: contributions of the extracellular domain and cytoplasmic tail to invasion and signaling
Gather in lobby at 18:30		
19:00 – 00:00	***Conference dinner and party at Kooperativet***	

Thursday 20th June

9:00 – 10:30	***Session 9: Mucins in Cancer***	***CHAIRS: Francois Boudreau and Nicolas Jonckheere***
9:00 – 9:30	Motohiro Kobayashi	Mucins in cancer: Potential role of sulfated glycans
9:30 – 10:00	Matthew Paszek	Resolving the role of mucins in extracellular vesicle biology
10:00 – 10:15	Lars Vereecke	Mucus and epithelial barrier defects promote pks+ driven colorectal cancer development through adhesin-mediated binding mechanisms
10:15 – 10:30	Cory L. Brooks	Structural Basis for MUC16 Recognition and Associated Anti-Pancreatic Cancer Activity of Humanized Antibody AR9.6
10:30 – 11:00	***Coffee break + Take down posters***	
11:00 – 12:30	***Session 10: Mucins in Infection and Inflammation***	***CHAIRS: Mindy Engevik and Jenny Gustafsson***

11:00 – 11:30	Ben Medoff	Mucus Plugging in Asthma – More than an Obstruction to Breathing
11:30 – 12:00	Annemieke Smet	The role of mucins and their isoforms in barrier dysfunction and patient stratification
12:00 – 12:15	Laura Campbell	Goblet cells and the circadian clock: a new regulatory mechanism
12:15 – 12:30	Marilena Bohley	Using mucinases to enhance oral delivery of macromolecules
12:30	Final remarks from the organizing committee	
12:30 – 13:30	***Lunch at Hotel 11***	
13:30	***Departure***	

Appendix 2

Mucins in Health and Disease

17-20 June 2024 • Gothenburg • Sweden

Abstracts for invited and selected talks

KEYNOTE SPEAKER

Titel

Carolyn R. Bertozzi[1]

1. Department of Chemistry, Stanford University, USA.

INVITED SPEAKER

Secreted mucins – The frontline of host defence

David J. Thornton[1]

1. Wellcome Centre for Cell-Matrix Research and Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, The University of Manchester

Mucus coats our mucosal surfaces and acts a gatekeeper denying access to harmful agents trying to enter the body. Mucus has bespoke properties at each mucosal site that are tailored to protect against diverse external challenges in terms of its physical and bioactive properties – for example in the colon there is a thick, bi-layered, adherent mucus. Whereas in the lung there much thinner, flowing mucus layer. Dysregulation of the mucus phenotype is associated with disease. For example, a change to a thin, patchy mucus layer in the gut is a feature of inflammatory bowel disease, while a change to a thick, adherent mucus in the lung is a feature of obstructive lung disease.

The structure and protective functions of mucus are provided by a family of closely related, network-forming, multi-domain glycoprotein polymers, the gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6 and MUC19). Animals deficient in gel-forming mucins have been shown to be compromised in host defence. A dominant feature of these mucins is their extensive modification with a diverse array of O-glycan chains, and the different mucins display distinct patterns of O-glycans. These glycans are key drivers of mucin function, including their regulation of microbes and immune cells.

While great progress has been made on many aspects of gel-forming mucin biology, for example, high-resolution structures are now available for the different protein domains within the mucins, the structural basis of mucus barrier formation by mucins, how mucins are synthesised and stored within secretory cells, the molecular components/pathways involved in their secretion, and that changes in mucins can result from external challenges, in particular by pathogens. However, we still lack a complete understanding of the regulation of mucus organisation, and knowledge of how mucins changes with age, infection, and inflammation is under-developed. Expanding our knowledge of these key areas is needed to understand how changes in mucins impact the protective function of mucus in health and disease.

INVITED SPEAKER

A Mucin Odyssey – Tales of MUC1 and other Transmembrane Mucins

Sandra Gendler[1]

1. Department of Immunology, Mayo Clinic, Mayo Clinic Arizona, Scottsdale and Phoenix, AZ, USA

The early years of studying mucins, in particular MUC1, were confusing. One of my earliest introductions to mucins was at the mucus club which was held at various locations in the UK in the mid1980s. Dr. John Sheehan was a pioneer in mucus studies, and he started one meeting with a slide showing a mucin strand extending through three slides in length. The size was a difficult concept, and many of us wondered what we were getting into.
We began the molecular search for mucin proteins with antibodies developed by Dr. Joyce Taylor-Papadimitriou in 1981. The antibodies, now known as HMFG-1 and HMFG-2, were made by immunizing mice with milk fat globule membrane from early lactation milk from women. The repetitive epitopes of the antibodies were confusing, but ultimately led to the discovery that mucins were made up of tandem repeats containing high levels of serine, threonine, and O-linked glycosylation. Similar stories can be told about several of the other large transmembrane mucins. The well-known CA125 antibody developed by Dr. Robert Bast in 1981 recognized the surface of ovarian tumors. The carboxy and amino termini of CA125 were identified 20 years later, in 2001, and described the exceedingly large mucin, MUC16, with a core protein weight of about 2.5 million Daltons and a potential glycosylated molecular mass of twice that. In 2024, long-read sequencing would change the number of repeats from 63 to 19. It seems that the exact molecular sequence and structure of MUC16 are not yet defined. Many of the mucins undergo alternate splicing, resulting in a functionally diverse repertoire of mucin proteins which makes them difficult to characterize. Understanding the gene structure of the transmembrane mucins will aid functional studies and expose therapeutic targets as many of these mucin proteins function in health and disease.

INVITED SPEAKER

Structural and genetic diversity in the secreted mucins, MUC5AC and MUC5B

Elizabeth Plender[1,2], Timofey Prodanov[4], PingHsun Hsieh[1], Evangelos Nizamis[1,3], William Harvey[1], Arvis Sulovari[1], Katherine Munson[1], Eli Kaufman[1,3], Paul Valdmanis[1,3], Wanda O'Neal[5], Tobias Marschall[4], Jesse Bloom[1,2,6], & Evan Eichler[1,7]

1. Department of Genome Sciences, University of Washington School of Medicine
2. Basic Sciences Division, Fred Hutch Cancer Research Center
3. Division of Medical Genetics, University of Washington School of Medicine
4. Institute for Medical Biometry and Bioinformatics, Heinrich Heine University Düsseldorf
5. Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill
6. Howard Hughes Medical Institute, Fred Hutch Cancer Research Center
7. Howard Hughes Medical Institute, University of Washington

Few loci in the human genome have been linked to severe respiratory disease outcomes; the mucin genes, however, are strong candidates due to their critical roles in pathogen entrapment. MUC5AC and MUC5B encode two secreted proteins with variable number tandem repeat sequences (VNTRs) that enable binding to pathogens. These loci have been difficult to assess due to the large and highly polymorphic protein-coding VNTRs. Here, we characterize the genetic structural diversity of MUC5AC and MUC5B by long-read sequencing and assembly of 206 human and 12 non-human primate haplotypes. We find that MUC5B is largely invariant in length (5761-5762aa) in humans; however, 7 haplotypes encode a much larger protein (6291-7019aa) due to a VNTR expansion. In contrast, we discovered 30 allelic variants of MUC5AC that encode 16 distinct proteins (5249-6325aa) with considerable variation in cys domain copy number and VNTR copy/domain number. We show that MUC5AC alleles can be grouped into three phylogenetic clades: H1 (46%, ~ 5654aa), H2 (33%, ~5742aa), and H3 (7%,~ 6325aa). Comparison to non-human ape gene models relative to the two most common human MUC5AC variants (5654aa and 5742aa) suggests an overall reduction in MUC5AC protein length during recent human evolution. Linkage disequilibrium (LD) and Tajima's D analysis reveal that East Asians carry exceptionally large MUC5AC LD blocks and an excess of rare variation ($p<0.05$ autosome-wide). We identified tagging SNPs (tSNPs) in high LD with haplogroups of MUC5AC ($r2 >0.85$; 37 for H1, 7 for H2, 67 for H3) to use for VNTR length imputation. We applied a structural variant genotyping tool called "locityper" to accurately genotype the haplotypes in 2,596 population samples from the 1000 Genomes Project. These findings confirm that the likely ancestral haplotype (H3) has been significantly reduced among Asians at the expense of H1/H2, which show signatures of positive selection. We predict population differences to airway associated disease and provide a more broadly applicable strategy to characterize the genetic impact of the hundreds of complex protein-encoding VNTRs that can now be resolved with long-read sequencing.

INVITED SPEAKER

A mechanism of gene evolution generating mucin function

Petar Pajic[1], Shichen Shen[1], Jun Qu[1], Alison J. May[1], Sarah Knox[1], Stefan Ruhl[1], and Omer Gokcumen[1]

1. Evolutionary Genetics, Biological Sciences, University at Buffalo, USA

How novel gene functions evolve is a fundamental question in biology. Mucin proteins, a functionally but not evolutionarily defined group of proteins, allow the study of convergent evolution of gene function. By analyzing the genomic variation of mucins across a wide range of mammalian genomes, we propose that exonic repeats and their copy number variation contribute substantially to the de novo evolution of new gene functions. By integrating bioinformatic, phylogenetic, proteomic, and immunohistochemical approaches, we identified 15 undescribed instances of evolutionary convergence, where novel mucins originated by gaining densely O-glycosylated exonic repeat domains. Our results suggest that secreted proteins rich in proline are natural precursors for acquiring mucin function. Our findings have broad implications for understanding the role of exonic repeats in the parallel evolution of new gene functions, especially those involving protein glycosylation.

SELECTED SPEAKER

AGR2 as a novel regulator of IRE1b in goblet cell quality control

Phaedra Guilbert[1,2], Eva Cloots[1,2], Mathias Provost[2,3], Farzaneh Fayazpour[1,2], Evelien Van de Velde[1,2], Delphine De Sutter[4,5], Savvas Savvides[2,3], Sven Eyckerman[4,5,*], Sophie Janssens[1,2,*]

1. Department of Pediatrics and Internal Medicine, Ghent University, Ghent, Belgium
2. VIB Center for Inflammation Research, Ghent, Belgium
3. Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
4. Department of Biomolecular Medicine, Ghent University, Ghent, Belgium,
5. VIB Center for Medical Biotechnology, Ghent, Belgium

*Contributed equally

The gastrointestinal tract is protected by a mucus layer, mainly consisting of the large glycoprotein MUC2. MUC2 misfolding leads to ER stress, which is mitigated by the Unfolded Protein Response (UPR). Aberrations in the UPR or mucin folding are associated with inflammatory bowel disease (IBD). Anterior Gradient 2 Homolog (AGR2) is a protein disulfide isomerase involved in maturation of mucins, and loss of function mutations have been identified in IBD. The goblet cell (GC)-specific UPR sensor Inositol-requiring Enzyme (IRE)1β protects ER integrity through degradation of excess Muc2 mRNA, but how IRE1β activity is regulated has remained unknown. We identified AGR2 as a regulator of IRE1β. AGR2 interacts specifically with IRE1β in both the human LS174T GC-like cell line and murine colon tissue and inhibits IRE1β through disruption of the catalytically active IRE1β dimer (Cloots et al, EMBO J, 2024; Neidhardt et al., EMBO J 2024; Bertolotti et al., 2024). We found that AGR2 mutants lacking their catalytic cysteine or displaying the disease-associated mutation H117Y were no longer able to dampen IRE1β activity. In vivo, Agr2 deficient mice exhibit depleted Muc2 mRNA levels, that are restored upon compound deficiency with IRE1β. This supports a model where AGR2 functions as a rheostat coupling IRE1β activity to the mucus folding load experienced by GC chaperones such as AGR2.

1. Cloots E, Guilbert P, Provost M, Neidhardt L, Van De Velde E, Fayazpour F, De Sutter D, Savvides S, Eyckerman S, Janssens S (2023). Activation of goblet cell stress sensor IRE1β is controlled by the mucin chaperone AGR2. The EMBO Journal Feb 2 PMID: 38177501 DOI: 10.1038/s44318-023-00015-y
2. Neidhardt L, Cloots E, Friemel N, Weiss CAM, Harding HP, McLaughlin SH, Janssens S, Ron D. The IRE1b-mediated unfolded protein response is repressed by the chaperone AGR2 in mucin producing cells (2023) The EMBO Journal Feb 2 PMID: 38177498 DOI: 10.1038/s44318-023-00014-z
3. Bertolotti A (2024) Keeping goblet cells unstressed. EMBO J Feb 2 PMID: 38308018 https://doi.org/10.1038/s44318-024-00041-4

SELECTED SPEAKER

MIA3 is a specialized trafficking protein that is required for MUC5AC production

Ana M Jaramillo[1], Eszter K Vladar[1], Christopher M Evans[1]

1. Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado School of Medicine

Mucus is required for airway defense, but in muco-obstructive diseases such as asthma, mucus is abundant, thick, and poorly transported. Mucus dysfunction is not adequately treated with existing treatments, making it essential to improve our mechanistic understanding of mucin biosynthesis and secretion.
Typically, secretory proteins are produced in two membrane bound intracellular compartments. First, in the endoplasmic reticulum (ER), proteins are folded and stabilized by disulfide bond formation. Late stages of biosynthesis occur in the Golgi apparatus, where sugars are added and secretory cargo is packaged into vesicles fated for secretion. For most secretory proteins, transport between these compartments is also mediated by vesicle transport. These folded proteins exit the ER via a conventional secretory pathway in vesicles whose sizes are restricted by the geometry of coat proteins (COPII coatamers) that limit vesicle diameters to size ranges of 60-90 nm diameter. Because of their massive sizes (500-1000 nm lengths), mucins are not able to fit into COPII vesicles. This task is made even more difficult when mucins are produced in excess, as they are in asthma.
We have identified a non-conventional pathway used by cells adapted to producing other large and polymeric proteins, such as collagen and fibrinogen. This pathway is led by MIA3, a transmembrane protein that localizes to the ER exit sites and binds to the COPII coatamer. Using immunohistochemistry in human lung asthmatic tissue, we found that MIA3 is expressed in goblet cells and in submucosal glands. We then used siRNAs to knockdown MIA3 in A549 cells, and we found that MIA3 is required for MUC5AC secretion. Furthermore, we used shRNA to knock down MIA3 in primary human bronchial epithelial cells (HBECs) grown at air-liquid interface conditions. We observed a 60% reduction in MUC5AC secretion. To fully knock out MIA3, we used a CRISPR/Cas9 approach and observed a severe phenotype characterized by suppressed mucous cell differentiation. Collectively, these findings identify MIA3 as a critical component in the mucin assembly pathway, with implications for MIA3 as a novel target for blocking mucus dysfunction in muco-obstructive diseases.
Currently, we are further dissecting roles of MIA3 in HBECs to test mechanistic functions of MIA3, and its two isoforms (long - 1,907 amino acids, and short - 785 amino acids). Both have been shown to assist in ER to Golgi transport during collagen synthesis. We are also generating conditional knockout mice to better understand the expression and function of MIA3 in vivo. These will be applied to studies of assembly and secretion of mucin and collagen.

SELECTED SPEAKER

Autophagy regulates lysosome-mediated degradation of mucin granules

Shailendra Mauyra[1], Katrina Kudrna[1], Elizabeth B. Staab[1], Paul Thomes[2], Bruton F. Dickey[3], John D. Dickinson[1]

1. University Of Nebraska Medical Center.
2. Auburn University
3. M.D. Anderson Cancer Center

Introduction: Airway diseases such as COPD and asthma are characterized by epithelial mucous metaplasia leading to hypersecretion of mucin granules and subsequent airway obstruction. The factors regulating resolution from mucous metaplasia are poorly understood. We hypothesize that the autophagy pathway is required to eliminate non-secreted mucin granules and present the concept of the mucin cycle to comprehensively explain the factors that regulate mucin granule content of secretory cells.

Methods: To understand the mechanism of mucin granule elimination across the spectrum of degradation pathway from the autophagosome to the lysosome, we first explored the role of autophagy regulatory genes. A secretory cell specific, tamoxifen (TAM) inducible mouse model was generated by crossing Scgb1a1-creERTM+/- with Atg5flox/flox mice (Atg5-/-). Atg5flox/flox mice without Cre (Atg5 +/+) were utilized as controls. Secondly, to assess the lysosomal end of the degradation pathway, we utilized Calu-3 cell lines and primary human airway epithelial cells (hAECs) treated with pharmacologic inhibitors of lysosomal function.

Results: To determine the role of autophagy on mucin granule degradation under homeostatic conditions, total lung Muc5b levels was measured in Atg5+/+ and Atg5-/- mice after Tam induction. We found small, non-significant increase in Muc5b levels in Atg5-/- mice. In response to T2 inflammatory stimuli, OVA-induced mucous metaplasia, we observed Atg5-/- mouse lungs had significantly increased Muc5ac levels. We found a similar increase in Muc5b in Atg5-/- mouse lungs using a IL-1 beta intra-nasal administration model. We next sought to address the back end of the degradation pathway by directly inhibiting lysosomal activity. Inhibition of lysosomal acidification (bafilomycin A1) or lysosomal cathepsin enzyme activity (PepstatinA1-E64D) led to an accumulation of mucin granules in both Calu-3 cells and hAECs. Inhibition of lysosome activity by PepstatinA1-E64D, led to a detection of increased fusion events between mucin granules and Lamp1 labeled lysosomes as detected by super-resolution microscopy and by transmission electron microscopy. This impact of lysosomal inhibition on mucin accumulation in hAECs was greatest during resolution of mucous metaplasia, after withdrawal of IL-13

Conclusion: We propose a new model, the mucin cycle for regulation of intracellular mucin granules in airway secretory cells. Loss of function studies both at the front and back of end of the autophagy-lysosome degradation pathway suggest that mucin granules are degraded. Minimal degradation occurs under homeostatic conditions. Degradation primarily occurs during resolution.

INVITED SPEAKER

Fiber-reinforced slime in hagfishes

Douglas Fudge[1], Dakota Piorkowski, Andrew Lowe, Sameh H. Tawfick, Kaleb Ducharme, Randy H. Ewoldt, Wonsik Eom, M. Tanver Hossain, Sergei Shalygin, Parastoo Azadi, and David C. Plachetzki

1. Schmid College of Science and Technology, Chapman University, USA

Hagfishes are an ancient group of eel-shaped marine animals that are notorious for defending themselves from predators using large volumes of fibrous slime that they produce within numerous slime glands. The exudate that is ejected from the slime glands consists of two main secretory products, mucous vesicles and slime threads, which are produced from distinct and specialized cell types. When a hagfish is attacked, ejected vesicles and coiled threads interact with seawater and expand by a factor of 10,000 in under 400 milliseconds. Slime deployment involves swelling of the mucous vesicles and unravelling of the coiled threads, resulting in a material that is exceptionally good at conforming to and clogging the gills of predators such as sharks. Hagfishes produce a second kind of fibrous slime from cells in their epidermis, which is produced when the skin is damaged. Epidermal slime also contains mucus and fibers, which are likely the evolutionary precursors of the same components in the glandular slime. In this talk, I will unpack the biophysical mechanisms underlying fibrous slime production and deployment in hagfishes and highlight some of our ongoing research in this area.

INVITED SPEAKER

A novel Cysteine-rich adaptor protein is required for mucin packaging and secretory granule stability in vivo

Liping Zhang[1], Kayla J. Muirhead[1,2], Zulfeqhar A. Syed[1,3], Emilios K. Dimitriadis[4] and Kelly G. Ten Hagen[1*]

Mucins are large, highly glycosylated extracellular matrix proteins that line and protect epithelia of the respiratory, digestive and urogenital tracts. Previous work has shown that mucins form large, interconnected polymeric networks that mediate their biological functions once secreted. However, how these large matrix molecules are compacted and packaged into much smaller secretory granules within cells prior to secretion is largely unknown. Here, we demonstrate that a small cysteine-rich adaptor protein is essential for proper packaging of a secretory mucin in vivo. This adaptor acts via cysteine bonding between itself and the cysteine-rich domain of the mucin. Loss of this adaptor protein disrupts mucin packaging in secretory granules, alters the mobile fraction within granules and results in granules that are larger, more circular and more fragile. Understanding the factors and mechanisms by which mucins and other highly glycosylated matrix proteins are properly packaged and secreted may provide insight into diseases characterized by aberrant mucin secretion.

SELECTED SPEAKER

MUC17 is an essential small intestinal glycocalyx component that is disrupted in Crohn's disease

Elena Layunta[1], Sofia Jäverfelt[1], Fleur C. van de Koolwijk[1], Molly Sivertsson[1], Brendan Dolan[1], Liisa Arike[1], Sara Thulin[1], Bruce A. Vallance2, and Thaher Pelaseyed[1*]

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Box 440, 405 30 Gothenburg, Sweden.
2. Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, BC Children's Hospital Research Institute, University of British Columbia, Vancouver, BC V5Z 4H4, Canada.

Crohn's disease (CD) is the chronic inflammation of the human ileum and colon triggered by unrestrained immune responses to bacteria, but insights into molecular perturbations at the bacteria-epithelium interface are limited. We report here that membrane mucin MUC17 protects small intestinal enterocytes against commensal and pathogenic bacteria. In the non-inflamed ileum of CD patients, reduced MUC17 levels correlated with a compromised apical glycocalyx barrier, allowing direct bacterial contact with enterocytes. Intestinal-specific Muc17 deletion in a new genetic mouse model, Muc17ΔIEC, rendered the small intestine prone to atypical infection by Citrobacter rodentium while maintaining resistance to experimental colitis. Additionally, the loss of Muc17 resulted in the spontaneous deterioration of epithelial homeostasis and extraintestinal translocation of viable bacteria. Finally, Muc17-deficient mice harbored specific small intestinal bacterial taxa observed in CD patients. In conclusion, our work uncovers a role for the MUC17-based glycocalyx as an essential defense system of the small intestine. The disruption of this system in the non-inflamed ileum of CD patients suggests that defective MUC17 biosynthesis or trafficking is an early epithelial defect that precedes inflammation. Given the limited number of mouse models for small intestinal inflammation, the Muc17ΔIEC model provides opportunities for understanding the molecular mechanisms underlying epithelial cell dysfunction in Crohn's disease.

SELECTED SPEAKER

The role of mucus and secreted mucins in the clearance of the normal airways

Brendan Dolan[1], Anna Ermund[1] and Gunnar C. Hansson[1]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden.

The airways are continuously exposed to external challenges, including dust, bacteria, fungi, and viruses. As such there is a requirement for an efficient system of clearance to maintain the essentially sterile nature of the distal airways. In the proximal airway mucus, liquid secretion and the beating of cilia are the key components of this defensive barrier. However, how these different elements of the airway mucus barrier work together remains to be fully elucidated. Through the use of high-resolution live imaging of explanted tracheal tissue from pigs and human donors we have studied the organisation and dynamic function of the proximal airway mucus barrier. In the normal airway the mucus barrier is organised in a hierarchical manner with the tissue surface protected by the beating of cilia and mucus threads, composed of MUC5AC. The airway surface fluid (ASF) contains large amounts of secreted mucus which continuously moves over the airway surface and finally the MUC5B-rich mucus bundles produced by the submucosal glands. Together these structures work to trap foreign material and efficiently remove it from the airways.
This study highlights the complex organisation and dynamic nature of the airway mucus systems. This barrier is composed of both liquid and mucus secretions and together they work in tandem to efficiently remove foreign material from the airways.

INVITED SPEAKER

New mechanisms in type 2 inflammation

Bart N. Lambrecht[1]

1. Director, VIB Center for Inflammation Research, Ghent University, Belgium

Spontaneous protein crystallization is a rare event in vivo, yet Charcot-Leyden crystals (CLC) consisting of the protein galectin-10 (Gal10) are frequently observed in asthma mucus plugs in humans. Pseudo-CLCs made up from other proteins are frequently seen in murine asthma. It is unclear if they exacerbate type 2 inflammation or are just markers for disease activity. Release of Gal10 and extracellular crystallization was associated with EEtosis of eosinophils in human primary eosinophils and patients with allergic mucin. We found that recombinant crystalline Gal10 was completely biosimilar to in vivo obtained CLCs and induced innate airway inflammation, whereas a soluble Gal10 engineered to resist crystallization was inert in the airways. When co-administered with harmless antigens, only crystalline Gal10 stimulated adaptive immunity, Th2 sensitization, goblet cell metaplasia and airway eosinophilia. In lung organoids of severe asthmatics, CLCs triggered mucus production. Transgenic mice engineered to overexpress human Gal10 in eosinophils (Galileo mice), ubiquitously (Galactic mice) or in the lungs only (GalacC) had enhanced features of asthma including mucus plugging and bronchial hyperreactivity. CLCs recruited neutrophils, which subsequently underwent NETosis, which contributed to mucus plug formation. To probe for the drugability of this pathway, we generated a panel of antibodies. Antibodies directed against key epitopes of the crystallization interface of Gal10 dissolved pre-existing CLC in patient-derived mucus within hours, and reversed crystal driven inflammation, goblet cell metaplasia, IgE synthesis and bronchial hyperreactivity in a humanized asthma model. Thus, Gal10 and CLC promote mucus formation and inflammation in asthma and can be targeted by crystal dissolving antibodies. In mice, other protein crystals made up of the chitinase like proteins Ym1 and Ym2 (encoded by Chil3 and Chil4) can form in the airways of asthmatic mice. These crystals are induced under the influence of IL-13 and these also enforce type 2 immunity and are drugable by antibodies. Thus protein crystallisation seems to be a unique feature of type 2 immune responses across species, acting to enforce type 2 immune responses, mucus plugging and neutrophil NETosis.

INVITED SPEAKER

Evolutionary Mechanisms for Generating Functional Diversity in Gel-Forming Mucins

Meital Haberman[1], Lev Khmelnitsky[1], Gabriel Javitt[1], Deborah Fass[1]

1. Department of Chemical and Structural Biology, Weizmann Institute of Science, Rehovot, Israel

The human secreted mucins are huge glycoproteins composed of characteristic amino- and carboxy-terminal folded regions and an extensive, intrinsically disordered, O-glycosylated central segment in which multiple CysD adhesion domains are, in some cases, embedded. During post-translational processing, individual mucin glycoproteins become linked by disulfide bonds to form covalent linear polymers. The mechanism for generating disulfide-bonded mucin polymers stepwise in the secretory pathway is shared by multiple mucins across different organ systems. However, mucus hydrogels comprising different mucins have distinct appearances and biophysical properties, with each mucin adapted to its bodily niche and physiological function. By studying large amino-terminal segments of the mucins MUC2, MUC5B, and, most recently, MUC5AC, we have gained insight into regions of these proteins that may tune the qualities of mucus by modulating covalent and non-covalent supramolecular assembly. Though MUC2, MUC5B, and MUC5AC all self-assemble into “beads-on-a-string” filaments when subjected to acidic pH conditions mimicking those of the late secretory pathway, we found that the filaments can further self-associate in different manners. Whereas the MUC2 amino-terminal segment forms isolated filaments, MUC5B forms bundles of filaments, and MUC5AC forms pairs of filaments arranged in a helix. Major determinants of higher-order mucin filament associations include the angles of bead-bead interactions in the filament and the docking sites of the CysD adhesion domains. CysD domain diversity is coupled to variation in the intrinsically disordered, O-glycosylated segments that separate CysD domains from other globular mucin regions and from one another. In particular, the PTS1 region, to which the key first CysD domain (CysD1) is tethered, has diverged in length and degree of conservation between MUC2, MUC5B, and MUC5AC. A new high-resolution structure of the MUC5AC beaded-filament helix enables a detailed comparison with MUC5B and analysis of the features that may produce functional diversity between these two respiratory mucins.

INVITED SPEAKER

The Unique Exocytosis Mechanism of Large Vesicles in Exocrine Tissues

Tom Biton[1,2], Nadav Scher[1], Shari Carmon[2], Yael Elbaz-Alon[1], Eyal D. Schejter[2], Ben-Zion Shilo[2*], Ori Avinoam[1*]

1. Department of Biomolecular Sciences, Weizmann Institute of Sciences, Rehovot, Israel

2. Department of Molecular Genetics, Weizmann Institute of Sciences, Rehovot, Israel

Exocrine cells that secrete mucinous cargos utilize large secretory vesicles (LSVs), reaching up to 10 μm in diameter. When LSVs fuse with the apical surface they often recruit an actomyosin meshwork that extrudes their content through dynamic fusion pores that initially dilate to large diameters and subsequently constrict without resealing. In this scenario, the vesicular and apical membranes remain distinct, and membrane homeostasis is maintained. However, the molecular mechanism regulating fusion pore dynamics remains largely uncharacterized. Using live-super-resolution and focused ion beam-scanning electron microscopy (FIB-SEM), we observe that the fusion pore of LSVs in the Drosophila larval salivary glands expand, stabilize, and constrict. Branched actin polymerization is essential for pore expansion and stabilization, while myosin II activity is essential for pore constriction. Furthermore, we identify several conserved Bin-Amphiphysin-Rvs homology (BAR) domain proteins that regulate fusion pore expansion and stabilization. Most notably, we show that the I-BAR protein Missing-in-Metastasis (MIM) localizes to the fusion site and is essential for pore expansion and stabilization in a dose-dependent manner. The MIM I-BAR domain is essential but not sufficient for localization and function. Taken together, our findings show that MIM is part of a dedicated machinery that acts in-concert with actin, myosin II and additional BAR-domain proteins to control fusion pore dynamics, mediating a distinct mode of exocytosis that facilitates content release without perturbing apical membrane homeostasis.

SELECTED SPEAKER

The generation of immunogenic gluten peptides occurs during their Goblet cell-mediated transport - Implications for Celiac Disease

Christian V. Recktenwald[1], Jenny K. Gustafsson[1], Brendan Dolan[1], Sjoerd van der Post[1], Sara Thulin[1], Gunnar C. Hansson[1]

1. Department of Medical Biochemistry, University of Gothenburg

Celiac Disease (CeD) is an immune-mediated enteropathy against dietary gluten derived from wheat, rye or barley and one of the most common lifelong food-related disorders worldwide. Morphologically, CeD is characterized by villous atrophy and crypt hyperplasia in the small intestine leading to the malabsorption of nutrients. The prevalence of the disease based on serological diagnosis can be as high as 1.4 % in the general population. The disease is triggered in genetically predisposed individuals harbouring the MHC class II phenotype HLA-DQ2 when tissue transglutaminase (TGM2) has deamidated specific glutamine residues in undigestable gluten peptides. This post-translational modification of the food antigen leads to a higher affinity and stabilization of the HLA-DQ2:Gliadin-complex on the cell surface of Antigen-presenting cells (APCs) and the subsequent activation of CD4+ T cells. When the disease has been established antibodies against deamidated gluten and auto-antibodies against the disease-triggering enzyme TGM2 are produced.
However, it is not understood how these gluten peptides are transferred from the gut lumen to the APCs in the lamina propria. Furthermore, it is not clear if the TGM2-catalyzed deamidation of these peptides takes place extra- or intracellularly. In this study, we have deciphered, by administering luminal injections of gluten peptides in the proximal small intestinal lumen of mice, that the molecular pathway of gluten uptake occurs via Goblet-cell associated antigen passaging (GAP). Furthermore, this pathway was also transferable to humans since the same phenomenon was observed when duodenal biopsies were exposed to labelled gluten peptides ex vivo. In addition, we could show that the TGM2-catalyzed deamidation of gluten peptides occurs both intra- and extracellularly. Surprisingly, the number of deamidated glutamines is increased extracellularly but these peptides do not seem to be taken up by the epithelium thereby suggesting that the GAP-mediated uptake of food antigens is restricted to non-charged molecules.

INVITED SPEAKER

Mucin Glycans and Microbial Virulence: Exploring Therapeutic Potential in Host-Derived Molecules

Katharina Ribbeck[1]

1. Department of Biological Engineering, Massachusetts Institute of Technology, USA

Mucus, a biological gel lining all wet epithelia including the mouth, lungs, and digestive tracts, is evolved to protect against pathogenic invasion. However, studies on microbial pathogenesis in these systems often neglect the natural, three-dimensional mucus gels, focusing instead on mucus-free environments that lack essential geometric constraints and microbial interactions. To address this gap, our laboratory has developed model test systems utilizing purified mucin polymers, the primary gel-forming constituents of the mucus barrier, and their associated glycans.

Through these models, we investigate the influence of the mucus barrier on microbial virulence and the mechanisms microbes use to breach this protection. Our findings reveal that mucin polymers, particularly their glycans, play a critical role in regulating microbial behaviors such as surface attachment, toxin secretion, quorum sensing, and biofilm formation. Furthermore, we are beginning to identify microbial sensors of mucins and intracellular pathways responsive to mucin regulation. These discoveries position mucin glycans as key regulators of microbial virulence, opening new therapeutic avenues leveraging these host-derived molecules.

INVITED SPEAKER

Mucus gels and innate lung defense

Daniel Song[1], Logan Kaler[1], Elizabeth Engle[1], Ethan Iverson[1], Maria Rife[1], Allison Boboltz[1], Margaret Scull[1], Gregg Duncan[1]

1. University of Maryland

Mucus is a biological gel within the lung designed to behave like an “escalator” with the ability to capture potentially harmful inhaled materials (e.g. pathogens, particulates) and carry these materials via mucociliary clearance up to the throat to be swallowed and sterilized. MUC5B and MUC5AC are large, gel-forming mucins that assemble to form airway mucus gels. MUC5B and MUC5AC are large, gel-forming mucins that assemble to form airway mucus gels. However due to the lack of appropriate models, it is not yet fully understood how MUC5B and MUC5AC individually or synergistically contribute to the biological function of mucus. To understand their unique roles in respiratory health, I will discuss our studies on the rheological properties and transport function of mucus in human airway tissue cultures genetically engineered to secrete either MUC5B or MUC5AC. A breakdown in lung mucus barrier function can lead to increased infections by respiratory viruses, such as influenza, rhinovirus, and coronaviruses, as they are not effectively removed from the airway. For these viral pathogens, it is important to understand the mechanisms through which viral particles avoid adhesion to the mucus barrier and transport to the underlying epithelium to cause infection. To address this, I will discuss our work to quantitatively measure respiratory virus diffusion through and trapping within the mucus barrier using high-speed fluorescent video microscopy and multiple particle tracking.

SELECTED SPEAKER

Peroxidase-mediated mucin cross-linking drives pathologic mucus gel formation in IL-13-stimulated airway epithelial cells

Maude A. Liegeois[1], Margaret Braunreuther[2], Annabelle Charbit[1], Wilfred W. Raymond[1], Gerald G. Fuller[2], and John V. Fahy[1,3]

1. Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, USA.
2. Department of Chemical Engineering, Stanford University, Stanford, CA, USA.
3. Division of Pulmonary and Critical Care Medicine, University of California, San Francisco, San Francisco, CA, USA.

Airway pathological mucus is a feature of airway type 2 inflammation and eosinophil peroxidase promote formation of oxidant acids that cross-link mucins to increase elastic behavior of mucus. Studies in cultured human bronchial epithelial cells (HBECs) show that IL-13 induces formation of pathologic mucus gels but the precise mechanisms underlying these changes remain incompletely elucidated. Here, we optimized a magnetic microwire rheometer (MMWR) to allow in situ characterization of mucus hydrogels secreted by HBECs grown at air liquid interface under control and IL-13-stimulated conditions. The MMWR device applies a force to a magnetic microwire that is placed on mucus secreted by HBECs. The resulting displacement of the microwire in response to the applied force is used to characterize mucus material properties and determine the steady-state compliance and zero-shear viscosity. Using this MMWR technology, we investigated if IL-13 upregulates epithelial cell peroxidases, lactoperoxidase (LPO) or thyroid peroxidase (TPO), to mediate mucin cross- linking and mucus gel elasticity. By applying creep compliance measurements with the MMWR device, we found that mucus secreted by HBECs under control conditions exhibits viscoelastic liquid behavior whereas mucus secreted by HBECs stimulated by IL-13 exhibits solid-like behavior, as revealed by decreased compliance and increased viscosity ($p<0.001$). When mucus from HBECs stimulated by IL-13 is treated with dithiothreitol (DTT) to cleave disulfide bridges between mucin polymers, the compliance and viscosity of the mucus normalize ($p<0.05$). HBECs express two peroxidases, LPO and TPO and we showed that both peroxidases are able to produce oxidant acids oxidizing free thiols into disulfide bridges. We investigated the peroxidase activity in HBECs and found that this activity is higher in IL-13 stimulated cells. To confirm that peroxidases were the mediator of cross-linking, a thiolated hydrogel was placed on IL-13-stimulated HBECs. The hydrogel shows increased stiffening that is prevented by a chemical inhibitor of LPO and TPO ($p<0.01$), confirming peroxidase activity at the apical side of the cells. Finally, gene expression for TPO increased in IL-13 stimulated HBECs as was higher than normal in epithelial brushings from patients with asthma. We conclude that IL-13 causes mucus cross-linking and mucus gel stiffening in HBECs via peroxidase-driven oxidant acid production and that TPO is a candidate mediator of airway mucus plugs in patients with asthma.

SELECTED SPEAKER

Topical reinforcement of the cervical mucus barrier to sperm

Ulrike Schimpf[1], Erika Caldas-Silveira[2], Ljudmila Katchan[3], Cécile Vigier-Carriere[3], Isabelle Lantier[2], Gilai Nachmann[1], Sebastian Gidlöf[4], Aino Fianu Jonasson[4], Lars Björndahl[4], Stéphane Trombotto[5], Xavier Druart[2], Thomas Crouzier[1,3]

1. Royal Institute of Technology, KTH
2. French National Institute for Agriculture, Food, and Environment, INRAE
3. Cirqle Biomedical Contraception ApS
4. Karolinska University Hospital
5. Polymer Materials Engineering, Lyon University 1

INTRODUCTION: Close to half of the world's pregnancies are still unplanned, reflecting a clear unmet need in contraception. Ideally, a contraceptive would provide the high efficacy of hormonal treatments, without systemic side effects. Our research group has been pioneering several mucus engineering, aims at using mucus and modifying the mucus in situ via topical treatments. Here, we studied topical reinforcement of the cervical mucus by chitosan mucoadhesive polymers as a form of female contraceptive.

METHODS: Using human ovulatory cervical mucus and human sperm, we studied the penetration of sperm into cervical mucus exposed to various chitosan solutions. Capillary tubes were filled with mucus and exposed to formulation and then to sperm. Sperm penetration was measured by microscopy. In vivo efficacy was assessed using the sheep model. Sheep were synchronized, and treated with vaginal gels containing chitosan, followed by artificial insemination with fluorescently labelled ram sperm. The distribution of sperm through the reproductive tract of the ewe was assessed by fluorescence confocal endomicroscopy. Safety of the treatments was assessed using three-dimensional vaginal epithelial cell cultures, by measuring kinematic parameters of sperm exposed to formulations, and by histological analysis of vaginal tissues of the ewes exposed to the formulation.

RESULTS: We found that chitosans larger than 7 kDa effectively cross-linked human ovulatory cervical mucus to prevent sperm penetration in vitro. We then demonstrated in vivo that vaginal gels containing chitosan could stop ram sperm at the entrance of the cervical canal and prevent them from reaching the uterus, whereas the same gels without chitosan did not substantially limit sperm migration. Chitosan did not affect sperm motility in vitro or in vivo, suggesting reinforcement of the mucus physical barrier as the primary mechanism of action. The chitosan formulations did not damage or irritate the ewe vaginal epithelium, in contrast to nonoxynol-9 spermicide. The demonstration that cervical mucus can be reinforced topically to create an effective barrier to sperm may therefore form the technological basis for muco-cervical barrier contraceptives with the potential to become an alternative to hormonal contraceptives.

INVITED SPEAKER

Cell-based Mucin Array for Discovery and Characterization of Mucinases and Carbohydrate Binding Modules

Yoshiki Narimatsu[1]

1. Copenhagen Center for Glycomics, Departments of Cellular and Molecular Medicine and School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Denmark

Mucins arguably represent the last frontier in the analytics of glycoproteins. Most mucins are extremely large and heterogeneous glycoproteins that are resistant to conventional glycoproteomics strategies dependent on proteolytic fragmentation and sequencing. Currently, there are no methods for obtaining human mucin molecules in reasonable purity with defined glycans, and this is a fundamental barrier and limitation for studies of mucins and their complex biology, particularly in the microbiome field. We, therefore, sought to capture the molecular information contained in the tandem repeat regions (TRs) of human mucin and mucin-like O-glycodomains for molecular dissection. We have developed a glycoengineered cell-based platform to display and produce representative mucin TRs with defined O-glycans using mucin reporters containing around 200 amino acids1. The cell-based mucin array enables molecular dissection of microbial interactions with mucin TR sequences and the attached O-glycan structures. Display of the mucin reporters on the cell surface provides the first cell-based display of the human mucins and we have used this to probe and dissect the binding specificities of microbial adhesins, influenza virus, and Siglecs2. We discovered that these adhesins show highly distinct binding preferences for O-glycan patterns displayed on distinct mucin TRs, providing a new level of complexity and diversity to interactions with the mucin glycome. A major power of the cell-based array platform is that it also allows the production of secreted homogenous reporters for wider studies of interactions with GBPs. This we have illustrated by producing a homogenous mucin fragment (MUC1 TRs, 150 amino acids with 25-28 trisaccharide O-glycans) that recently enabled the first single-molecule atomic imaging of glycoproteins by low-temperature scanning tunneling microscopy after soft-landing electrospray ion beam deposition3. We have also shown that the mucin display platform is ideal for the discovery and exploration of mucin-degrading enzymes as well as mucin-binding modules. Using the mucin display, we originally identified a small mucin-binding module (X409)1 on the mucinase StcE which does not bind simple oligosaccharides and hence cannot be discovered by traditional glycan arrays. We also discovered new families of mucinases (O-glycoproteases), including the di-glutamate mucinase HC7, which recognizes dense clusters of O-glycans4 and has family members in eukaryotes. The cell-based glycan and mucin arrays are sustainable resources that we hope to make widely available to the community

References

1. Nason R, Büll C, Konstantinidi A, Sun L, Ye Z, Halim A, Du W, Sørensen DM, Durbesson F, Furukawa S, Mandel U, Joshi HJ, Dworkin LA, Hansen L, David L, Iverson TM, Bensing BA, Sullam PM, Varki A, Vries E, de Haan CAM, Vincentelli R, Henrissat B, Vakhrushev SY, Clausen H, Narimatsu Y. Display of the human mucinome with defined O-glycans by gene engineered cells. (2021) Nat Commun 1;12(1):4070.
2. Büll C, Nason R, Sun L, Van Coillie J, Madriz Sørensen D, Moons SJ, Yang Z, Arbitman S, Fernandes SM, Furukawa S, McBride R, Nycholat CM, Adema GJ, Paulson JC, Schnaar RL, Boltje TJ, Clausen H, Narimatsu Y. Probing the binding specificities of human Siglecs by cell-based glycan arrays. (2021) Proc Natl Acad Sci U S A. Apr 27;118(17):e2026102118.
3. Anggara K, Sršan L, Jaroentomeechai T, Wu X, Rauschenbach S, Narimatsu Y, Clausen H, Ziegler T, Miller RL, Kern K. Direct observation of glycans bonded to proteins and lipids at the single-molecule level. (2023) Science 382(6667):219-223. doi: 10.1126/science.adh3856.
4. Narimatsu Y, Christian Büll, Víctor Taleb, Qinghua Liao, Ismael Compañón, David Sánchez-Navarro, Fabien Durbesson, Renaud Vincentelli, Francisco Corzana, Carme Rovira, Bernard Henrissat, Henrik Clausen, Hiren J. Joshi, Ramon Hurtado-Guerrero*. A Family of Di-Glutamate Mucin Degrading Enzymes that Bridges Glycan Hydrolases and Peptidases. (2024) Nat Catalysis in press.

INVITED SPEAKER

Recent developments in the MS-based analysis of mucin-domain glycoproteins

Stacy Malaker[1]

1. Department of Chemistry, Yale University, USA

Mucin-domain glycoproteins are densely O-glycosylated and play key roles in a host of biological functions. However, their dense O-glycosylation remains enigmatic both in glycoproteomic landscape and structural dynamics, primarily due to the challenges associated with studying mucin domains. Here, we present advances in the mass spectrometric analysis of mucins, including the characterization of mucinases, enrichment techniques, and complete mucinomic mapping of translationally relevant mucin proteins.

SELECTED SPEAKER

Mucin O-glycan degradation patterns of Akkermansia muciniphila, Ruminoccocus torques, and a synthetic gut-associated microbial community

Carol de Ram[1], Maryse Berkhout[2], Clara Belzer[2], Guido Hooiveld[3], Henk Schols[1]

1. Laboratory of Food Chemistry, Wageningen University
2. Laboratory of Microbiology, Wageningen University
3. Human nutrition and Health, Wageningen University

Mucin O-glycans are degraded by the diverse human gut microbiota and used as an important nutrient source. However, the various gut bacteria each have different enzymes and approaches to degrade these O-glycans. Furthermore, the presence of specific O-glycan structures may steer the microbiota composition. Certain bacteria are generalists and have a rather broad enzyme repertoire to degrade numerous different types of O-glycan structures whilst others are specialists and only capable of degrading specific types of sugar linkages within the O-glycans. In this study we investigated the O-glycan degradation patterns of two common gut-associated bacteria Akkermansia muciniphila and Ruminococcus torques by analysing the remaining O-glycan structures in time during mucin fermentation. We compared these degradation patterns with those of a synthetic bacteria community consisting of a diverse range of gut-associated bacteria tin order to recognize dominant mucin degraders and possible cross-feeding. To this end, the bacteria were grown on porcine stomach mucin for 24 hours and sampled every 3 hours. O-glycans were released from the bacterial supernatant samples and analysed using PGC-UPLC-MS/MS. Akkermansia muciniphila showed a slower consistent degradation of all O-glycan structures whilst Rumminococcus torques displayed a preference for targeting individual or specifically fucosylated glycans first. The synthetic community exhibited a faster and more complete degradation compared to the individual bacteria stressing the importance and efficiency of cross-feeding. These findings provide an insight in the mechanisms of gut-associated bacteria in their quest for nutrients via glycan degradation.

SELECTED SPEAKER

Unraveling the role of α-(1,2)-fucosylation in bacterial pneumonia

Cengiz Goekeri[1,2], Sebastian M. K. Schickinger[1], Alina Nettesheim[1], Kerstin A. K. Linke[1], Leila Bechtella[3], Gaël M. Vos[3], Elena Lopez-Rodriguez[4], Vladimir Gluhovic[4], Anne Voß[5], Sandra Kunder[5], Jade Bath[6], Katharina Ribbeck[6], Kevin Pagel[3], Achim D. Gruber[5], Matthias Ochs[4,7], Peter H. Seeberger[8], Martin Witzenrath[1,7], Geraldine Nouailles[1]

1. Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Infectious Diseases, Respiratory Medicine and Critical Care, Berlin, Germany.
2. Cyprus International University, Faculty of Medicine, Nicosia, Cyprus.
3. Freie Universität Berlin, Institute of Chemistry and Biochemistry, Berlin, Germany.
4. Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Functional Anatomy, Berlin, Germany.
5. Freie Universität Berlin, Institute of Veterinary Pathology, Berlin, Germany.
6. Massachusetts Institute of Technology, Cambridge, MA, USA.
7. German Center for Lung Research (DZL), Berlin, Germany.
8. Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, Potsdam, Germany.

Community-acquired pneumonia (CAP) remains a leading contributor towards global communicable disease-mediated mortality and Streptococcus pneumoniae (Spn) is the most frequently detected pathogen in CAP. Mucosal epithelia and biointerfaces, including those of the lungs, are rich in cell-bound and secreted N- and O-glycans, which harbor terminally fucosylated glycans. This study investigates how terminal fucosylation and α-(1,2)-fucosylated glycans influence Spn infection. Pneumococci express fucose utilization genes, which are upregulated upon interaction with fucosylated MUC5AC in vitro. Murine lungs express fucosylated glycans, which are shed into the bronchoalveolar lavage (BAL) during Spn infection. Systemic treatment of mice with 2-deoxy-D-galactose (2-DGal) resulted in incorporation of 2-DGal into the murine glycome and inhibition of fucosylation in vivo. Mice treated with 2-DGal displayed markedly improved physiological parameters such as infection-induced body weight and temperature loss. Treatment with 2-DGal also led to reduced bacterial burden in BAL and systemic spread following Spn infection. Flow cytometry revealed that following 2-DGal treatment, neutrophil recruitment was at similar levels 24 hours post-infection (hpi), but neutrophilic inflammation resolved quicker than sham-treated mice. Moreover, 2-DGal treatment markedly improved lung barrier permeability and led to decreased IL-6 and TNF-α production in Spn-infected mice 48 hpi. Taken together, we conclude that 2-DGal treatment prevents the establishment of pneumococcal infection in mice and alleviates murine pneumonia.

INVITED SPEAKER

Mucin glycan foragers in the human gut: the case of Ruminococcus gnavus

Nathalie Juge[1]

1. Quadram Institute Bioscience, UK

Ruminococcus gnavus is a prevalent human gut symbiont part of the normal gut microbiota and disproportionately represented in intra- and extra-intestinal diseases from inflammatory bowel diseases to neurological disorders. There is therefore great interest in understanding the mechanisms by which R. gnavus adapt to the gut and communicate with the host.
We showed that R. gnavus' ability to utilise mucin glycans is strain dependent and associated with the utilisation of terminal glycan epitopes. Using a combination of molecular microbiology, biochemical and structural approaches, we uncovered a novel sialic acid metabolic pathway in gut bacteria, which is essential to the capacity of R. gnavus strains to colonise the intestinal mucus niche. From an ecological point of view, since R. gnavus is the only strain reported to produce 2,7-anhydro-Neu5Ac in the gut, the strict specificity of its sialic acid transporter confers a nutritional advantage while supporting the microbial community by enabling access to the uncapped mucin glycan chain.
The interaction of R. gnavus strains with the host at the mucosal interface was further investigated using mouse and human gut-on-chip models, underscoring the importance of studying the health impact of bacteria at the strain level.

INVITED SPEAKER

The adaptation hallmarks of Akkermansia muciniphila enzymes to human O-glycans

Mathias Jensen[1], Linn Stenfelt[1,2], Michael J. Pichler[1], Bashar Shuoker[1,3], Chunsheng Jin[4], Jennifer Ricci Hagman[2,5], Annika Hult[5], Hiroka Sakanaka[1], Haiyang Wu[6], Ana Martínez Gascueña[6], Jining Liu[7], Julia Weikum[1], Tine Sofie Nielsen[1], Jan Holgersson[7], Eva Nordberg Karlsson[3], Nathalie Juge[6], Sebastian Meier[8], Niclas G. Karlsson[4], Jens Preben Morth[1], Martin L. Olsson[2,5] and Maher Abou Hachem[1]

1. Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, 2800, Denmark. maha@bio.dtu.dk.

Akkermansia muciniphila, a core human gut microbiota species, which is a dedicated mucin degrader, relies on mucin both as a carbon and nitrogen source. The relative abundance of A. muciniphila is inversely correlated to the proliferation of mucin-degrading pathobionts, whereas it is positively associated with gut barrier and metabolic health. Despite its pivotal role in mucin turnover and physiological importance, our insight into the mucin O-glycan degradation apparatus of A. muciniphila remains underexplored.
We have recently investigated the initial steps of mucin degradation by A. muciniphila, demonstrating that removal of sialic acid and fucose decapping mediates nutrient sharing to other microbiota groups and is crucial for growth. Inspired by the observed A. muciniphila ability to break down ABO, H and Lewis blood group antigens on mucin, we investigated the adaptation to host-derived ABO antigens on O-glycans. We showed that A. muciniphila possesses exceptionally efficient enzymes to degrade not only the A and B antigens, but additional extensions of these epitopes, which have hitherto received no attention in the context of blood transfusion. Interestingly, the removal of canonical and extended ABO antigens on red blood cells by A. muciniphila enzyme blends significantly improved compatibility with group O plasmas, compared to conversion of A or B antigens alone. Our findings revive hopes for enzymatic production of ABO-universal blood via targeting the hitherto overlooked extended A and B antigens and highlight hallmarks of enzymatic efficiency on host O-glycans by A. muciniphila.

1Department of Biotechnology & Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark.
2Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden.
3Biotechnology, Department of Chemistry, Lund University, Lund, Sweden.
4Proteomics Core Facility at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
5Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Region Skåne, Sweden.
6Quadram Institute, Bioscience, Norwich, UK.
7Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
8Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark.

SELECTED SPEAKER

Maturation of Human Intestinal Epithelia involves Fortification of the Apical Surface against Salmonella Attack

Jorik M. van Rijn[1,2,#], Ana C. C. Lopes[1,#], Merve Ceylan[3], Jens Eriksson[1], Alexandra Florbrant[1], Angeliki Ntokaki[1], Rebekkah Hammar[3], Dominic-Luc Webb[4], Per M. Hellström[4], Wilhelm Graf[5], Martin Skogar[5], Magnus Sundbom5, Per Artursson[3], Thaher Pelaseyed6, Maria Letizia Di Martino[1], and Mikael E. Sellin[1,7,*]

1. Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden
2. Princess Máxima Center for pediatric oncology, Utrecht, Netherlands
3. Department of Pharmacy, Uppsala University, Sweden.
4. Department of Medical Sciences, Uppsala University, Sweden
5. Department of Surgical Sciences, Uppsala University, Sweden
6. Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Sweden.
7. Science for Life Laboratory, Sweden

#Contributed equally.
*Correspondence: mikael.sellin@imbim.uu.se

The active invasion of intestinal epithelial cells (IECs) constitutes a key event in the infection cycle of many gut bacterial pathogens. Studies of how the prototype enterobacterium Salmonella enterica Typhimurium (Salmonella) invades transformed cell lines has generated a paradigm for the near-instant, efficient, and type-three-secretion-system (TTSS-1)-driven IEC invasion process, fueled by expansive membrane ruffles. However, recent comparative studies suggest that non-transformed IECs in their native gut context comprise a significantly more challenging target for the bacterial attack. The molecular and cellular features that explain these discrepancies are largely undefined. By live-cell imaging of infections in enteroid- and colonoid-derived epithelial cell layers, our work reveals that the maturation state of human gut epithelia dramatically impacts permissiveness to Salmonella invasion. IEC layers kept under stem- and progenitor-cell-promoting conditions remain permissive to Salmonella invasion, whereas maturation towards an enterocyte/colonocyte end fate markedly reduces the frequency of bacterium-induced epithelial entry structures, and lowers the overall invasion efficiency by up to 10-fold. This phenotypic shift appears coupled to an altered expression of actin regulatory proteins implicated in the Salmonella invasion process, and an increased dependence on the TTSS-1 effector SipA for successful bacterial entry. IEC maturation also involves upregulation of cell surface mucins and distinct shifts in the apical surface glyco-composition, as revealed by multiple lectin stainings. Most notably, enzymatic digestion of this apical glycocalyx converts mature IEC layers back to the Salmonella-invasion-permissive phenotype of their immature counterparts. Taken together, these results showcase how the maturation state of human intestinal epithelia dictate the efficiency, as well as the mechanistic and temporal progression, of the Salmonella invasion process.

SELECTED SPEAKER

Characterization of the founding member of the first CBM family known to target sulfated glycans

Elena Fekete[1], Grete Raba[1], Alan Cartmell[2], Ana Luis[1]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg. Gothenburg, Sweden
2. Department of Biology, York University. York, UK

The human gut is colonized by a dense and diverse microbial community: the microbiota. In the healthy intestines, mucus creates a habitat for the commensal microbiota, as well as a barrier to separate the microbiota from the intestinal epithelium(1). Mucin glycoproteins are the major component of intestinal mucus. Mucins are decorated with complex and diverse O-glycans that protect the glycoprotein from degradation by most commensal bacteria. However, some microbiota members, including Akkermansia muciniphila (Akk) can degrade and utilize mucin O-glycans as a carbon source(2). Interestingly, our results show that this bacterium is able to utilize human colonic mucins, but is unable to utilize released mucin O-glycans. This indicates that recognition of the native mucin structures has a key role in mucin utilization by Akk. Understanding the mechanisms of interaction between Akk and mucin glycans will contribute to better understanding of how this bacterium degrades mucins and colonizes the human gut. Previously, it has been shown that some microbiota members express sulfatases that play a key role in O-glycan utilization and in vivo intestinal colonization. A bioinformatic analysis of Akk sulfatases revealed a protein (Amuc_0953) closely related to the key 3S-galactose (3S-Gal) sulfatase identified in the mucin-degrading commensal Bacteroides thetaiotaomicron(3). Amuc_0953 displays a N-terminal domain of unknown function. The crystal structure of this domain reveals a right-handed parallel b-helix fold with 16 turns. Structural alignments show a low similarity to pectin lyases, proteases and the previously characterized CBM89. Using a combination of biochemical and immunofluorescent techniques, we disclosed that the N-terminal domain of Amuc_0953 is the founding member of a novel CBM family that specifically binds 3S-Gal epitopes. Site-directed mutagenesis studies revealed that the recognition of negatively charged sulfated substrates is mediated by positively charged residues. Moreover, this novel CBM potentiates the enzymatic activity of the sulfatase enzyme by increasing binding to the target substrate. Additionally, due to the lack of specific binding proteins that specifically recognize sulfated glycans, we explored this novel CBM as a probe to detect glycosylation alterations associated with cancer, and observed that this binding protein specifically binds to ovarian cancer tissues, known to overexpress 3S-Gal epitopes.
The characterization of this novel CBM revealed the first binding protein that specifically recognizes a sulfated epitope. The expression of these binding proteins in Akk increase binding to glycans epitopes found in mucins, a key step to the utilization of this substrate by Akk. Additionally, this CBM shows the potential to be utilized as a novel probe to target specific epitopes in biological samples.

SELECTED SPEAKER

Salmonella enterocyte invasion through MUC1: contributions of the extracellular domain and cytoplasmic tail to invasion and signaling

Jinyi Su[1], Xinyue Li[1], Evelien Floor[1], Karin Strijbis[1]

1. Utrecht University, Utrecht Biomolecular Health Sciences, The Netherlands

Salmonella Enteritidis (Salmonella) is a common food-borne enteropathogenic bacterium that can bypass the mucus layer and invade intestinal epithelial cells. Highly glycosylated mucin proteins are expressed on the apical surface of enterocytes, together called the glycocalyx. Previously, we discovered that the Salmonella giant adhesin SiiE interacts with the glycosylated transmembrane mucin MUC1 which induces apical invasion into enterocytes. Here, we investigate if the MUC1 glycosylated extracellular domain (ED) and/or the cytoplasmic tail (CT) with signaling capacity contribute to Salmonella invasion and/or induction of downstream signaling. As our model, we use HT29-MTX (MUC1-WT) cells, CRISPR/Cas9-MUC1 knockout cells (ΔMUC1), CRISPR/Cas9-MUC1-CT (MUC1-ΔCT) cells that lacked the CT and mucinase-cleaved MUC1-ED (cleaved-ED MUC1) cells leaving the transmembrane domain and CT intact. A significant reduction in bacterial infection was observed in ΔMUC1 and cleaved-ED MUC1 cells, suggesting that MUC1-ED has an essential receptor function. No difference in bacterial infection was observed between MUC1-WT and MUC1-ΔCT cells, showing that the CT is not essential for SiiE-MUC1 invasion. The MUC1-CT was also not required for secretion of IL-8 as measured by ELISA. To determine the contribution of the MUC1-CT, we performed a large RNAseq experiment with uninfected and infected cells. The most striking observation was that the regulation of certain genes by the NFκB family was dependent on the presence of the MUC1-CT. Immunoblot analysis demonstrated that the NFκB transcriptional subunits p50, p52, p65, RELB and c-REL are equally expressed and translocated in all cell types. However, the NFκB cytoplasmic inhibitory subunits p100, p105 and IκBα are significantly upregulated in MUC1-ΔCT and ΔMUC1 cells compared to MUC1-WT cells in the absence of Salmonella but are equally upregulated after Salmonella invasion. Based on these results, we conclude that the MUC1 extracellular domain is essential for Salmonella invasion and MUC1-CT has an anti-inflammatory function by suppressing the NFκB pathway.

INVITED SPEAKER

Transcriptional regulation of invasive mucinous adenocarcinoma of the lung

Motohiro Kobayashi[1]

1. Department of Tumor Pathology, Faculty of Medical Sciences, University of Fukui, Japan

It is important to recognize that tumors are not made up of neoplastic tumor cells alone. Tumor tissue consists of neoplastic tumor cells as well as blood vessels, lymphatic vessels, fibroblasts and immune cells such as macrophages and lymphocytes. As a carbohydrate moiety of mucins, sulfated glycans are expressed not only on neoplastic tumor cells but also on these stromal cells. In this talk, the potential role of sulfated glycans expressed on these two cell types constituting cancer tissue will be discussed..

INVITED SPEAKER

Resolving the role of mucins in extracellular vesicle biology

Matthew Paszek[1]

Cancer cells deploy extracellular vesicles (EVs) to reprogram the local microenvironment and prepare distant niches for metastatic spread. We find that expression of cancer-associated mucins is associated with a progressive increase in EV biogenesis. The mucin induced EVs have physical and biochemical characteristics that are distinct from canonical exosomes and microvesicles, the two primary classes of EVs that are implicated in transmission of molecular messengers between tumor cells and recipient cells. Notably, mucin induced EVs are highly enriched in tetraspanins that are classical biomarkers for exosomes but display size distributions that are more typical of microvesicles. We apply various super-resolution imaging strategies and biophysical techniques to resolve the nanoscale biology of the mucin induced EVs and their cargo selection. Our studies implicate mucins in the generation of membrane curvature, which recruits tetraspanins, including CD63, to the plasma membrane and packaging into EVs. The CD63-containing EVs display a conspicuous mucin corona with broad implications for EV stability, extracellular matrix interactions, and biological function. Together, our results suggest that mucins have a critical but underappreciated role in cancer-associated EV biogenesis.

SELECTED SPEAKER

Mucus and epithelial barrier defects promote pks+ driven colorectal cancer development through adhesin-mediated binding mechanisms

Maude Jans[1,2], Magdalena Kolata[4,5], Gillian Blancke[1,3], Maarten Ciers[1,2,3], Anders B. Dohlman[6], Takato Kusakabe[7], Mozes Sze[1,2], Alexandra Thiran[1,3], Geert Berx[2], Sabine Tejpar[8], Geert van Loo[1,2], Iliyan D. Iliev[7], Han Remaut[4,5], Lars Vereecke[1,3]

1. VIB Center for Inflammation Research, B-9052 Ghent, Belgium
2. Department of Biomedical Molecular Biology, Ghent University, B-9052 Ghent, Belgium
3. Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium
4. Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium
5. Structural & Molecular Microbiology, VIB-VUB Centre for Structural biology, Brussels, Belgium
6. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA
7. Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA; The Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, NY 10065, USA
8. Department of Oncology, University Hospital Leuven, KU Leuven, Leuven, Belgium

Various bacteria are suggested to contribute to colorectal cancer (CRC) development, including pks+ E. coli which produce the genotoxin colibactin that induces characteristic mutational signatures in host epithelial cells. It remains unclear how the highly unstable colibactin molecule is able to access host epithelial cells and its DNA to cause harm. We use experimental and transgenic mouse models to study microbial tumor-driving mechanisms, including Zeb2-transgenic mice, which are characterized by mucus and epithelial barrier defects. We found that pks+ E. coli drives CRC exacerbation and tissue invasion in a colibactin-dependent manner. Using isogenic mutant strains, we further demonstrate that CRC exacerbation critically depends on expression of the E. coli type-1 pilus adhesin FimH and the F9-pilus adhesin FmlH. Blocking bacterial adhesion using a pharmacological FimH inhibitor attenuates colibactin-mediated genotoxicity and CRC exacerbation. Using fluorescently labeled recombinant FimH and FmlH, we characterized E.coli docking sites in healthy and CRC tissue, and found distinct binding patterns associated to mucus and goblet cell subsets. Together, we show that the oncogenic potential of pks+ E. coli critically depends on bacterial adhesion to host epithelial cells and mucus, and is critically mediated by specific bacterial adhesins. Adhesin-mediated epithelial binding subsequently allows production of the genotoxin colibactin in close proximity to host epithelial cells, which promotes DNA damage and drives CRC development. These findings present promising therapeutic avenues for the development of anti-adhesive therapies aiming at mitigating colibactin-induced DNA damage and inhibiting the initiation and progression of CRC, particularly in individuals at risk for developing CRC. Furthermore, my lab has generated various tools for goblet cell research based on our Muc2Cre mouse line, including goblet cell reporter and diphtheria-toxin based depletion models, which we study in the context of infection, inflammation and CRC development.

SELECTED SPEAKER

Structural Basis for MUC16 Recognition and Associated Anti-Pancreatic Cancer Activity of Humanized Antibody AR9.6

Eric N. Aguilar[1], Satish Sagar[2,3], Brandy Murray[1], Christabelle Rajesh[2,3], Eric K. Lei[4], Sarah A. Michaud[5], David R. Goodlett[5], Thomas C. Caffrey[2,3], Paul M. Grandgenett[2,3], Benjamin J Swanson[6], Teresa M. Brooks[1], Adrian Black[2,3], Henk van Faassen[4], Greg Hussack[4], Kevin A. Henry[4,7], Michael A. Hollingsworth[2,3], Prakash Radhakrishnan[2,3], and Cory L. Brooks1

1. Department of Chemistry and Biochemistry, California State University Fresno, Fresno, CA, USA.
2. Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE, USA.
3. Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, USA.
4. Human Health Therapeutics Research Centre, National Research Council Canada, Ottawa, ON, Canada.
5. University of Victoria-Genome BC Proteomics Centre, Victoria, BC, Canada.
6. Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA.
7. Department of Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON, Canada.

Mucin-16 (MUC16), is an attractive target for antibody-mediated immunotherapy due to its central role in oncogenic signaling in pancreatic ductal adenocarcinoma (PDAC). The MUC16 specific monoclonal antibody AR9.6 has shown promise for immunotherapy and imaging of PDAC. Here we report the structural and biological characterization of the humanized AR9.6 antibody (huAR9.6). The structure of humanized antibody huAR9.6 was determined in complex with a MUC16 SEA domain. Binding of huAR9.6 to recombinant, shed and cell-surface MUC16 was characterized and the anti-PDAC activity was evaluated in vitro and in vivo. huAR9.6 bound a discontinuous, conserved epitope with an affinity of ~90 nM. Binding affinity depended on the specific SEA domain(s) present and glycosylation enhanced affinity 3-7-fold. The enhanced affinity was driven by favorable changes in entropy and enthalpy, while transition state thermodynamics revealed that binding to the glycosylated domain proceeded though a distinct pathway. Treatment with huAR9.6 reduced viability, migration, invasion and proliferation of MUC16+ PDAC cells in vitro, disrupted ErbB and AKT signaling and induced apoptosis in cells from patient-derived PDAC xenografts. Tumor burden in PDAC xenograft models was reduced. In addition, huAR9.6 elicited antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Dense avid binding of huAR9.6 to homologous SEA domains on MUC16 may facilitate ADCC and CDC. The avid interaction may sterically inhibit activation of ErbB receptors by MUC16, or alternatively, AR9.6 binding may specifically inhibit ErbB-MUC16 interactions. Either mechanism explain disruption of oncogenic signalling. The results of this study validate the translational therapeutic potential of huAR9.6 against MUC16-expressing PDACs.

INVITED SPEAKER

Mucus Plugging in Asthma – More than an Obstruction to Breathing

Benjamin Medoff[1]

1. Division of Pulmonary and Critical Care Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA

Mucus hypersecretion is a major feature of airway diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. In allergic asthma, goblet cell hyperplasia and type 2 airway inflammation are important pathophysiologic mechanisms that drive mucus production and the formation of plugs in the airways. It has long been recognized that extensive mucus plugging of the airways in asthma may contribute to airway obstruction and when severe may cause respiratory failure and death in acute exacerbations. More recently it has been shown that the presence of mucus plugs in asthmatics is associated with greater type 2 inflammation and more frequent severe exacerbations. There is also evidence that mucus plugs persist in the same airways over time. Recent studies comparing allergic asthmatics to allergic non-asthmatic control subjects suggest that there is a direct link between inflammation and mucus production in asthmatics that is uncoupled in allergic controls. Furthermore, these studies demonstrate that airway goblet cells are dynamic and develop a pro-inflammatory phenotype with allergen challenge. These data suggest that mucus plugs are more than just physical barriers to airflow and may, along with abnormal goblet cells, provide feed-forward signals that propagate an abnormal airway microenvironment that drives a severe asthma phenotype. Thus, the therapeutics that directly target mucus in the airways may have benefit beyond relieving airway obstruction.

INVITED SPEAKER

The role of mucins and their isoforms in barrier dysfunction and patient stratification

Annemieke Smet[1]

1. Laboratory of Experimental Medicine and Pediatrics, University of Antwerp, Antwerp, Belgium

Mucins are indispensable for the maintenance of mucosal barrier integrity but are still often considered as passive mediators, understating their importance in mediating epithelial barrier function. Furthermore, they act as dual-faceted proteins providing on the one hand an essential nutrient source for the gut microbiota, shaping its community composition and function and regulate epithelial permeability. On the other hand, aberrant mucin expression compromises microbial homeostasis and mucosal barrier integrity further promoting chronic inflammation and even tumourigenesis. Given that abnormal mucin expression is frequently observed in gastrointestinal pathologies, including inflammatory bowel diseases (IBD) and cancer, mucins must be considered as active players impacting mucosal barrier function and, thus, in the onset and course of these diseases. Here, we will provide mechanistic insights on how aberrant MUC13 expression, in cooperation with MUC1, impacts intestinal barrier function upon inflammation and demonstrate that aberrant mucin signatures associate with IBD presentation and gastric cancer outcome. In addition, mucins are highly polymorphic and the presence of genetic differences in mucin genes can give rise to a large repertoire of structurally diverse mRNA isoforms via alternative splicing. While most mRNA isoforms produced from the same mucin gene locus encode similar biological functions, others have the potential to alter protein function resulting in progression towards disease. Using a novel targeted mucin mRNA isoform sequencing approach in conjunction with deep learning and external validation, we unveiled the mucin mRNA isoform landscape in the intestinal tract of IBD and control patients encompassing distinct mucin RNA isoform panels that accurately stratified the heterogeneous IBD patient population in multiple subpopulations based on inflammation, IBD subtype and anatomical location in the intestinal tract.

SELECTED SPEAKER

Goblet cells and the circadian clock: a new regulatory mechanism

Laura Campbell[1], Michal Dudek[2], Richard K. Grencis[1], Qing-Jun Meng[2] and David J. Thornton[1]

1. Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester Academic Health Science Centre, Manchester M13 9PL, UK
2. Wellcome Centre for Cell Matrix Research, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester M13 9PL, UK

The intestinal mucus barrier provides frontline protection to the epithelium and controls daily access of particulates, microbes, and toxins to the underlying mucosal tissues. Specialised epithelial cells, goblet cells, instruct and maintain the protective mucus barrier through the highly dynamic production of gel-forming mucin glycoproteins. A potentially important intrinsic regulator of goblet cell function is the control by the circadian clock. This cell-intrinsic timing mechanism controls rhythmic outputs of gene expression and protein activity in a tissue-specific manner, which allow an organism to anticipate and respond to daily rhythmic changes in their environment, such as feeding/fasting cycles. Research has shown essential roles of circadian rhythms in regulating intestinal tissue homeostasis and microbiota, however, little is known about circadian rhythms in goblet cells. Here, we show that intestinal mucus-associated properties change over a diurnal 24-hour cycle in mice, including the number of goblet cells/crypt and mucin glycosylation. We show that intestinal goblet cells express key components of the molecular clock and that their expression changes over a 24-hour period, supporting a major role for a goblet cell intrinsic clock in the control of mucin production/properties. To assess the importance of the goblet cell clock, we have generated a unique goblet cell “clockless” mouse model (specific knockout of the core clock transcription factor Bmal1 in intestinal goblet cells; Muc2-iCre:Bmal1fl/fl). Bmal1 KO mice show an altered mucus barrier, microbial dysbiosis and a susceptibility rather than resistance to a challenge with the intestinal dwelling helminth, Trichuris muris. These data have identified goblet cell circadian rhythm as a temporal regulatory mechanism for mucin production, revealing fundamental daily regulation at this critical site of defence against inflammation and infectious challenge.

SELECTED SPEAKER

Using mucinases to enhance oral delivery of macromolecules

Marilena Bohley[1], Angela Steinauer[2], Jean-Christophe Leroux[3]

1. Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland
2. Institute of Chemical Sciences and Engineering, Ecole polytechnique fédérale de Lausanne, Switzerland
3. Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

Peptide drugs hold great therapeutic potential, but their oral delivery often results in subtherapeutic concentrations due to the various gastrointestinal barriers such as mucus, epithelial linings, and digestion processes.1 Despite the use of permeation increasing strategies, clinical success in improving oral bioavailability of peptides remains limited (<1%).1,2 Here, we employ mucinases to reduce the barrier properties of gastrointestinal mucus.3 We evaluated three different mucinases (StcE, BT4244, and AM0627), and a broad-acting O-glycoprotease (OgpA) for their efficacy in degrading the mucin network and reducing the viscosity of porcine small intestinal mucus.4, 5 StcE was identified as the most effective mucin-degrading enzymes, significantly reducing both viscous and elastic properties of mucus within 5 min of application. Using fluorescence recovery after photobleaching (FRAP), we demonstrated that StcE treatment notably increases the diffusivity of peptide surrogates (Cy5-labelled poly(ethylene glycol)s (PEGs); 1, 5, and 20 kDa) through mucus, enhancing both mobile and immobile macromolecule fractions. Subsequently, we assessed the efficacy of StcE and peptide co-delivery in vivo. To that end, we lyophilized StcE and incorporated it together with a novel permeation enhancer and a model drug (desmopressin acetate, ~1 kDa) in commercial enteric capsules. Upon oral administration to dogs, plasma concentration of desmopressin was assessed and compared to those achieved with a control capsule only containing desmopressin acetate. Results achieved with the combination of StcE and permeation enhancer indicated a relative increase in desmopressin plasma levels and area under the curve (AUC) compared to the control, showing a trend towards improved oral bioavailability, however with notable inter-individual variability. Overall, StcE can be employed for reducing the gastrointestinal mucus barrier, enhancing the permeability of macromolecules. StcE has the potential, especially when combined with other delivery strategies, to enhance oral delivery of macromolecules, ultimately improving oral bioavailability and therapeutic efficacy. This research was supported by the ETH Zurich Postdoctoral Fellowship program (Grant No. 22-1 FEL-12).

References:
1. Brown, T. D. et al. Materials for oral delivery of proteins and peptides. Nat Rev Mater (2020) 5, 127–148
2. Moroz, E. et al. Oral delivery of macromolecular drugs: Where we are after almost 100 years of attempts. Adv. Drug Deliv. Rev. (2016) 101, 108–121.
3. Murgia, X. et al. The role of mucus on drug transport and its potential to affect therapeutic outcomes. Advanced drug delivery reviews (2018) 124, 82–97.
4. Lai, S. K. et al. Micro- and macrorheology of mucus. Advanced drug delivery reviews (2009) 61, 86–100.
5. Shon, D. J. et al. Classification, structural biology, and applications of mucin domain-targeting proteases. The Biochemical journal (2021) 478, 1585–1603.

Appendix 3

Mucins in Health and Disease

17-20 June 2024 • Gothenburg • Sweden

Abstracts for poster presentations

Adaptation of mucins and mucous cells

Study of goblet and Paneth cell biology in health and disease using a novel Muc2iCre line

Alexandra Thiran[1-4] , Gillian Blancke[1-4], Ioanna Petta[1-4], Maarten Ciers[1-4], Maude Jans[1-4], Marie Thorp[1-4], Niels Vandamme[1,2,4], Peter Borghgraef[1], Mahadevan V. Subramani[5], Wendy Toussaint[1,4], Bart Lambrecht[1,2,4], Geert Van Loo[1-4], George Birchenough[5], Lars Vereecke[1-4]

1. VIB Center for Inflammation Research (IRC), 9052 Ghent, Belgium.
2. Ghent Gut Inflammation Group (GGIG), Ghent University , Ghent , Belgium.
3. Department of Internal Medicine and Pediatrics, Ghent University, 9000 Ghent, Belgium.
4. Cancer Research Institute Ghent (CRIG), 9000 Ghent, Belgium.
5. Department of Medical Biochemistry and Cell biology, University of Gothenburg, 40530 Göteborg, Sweden

The state of symbiosis between host and gut microbiota is established through an effective intestinal epithelial barrier: a single cell layer of intestinal epithelial cells (IECs) covered with mucus separates the microbiota from underlying host tissue. Goblet cells, specialized secretory cells with pleiotropic functions in the gut, synthesise and secrete mucus, sample and present luminal antigens via cell-associated antigen passages (GAPs) and produce immune polarising chemokines and cytokines. Multiple goblet cell subpopulations have already been identified, each with a specific expression profile and function. In the small intestine, Paneth cells produce antimicrobial peptides (AMP's) and stem-cell supporting factors. Defects in goblet and Paneth cells are associated to multiple inflammatory and metabolic diseases and colorectal cancer. The Host-Microbiota Interaction (HMI) lab generated a new transgenic mouse line which expresses the improved Cre (iCre) recombinase under the control of the rat Muc2-promotor. Muc2iCre transgenic mice were crossed with RFP and YFP reporter mice, in order to characterise cell specificity, and to study sorted goblet cells by scRNA and bulkRNA sequencing, both in steady state and upon infection with enteric bacteria (Citrobacter rodentium) and helminths (Trichuris muris). We also generated conditional and constitutive goblet cell Diphtheria-toxin based depletion models, in order to study the physiological consequences of temporal and constitutive loss of Paneth, goblet cells and the protective mucus layer, respectively. Constitutive goblet and Paneth cell loss induces the development of chronic intestinal inflammation and colorectal cancer overtime, which is driven by bacterial-epithelial interactions. The Muc2iCre line thus represents a unique tool to perform Paneth and goblet cell-specific gene-editing, sorting and depletion experiments in order to acquire more insights in Paneth and goblet cell biology in various physiological conditions.

Adaptation of mucins and mucous cells

Circadian dynamics of the colonic barrier and glycome

Alex Bennett[1], George Birchenough[1], Daniel Bojar[1]

1. University of Gothenburg, University of Gothenburg, University of Gothenburg,

Circadian rhythms govern crucial biological processes, and recent investigations have unveiled the diurnal dynamics of the colonic mucus barrier. Yet, gaps persist in understanding the temporal behaviors of the colon, particularly concerning mucin glycosylation and goblet cells. This study aims to characterize temporal rhythms in the colonic-mucin-glycome and host behaviors related to mucus production and glycosylation. We employ novel glyco-informatic techniques to analyze temporal glycomics data, integrating it with host transcriptome and proteome analyses, as well as characterizing the fecal and mucosal-associated microbiome in mice. By bridging circadian biology, mucus biology, and glycomics, our research elucidates mechanisms underpinning the circadian dynamics of colonic host-microbe interactions in health and disease, addressing a fundamental gap in current knowledge.

Adaptation of mucins and mucous cells

Pre-weaning microbiota colonization drives maturation of primary and secondary goblet cell mediated protection in the neonatal colon

Åsa Johansson*[1], Mahadevan Venkita Subramani*[1], Bahtiyar Yilmaz[2], Felix Sommer[3], Lars Vereecke[4], Andy Wullaert[4], Malin Johansson[1], George Birchenough[1]

1. Institute of Biomedicine, University of Gothenburg
2. Department for BioMedical Research, University of Bern
3. Institute of Clinical & Molecular Biology, Kiel University
4 VIB Center for Inflammation Research, Ghent

Mucus secreting goblet cells (GCs) primarily confer protection from luminal microorganisms via generation of a sterile inner mucus layer (IML) barrier structure. Bacteria-sensing sentinel goblet cells (senGCs) are thought to provide a secondary defensive mechanism that orchestrates mucus secretion in response to microbes that breach the IML barrier. Examination of adult GF mice has previously reported IML deficiencies, thus implicating a role for the microbiota in IML function; however, the natural development of the IML in response to postnatal colonization and the potential role of the microbiota in guiding senGC development has yet to be confirmed. In the current study, we have combined in vivo and ex vivo analysis of pre- and post-weaning IML and senGC maturation to demonstrate dynamic, microbiota-dependent development of both primary and secondary GC-intrinsic protective functions in the pre-weaned neonatal colon, thus further highlighting the importance of this period for the development of mucosal barrier function.

Adaptation of mucins and mucous cells

Role of the transcription factor HNF4A in controlling the production of intestinal goblet cells following bacterial infection

Dianne Pupo Gomez[1], Vilcy Reyes Nicolas[1], Gisela Marrero Cofino[2], Nathalie Perreault[1], Alfredo Menendez[2], Francois Boudreau[1]

1. Department of Immunology and Cell Biology, Universite de Sherbrooke, Sherbrooke, QC, Canada
2. Department of Microbiology and infectious disease, Universite de Sherbrooke, Sherbrooke, QC, Canada

Inflammatory bowel diseases (IBD) are multifactorial, depending on genetic, immune, and environmental dysregulations. The intestinal barrier, both physically and functionally, plays an important role in maintaining intestinal homeostasis. Now, it is well-established that perturbations in the integrity of the barrier have become a hallmark in the development of these diseases. On the other hand, our laboratory has shown that the conditional deletion of the HNF4A nuclear receptor in mouse's intestinal epithelium leads to intestinal chronic inflammation. However, the impact of the loss of this transcriptional factor on the epithelial barrier is still controversial. Aim: To evaluate the impact of Hnf4a deletion on the epithelial barrier during bacterial infections. Methods: We used a tamoxifen-inducible CreER-loxP system to delete the Hnf4a gene in the intestinal epithelium of 2–4-month-old mice (Hnf4aΔIEC-ind). Intestinal permeability was assessed in Hnf4aΔIEC-ind and control mice from collected serum after oral gavage of FITC-dextran. Hnf4aΔIEC-ind and control littermate were infected with Salmonella Typhimurium (SB103), an invasion-attenuated strain. S. Typhimurium loads were scored in the feces, liver, and spleen tissues. Histological examinations were carried out by different staining, and gene expression of selected targets was assessed by RT-qPCR between mutant and control mice. Results: In Hnf4aΔIEC-ind mice, an increase in the passage of FITC-dextran into the blood circulation revealed that the intestinal epithelium presented an enhancement in permeability associated with the mutation. Interestingly, oral infection with an invasion-deficient Salmonella Typhimurium strain did not show difference in the bacterial load or epithelial damage between Hnf4aΔIEC-ind and control mice. However, histological examinations showed that at 4 days post-infection (dpi), Hnf4aΔIEC-ind mice presented not only a superior number of goblet cells in the ileal crypt, but also an increase in the average size of these cells. Following the infection, gene expression analysis showed that Hnf4aΔIEC-ind mice showd increased expression of genes related to the protection and integrity of the epithelial barrier, such as Retlnb, Muc2, as well as the anti-microbial peptides Defa5 and Defa20. Moreover, Hnf4a deletion not only impacted the expression patterns of fucosylation in the ileum after infection, but also appeared to influence the expression of Math1 and Spdef genes associated with the specification and differentiation of secretory cells. Bacterial localization using the general bacterial probe EUB388-cy3 (red) staining showed that infected mutant mice tended to enhance barrier mucus layer thickness compared to the controls. Conclusion: Altogether, these results support that HNF4A could play a crucial adaptive role in modulating the intestinal epithelial barrier function in the face of enteropathogenic infections.

Adaptation of mucins and mucous cells

Adenosine receptor signalling drives mucin expression in the intestine

K Clare[1,2], L O'Connell[1,2], M Stepanova[1,2], CM Aherne[1,2]

1. School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland.
2. UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland.

Loss of mucosal barrier integrity and inappropriate perpetuation of the inflammatory response are key features of ulcerative colitis (UC). Increased permeability and disruption of the mucus gel layer (MGL) lining the colonic intestinal epithelium is observed prior to the onset of colitis in UC and murine colitis models. Therefore, understanding how the integrity of the MGL is maintained during health and disease may lead to novel therapeutic approaches for UC.
The major constituents of the MGL are mucins which are secreted by the intestinal epithelium. Extracellular adenosine signalling can mediate mucin hypersecretion in asthma and other inflammatory respiratory conditions. Studies suggest that signalling via the Adora2a adenosine receptor (A2AAR) and Adora2b adenosine receptor (A2BAR) is protective in acute gastrointestinal inflammation such as colitis, although the mechanism by which this occurs, especially for the A2BAR, is unclear. We hypothesised that as in the respiratory epithelium, adenosine signalling in the gut may stimulate mucin synthesis, thus enhancing the protective efficacy of the MGL.
Treatment of Caco-2 and T84 colonic epithelial cell lines with an A2B selective receptor agonist induced upregulation of MUC2 and MUC5AC mRNA, which was abolished by blockade of receptor signalling and inhibition of second messenger pathways. Similar results were observed using MUC5AC promoter reporter assays. In the mucus producing HT29-MTX-E12 intestinal epithelial cell line treatment with an A2BAR agonist significantly induced injury associated increases in MUC5AC mRNA and protein expression. A non-specific A2 adenosine receptor agonist was unable to significantly alter MUC2 or MUC5AC mRNA expression in intestinal epithelial cell lines.
Our preliminary findings suggest that adenosine receptor signalling can increase secreted mucin expression in the injured intestine and implicate the A2BAR as the specific receptor responsible for increased mucin expression.

Adaptation of mucins and mucous cells

AGR2 and IRE1β: a dream team in intestinal goblet cell quality control

Phaedra Guilbert[1,2], Eva Cloots[1,2], Mathias Provost[2,3], Farzaneh Fayazpour[1,2], Evelien Van de Velde[1,2], Delphine De Sutter[4,5], Savvas Savvides[2,3], Sven Eyckerman[4,5], and Sophie Janssens[1,2]

1. Department of Pediatrics and Internal Medicine, Ghent University, Ghent, Belgium
2. VIB Center for Inflammation Research, Ghent, Belgium
3. Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
4. Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
5. VIB Center for Medical Biotechnology, Ghent, Belgium

The gastrointestinal tract is characterized by a protective mucus layer, secreted by intestinal goblet characterized by a complicated maturation and secretion process. It is well-known that misfolding of such proteins leads to a state of endoplasmic reticulum (ER) stress in secretory cells, which is then mitigated by an orchestrated transcriptional response called the Unfolded Protein Response (UPR). Aberrations in the UPR or in the mucin folding machinery can contribute to the development of inflammatory bowel disease (IBD). Anterior Gradient 2 Homolog (AGR2) is a protein disulfide isomerase involved in maturation of mucins in the ER, and loss of function mutations have been identified in individuals with early onset IBD. Still, our understanding of the quality control mechanisms for goblet cells and intestinal MUC2 folding and maturation is incomplete. We discovered that the MUC2 chaperone AGR2 regulates the goblet cell-specific ER stress sensor Inositol-requiring Enzyme (IRE)1β. IRE1β is a transmembrane signaling protein in the goblet cell ER, characterized by a cytoplasmic endonuclease domain. It is thought to protect ER integrity by regulating the amount of unfolded MUC2 polypeptide chains that enter the ER, through degradation of excess Muc2 mRNA via its endonuclease domain. How IRE1β activity was regulated has however remained obscure since its discovery over 20 years ago. We now identified AGR2 as a main regulator of IRE1β activity. AGR2 interacts specifically with IRE1β in both the human LS174T goblet cell-like cell line and murine colon tissue. The interaction does not require AGR2 dimerization but does require its catalytic cysteine (C81) to be intact. A recently identified AGR2 mutation associated with early-onset IBD, H117Y, also disrupts the interaction between AGR2 and IRE1β. Co-expression of wild-type AGR2, but not the C81S or H117Y mutants, with IRE1β restricts IRE1β endonuclease activity. Using competition assays and size exclusion chromatography, we demonstrate that AGR2 exerts this inhibitory effect on IRE1β through disruption of the catalytically active IRE1β dimer. In vivo, Agr2 deficient mice exhibit depleted Muc2 mRNA levels that are restored upon compound deficiency with IRE1β. This supports a model where AGR2 functions as a molecular switch coupling endonuclease activity on Muc2 mRNA to the mucus folding load experienced by mucin chaperones such as AGR2.
In conclusion, we report a novel link between two goblet cell proteins involved in mucin barrier regulation, leading to new insights into their role in mucin quality control and the potential impact of this UPR axis in IBD.

Adaptation of mucins and mucous cells

Role of free-fatty acid receptors in regulating intestinal goblet cell intrinsic defense

Akshi Singla[1], George Birchenough[1]

1. Department of Medical Chemistry and Cell Biology, University of Gothenburg

Sufficient evidence has emerged to establish that goblet cells play a vital role in sustaining the intestinal homeostasis through mucus secretion via more than one mechanism and intestinal microbiota play a key role in goblet cells development and maturation. However, there is still no causative data linking specific microbiota-host signals to the regulation of mucus layer. G-protein coupled receptors are critical regulators of responses to extracellular signals, and mRNA sequencing of isolated murine enterocytes and goblet cells shows that the short and long chain fatty acid (SCFA/LCFA) receptors Ffar2/Ffar4 are largely restricted to goblet cells in the ileum and colon. Therefore, we are investigating microbiota-host signaling pathways in response to SCFA/LCFA using in vivo and cutting-edge ex vivo tools that enable the quantification of mucosal barrier properties and mucus secretion in live tissue. We have employed transgenic mice expressing Cre recombinase under Muc2 promoter control (Muc2-iCre) and used these to generate goblet cell specific FFAR2/FFAR4 knock-out mice.

Adaptation of mucins and mucous cells

Investigating the mechanism of goblet cell-antigen presenting cell interactions in regulation of colonic immunity

Victor Pettersson[1], Jenny Gustafsson[1]

1. Institute of Neuroscience and Physiology, Department of Physiology, University of Gothenburg, Gothenburg, Sweden.

Colonic goblet cells (GCs), known to produce and secrete mucins, have been shown to also sample and deliver luminal antigen to lamina propria antigen-presenting cells (LP-APCs) by the formation of goblet cell associated antigen passages (GAPs). GC mediated sampling is an important process to support the induction and maintenance of tolerance. We have previously shown that approximately 10% of GCs form GAPs at steady state, primarily in the lower half of the colonic crypts. Whether regulation of colonic immunity is restricted to GCs forming GAPs is currently unknown. To address this question, we quantified GC – LP-APC interactions along the crypt – surface axis using confocal microscopy. Interaction patterns were altered, by stimulation of the muscarinic receptor using carbachol and atropine, and by the administration of mixed antibiotics (Abx). Our observations show that at steady state, 20% of GCs interact with LP-APCs, and that the occurrence is higher in the surface GCs. Carbachol significantly increased interactions in the upper crypt region and surface epithelium, an effect that was reversed by atropine, suggesting the involvement in facilitating either interactions or the recruitment of LP-APCs. A suppression of the microbiota instead reduced interactions in the same regions suggesting that the microbiota is a driving factor. Taken together, these results show that GC – LP-APC interactions are not restricted to GCs forming GAPs and occur at a higher frequency in the upper distal colon crypt – surface axis. The physiological role of the interactions between GCs not forming GAPs and LP-APCs remains to be determined.

Adaptation of mucins and mucous cells

Regulation of epithelial renewal in the colon by microbial metabolites and immune signals

Iwona T. Myszor[1], Hannah Sträussnigg[1], Sjoerd van der Post[1]

1. University of Gothenburg, Department of Medical Biochemistry and Cell biology, Gothenburg, Sweden

Proliferation of adult stem cells maintains epithelial renewal in the colon. Effects of individual microbial metabolites and immune components on colonic stem cell proliferation and the regulatory mechanism are poorly characterized as effects of single metabolites in the complex network of microbial metabolites are challenging to study in vivo.
The main objective of this work is to determine how microbial metabolites and immune signals regulate epithelial renewal and function in the colon. Our previous findings show that stem cell proliferation in the colon coincidence with an increased generation of reactive oxygen species (ROS) generated by NADPH oxidase 1 (Nox1). We have established a screening platform to test individual compounds from the library of microbial metabolites and selected cytokine/chemokines simultaneously measuring proliferation and ROS. We used organoids from germ-free and conventionalized mice to assess stem cell proliferation upon microbiota colonization and determine if microbial metabolites imprint epithelial memory in adult stem cells. Analyses of imprinting epithelial memory include differentiation-like memory and imprinting innate immune responses in differentiating epithelial cells. Among microbial metabolites, we identified e.g., polyphenols and tryptophan metabolites that have pronounced effect on stem cell proliferation and differentiation. We further characterized the protein networks regulated during stem cell renewal and front-line defenses by mass spectrometry-based proteomics.
Our work brings new knowledge to the field of mucosal immunology by identifying novel regulators and mechanisms contributing to the epithelial turnover and trained immunity-like memory in the intestinal epithelium in response to microbial metabolites and immune signals.

Adaptation of mucins and mucous cells

Proteolysis of the cell surface glycocalyx

Gabrielle Tender[1], Angel Kuo[1], William Chow[1], Ke (Chloe) Wen[2], Nicholas Riley[1], Carolyn Bertozzi[1,3]

1. Department of Chemistry and Sarafan ChEM-H, Stanford University, Stanford, CA, USA
2. Department of Chemical Engineering, Stanford University, Stanford, CA, USA
3. Howard Hughes Medical Institute, Stanford, CA, USA

All characterized mucin-selective O-glycoproteases (mucinases) are bacterial enzymes, evolved mainly from members of the gut microbiota. However, human cathepsin D was previously characterized to catabolize fully glycosylated mucin domains. Cathepsin D is one of 15 cathepsins, a class of highly abundant lysosomal proteases, spanning serine, cysteine, and aspartic proteases.

We identified that multiple human cathepsins degrade purified and recombinant mucins in in vitro digestions. Cathepsin K, and not cathepsin D, degraded multiple cell surface mucins on cancer cells within the physiological pH range of human tumor microenvironments. Cathepsin K can cut mucins within the densely O-glycosylated mucin domains and can tolerate, but not require, glycans near the cleavage site, as identified via mass spectrometry characterization of cathepsin K cleavage of multiple recombinant and purified mucins. Cathepsin K treatment of cancer cell lines additionally reduced heparan sulfate and polysialic acid levels, further debulking the cancer cell surface glycocalyx.

Cathepsin K is a papain-like cysteine protease predominantly secreted by osteoclasts to degrade collagen. However, cathepsin K has also been characterized to be secreted in multiple cancers, primarily enhancing invasiveness and metastasis.

Prior published work has demonstrated that increased mucin expression and glycocalyx bulk enhances cancer cell survival and metastasis. Furthermore, removing mucins via a targeted-mucinase blunted primary tumor burden and metastatic outgrown in mouse models of breast cancer progression. Therefore, repurposing cathepsin K is a promising tool for debulking cancer cells using a human enzyme.

Adaptation of mucins and mucous cells

The impact of MUC1 in breast milk on maturation of the gut microbiota and epithelial cell function in infants

Fleur C. van der Koolwijk[1], Nirary Nissan[1], Fabiola Puértolas Balint[2], Bjoern O. Schroeder[2], and Thaher Pelaseyed[1]

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Box 440, 405 30 Gothenburg, Sweden.
2. Department of Molecular Biology and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Umeå, Sweden.

The human digestive tract is colonized with trillions of bacteria, the gut microbiota, that transmit cues to the intestinal epithelium and the underlying immune cell compartment to establish tolerance towards commensal bacteria while remaining vigilant towards pathogens. Upon birth, the infant digestive tract is instantly seeded by bacteria derived from its mother and the surrounding environment. The mother in turn provides breasy milk carrying antibodies and milk oligosaccharides that protect the infant against bacterial gut pathogens. Importantly but often neglected, breast milk is also a major source of sugar-laden proteins called mucins, the impact of which on the gut microbiota and intestinal defense systems is severely understudied. MUC1 is a major membrane-attached mucin expressed on the mammary epithelium during lactation and then transferred to breast milk where it presents thousands of O-glycan epitopes to the infant gut microbiota. However, its role in shaping the composition of the infant gut microbiota is totally unknown.

Here, we aimed to define the impact MUC1 in milk on the composition of the gut microbiota and the maturation of the infant intestine. Using a genetic mouse model, we demonstrated that newborn mice fostered by Muc1 knockout (KO) females exhibited weight loss during postnatal development. An assessment of microbioal composition in the small intestine revealed increased abundance of the known mucin-utilizers *Akkermansia*, *Muribaculaceae*, and *Coriobacteriaceae* in mouse pups fostered by Muc1 KO females. Strikingly, epithelial profileration and glycocalyx barrier formation were delayed in mouse pups deprived of Muc1. To gain mechanistic insights into these findings, we used a recombinant murine Muc1, with bioorthogonally labeled O-glycans, to monitor the biological faith of Muc1 in the neonatal intestine. Ingested recombinant Muc1 was resistant to host digestive enzymes as well as enzymes produced by small intestinal bacteria, but was completely degraded by bacteria residing in the distal colon. Moreover, small intestinal enterocytes, which engage in macropinocytosis, absorbed large quantities of labeled Muc1, suggesting that mucins in breast milk not only influence the infant gut microbiota but may also regulate epithelial cell funcitions during postnatal development.

Our results demonstrate that MUC1 carried in breast milk has a profound effect on the maturation of the infant gut microbioa och intestinal defense systems. Mode of delivery, infant formula, and antibiotics reshape the in infant gut microbiota and impose short-term and long-term adverse health outcomes such as infections and allergies. Breast milk mucins present a novel concept for understanding the mechanisms for maintenance of a healthy infant gut microbiota. Notably, mucins offer a new therapeutic approach for supporting beneficial bacterial communities that promote intestinal defenses and establish colonization resistance against bacterial infections early in life.

Models in Mucin Research

Air-liquid interface Caco-2 culture with vasointestinal peptide mimicks gut mucosal barrier function in permeability and bacterial infection

Evelien Floor[1$] , Jinyi Su[2$], Maitrayee Chatterjee[2 $*], Elise Kuipers[2], Noortje Ijssennagger[3], Laura Giordano[4], Silvia M. Mihăilă[4], Daphne Stapels[2], Yvonne Vercoulen[1*], and Karin Strijbis[2]

1. Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, 3584 CX Utrecht, The Netherlands.
2. Department of Biomolecular Health Sciences, Division of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands
3. Danone Nutricia Research, The Netherlands
4. Div. Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands.
$ Equal contribution
*current address: The TIM company, The Netherlands
* Corresponding authors.

The intestinal mucosal barrier is a dynamic system that allows nutrient uptake, stimulates healthy microbe-host interactions, and prevents invasion by pathogens. The mucosa consists of an epithelial layer covered by a viscous mucus layer that plays an important role in host-microbiome interactions: Mucus prevents direct contact with the intestinal microbiota and pathogenic invasion, but also provides binding sites and nutrients for commensal bacteria. The underlying epithelial cells are connected by tight and adherence junctions that regulate the passage of nutrients and immune cells. Mimicking the intestinal mucosa for in vitro assays, in particular the generation of a viscous mucus layer, has proven to be challenging. Hence, there is a need to develop robust and representative in vitro culture models that contain a functional mucus layer and allow mechanistic studies of host-microbiome interactions. Here, we present a novel in vitro intestinal culture model that produces a robust mucus layer and is based on the widely used intestinal Caco-2 cell line. We investigated the effects of air-liquid interface (ALI) culturing compared to liquid-liquid interface (LLI) on Caco-2 cells grown under low-glucose conditions in Transwell plates. In addition, we determined the impact of vasointestinal peptide (VIP) on mucus secretion, epithelial barrier properties and microbe-mucus interactions. A combination of ALI-VIP culturing led to formation of a robust mucus layer on the apical surface of the Caco-2 confluent layer. ALI-VIP culturing induced high expression and secretion of MUC2, the highly O-glycosylated mucin which is the main structural component of the intestinal mucus layer. Robust MUC2 production was also observed in Caco-2 cells under ALI/VIP conditions in a gut-on-a-chip model. RNAseq analysis demonstrated upregulation of unique gene clusters in response to ALI and VIP conditions, but the ALI-VIP combination treatment resulted in a significant upregulation of multiple mucin genes and proteins including MUC2, MUC13 and MUC17. Expression of tight junction proteins was significantly altered in the ALI-VIP condition. Under these conditions, in the presence of the mucus layer, the Caco-2 cell layer was more permeable to small molecules as measured by TEER and tracers ranging from 0.5 kDa to 70 kDa compared to LLI conditions or conditions without VIP. In infection experiments with commensal Lactobacillus plantarum, pathogenic Salmonella enterica serovar Enteritidis, or enterotoxigenic Escherichia coli (ETEC), we could demonstrate that the ALI-VIP mucus layer separates the bacteria from the underlying epithelium and that the bacteria differentially interact with the mucus layer. In conclusion, ALI-VIP culture of Caco-2 cells provides an attractive in vitro model to study the function of the intestinal mucosal barrier and pathogenic and commensal microbe-host interactions.

Models in Mucin Research

3-D bioinspired mucin-based biomaterials to model mucosal barrier

L. Sardelli[1], E. Frasca[1], O. G. Garbero[1], C. Medana[1], C.Butnarasu2 and S. Visentin[1]

1. Dept. Department of Molecular Biotechnology and Health Science, University of Torino, Torino, Italy
2. Institut für Pharmazie Biopharmazeutika, Freie Universität Berlin

Human mucins create polymeric networks in distinct tracts of our bodies thus forming mucus layers that act as the first biological barrier for molecules, including nutrients and drug. This role is expressed thanks to specific compositional, microstructural and rheological properties, which actively filters molecules depending from their chemical structures and features1. In the perspective to study the passage of molecules from the external environment to the human tissue, in vitro systems may represent a fundamental asset, as they can provide information about molecules permeability in controlled conditions, outside of the confounding complexity of the human body. Surprisingly, mucins and mucus in these systems remained limitedly considered2,3.
In our work, we developed and applied new mucins-based biomaterials suitable for the study of molecules permeability within high throughput systems (HTS) modelling the environment of different human mucosal tracts. The mucins-based biomaterials address the viscoelastic properties of the target mucus layers (e.g., lung, intestinal and vaginal mucus layers) while maintaining a bioinspired mucins content within a stable 3D microarchitecture. The coupling of the mucin-based biomaterials with in vitro permeability assay that relies on cell-free membranes (like PAMPA or Permeapad) enabled the investigation of the mucus role in different pathological scenarios. For example, we quantified in vitro the permeability of immune-triggers (e.g., P. aeruginosa quorum sensing molecules, including pyocyanin) in the context of infections, observing the possible and intriguing pathological role that mucus may express by masking immune-activating molecules and hence delaying the fight of infection. Similarly, we used mucins-based biomaterials to screen molecules (> 10) acting on a specific cytoplasmic receptor, the AhR, having a role in the pathological scenario such as cystic fibrosis (CF) and hence recently selected as possible terapeutic target. We computed the apparent permeability coefficients in in vivo-like CF-condition, thus investigating the impact of the pathological mucins microenvironment on the capability of the selected molecules to reach their target (AhR). Importantly, we observed that CF-mucus have a potent influence of AhR targeting, with 70% of the permeability coefficients of the molecules that were modified if compared to the physiological-like in vitro conditions.
Overall, the proposed mucins-based biomaterials could be promising in vitro tools to model the environmental complexity of mucosal tissues and hence open the possibility to study in an in-vivo-like in vitro system the effect of the mucins network in different biological context, including pathophysiological molecular mechanisms and drug-permeability profiling.

1. Sardelli, L. et al. RSC Adv 9, 15887–15899 (2019).
2. Butnarasu, C. et al. Pharmaceutics 15, 380 (2023).
3. Butnarasu, C. et al. Mol Pharm 19, 520–531 (2022).

Models in Mucin Research

Recombinant mucin tandem repeats with tunable repeat number and glycosylation as a source to build novel antiviral biomaterials

Robyn Diehn[1], Cosmin Butnarasu[1], Jolly Thomas[1], Fabian Nußhardt[1], Daniel Lauster[1]

1. Institute of Pharmacy, Freie Universität Berlin, Germany

Mucins are a valuable source for biomaterials targeting pathogen-host interactions. Especially the highly glycosylated regions harbor the potential to interfere with virus infections based on inhibitory virus-glycans interactions. However, mucins represent a highly polydisperse biopolymer with differences in the number and size of tandem repeats, as well as in the amount and kind of glycosylation. In order to obtain defined, but functionally intact mucin regions, proteolytic digest of mucins is a meaningful strategy, but heavily glycosylated mucin regions restrict accessibility to the protein backbone leading to large, heterogeneous fragments. To tackle this challenge, sophisticated recombinant versions of small tandem repeats of MUC5B were designed to obtain defined repeat numbers and glycosylations (O-glycans or N-glycans) alongside with protease cleavage sites. We could achieve yields up to 18 mg/L of these biomolecules in HEK293Expi cell cultures and found by glycoanalysis a human-like glycopattern as found on mucins. From these mini-mucins we were able to obtain by proteolytic treatment small defined glyopeptides, which in the future will be used to functionalize nanoparticles for the buildup of new antiviral drugs.

Models in Mucin Research

Generating extracellular vesicles for biomedical applications using genetically encoded glycoengineered mucins

Erik Chow, Raviv Dharan, LaDeidra M Roberts, Joe CH Kuo, Sangwoo Park, Raya Sorkin, Matthew J Paszek

1. Dept. of Biomedical Engineering, Cornell University, Ithaca, NY, United States
2. School of Chemistry, Tel Aviv University, Tel Aviv-Yafo, Israel
3. Dept. of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, United States
4. Dept. of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, United States
5. Dept. of Biophysics, Cornell University, Ithaca, NY, United States
6. School of Chemistry, Tel Aviv University, Tel Aviv-Yafo, Israel
7. Dept. of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, United States

Extracellular vesicles (EVs) have inspired growing interest in biomedical research for their role in intercellular communication via the transport of DNA, RNA, proteins, and small molecule payloads. However, the role of the cell glycocalyx in regulating EV-mediated intercellular communication remains poorly understood. Furthermore, there is an unmet need for the ability to engineer EVs with desired biochemical and biophysical properties for biomedical applications. The Paszek Group has shown previously that altering expression of the mucin glycoprotein MUC1 in the cell glycocalyx can influence the generation of EVs. Here, we combine a genetic library for induced expression of engineered cell surface mucins with CRISPR/Cas9 glycoengineering strategies to engineer EVs and investigate the hypothesis that MUC1 expression regulates EV biogenesis by affecting cell membrane curvature.

Increasing cell-surface mucin expression resulted in a corresponding increase in EV secretion. Surprisingly, flow cytometry analysis and immunofluorescence imaging of EV parent cells showed an increase in cell-surface localization of CD63, a canonical EV biomarker more commonly associated with intracellular organelles like late endosomes and multivesicular bodies. Collaborating researchers at Tel Aviv University showed that CD63 is preferentially sorted into regions of high membrane curvature. This behavior offers a possible explanation for the change in CD63 localization observed in the mucin-overexpressing cells, as the Paszek Group previously showed that increasing mucin expression in the cell glycocalyx promotes dramatically curved membrane morphologies. Proteomics analysis of mucin-induced EVs identified multiple proteins known to interact with CD63, suggesting that MUC1-driven sorting of CD63 may promote cargo-loading and subsequent secretion of EVs. Notably, mucin-induced EVs were found to have mucin polymer coatings and to carry markers of both classical exosomes and microvesicles, suggesting this strategy could be applied to engineer EVs with inherent biopolymer coatings. Mucin-coated EVs exhibited improved stability at 4℃, showing that such coatings could be leveraged to confer useful features for biomedical applications.

To our knowledge, this study marks the first effort to closely investigate the relationship between mucin expression and EV biogenesis. Because the majority of solid-tumor cells are known to overexpress MUC1 and to generate more EVs than healthy cells, exploring this relationship will deepen our understanding of EV-mediated intercellular communication in human health and disease. Further studies are needed to more thoroughly unravel the biogenesis pathway of mucin-induced EVs and to verify these findings in tumor cells. Additionally, these results establish a genetically encoded platform for the production of EVs with inherent surface coatings. Future studies will expand this platform by exploring strategies to functionalize EV mucin coatings.

Models in Mucin Research

Investigating gut microbiota-mucin interactions within an engineered colonic simulator

Serena Berberolli[1], Emily Dewhurst[2], Ines Moura[3], Dr Celia Ferreira[1], Prof Daniel Read[4], Nik Kapur[2], Mar Collado González[5], Dr. Jin Seo[6], Dr. Mariam Hussain[7], Dr. Gabriele Gross[8], Prof Francisco Goycoolea[1,5], Dr Anthony Buckley[1]

1. School of Food science and Nutrition, University of Leeds, UK
2. School of Mechanical Engineering, University of Leeds, UK
3. Leeds Institute of Medical Research, Faculty of Medicine and Health, University of Leeds, UK
4. School of Mathematics, University of Leeds, UK
5. Department of Cell Biology and Histology, University of Murcia, Spain.
6. Reckitt Health US LLC, U.S.A
7. Reckitt U.K
8. Reckitt / Mead Johnson Nutrition Institute Netherlands

The colonisation of the gastrointestinal mucosal layer by the gut microbiota and the interaction of commensal bacteria with mucins play a fundamental role in regulating host homeostasis and disease susceptibility. However, the limitations of current in vitro models pose a challenge to deepening our understanding of the dynamics of microbial populations in a physiologically representative context. The aim of this study was to evaluate the role of mucin coating in MiGut®1, an innovative engineered simulator of the colonic environment, on mucin-utilising microbial communities. To this end, 3D printed scaffolds designed to support gut relevant biofilm formation were coated with mucin and placed into the vessels of the MiGut® platform. Through precise regulation of pH and temperature, and the continuous bubbling of nitrogen to uphold an anaerobic environment, it is possible to culture gut bacteria in a context that closely represents physiological conditions in the human colon.
A spatiotemporal analysis was conducted by sampling different modelled sections of the colon within MiGut®1at different time points. A total of ten different mucosal bacterial species were quantified via qPCR, including Akkermansia. Our results showed a notable increase in biofilm formation on mucin coated scaffolds vs untreated scaffolds, highlighting the pivotal role of mucin in promoting bacterial
adhesion and colonisation, and in shaping the microbial composition in the colonic environment. This included significant increases in Akkermansia spp. (5.85 log10 CFU/mL) and Bifidobacterium spp. (8.55 log10 CFU/mL) on the mucin coating vs plain coated biofilms (4.90 log10 CFU/mL and 7.04 log10 CFU/mL respectively).
These findings obtained from this optimized model contribute to an improved understanding of the gut microbial ecology, paving the way for more complex studies that aim to evaluate the effect of exogenous factors on mucus-associated species and, ultimately, develop strategies to improve gastrointestinal health.

1. Davis Birch WA, Moura IB, Ewin DJ, et al. MiGut: A scalable in vitro platform for simulating the human gut microbiome—Development, validation and simulation of antibiotic-induced dysbiosis. Microb Biotechnol. 2023.

Models in Mucin Research

Studying host-microbe interactions in engineered human gut tissues

Ruben Lopez Sandoval[1], Marius Harter[1], Mikhail Nikolaev[1], Julien Aubert[1], irineja Cubela[1], Nikolche Gjorevski[1], Gray Camp[1]

1. Institute of Human Biology (IHB), Basel, Switzerland

The human gut microbiome comprises a complex community of microorganisms that play an essential role in health and disease. However, the mechanisms that govern the interaction between microbes, the epithelium and the immune system are not yet fully understood. Current methods to study this relationship are mainly focused on the analysis of stool samples, the use of gnotobiotic animals and in vitro models. Among these, intestinal organoids represent one of the most advanced systems to date but their high variability and the challenging access to the luminal space by microinjection techniques make them suboptimal for microbiota research. In contrast, lab-on-chip devices and in particular the use of engineered hydrogel scaffolds with crypt-villus architecture overcome the limitations of intestinal organoids and recreate a morphology close to the in vivo. Here, we aim to combine such engineered tissues with single-cell genomic technologies to grow a differentiated human intestinal epithelium and characterize the gene regulatory network (GRN) responses that dominate host-microbe interactions in homeostasis and inflammation. Our results show how human intestinal stem cells are able to populate the crypts homogeneously and form an epithelial monolayer containing differentiated cell types arranged in a patterned distribution similar to the in vivo counterpart. In addition, the monolayer presents an apical-basal polarity and a thick mucus layer that is physiologically relevant to protect the epithelium from the luminal compartment and bacteria. Preliminary experiments incorporating live commensal bacterial species indicate the importance of the mucus layer to avoid bacterial overgrowth and allow the homeostatic co-culture for several days. Overall, these results validate the human gut-on-chip and co-culture model and encourage to continue analyzing the mucus layer and its protective role to allow homeostatic long-term co-cultures that could pave the way to more physiologically relevant host-microbe in vitro studies.

Models in Mucin Research

Developing a platform for the identification of target genes regulating MUC5AC production and secretion in chronic airway disease

Luke R Bonser[1], Xavier Romero Ros[2], Henric Olsson[3], and Suzanne Cohen[2]

1. Translational Science and Experimental Medicine, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK
2. Bioscience Asthma and Skin Immunity, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK
3. Translational Science and Experimental Medicine, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

Introduction
Airway mucus is essential for health, while excessive mucin production and secretion contributes to plugging in asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis. MUC5AC, an integral component of airway mucus, is increased in chronic airway disease, yet our understanding of MUC5AC production and secretion is incomplete.

Methods
To systematically identify novel regulators of MUC5AC production and secretion we sought to develop a strategy for the high-throughput CRISPR-based functional characterisation of genes contributing to MUC5AC production and secretion in primary human bronchial epithelial cells (HBECs) grown at air-liquid interface. We implemented a series of assays including flow cytometry with cell sorting to enrich MUC5AC+ cells, lentiviral transduction of HBECs, and CRISPR-based protocols to enable the screen.

Results
A flow cytometry protocol to isolate TSPAN8+MUC5AC+ cells will be employed in conjunction with RNA-seq to catalogue goblet cell specific transcripts and enumerate sgRNAs depleted post-transduction. Approaches for lentiviral transduction of HBECs with CRISPR-Cas9 and CRISPRi will enable the screen and validation of individual targets respectively.

Conclusion
Our method will enable discovery of novel gene product targets that contribute to MUC5AC production and secretion, providing novel insights into the biosynthesis of MUC5AC and identification of novel therapeutic targets to unplug the airways in chronic airway disease.

Models in Mucin Research

Biocompatibility analysis of porcine gastric mucin in two cell lines derived from *Sparus aurata*

Mar Collado-González[1], Nora Albadalejo-Riad[1], Lucía Maquilón-Garrido[1], Yulema Valero[1], Alberto Cuesta[1], Maria Ángeles Esteban[1]

1. Immunobiology for Aquaculture, Cell Biology and Histology Department, Faculty of Biology, University of Murcia

According to the Spanish Aquaculture Business Association (APROMAR), in 2022 Spain was the second most productive country in aquaculture in the EU, with 326,520 tonnes produced valued at 760.7 million euros. Despite these data and the advances in this field, high mortality rates are recorded in aquaculture due to factors such as the high density of farmed specimens or the low water quality parameters, which in turn facilitates the spread of infections. To overcome this fact, the development of drug delivery systems (DDS) that can entrap bioactive molecules of interest has been proposed as a promising biotechnological strategy. In this scenario, porcine gastric mucin (PGM) has been proposed as an interesting material for the preparation of DDS. In our lab, we have analysed the biocompatibility of PGM in two different Sparus aurata cell lines, namely SAF-1 (fin) and SaB-1 (brain). Surprisingly, viability assays found that while PGM is cytotoxic to SAF-1 cells at high concentration after 72 h of exposure, whilst it showed biocompatibility when applied in SaB-1 cells under the same conditions. Furthermore, the production of reactive oxygen species varied between the two cell lines when exposed to PGM at different concentrations and different time points. Further analyses are required to understand the differential effects of PGM on both cell lines.

MCG acknowledges the funding from the Séneca Foundation-Science and Technology Agency of the Region of Murcia within the framework of the Young Leaders in Research call (22010/JLI/22) and the Ramón y Cajal scholarship (RYC2021-034184-I) from the State Research Agency of the Ministry of Science and Innovation (Spain). This study is part of the ThinkInAzul program supported by MCIN with funding from the European Union Next Generation EU (PRTR-C17.I1) and by the Autonomous Community of the Region of Murcia-Seneca Foundation (Spain).

Mucin Biosynthesis and Processing

Structural and genetic diversity in the secreted mucins, MUC5AC and MUC5B

Elizabeth Plender[1,2], Timofey Prodanov[4], PingHsun Hsieh[1], Evangelos Nizamis[1,3], William Harvey[1], Arvis Sulovari[1], Katherine Munson[1], Eli Kaufman[1,3], Paul Valdmanis[1,3], Wanda O'Neal[5], Tobias Marschall[4], Jesse Bloom[1,2,6], & Evan Eichler[1,7]

1. Department of Genome Sciences, University of Washington School of Medicine
2. Basic Sciences Division, Fred Hutch Cancer Research Center
3. Division of Medical Genetics, University of Washington School of Medicine
4. Institute for Medical Biometry and Bioinformatics, Heinrich Heine University Düsseldorf
5. Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill
6. Howard Hughes Medical Institute, Fred Hutch Cancer Research Center
7. Howard Hughes Medical Institute, University of Washington

Few loci in the human genome have been linked to severe respiratory disease outcomes; the mucin genes, however, are strong candidates due to their critical roles in pathogen entrapment. MUC5AC and MUC5B encode two secreted proteins with variable number tandem repeat sequences (VNTRs) that enable binding to pathogens. These loci have been difficult to assess due to the large and highly polymorphic protein-coding VNTRs. Here, we characterize the genetic structural diversity of MUC5AC and MUC5B by long-read sequencing and assembly of 206 human and 12 non-human primate haplotypes. We find that MUC5B is largely invariant in length (5761-5762aa) in humans; however, 7 haplotypes encode a much larger protein (6291-7019aa) due to a VNTR expansion. In contrast, we discovered 30 allelic variants of MUC5AC that encode 16 distinct proteins (5249-6325aa) with considerable variation in cys domain copy number and VNTR copy/domain number. We show that MUC5AC alleles can be grouped into three phylogenetic clades: H1 (46%, ~ 5654aa), H2 (33%, ~5742aa), and H3 (7%,~ 6325aa). Comparison to non-human ape gene models relative to the two most common human MUC5AC variants (5654aa and 5742aa) suggests an overall reduction in MUC5AC protein length during recent human evolution. Linkage disequilibrium (LD) and Tajima's D analysis reveal that East Asians carry exceptionally large MUC5AC LD blocks and an excess of rare variation ($p<0.05$ autosome-wide). We identified tagging SNPs (tSNPs) in high LD with haplogroups of MUC5AC ($r2 >0.85$; 37 for H1, 7 for H2, 67 for H3) to use for VNTR length imputation. We applied a structural variant genotyping tool called "locityper" to accurately genotype the haplotypes in 2,596 population samples from the 1000 Genomes Project. These findings confirm that the likely ancestral haplotype (H3) has been significantly reduced among Asians at the expense of H1/H2, which show signatures of positive selection. We predict population differences to airway associated disease and provide a more broadly applicable strategy to characterize the genetic impact of the hundreds of complex protein-encoding VNTRs that can now be resolved with long-read sequencing.

Mucin Biosynthesis and Processing

The Cl-/H+ antiporter ClC-5 modulates MUC1 trafficking and maturation to regulate renal proximal tubule epithelial cells differentiation

Mònica Duran[1], Gema Ariceta[1,2], Anna Meseguer[1], Gerard Cantero-Recasens[1]

1. Renal Physiopathology Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain
2. Pediatric Nephrology Department, Vall d'Hebron University Hospital, Barcelona, Spain

ClC-5 (Cl-/H+ exchange transporter 5), encoded by CLCN5 gene, is mainly expressed in renal epithelial cells of proximal tubule (PTC), where it controls endosomal acidification by cooperating with the V-ATPase and regulates protein trafficking and recycling to the plasma membrane. Mutations on CLCN5 cause Dent's Disease type 1 (DD1), a rare renal disease that progresses to renal fibrosis and kidney failure and is characterized by hypercalciuria and low molecular weight proteinuria. To decipher the mechanisms linking ClC-5 loss-of-function with proximal tubule dysfunction, our group generated cell models of the disease that carry different ClC-5 pathogenic variants. Analysis of gene expression profiles revealed several pathways related to epithelial dedifferentiation that could explain DD1 pathophysiology. In fact, many epithelial markers were downregulated in our DD1 cellular models, being the transmembrane Mucin-1 (MUC1) one of the most dysregulated genes. Our results showed that MUC1 levels are decreased and its location at the plasma membrane (PM) is strongly reduced in cells lacking a functional ClC-5. Moreover, treatment of control cells with bafilomycin, which impairs endolysosomal acidification mimicking ClC-5 loss-of-function, also caused a reduction of MUC1 levels and altered its trafficking to PM. Importantly, MUC1 altered expression and localisation leaded to β-catenin release from the PM and its translocation to the nucleus facilitating epithelial-mesenchymal transition (EMT). Amongst others, collagen transcription was enhanced, which, together with impaired degradation due to endolysosomal acidification defects, ended in a massive increase of collagen type I and IV intracellular and extracellular levels. Analysis of renal tissue from Clcn5-/- mice confirmed this effect on MUC1 localization and levels, which correlated with higher Col IV staining and a pre-fibrotic phenotype. Finally, analysis of MUC1 levels in urine samples from DD1 patients showed not only an alteration of their levels compared to control subjects, but also a decrease of its molecular weight, suggesting defects in MUC1 glycosylation. Altogether, our data provide a possible mechanism linking ClC-5 loss-of-function to MUC1 trafficking regulation and DD1 progression to renal fibrosis.

Mucin Biosynthesis and Processing

Composition and Function of the Airway Mucin Exocytic Machinery

Oanh Hoang[1], Brianne Wharton[1], Valerie Oren[1], Boxuan Yang[1], Emilia Nunez[1], Sunu Kim[1], Michael Tuvim[1], Burton Dickey[1]

1. Department of Pulmonary Medicine, MD Anderson Cancer Center, Houston, Texas USA

Mucins are released into the airway lumen to form mucus by the exocytic fusion of secretory granules with the plasma membrane. We have been studying the molecular mechanism of this exocytic process for twenty-five years by deleting genes encoding candidate proteins in mice. Through this work we have identified six proteins that together comprise the core exocytic machinery. These include three SNARE proteins that drive membrane fusion (Syntaxin3, SNAP23, VAMP8), a Munc18b scaffold upon which the SNARE proteins assemble, a Munc13-2 priming protein that opens Syntaxin3 for interaction with the other SNAREs, and the fast calcium sensor Synaptotagmin2. Two years ago, calcium-dependent exocytic fusion of artificial membranes was biochemically reconstituted using these six recombinant proteins, confirming their sufficiency for this process (Lai Y, et al, Nature, 2022). However, we have also obtained evidence that besides this molecular machine that mediates exocytosis at high cytoplasmic calcium concentrations in response to stimulation by extracellular agonists, a distinct molecular machine mediates exocytosis at low baseline calcium concentrations. The strongest evidence for this is that Munc18a rather than Munc18b is the major scaffold for SNARE assembly in baseline mucin secretion (Jaramillo AM, et al, JCI Insight, 2019). To identify other components of the baseline exocytic machine, we have obtained conditional deletant mice for candidate partners of Munc18a and are studying their subcellular localization and function. Results from these studies will be presented at the time of the meeting. Understanding the full composition of baseline and stimulated exocytic machines and how these interact functionally is important in order to know whether secretory products including mucins can be differentially released, and how to minimize rapid stimulated mucin release that can lead to lumenal mucus occlusion while maintaining slow baseline release that is required for lung health.

Mucin Biosynthesis and Processing

Airway Secretory Cells Contain Both a Perinuclear Golgi Ribbon and Numerous Widely Dispersed Golgi Outposts

Oanh N. Hoang[1] , Burton F. Dickey[1], Michael J. Tuvim[1]

1. Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

We have long studied the molecular mechanism and pathophysiologic significance of airway mucin secretion. As part of this work, we recently reported the packaging of MUC5AC and MUC5B within secretory granules, finding that most granules contain both mucins interdigitating, and that smaller numbers of granules contain exclusively one or the other mucin (Hoang ON, et al., Am J Respir Crit Care Med, 2022). To understand how secretory granules, including lumenal mucins and surface exocytic proteins, are assembled, we examined the distal (trans) surface of the Golgi apparatus in airway secretory cells where granule assembly presumably takes place. Fluorescence microscopy using an antibody against TGN46, which localizes to the trans-Golgi network (TGN), revealed numerous puncta widely distributed throughout the cytoplasm, rather than a few puncta close together in a perinuclear distribution as expected for the Golgi ribbon of a typical mammalian cell. Laser confocal microscopy showed 87 puncta in naïve uninflamed secretory cells compared to 11 in ciliated cells. In airways with mucous metaplasia induced by instillation of IL-13, there were 113 puncta in metaplastic secretory cells compared to 11 in ciliated cells. Besides the difference between secretory and ciliated cells in the number of puncta, TGN46 was widely distributed throughout the cytoplasm of secretory but not ciliated cells, extending all the way to the apical surface of tall metaplastic secretory cells distended with mucin granules. The cis-Golgi marker GM130 was occasionally found associated with dispersed TGN46 puncta, but was mostly confined to the perinuclear region. For comparison to a cell with a conventional Golgi structure, we imaged submucosal fibroblasts that showed TGN46 puncta adjacent to one side of the nucleus, interspersed with GM130 puncta that mark the cis-Golgi. By electron microscopy, we observed a conventional Golgi ribbon in both naïve and metaplastic airway secretory cells, generally located close to the apical side of the nucleus. In addition, numerous isolated Golgi stacks were observed among immature secretory granules in the middle third of metaplastic secretory cells and occasionally among mature secretory granules in the apical third of cells. These dispersed Golgi stacks are termed “outposts” in reference to such dispersed stacks seen in other specialized cell types (Wei JH, Seeman J. Curr Opin Cell Biol, 2017). In both fluorescent and EM images, Golgi outposts were often observed in close apposition to mucin granules. Human airway secretory cells showed a similar distribution to mouse airway cells of TGN46, GM130, MUC5AC and MUC5B. In summary, airway secretory cells of mice and humans contain numerous dispersed Golgi outposts, rich in trans- but not cis- Golgi markers, which are closely associated with immature mucin secretory granules. Whether these Golgi outposts participate in mucin synthesis as well as granule assembly is a focus of ongoing studies.

Mucin Biosynthesis and Processing

A missense mutation in goblet cell IgGFc-binding protein disrupts gut MUC2 mucus protective functions

Hayley Gorman[1], Elise Beaupré[1], France Moreau[1], Wesley Zandberg[2], Kirk Bergstrom[3], Kris Chadee[1]

1. Department of Microbiology, Immunology and Infectious Diseases, University of Calgary
2. Department of Chemistry
3. Department of Biology, University of British Columbia Okanagan Campus

MUC2 mucin and FCGBP are major goblet cell glycoproteins that form the colonic mucus barrier in innate host defence. Here, we investigated if a missense mutation in FCGBP could alter the glycosylation of LS174T goblet cell MUC2 mucin and susceptibility to Salmonella enterica infection. To quantify if FCGBP-Mut led to a loss in mucus barrier function, S. enterica adherence, invasion, and cytotoxicity in WT and FCGBP-Mut monolayers were quantified by the gentamicin protection assay and LDH release. MUC2 and FCGBP mRNA and protein expression induced by S. enterica were analysed by RT-PCR and immunoblotting. To determine if the differences in S. enterica adherence and penetrability to the mucus layer were caused by differences in MUC2 glycosylation, sensitive glycomics analyses were performed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) and capillary electrophoresis with laser-induced fluorescence detection (CE-LIF). RT-PCR was used to determine if differences in the glycomics profiles was caused by differences in glycosyltransferases. Adherence of S. enterica was significantly increased temporally in FCGBP-Mut but not in WT cells and induced robust MUC2 mRNA expression, mucus secretion and pro-inflammatory cytokine release. FCGBP-Mut cells were readily invaded by S. enterica that resulted in increased cytotoxicity as compared to WT cells. FCGBP-Mut cells exhibited an altered glycomics profile dominated with an increase in sialyltransferases expression and sialylated glycans. These data demonstrate that a single missense mutation in FCGBP resulted in an increase in sialylated proteins that altered penetrability of the mucus layer to S. enterica-induced invasion and cytotoxicity.

Mucin Biosynthesis and Processing

Understanding and characterising the A4GNT-TFF axis in mucus biology

Shiyi Xi[1], Ethan D Goddard-Borger[1]

1. Walter and Eliza Hall Institute, Parkville, VIC, Australia

Background:
The mucin glycoproteins are the main contributors to mucus biology and are characterised by the heavily O-glycosylated repetitious PTS-domains. In a previous study of our lab, we showed that the αGlcNAc displayed on the termini of the mucin O-glycans are recognised by a family of extracellular peptides named the trefoil factors (TFFs). These TFF peptides are divalent lectins that bind to two αGlcNAc residues and therefore crosslink two mucin chains. The synthesis of the TFF sugar ligand, αGlcNAc, is performed by the glycosyltransferase α-1,4-N-acetylglucosaminyltransferase (A4GNT). In this study, we seek to understand the biosynthesis and regulation of αGlcNAc-capped mucin O-glycans and how this impacts mucus biology in health and disease.

Methods & Results:
The catalytic domain of human A4GNT (HsA4GNT) was recombinantly produced in insect cells and used for enzymology studies. The substrate preference of A4GNT was revealed by in vitro enzyme activity assays using a range of p-nitrophenol conjugated acceptor sugar substrates. The Michaelis-Menten kinetics was then determined for each substrate. Our data showed that A4GNT has a high specificity towards β-linked Gal against the α-linked one. The enzyme displayed a moderate preference towards a β-1,3-linked Gal rather than a β-1,4-linked one, while similar catalytic efficiencies were observed for the linear and branched substrates.
To better understand the substrate recognition, we sought to determine the structure of A4GNT at a ligand-bound state. Seven nanobodies (Nbs) were generated against HsA4GNT to promote protein stability and facilitate structural determination. A Nb-bound HsA4GNT was successfully co-crystallised with Mn^{2+} and UDP, giving a crystal structure at 2.5 Å. Our structural data uncovers the enzyme active site, where the divalent Mn^{2+} displays the characteristic octahedral coordination with the pyrophosphate group from UDP and three key residues from A4GNT, namely Asp162, Asp164 and His283.
The enzymology combined with the structural data suggested that HsA4GNT does not have a notable intrinsic preference for one glycan substrate over another. Therefore, the specificity of A4GNT may be driven by other factors, such as subcellular localization. We thereby established a lentiviral-transfected HT29-MTX cell line overexpressing HsA4GNT and performed confocal microscopy studies. Strikingly, our immunostaining results revealed that A4GNT does not co-localize with the Golgi markers. Instead, A4GNT was observed extensively at the secreting vesicles, where it possibly co-localizes with the secreting mucins.

Conclusion:
Our studies characterized the A4GNT-TFF axis in mucus biology by examining different players involved. The upstream A4GNT governs and regulates αGlcNAc synthesis, whereas the downstream TFFs crosslink the mucin chains and hence mediate the mucus rheology. The orchestration of these players together contributes to a healthy mucosal environment.

Mucin Biosynthesis and Processing

VWD domain stabilization by autocatalytic Asp-Pro cleavage

Noa Yeshaya[1], Prashant Kumar Gupta[2], Orly Dym[3], David Morgenstern[4], Dan Thomas Major[2], Deborah Fass[1]

1. Department of Chemical and Structural Biology, Weizmann Institute of Science, Rehovot, Israel
2. Department of Chemistry and Institute for Nanotechnology & Advanced Materials, Bar-Ilan University, Ramat-Gan, Israel
3. Department of Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel
4. De Botton Institute for Protein Profiling, Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel

Multiple copies of von Willebrand factor type D (VWD) domains are present in extracellular and cell-surface proteins, including mucins, von Willebrand factor, and various signaling molecules and receptors. Some of these proteins have crucial roles in protecting mucosal tissues. Many VWD domains, including in MUC2, MUC4, and MUC5AC, contain a glycine-aspartate-proline-histidine (GDPH) amino acid sequence, which is cleaved auto-catalytically between the aspartate (Asp) and proline (Pro) residues. While selective cleavage at Asp-Pro bonds in other polypeptides can be induced in vitro by high temperature and low pH, typically outside physiological ranges, in vivo cleavage of the GDPH motif in VWD domains occurs at body temperature and at the mildly acidic pH of the Golgi apparatus or secretory granules, or even at the approximately neutral pH of the endoplasmic reticulum. However, the physiological role and mechanism of GDPH cleavage were unknown. We studied the structural and biophysical consequences of Asp-Pro peptide cleavage in the Fc IgG binding protein (FCGBP), found in intestinal mucus and other body secretions. FCGBP contains 13 VWD domains, 11 of which have a GDPH cleavage site. We found that endogenous Asp-Pro cleavage in a representative domain of FCGBP stabilizes the protein and increases resistance to degradation by exogenous proteases. Moreover, we determined the structure of an FCGBP segment containing the cleaved VWD domain, providing insights into the cleavage mechanism and the local structural reorganization that occurs upon cleavage. We also observed global differences in domain orientation when comparing the FCGBP segment structure with numerous other mucin structures containing the same set of domains. These findings illuminate the outcome of GDPH cleavage and demonstrate the plasticity of proteins with VWD domains, which may contribute to their evolution for functioning in dynamic extracellular environments.

Mucin Biosynthesis and Processing

The structure of the second CysD domain of MUC2 and role in mucin organization by transglutaminase-based cross-linking

Christian V. Recktenwald[1], Göran Karlsson[2], Maria-Jose Garcia-Bonete[1], Gergely Katona[3], Maja Jensen[3], Richard Lymer[4], Malin Bäckström[4], Malin E.V. Johansson[1], Gunnar C. Hansson[1] and Sergio Trillo-Muyo[1]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden
2. Swedish NMR Centre, University of Gothenburg, Gothenburg, Sweden
3. Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden
4. Mammalian Protein Expression core facility, University of Gothenburg, Gothenburg, Sweden

The MUC2 mucin protects the colonic epithelium by a two-layered mucus with an inner attached bacteria-free layer and an outer layer harboring commensal bacteria. CysD domains are 100 amino acid long sequences containing 10 cysteines that separate highly O-glycosylated PTS regions in mucins. The structure of the second CysD, CysD2, of MUC2 has now been solved by NMR. CysD2 shows a stable stalk region predicted to be partly covered by adjacent O-glycans attached to neighboring PTS sequences, whereas the CysD2 tip with three flexible loops is suggested to be well exposed. It shows transient interactions at acidic pH, that are weakened at physiological pH. This transient interaction can be stabilized in-vitro and in-vivo by transglutaminase 3-catalyzed isopeptide bonds, preferring a specific glutamine residue on one flexible loop. This covalent dimer is modelled suggesting that CysD domains act as connecting hubs for covalent stabilization of mucins to form a protective mucus.

Mucin Biosynthesis and Processing

An unexpected role for Rab7 governing intestinal homeostasis via mucin protease CLCA1

Yesheswini Rajendran, Preksha Gaur, C.V. Srikanth

1. Regional Centre for Biotechnology, Faridabad, India
2. Regional Centre for Biotechnology
3. Regional Centre for Biotechnology

The mucus layer of gut is an important insulation that prevents unwarranted immune activation against the vast diversity of resident microbiota. Goblet cell, which secrete the mucin proteins, an important constituent of the mucus layer, are known to be depleted in Ulcerative colitis, a form of inflammatory bowel disease (IBD). IBD is a chronic form of intestinal auto immune disorder. IBD patients lead a compromised lifestyle throughout, particularly owing to no available cure. How the GC number and the mucus layer formed by them is regulated in healthy versus UC gut is not fully understood. Cellular signaling involved in goblet cell regulation, especially mucus homeostasis in the aspect of IBD remains unknown.
In the current work we identified a mucin protease, CLCA1 (Chloride channel accessory1to be excessively secreted by GCs, leading to thinning of mucus barrier, in inflammatory conditions.
Rab7, a protein constituent of cellular vesicular transport pathway, was seen to regulate CLCA1 in goblet cell via lysosomal degradation
Rab7 mediated regulation of CLCA1 secretion and mucus layer maintenance was also seen in actual UC patient endoscopy samples and mechanistically characterized in murine and in vitro goblet cell model systems.
Overall, our work establishes a role for Rab7 dependent control of CLCA1 secretion required for maintaining mucosal homeostasis.

Mucin Biosynthesis and Processing

Evaluation of interaction between polymeric mucin and drugs

Hisanao Kishimoto[1], Caroline Ridley[2], Katsuhisa Inoue[1], and David J. Thornton[2]

1. Department of Biopharmaceutics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Japan.
2. Wellcome Centre for Cell-Matrix Research, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Sciences Centre, The University of Manchester, UK.

Background and Purpose: Mucosal-delivered drugs have to pass through the mucus layer that covers the surface of the secretory epithelia such as intestinal and respiratory epithelium. The polymeric mucins (MUC2, MUC5AC, MUC5B), a major structural protein of mucus, underpin the mucus gel network and also limit drug diffusion toward the surface of epithelial cells. Mucins potentially act as important physiological regulators of mucosal drug absorption, however, the molecular basis of this regulation has remained unclear. In this study, we have investigated the interaction between human polymeric mucins and various drugs with different physicochemical properties (e.g., molecular weight, lipophilicity, and physiological charge).

Methods: Human MUC2, MUC5AC, and MUC5B were purified from LS174T and A549 cells (MUC5B or MUC5AC knockdown cell lines). Three cyclic peptides (daptomycin, polymyxin B, and cyclosporin A) and six drugs (antipyrine, 5-fluorouracil, griseofulvin, theophylline, paclitaxel, and rifampicin) were selected as model drugs. The effect of various drugs on the sedimentation behavior of purified mucins under physiological conditions was assessed using rate-zonal centrifugation. To estimate the binding affinity of various drugs for polymeric mucins, changes in mucin-derived fluorescence intensity after incubation with model drugs were measured. To assess the potential effects of mucin-drug interaction on drug efficacy, we examined the impact of mucin depletion on cell cytotoxicity.

Results: We demonstrated that cyclosporin A and paclitaxel induce the aggregation of polymeric mucins. Especially, the interaction of cyclosporin A with MUC5B was shown to mediate aggregation in both the N-terminal and C-terminal domains of the MUC5B polymer, with an enhanced effect observed under low pH conditions (pH 5.5) for the N-terminal region. On the other hand, we showed that the binding affinities of drugs for mucins were varied, not only among individual drugs but also among mucin subtypes. Lastly, we showed that deletion of mucin production in A549 cells increased the cell cytotoxicity of cyclosporin A and paclitaxel likely due to loss of mucin-drug interaction.

Conclusion: These data suggested that mucins are an important physiological factor regulating mucosal permeation of various drugs. Therefore, we have shown the necessity of considering both the potential for mucin aggregation and the binding ability of drugs to mucins. Our data contribute to the understanding of mucin-drug interactions, and it likely ensures the efficacy and safety of drugs.

Mucin Biosynthesis and Processing

Structure of MUC5AC N-terminal region

Meital Haberman[1], Deborah Fass[1]

1. Department of Chemical and Structural Biology, Weizmann Institute of Science, Rehovot, Israel

The three major gel-forming mucins are MUC2, MUC5AC, and MUC5B. Recent research has demonstrated that the two lung mucins, MUC5AC and MUC5B, generate differently structured hydrogels, but the mechanistic basis for this difference is not yet known. Inspection of the protein sequences of MUC5AC and MUC5B reveals elements of primary structure that have diverged between these two mucins and may have contributed to the diversification of their hydrogels. Specifically, MUC5AC and MUC5B have different numbers and distributions of CysD domains and different lengths of intervening proline-, threonine-, and serine-rich (PTS) regions. Our lab showed that mucin constructs spanning the N-terminal D assemblies through the first PTS segment and CysD domain self-assemble into chains of beads in pH ranges that correspond to Golgi pH. The chains of beads are composed of numerous, intertwined copies of the mucin molecules. Notably, despite the similarity of the structures of individual beads, the beads arrange into filaments in different manners for the different mucins. This observation shows that the divergence between the N-terminal sequences is sufficient to produce distinct supramolecular structures. We suggest that the evolution of distinct mucin supramolecular assembly modes may have contributed to tuning hydrogel properties. We have recently determined the high-resolution structure of a supramolecular assembly of the MUC5AC N-terminal region, which is composed of a beaded filament structure with a unique helical arrangement not seen previously for any homologous mucin. A comparison with the MUC5B beaded filament structure reveals how apparently minor differences in primary structure can produce highly divergent supramolecular assemblies.

Mucin Biophysics

Surface layer adsorption and bulk association of mucins in human airway mucus

Scott Danielsen[1], David Hill[2], Richard Boucher[2], Michael Rubinstein[3]

1. Georgia Institute of Technology
2. University of North Carolina
3. Duke University

The airway surface layer lines the respiratory tract, simultaneously trapping inhaled particulates and facilitating their removal from the lung. Secreted gel-forming mucins, which possess both hydrophilic glycosylated domains and hydrophobic globular domains, interact with a wide range of proteins and nucleic acids and are responsible for the characteristic biophysical properties of mucus. The mucin hydrophobic domains promote: 1) self-association in the bulk phase; and 2) strong adsorption at the air-mucus interface with formation of a thin, viscoelastic skin layer that effectively separates the mucus layer itself into two distinct sublayers. Measuring the modulus of mucus using multiple rheometric geometries with varying surface-to-volume ratios, we measure different apparent viscosities with different contributions of surface to bulk. A simple model deconvolutes these contributions, permitting assessment of the bulk and surface properties. Surfactants are shown to reduce the associative interactions of mucins, lowering the degree of association/adsorption and overall viscoelastic modulus. Understanding of the structural organization of mucus in the airway and resultant mechanical properties will enable the development of therapeutic approaches to improve mucus clearance in muco-obstructive lung diseases.

Mucin Biophysics

Impact of electronic cigarette liquid solvents on mucin swelling kinetics released from lung epithelium cells

Carlos Vazquez[1], Rue Feng[1], Wei-Chun Chin[1]

1. Bioengineering, School of Engineering, University of California at Merced, Merced, CA 95343, USA

Electronic cigarettes (E-cigs) have grown in popularity, yet their potential risks to pulmonary health, especially in conditions like chronic obstructive pulmonary disease (COPD), cystic fibrosis, and asthma, remain a concern. Central to these diseases are the challenges of airway mucin hypersecretion and the presence of highly viscous mucus. While E-cig usage is implicated in predisposing individuals to lung inflammation and potentially elevating COPD risks, the direct effects of E-cig liquids on the viscoelastic properties of mucus remain understudied. In this work, we investigate the influence of E-cig liquids solvents, specifically Vegetable Glycerin (VG) and Propylene Glycol (PG), on mucin swelling kinetics in A549 lung epithelial cells. Utilizing video microscopy, we establish that mucin diffusivity is inversely proportional to VG and PG concentrations. Furthermore, direct observations of mucin-swelling kinetics post-exocytosis reveal a ~50% reduction in mucin diffusivity at a solvent concentration of 5mg/mL for both VG and PG. Interestingly, VG consistently exhibited slightly lower diffusivity across all tested concentrations compared to PG. Our findings underscore the importance of understanding E-cig solvent effects on mucus properties, with potential implications for respiratory health.

Mucin Biophysics

Dual-functional nanoparticles achieve simultaneous mucus penetration and epithelial cell targeting

Corey A. Stevens[1,2], Boris Sevarika[1,3], Chia-Ming Wang[4], Brian Wilson[5], Angela Belcher[2], Claus Michael Lehr[3], Rebecca Carrier[4], Katharina Ribbeck[1], Robert Prud'homme[5]

1. Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139
2. Department of Biological Engineering, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139
3. Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), D-66123 Saarbrücken, Germany, Department of Pharmacy, Saarland University, D-66123 Saarbrücken, Germany
4. Department of Chemical Engineering, Northeastern University, Boston, MA 02115
5. Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA 08544

Mucosal drug delivery hinges on balancing efficient mucus penetration and targeted cellular interaction. Existing materials often excel at one over the other. Here, we introduce an innovative nanoparticle design with a hybrid surface that achieves both functionalities simultaneously. Nanoparticles are assembled using the kinetically controlled, block copolymer-directed assembly process Flash NanoPrecipitation. The process enables the single-step preparation of nanoparticles with dense polymer brush layers comprising poly(ethylene glycol) (PEG) for mucus transport and polycationic dimethylaminoethyl methacrylate (PDMAEMA) for cell targeting at an optimized ratio. A native mucus hydrogel, a novel gut organoid system, single-particle tracking, and cell adhesion assays validated a 50-fold increase in cell targeting efficacy and 100-fold faster mucus transport compared to single-functionality particles. Our work demonstrates the feasibility of tuning both transport and targeting within a single nanoparticle. Unlike existing materials, ours achieve rapid mucus penetration while maintaining targeted cellular interaction. This approach presents a promising strategy for improved mucosal drug delivery by enabling independent optimization of both functionalities on the same platform, potentially overcoming longstanding challenges.

Mucin Biophysics

Chemoenzymatic synthesis of synthetic mucins

Amanda Wood, Jessica Kramer

1. Department of Biomedical Engineering, University of Utah, Salt Lake City, Utah 84102, USA
2. Department of Molecular Pharmaceutics, University of Utah, Salt Lake City, Utah 84102, USA

Physical barriers are the first line of defense against pathogenic infection, including over 400m2 of the mucosal glycocalyx. Mucus and the glycocalyx are comprised of glycoproteins called mucins that form bottlebrush-like structures of amino acid backbones and glycan sidechains. Mucins are inherently heterogeneous across species, between members of the same species, and even across tissues. Although heterogeneous, distinct mucin glycosylation motifs are correlated across individuals with specific pathogenic infections. Mucin heterogeneity renders the mucosal immune system difficult to study and there is not currently a method to accurately portray the chemical and physical properties of mucins in disease models. This leads to inaccurate depictions of pathogen transmission and infection, where mucins are either ignored entirely or modelled with poor surrogates. Mucins appear to play an integral role in pathogen interaction with host cells. My project will tackle the lack of information available surrounding mucin glycan interaction with pathogens in the context of mucosal immunity. Tunable synthetic mucins (synMUCs) will be generated to allow study of the effect of distinct mucin compositions on pathogen infectivity and potential tropism. SynMUC backbones will be generated via N-carboxyanhydride polymerization followed by enzymatic addition of the terminal glycans fucose and sialic acid at defined chemical linkages, orientations, and densities. SynMUCs were characterized via circular dichroism and lectin assay to determine secondary conformation and glycan linkage, respectively. Overall, I aim to develop a streamlined method to generate disease-associated mucins that are defined at the molecular level to create better in-vitro models of pathogen transmission, host cell binding, and infection.

Mucin Biophysics

Interaction of denatured lactoferrin with mucin: a sticky affair

Bianca Hazt, Daniel Read, Oliver Harlen, Wilson Poon, Adam O Connell, Anwesha Sarkar

1. Food Colloids and Bioprocessing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK
2. School of Mathematics, University of Leeds, Leeds, LS2 9JT, UK
3. School of Mathematics, University of Leeds, Leeds, LS2 9JT, UK
4. School of Physics and Astronomy, University of Edinburgh, Edinburgh, EH9 3FD, UK
5. Reckitt Benckiser Healthcare UK Ltd, Hull, HU8 7DS, UK
6. Food Colloids and Bioprocessing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK

Mucoadhesion happens when a material adheres to soft mucosal tissues. Some key aspects of using mucoadhesives include the possibility of coating and protecting damaged tissues, and the localized delivery of agents. The structure of secreted mucins plays a major role in dictating whether a material will behave as a mucoadhesive or not, and while there has been a significant interest in how polysaccharide and synthetic-based polymers interact with mucins and therefore promote mucosal adhesion, some proteins remain less-studied. Here we focus on understanding how the denatured proteins can generate such mucoadhesive materials using lactoferrin (LF), a cationic bovine milk protein as a model system. With lactoferrin's tendency to denature at temperatures higher than 65 °C, we explore the impact of thermal processing on unfolding and exposure of hydrophobic groups and stickiness to negatively-charged bovine submaxillary mucin. A combination of circular dichroism (CD), rotational rheology, dynamic light scattering (DLS), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and quartz crystal microbalance with dissipation monitoring (QCM-D) was used to elucidate the stickiness of denatured lactoferrin undergoing various degrees of thermal denaturation to mucin. Results show that post-denaturation, the viscosity of a diluted lactoferrin sample (1 wt%) remains unchanged, though circular dichroism indicates alterations in secondary structure content, as α-helix structures were converted into β-sheet. DLS measurements showed an increase in particle size, while SDS-PAGE confirms higher molecular weight oligomers (<260 kDa) after the thermal treatment of LF. Notably, the denatured lactoferrin interacted more with bovine submaxillary mucin (BSM) when compared to its non-denatured counterpart, as observed through adsorption measurements on a QCM-D sensor. Increased viscosity values for lactoferrin-BSM complexes also show the rheological synergism associated with mucoadhesive materials. These experimental investigations underscore the enhanced mucoadhesivity of denatured lactoferrin, which interacts with mucin due to its positive surface charge, surface hydrophobicity, and increased access of thiol groups for the resulting stickiness with mucins. This research thus offers valuable insights into the dynamic interplay between lactoferrin and mucin as a function of thermal denaturation, paving the way for innovative applications in various biomedical, chemical, and allied sectors.

Mucin Biophysics

Mucus as a physicochemical barrier to Influenza A fiffusion

Elizabeth Engle[1,2], Logan Kaler[2,3], Maria Rife[1,4], Ethan Iverson[1,4], Allison Boboltz[2], Maxinne Ignacio[4], Margaret Scull[1,4], Gregg Duncan[1,2,3]

1. Molecular and Cellular Biology Program
2. Fischell Department of Bioengineering
3. Biophysics Program
4. Department of Cell Biology & Molecular Genetics
5. Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20740

It has been well characterized that Influenza A virus (IAV) penetrates the airway mucosal barrier through the coordination of sialic acid binding viral envelope protein, hemagglutinin (HA), and sialic acid cleaving viral envelope protein, neuraminidase (NA). However, the contribution of other host and viral factors to effectively trap IAV in mucus remains poorly understood. Using mucus harvested from 3 distinct airway epithelial culture models, this study investigated how the physicochemical properties of mucus influences effective trapping of IAV with varying sialic acid preferences using fluorescence video microscopy and multiple particle tracking. We discovered that mucus containing pore sizes on the size scale of IAV was most effective at physically reducing IAV diffusion. Furthermore, the availability of sialic acid for IAV binding increased the degree of mucus trapping, but sialic acid preference did not alter the percentage of IAV particles expected to penetrate the mucus barrier. Overall, this study reveals the dual importance of both physical mucosal restriction as well as reinforces the importance of sialic acid binding in IAV diffusion inhibition.

Mucin Biophysics

Evaluation of the stability of the resulting structures obtained after the interaction between mucin and chitosan-coated oil-in-water nanocapsules

Mar Collado-González[1,2], Gurmeet Kaur[1], Yadira González-Espinosa[1], Rebecca Brooks[1], and Francisco M Goycoolea[1,2]

1. School of Food Science & Nutrition, University of Leeds, Leeds LS2 9JT, United Kingdom
2. Universitry of Murcia, Faculty of Biology, Campus de Espinardo, 30100, Murcia, Spain

Chitosan-coated oil-in-water nanocapsules have been proposed for treating oral infections since they can load lipophilic bioactive molecules and interact with mucosa where they can release their payload. Chitosan-coated oil-in-water nanocapsules can interact with saliva mucins and be coated during their administration to mucosal surfaces. Therefore, it was of interest to investigate the nature of the interactions at play and their stoichiometry. It is also to be noted that the use of mucin as raw material for the preparation of drug delivery systems (DDS) has gained traction especially when considering that mucin-based systems show an improvement in its residence time in mucosal tissues. In this work, we have studied the interaction between hydrosoluble porcine gastric mucin from Sigma-Aldrich and chitosan-coated oil-in-water nanocapsules by dynamic light scattering (DLS). Size and zeta potential of the resulting complexes were analysed immediately after mixing nanocapsules and mucin and up to 24 h later. The mixtures were prepared at mucin:chitosan-coated oil-in-water nanocapsules ratios of 0.67, 0.34, 0.17 and 0.08 (w/w). A mucin corona is formed on the surface of nanocomposites at mucin:chitosan-coated oil-in-water nanocapsules ratio higher than 0.08, as evidenced by the zeta potential results. According to the size results, composites prepared at mucin:chitosan-coated oil-in-water nanocapsules ratio equals to 0.34 showed nanometric size after 24 h of incubation, which could be interpreted as stable nanocomposites against aggregation. Further studies are required to evaluate the stability of these systems in physiological relevant contexts as well as the payload in vitro release and antimicrobial activity, followed by in vivo proof-of-concept of their potential therapeutic efficacy.

M.C.G. acknowledges the funding from the Séneca Foundation-Science and Technology Agency of the Region of Murcia within the framework of the Young Leaders in Research call (22010/JLI/22) and the fellowship for postdoctoral training (20381/PD/17) and the Ramón y Cajal scholarship (RYC2021-034184-I) from the State Research Agency of the Ministry of Science and Innovation (Spain). G.K. acknowledges the Newton International Fellowship (NIFnRIn180856) from the Academy of Medical Sciences, United Kingdom. F.M.G. acknowledges a Beatriz Galindo Fellowship (BG22/00138) from the Ministry of Universities (Spain).

O-glycosylation of Mucins

The impact of B3GNT7 on mucin glycosylation

Mary Burns[1] and Jennifer Kohler[1]

1. Department of Biochemistry, UT Southwestern Medical Center, 2. Department of Biochemistry, UT Southwestern Medical Center

β1-3-N-acetylglucosaminyltransferase 7 (B3GNT7) is a Golgi-resident glycosyltransferase that adds GlcNAc to Gal acceptors and thereby participates in polyLacNAc biosynthesis. Importantly, these poly-LacNAc chains can go on to be further modified by fucose, sialic acid, and sulfate. We previously reported that that IL-22, a cytokine critical for maintaining intestinal epithelial homeostasis, promotes STAT3-dependent B3GNT7 expression, increases fucosylated O-glycans, and increases poly-LacNAc expression on differentiated Caco2 Bbe1 cells. Furthermore, we found that overexpression of B3GNT7 is sufficient to increase cell surface fucosylation. These data position B3GNT7 as an important modulator of intestinal mucus glycosylation, and we are now elucidating how B3GNT7 functions to regulate mucin glycan structure, as well as the biochemical and biophysical properties of mucus.

O-glycosylation of Mucins

Site-specific assignment of the histo-blood group antigen (HBGA) Type 1 and Type 2 glycan structures in LC-MS/MS based glycoproteomics

Jonas Nilsson[1], Ulrika Westerlind[2], Göran Larson[3]

1. Proteomics core facility, University of Gothenburg
2. Department of Chemistry, Umeå University
3. Department of Laboratory Medicine, University of Gothenburg

The ABO histo-blood group antigen (HBGA) glycan structures are well established as host attachment factors for several virus infections, e.g. rota- and norovirus, the main causes of viral gastroenteritis. The ABO HBGAs contain the linkage isomers Fuc alpha1-2Gal beta1-3GlcNAc (H Type 1) or Fuc alpha1-2Gal beta1-4GlcNAc (H Type 2). The biosynthesis of these structures is dependent on two alpha-1,2-fucosyltransferases, coded for by the FUT1 (H) and FUT2 (secretor) genes typically but not exclusively expressed in hematopoetic stem cells and in mucosal epithelial cells, respectively. Individuals lacking an active FUT2 gene (non-secretors) do not synthesize the H Type 1 glycan and are not susceptible to the most common variants of norovirus although they can still produce the H type 2 glycan through the action of the FUT1 gene.

The HBGA glycan structures are presented extracellularly on both glycosphingolipids and on glycoproteins including mucins. LC-MS/MS identification of glycopeptides (glycoproteomics) has recently evolved to become an efficient tool to investigate the site-specific glycosylation of proteins, and progress is also made with respect to mucins. Glycoproteomics is normally not aimed at determining glycan isomer structures. However, we have previously shown that the isomeric structures GalNAc vs GlcNAc; and Neu5Ac alpha2-3 vs Neu5Ac alpha2-6 could be discriminated in the LC-MS/MS of glycopeptides by careful analysis of diagnostic oxonium ion patterns.

Here, we have continued our endeavors to study isomeric structures of glycopeptides and show that synthetically prepared MUC1 O-glycopeptides with well-defined H Type 1 and H Type 2 structures produce different oxonium ion profiles that can be used to distinguish them by MS/MS. In addition, the isomeric structures Lewis a and Lewis b (Type 1 chain); and Lewis x and Lewis y (Type 2 chain) could be distinguished. Technically, the use of these fucosylated glycopeptides enabled us to investigate the complication of “fucose migration”, and how it may be avoided using specific settings for the MS/MS analyses.

This methodology should be beneficial to be applied on both N- and O-glycopeptides carrying ABO and Lewis HBGA structures and will be important to use in the site-specific glycan identification of biomedically important glycoproteins, especially with respect to virus infections.

O-glycosylation of Mucins

In Atlantic salmon skin infected with salmon lice, elevated seawater temperatures change gene expression and mucin *O*-glycosylation, which promotes pathogen binding

Kristina A. Thomsson[1], Lene Sveen[2], John Benktander[1], Binyam Dagnachew[2], Macarena P. Quintana-Hayashi[1], Lill-Heidi Johansen[2], Mette Breiland[2], Celeste Jacq[2,3], Elisabeth Ytteborg[2], Sara K. Lindén[1]

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Box 440, Medicinaregatan 9C, 405 30 Gothenburg, Sweden
2. Nofima, Muninbakken 9-13, Breivika Postboks 6122 Langnes NO-9291 Tromsø, Norway
3. Blue Analytics AS, Kong Christian Frederiks Plass 3 5006 Bergen, Norway

Skin barrier function is of paramount importance for fish welfare and health. Salmonid skin produces a mucus layer mainly composed of mucin glycoproteins. Mucin glycans regulate interactions with pathogens, including binding to host cells, pathogen quorum sensing, and regulation of virulence genes. Owing to predicted increases in seawater temperature, it is important to understand how temperature affects the skin, mucus, and mucin O-glycan repertoire in fish. In this work the primary objective was to understand the mucosal responses of the skin in response to temperature and lice. A simultaneous lice and temperature challenge trial with Atlantic salmon (50 – 60 g) was performed, at, low (5 °C), medium (10 °C), and high (17 °C) temperatures.
Histology demonstrated that temperature affected skin morphology, with a thinner outer epidermal layer with fewer mucous cells at 17 oC than at 5 °C. Liquid chromatography–mass spectrometry showed that the skin mucin O-glycome changed with temperature, and the most pronounced glycan changes were a decrease in the disaccharide Sialyl-Tn and an increase in the tetrasaccharide NeuAcα2-3Galß1-3[NeuAcα2-6]GalNAcol and sulfated glycans at 17 oC. Principal component analysis of transcriptomic data clustered the samples according to the temperature treatments, and changes in the expression of homologues of human sialyl-, core 1-, Gal, and GalNAc transferase genes were proposed to be linked to the glycan changes observed by mass spectrometry. Finally, we showed that Aeromonas salmonicida had a higher ability to bind to mucins from fish kept at 17 °C than at 5 °C, demonstrating functional effects of temperature related glycosylation changes on host-pathogen interactions.

O-glycosylation of Mucins

The protective effect of a recombinant protein vaccine candidate against HSV-2 is dependent on its glycosylation

Ebba Samuelsson[1], Carolina Gustafsson[1], Ekaterina Mirgorodskaya[2], Jonas Nilsson[2], Staffan Görander[1], Jan-Åke Liljeqvist[1], Rickard Nordén[1]

1. Section of Virology, Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
2. Proteomics Core Facility, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Herpes Simplex Type 2 (HSV-2) constitutes a global problem with an estimated 500 million individuals infected worldwide, still no vaccines are approved for commercial use. Here, we have used a recombinant protein vaccine candidate based on one HSV-2 envelope glycoprotein with putative mucin like domains. We dissected the glycan composition and assessed the impact of glycosylation on the protective effect in a murine model.
Using LC-MS/MS we defined all glycan sites and glycoform distribution of the recombinant glycoprotein, identifying two N-linked glycans of primarily complex- and high mannose type and eleven core 1 O-linked glycans, extensively decorated with sialic acids.
We modulated the glycan content of the recombinant glycoprotein, obtaining a total of five vaccine candidates with distinct glycosylation profiles. The protective effect was assessed in a mouse model, along with measurements of antibody production and antibody reactivity. Recombinantly expressed glycoprotein with intact glycosylation profile showed 92.3 % protection (24/26 mice survived viral challenge) when mice were challenged with a lethal dose of HSV-2 strain 333. Removal of both O- and N-linked glycans from the vaccine reduced the protection with 48.5 % (7/16 mice survived viral challenge, $p = 0.0025$). Removal of distinct types of glycan structures or only the terminating sialic acid of the glycan chains conferred a marginal reduction in protection.
An intact glycosylation profile was essential for protection, but the induced IgG levels were similar for all vaccine candidates. However, antibody recognition of the fully glycosylated glycoprotein was reduced in sera from mice that received the completely deglycosylated vaccine candidate ($p = 0.0026$). This indicates that immunization vaccine a candidate devoid of glycan structures generates an antibody pool that fail to bind to fully glycosylated protein, possibly due to glycan shielding of potent antibody epitopes.
Infectious HSV-2 virus particles contain glycoproteins which are densely glycosylated. Thus, a possible explanation to the reduced protective effect of the deglycosylated vaccine candidate would be that the antibody pool is skewed towards epitopes that are not accessible in the fully glycosylated vaccine candidate or the infectious viral particles. Another hypothesis could be that certain glycan structures together with the peptide backbone constitute antibody epitopes that are necessary for eliciting a proper protective response.

O-glycosylation of Mucins

Decoding the molecular basis of tumor-associated mucin *O*-glycan recognition by the enigmatic macrophage galactose C-type lectin

Filipa Marcelo[1,2]

1. UCIBIO- Applied Molecular Biosciences Unit, NOVA School of Science and Technology, 2829-516 Caparica, Portugal
2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, 2829-516 Caparica, Portugal

Abnormal mucin O-glycosylation signature is a common feature of cancer that yields tumor-associated mucin O-glycans.[1] Among others, Tn- (GalNAcα1-O-Ser/Thr) and its sialylated form, the sTn-antigen (Neu5Acα2–6GalNAcα1-O-Ser/Thr), are tumor-associated mucin O-glycans exclusively exposed in cancers cells. Tn- and sTn-antigens interact with lectins enrolled in tumor immune surveillance and this recognition process has been identified to dampen anti-tumor immune responses.[2] Specifically, the macrophage galactose-type lectin (MGL) expressed by immune cells binds these tumor-glycans[3] and mediate anti-tumor immune suppression.[4] In this perspective, molecules able to interfere with the aberrant MGL/tumor-glycans interactions axis could have the potential to modulate MGLinduced anti-tumor immunity for immunomodulation strategies in cancer. Through an integrative and multidisciplinary approach, we revealed that the carbohydrate recognition domain (CRD) of MGL is highly dynamic and is strongly dependent of the structure and presentation of the precise GalNAc-containing antigen, [5-7] which might explain the capacity of MGL to modulate tolerance versus immunity responses. Herein, our latest advances in deciphering the molecular recognition of distinct mucin-derived tumour-associated glycans by MGL will be described.[8] Furthermore, strategies exploiting MGL/tumourassociated mucin O-glycans will be presented.[8]

References

[1] S. S. Pinho, C. A. Reis. Nat Rev Cancer. 2015, 15, 540-555.
[2] E. Rodríguez, et al. Nat Rev Immunol. 2018, 18, 204–211.
[3] F. Marcelo, et al. J Biol Chem., 2019, 294, 1300-1311.
[4] K. Lenos, et al. Oncotarget. 2015, 6, 26278–26290.
[5] A. Diniz A, et al. Chem. Eur. J., 2019, 25, 13945-13955.
[6] A. Gabba et al.; Biochemistry 2021, 60, 1327-1336.
[7] C.D. L.Lima.; Chem. Eur. J. 2021, 29, 7951-7958.
[8] in preparation

Acknowledgements: Filipa Marcelo acknowledges Fundação para a Ciência e Tecnologia (FCT-Portugal) for the funding projects: MGL4Life PTDC/QUI-OUT/2586/2020 (http://doi.org/10.54499/PTDC/QUI-OUT/2586/2020), UCIBIO project UIDP/04378/2020 and UIDB/04378/2020 and i4HB project LA/P/0140/2020). Filipa Marcelo thanks FCT-Portugal for the CEEC contract CEECINST/00042/2021. The NMR spectrometers are part of the National NMR Facility supported by FCT-Portugal (ROTEIRO/0031/2013-PINFRA/22161/2016, cofinanced by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). Filipa Marcelo acknowledges the project HORIZON-WIDERA-2021-101079417- GLYCOTwinning funded by European Commission.

Mucin-Microbe Interactions

Akkermansia muciniphila drives mucin glycan degradation in a cooperative synthetic in vitro mucosal microbial community of the human gut

Maryse Berkhout[1], Caroline Plugge[1], Clara Belzer[1]

1. Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands [all authors have the same affiliation]

A distinct microbial community resides in the human gut outer mucosal layer. Some of these microbes can degrade mucin glycans. This microbial mucin degradation is part of the normal turnover process of mucus and results i.a. in the production of beneficial short-chain fatty acids (SCFA) near the host epithelium. Due to the complexity and diversity of mucin glycans, microbial mucin degradation requires a broad range of bacterial extracellular glycan degrading enzymes. Consequently, we hypothesised that microbial mucin degradation occurs in a network of mucosal microbes with concerted action of various glycan degrading enzymes. Therefore, we set out to assemble and study an in vitro synthetic mucin-degrading community in anaerobic bioreactors.

In this study, we created a synthetic community of microbes of interest to model the ecological interactions between microbes and mucus. This 15-member mucin-degrading synthetic community (MDSC) consisted of seven mucin degraders and eight cross-feeding microbes. The community was grown in triplicate anaerobic bioreactors with continuous mucin supply for 120 hours. We tracked the relative abundance of individual species through a combination of 16S rRNA gene amplicon sequencing and qPCR, we followed metabolite production with HPLC and we evaluated community function with metaproteomics. Mucin degradation was assessed by LC-MS/MS and MALDI-TOF MS.

The community reached a stable state at t=72h. During this stable state (t72-t120), the community was dominated by specialist mucin degraders Akkermansia muciniphila and Ruminococcus spp, and generalist glycan degraders from the Bacteroides genus. Butyrate-producing bacteria and hydrogen-consuming microbes were able to cross-feed on the products of mucin degradation. The community consistently produced SCFAs acetate, propionate and butyrate. During the stable state, we observed near complete degradation of the mucin glycans by this community. To access the mucin glycans, the community expressed a plethora of mucin-targeting enzymes, including sialidases, fucosidase, galactosidases, hexosaminidases, sulfatases and peptidases.

Overall, we established a synthetic mucin-degrading microbial community that can be used to model ecological interactions that occur in the human gut mucosal layer. Specialist mucin degrader A. muciniphila plays a key role in this community, but we also observed that other mucin degraders and cross-feeding microbes are able to occupy their own niche. Taken together, this study provides evidence for a network of collaborating microbes in the human gut mucus layer.

Mucin-Microbe Interactions

Functional characterization of mucus-associated bacteria in health and disease

Melissa C. Kordahi[1], Noëmie Daniel[1], Andrew T. Gewirtz[2], Benoit Chassaing[1]

1. INSERM U1016, Team “Mucosal microbiota in chronic inflammatory diseases”, CNRS UMR 8104, Université Paris Cité, Paris, France.
2. Institute for Biomedical Sciences, Centre for Inflammation, Immunity and Infection, Digestive Disease Research Group, Georgia State University, Atlanta, GA, USA.

The gastrointestinal tract is normally protected from its microbiota via various adaptive and innate immune mechanisms, including a multilayered mucus structure that covers its surface and keeps the vast majority of intestinal bacteria at a safe distance from the intestinal epithelium. We and others have previously reported that certain food additives, such as dietary emulsifiers, can disrupt the mucus-microbiota interactions and promote encroachment by select microbiota members within the inner mucus layer, in a way that associates with chronic intestinal inflammation and metabolic dysregulations in the host. Here, we hypothesized that bacteria invading the normally sterile mucus layer are the key player in driving such detrimental consequences on their host. Thus, the aim of this study was to isolate mucus-associated bacteria and characterize their ability to directly drive chronic intestinal inflammation and downstream metabolic dysregulations. We observed that dietary emulsifiers consumption reproducibly causes low-grade intestinal inflammation and metabolic dysregulations in a way that associates with microbiota encroachment and compositional alterations in the mucus-associated microbial communities. Moreover, we report that transplantation of mucus-associated microbiota into germfree mice is sufficient to transfer microbiota-encroachment phenotype as well as associated chronic low-grade intestinal inflammation and downstream metabolic dysregulations. Altogether, these data suggest that the mucosal microbiota plays a central role in regulating intestinal inflammatory tone and metabolism. These findings also suggest that strategies aimed at modulating the mucosal microbiota could constitute innovative therapeutic approaches to treat and/or prevent various chronic inflammatory diseases.

Mucin-Microbe Interactions

Structural basis of mucin processing in the human gut by the *O*-glycopeptidase OgpA and the fucosidase FucOB from *Akkermansia muciniphila*.

Marcelo Guerin[1]

1. Structural Glycobiology Laboratory, Department of Structural and Molecular Biology, Molecular Biology Institute of Barcelona (IBMB), Spanish National Research Council (CSIC), 08028, Barcelona, Spain.

Akkermansia muciniphila is a mucin-degrading bacterium found in the human gut that promotes a beneficial effect on health, likely based on the regulation of mucus thickness and gut barrier integrity, but also on the modulation of the immune system. A. muciniphila is one of the few gut microbiota members growing on mucin as a sole carbon source. Mucin O-glycans can be very heterogeneous, with numerous different chains present in some glycoproteins. A. muciniphila genome encode the apparatus to orchestrate the hydrolysis of peptide and glycosidic linkages to process mucins in the large intestine, including a large repertoire of Carbohydrate-Active enZymes.

We focused in OgpA from A. muciniphila, an O-glycopeptidase that exclusively hydrolyzes the peptide bond N-terminal to serine or threonine residues substituted with an O-glycan. We determined the high-resolution X-ray crystal structures of the unliganded form of OgpA, the complex with the glycodrosocin O-glycopeptide substrate and its product, providing a comprehensive set of snapshots of the enzyme along the catalytic cycle. In combination with O-glycopeptide chemistry, enzyme kinetics, and computational methods we unveiled the molecular mechanism of O-glycan recognition and specificity for OgpA. The experimental data also contribute to progress the analysis of post-translational O-glycosylation events in proteins.

We discovered FucOB from A. muciniphila as an α-1,2-fucosidase able to hydrolyze Type I, Type II, Type III and Type V H antigens to obtain the afucosylated Bombay phenotype in vitro. X-ray crystal structures of FucOB show a three-domain architecture, including a GH95 glycoside hydrolase. The structural data together with site-directed mutagenesis, enzymatic activity and computational methods provide molecular insights into substrate specificity and catalysis. Furthermore, using agglutination tests and flow cytometry-based techniques, we demonstrate the ability of FucOB to convert universal O type into rare Bombay type blood, providing exciting possibilities to facilitate transfusion in recipients/patients with Bombay phenotype.

References

1. Anso I, Naegeli A, Cifuente JO, Orrantia A, Andersson E, Zenarruzabeitia O, Moraleda-Montoya A, García-Alija M, Corzana F, Del Orbe RA, Borrego F, Trastoy B, Sjögren J, Guerin ME. "Turning universal O into rare Bombay type blood." Nat Commun. 14(1):1765 (2023). doi: 10.1038/s41467-023-37324-z.

2. Trastoy B, Naegeli A, Anso I, Sjögren J, Guerin ME. Structural basis of mammalian mucin processing by the human gut O-glycopeptidase OgpA from Akkermansia muciniphila. Nat Commun. 11(1):4844 (2020). doi: 10.1038/s41467-020-18696-y.

Mucin-Microbe Interactions

Comparative characterization analysis of two novel mucin-degrading proteases, MdpL and MdpS, from different oral bacteria

Fredrik Leo[1,2], Rolf Lood[2,3], Gunnel Svensäter[1], Claes Wickström[1]

1. Department of Oral Biology and Pathology, Faculty of Odontology, Malmö University, Malmö, Sweden
2. Genovis AB, Kävlinge, Sweden
3. Department of Clinical Sciences Lund, Division of Infection Medicine, Faculty of Medicine, Lund University, Lund, Sweden

While several studies have contributed to our understanding of the microbial composition within an eubiotic oral flora, the pivotal role of enzymes and their interactions with salivary glycoproteins has mainly been disregarded. Recently, two novel proteases, MdpL and MdpS (Mucin Degrading Protease from Limosilactobacillus fermentum and Streptococcus oralis respectively) were characterized in regards of their sequence homology, physicochemical properties, substrate and amino acid specificity, cellular localization, and their hydrolytic interactions with MUC5B. Both enzymes exhibit a remarkable conservation of their protein backbone within their respective species and share similarities with streptococcal species that rely on mucins for attachment and nutrition. These enzymes operate extracellularly, degrading MUC5B into smaller protein fragments, albeit with differing levels of efficiency. Additionally, they hydrolyze other O-glycoproteins independently of the O-glycan presence but lacks activity towards non-glycosylated substrates. MdpL functions optimally under reducing conditions and showcases tolerance across various temperatures, salt concentrations, and pH values. It exhibits an amino acid preference for C-terminally located hydrophobic residues, suggesting a limited sequence preference. Conversely, MdpS acts as a serine protease with strict physicochemical properties, displaying sensitivity to increased sodium chloride and reducing agent concentrations, and operates within a narrow pH window. Uniquely, MdpS demonstrates activity towards IgA1/2 and IgM, indicating potential immunomodulatory effects. Its hydrolytic preference appears to be around Ser/Thr residues, elucidating its specificity for O-glycoproteins. In comparison, MdpL and MdpS offer distinct insights into mucin degradation, mirroring the diverse biofilm environments of L. fermentum and S. oralis. While MdpL showcases broader physicochemical preferences, MdpS exhibits selectivity while significantly enhancing MUC5B degradation. Understanding the intricate interplay between L. fermentum, S. oralis and MUC5B holds significant implications for managing a healthy eubiotic oral microenvironment. This comprehension offers potential targets for intervention aimed at modulating the composition and succession of oral biofilms.

Mucin-Microbe Interactions

Mucin *O*-glycans degradation by gut *Bacteroides* requires multiple key enzymes

Chunsheng Jin[1], Grete Raba[1], Sadie Gugel[2], Gabriel Pereira[2], Eric C. Martens[2], Ana S. Luis[1,3]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg. Gothenburg, Sweden
2. Department of Microbiology and Immunology, University of Michigan. Ann Arbor, USA
3. SciLifeLab, Gothenburg, Sweden

The gastrointestinal mucus layer provides a critical barrier that separates gut microbes from the intestinal epithelium. Mucus is mainly composed of mucins glycoproteins containing ~102 different O-linked glycan structures. Some microbiota members are able to utilize O-glycans. The combination of increased mucin degrading bacteria and the corresponding disruption of the mucus barrier have been proposed to promote inflammatory bowel disease (IBD). Bacteroides thetaiotaomicron (B. theta), a dominant member of human microbiota, has numerous Polysaccharide Utilization Loci (PULs) encoding dozens of predicted mucin-degradation enzymes. Significantly, the enzymatic mechanisms of mucin degradation by this and other gut bacteria remain unclear.
We hypothesized that “early” steps in depolymerization of O-glycans exist, which could block downstream metabolism of mucin glycans and may represent drug targets to block mucus degradation by the microbiota. Using biochemical and genetic approaches, we disclosed the first model of colonic O-glycan depolymerization by a single human gut bacterium. We established that utilization of O-glycans by B. theta can require the sequential action of at least 36 enzymes [glycoside hydrolases (GHs) and sulfatases]. Investigation of 30 GHs revealed the substrate specificity of these enzymes on O-glycans. The characterization of a novel endo-active enzyme that targets sulfated O-glycans revealed that initial steps of mucin degradation can required the action of multiple endo-enzymes. Unexpectedly, in vivo studies of B. theta mutants revealed that multiple exo-active enzymes act as key enzymes in O-glycan utilization. Simultaneous deletion of fucosidases revealed a critical role for these enzymes in growth on O-glycans and in vivo gut fitness. Additionally, the deletion of a sialidase revealed that this enzyme has a major role in gut colonization. The characterization of the model of degradation O-glycans provides novel insights into the mechanism of mucin degradation by the microbiota allowing the identification of potential drug targets in the treatment of IBD.

Mucin-Microbe Interactions

Structural and functional insights into the mechanism of a *Toxoplasma gondii* mucin-type *O*-glycosyltransferase

Pranav Kumar[1], Tadakimi Tomita[2], Thomas A. Gerken[3], Collin J. Ballard[3], Yong Sok Lee[4], Louis M. Weiss[2,5], Nadine L. Samara[1]

1. Structural Biochemistry Unit, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, 20892, USA.
2. Department of Pathology, Albert Einstein College of Medicine, Bronx, 1300 Morris Park Avenue, New York, 10461, USA.
3. Departments of Biochemistry and Chemistry, Case Western Reserve University, Cleveland, OH, 44106, USA.
4. Bioinformatics and Computational Biosciences Branch, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20892, USA.
5. Department of Medicine (Infectious Disease), Albert Einstein College of Medicine, Bronx 1300 Morris Park Avenue, New York, 10461, USA.

Mucin-type O-glycosyltransferases (GalNAc-Ts) catalyze the attachment of N-acetylgalactosamine (GalNAc) onto Serine or Threonine residues on protein substrates to initiate mucin-type O-glycosylation, an abundant and complex post-translational modification that regulates protein stability, structure, and function. In multicellular organisms, densely O-glycosylated proteins at the epithelium protect underlying layers from infection and modulate interactions with the microbiome. Mucin-type O-glycosylation also occurs in a subset of pathogenic Apicomplexan protozoa, including the parasite Toxoplasma gondii (T. gondii), which has five GalNAc-Ts: TxgGalNAc-T1 to T5. TxgGalNAc-T3 is expressed in T.gondii bradyzoites that reside in tissue cysts associated with latent toxoplasmosis and O-glycosylates a subset of cyst wall proteins containing mucin domains. Deletion of TxgGalNAc-T3 results in a fragile cyst wall and decreases bradyzoite persistence. A low sequence similarity between TxgGalNAc-T3 and metazoan homologues hints at a divergence in enzyme function, suggesting that specifically targeting this enzyme could weaken bradyzoites that have thus far been resistant to therapies. To gain insight into TxgGalNAc-T3 function, we solved X-ray crystal structures of TxgGalNAc-T3 alone and in complex with cyst wall peptides. The structures reveal unique features that are strictly conserved among Apicomplexan homologues of TxgGalNAc-T3, including a second metal binding site that influences O-glycosylation in vitro and in vivo. Additional features illustrate the divergence from host to pathogen GalNAc-Ts and lay the framework for specifically inhibiting TxgGalNAc-T3 in toxoplasmosis.

Mucin-Microbe Interactions

Exploring the mucus adhesion potential of vaginal *Lactobacilli* from the Isala citizen science project

Inas Rahou[1], Matisse Van Hee[1], Sarah Ahannach[1], Camille Nina Allonsius[1], Ilke De Boeck[1], Stijn Wittouck[1], Annemieke Smet[2], Sarah Lebeer[1]

1. University of Antwerp, Belgium, Laboratory of Applied Microbiology and Biotechnology, Department of Bioscience engineering
2. University of Antwerp, Laboratory of Experimental Medicine and Pediatrics, Department of Translational Research in Immunology and Inflammation

Cervicovaginal mucus is vital for women's health and reproductive outcomes. It is home to a complex and dynamic microbial community, and balances between sheltering beneficial bacteria and maintaining a barrier for pathogens and harmful molecules. Despite its importance, the mechanisms by which bacteria interact with cervicovaginal mucus remain poorly understood. Previous research has shown the presence of mucus-binding (MUB) proteins within lactic acid bacteria, particularly prominent in lactobacilli inhabiting the gastrointestinal tract. However, these proteins remain largely unexplored in the cervicovaginal microbiome. Lactobacilli, in particular Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus gasseri and Limosilactobacillus taxa, play a key role in maintaining a healthy ecosystem, but the genes and molecules behind these beneficial functions are largely unknown.

To obtain a better understanding of the functionality of lactobacilli in the human vagina, a large-scale citizen-science project named Isala, was set up, in which more than 3,300 vaginal swabs were obtained from adult women in Belgium (https://isala.be/en/). In a first step, the microbiome of these self-sampled swabs was profiled with 16S amplicon sequencing, and a large subset was also subjected to a culturomics approach. So far, more than 3,000 vaginal bacterial isolates have been obtained and identified, with almost 1,000 Lactobacillaceae isolates of which more than 500 are whole genome sequenced. These genomes were then screened for putative MUB proteins using a hidden Markov model (HMM) profile based on the MUB domains of the previously described MUB protein of Limosilactobacilus reuteri 1063. Subsequently, we searched these proteins for sequence motifs often associated with MUB proteins, including an N-terminal signal peptide, C-terminal sortase recognition site, proline-rich amino acid stretches, and the MUB-associated domain from L. reuteri 1063. Bioinformatic tools used for this analysis included HMMER, ClustalW for multiple sequence alignment, MEME and MAST for motif discovery and scanning.

Overall, we observed a large variation in both the number and size of MUB domains across different Lactobacillus species, spanning from 3 to 16 per MUB protein and ranging in size from approximately 100 to 200 amino acids. In more than half of the putative MUB proteins identified, we found an N-terminal signal peptide, LPxTG sortase motif and PxxP region, either flanking or inserted in a MUB domain. Particularly intriguing was the identification of a putative MUB protein in one L. crispatus strain, which besides 13 MUB domains harbored a MUB-associated domain. This could be associated with an enhanced mucus adhesion potential. Further functional analyses will shed new light onto the host-adapted nature of L. crispatus and related taxa in the cervicovaginal environment.

Mucin-Microbe Interactions

Effects of airway disease on the ability of mucins to bind influenza virus.

John Benktander[1], Macarena Paz Quintana Hayashi[1], Lisa Pettersson[1], Rickard Nordén[2] and Sara K. Lindén1

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Box 440, Medicinaregatan 9C, 405 30 Gothenburg, Sweden
2. Section of Virology, Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Influenza virus is an RNA virus of the Orthomyxoviridae family whose seasonal variants annually affects around a billion people, causing 290.000-650.000 death. There are four types of influenza viruses, with type denoted as A, B, C or D. Of these types, the type A viruses is the only known to cause pandemics. The influenza A type is further divided into subtypes based on cell surface proteins. The subtypes are named based on the hemagglutinin (H) and neuraminidase (N). The hemagglutinin is responsible for helping the virus to attach to the host cell to enable entry into the cell while the neuraminidase is an exo-glycosidase that facilitates cleavage of the α-ketosidic linkage of sialic acids. Generally, influenza A hemagglutinin of avian or equine origin binds sialic acid with an α2-3 linkage to galactose while human influenza A preferably binds to sialic acids with an α2-6 linkage. For human influenza viruses the neuraminidase often cleaves both α2-3 and α2-6 linked sialic acids while influenza viruses of avian origin cleaves only α2-3 linked sialic acids. For effective infection, the hemagglutinin and the neuraminidase need to be in balance to enable binding to the cell membrane but not to the decoys in the innate immune system.
To investigate the ability of mucins to inhibit influenza A infection, the binding ability and the inhibitory properties of mucins were studied. Peripheral airway mucin samples from healthy non-smokers, long term smokers without and with COPD (chronic obstructive pulmonary disease) and pneumonia patients were investigated. In addition, healthy salivary mucins were examined. We found that H1N1 virus bound to mucins from smokers with and without COPD and the saliva, while H3N2 virus bound to all tested mucins. The neuraminidase inhibitor oseltamivir caused an increased binding to all samples which was more apparent for the H1N1 virus. Mass spectrometry analysis of the O-glycans of the mucins showed increasing levels of sialic acids from healthy>smoker>COPD>pneumonia. H1N1 virus binding was correlated to the level of α2-6 linked sialic acid while the H3N2 strain correlated to the amount of α2-3 linked sialic acids.
By testing α2-3 and α2-6 linked sialic acid reference binding to the influenza virus with and without oseltamivir, we could determine the neuraminidase activity. The results concluded that the H1N1 neuraminidase cleaves both α2-3 and α2-6 linked sialic acids and H3N2 only cleaves the α2-6 linked sialic acid. To test the ability of mucins to inhibit infection, A549 cells were infected with/without mucin. Results showed an inhibitory effect with 10 mg/ml mucin for both virus strains. 1 mg/ml could also inhibit H1N1 virus.
These results concludes that mucins can have an inhibitory effect on the infection of influenza
viruses, with the type of virus strain and sialic acids present on the mucins determining to what degree inhibition occurs.

Mucin-Microbe Interactions

Novel insights into mucin *O*-glycans recognition by commensal bacteria from the human gut microbiome

Viviana G, Correia[1,2], Raquel L Costa[1,2], Filipa Trovão[1,2], Sandra Behren[3], Antonio Di Maio[4], Ulrika Westerlind[3], Ten Feizi[4], Wengang Chai[4], Yan Liu[4], Benedita A Pinheiro[1,2], Ana Luísa Carvalho[1,2], Angelina S Palma[1,2,4]

1. UCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry/Department of Life Sciences, School of Science and Technology, NOVA University Lisbon, Portugal
2. Associate Laboratory i4HB, Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Portugal
3. Umeå University, Department of Chemistry, Sweden
4. Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, United Kingdom

The human gut microbiome, a microbial community that coexists within our digestive tract, plays a vital role in targeting and utilising a diverse range of glycans derived from our diet, but also mucin glycans from the protective mucosal intestinal layer, significantly impacting host nutrition, immunity, and susceptibility to infection [1,2]. How mucin O-glycans are differentially exploited by intestinal commensal or pathogenic bacteria and influence the crosstalk with the human host largely remains to be elucidated at the molecular level.
Abundant colonic bacteria such as Bacteroides species, have extensive sets of co-localized genes – the polysaccharide utilization loci (PULs), that enable bacterial adaptation to structural variations of the glycan substrates [1,2]. Each PUL encodes the necessary proteins for recognising and breaking down specific glycans, including carbohydrate-active enzymes (CAZymes) with carbohydrate-binding modules (CBMs) and other non-catalytic glycan-binding proteins displayed on the cell surface, which often reflect the specificity of the entire system.
In this communication, we report the functional and structural characterization of newly identified glycan-binding proteins from Bacteroides thetaiotaomicron with increased activity on mucin O-glycans in conditions of a low-fiber diet. Following an integrative strategy, we combined i) bioinformatic analysis of bacterial genomes and high-throughput production of putative glycan binding proteins with ii) ligand discovery using microarrays of human mucin-type glycoproteins, glycopeptides and sequence-defined glycans [3-5], and iii) structural characterization of protein-glycan complexes by X-ray crystallography [6]. Our findings elucidate the molecular basis for the unique specificities of glycan-binding proteins targeting mucin O-GalNAc-Thr/Ser cores and fucosylated Lewis A structures. Uncovering the molecular determinants for mucin O-glycan recognition by the bacterial systems can be used to understand the role of commensals in gut health and to design new therapeutic and diagnostic strategies.

Work supported by FCT-MCTES project grants (PTDC/BBB-BEP/0869/2014, PTDC/BIA-MIB/31730/2017, 2022.06104.PTDC), fellowships & contracts (PD/BD/105727/2014, PD/BD/135517/2018, SFRH/BD/143494/2019, DL-57/2016), projects (, UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020 and RECI/BBB-BEP/0124/2012); HORIZON-WIDERA-2021-101079417-GLYCOTwinning; and by Wellcome Trust Biomedical Resource grants (099197/Z/12/Z, 108430/Z/15/Z, and 218304/Z/19/Z).

[1] E. C. Martens et al (2018), Nat. Rev. Microbiol. (16) 457-470.
[2] M. S. Desai et al (2016), Cell, (167) 1339-1353.
[3] Z. Li et al (2018), Mol. Cell. Proteomics (17) 121-133.
[4] C. Li et al (2021), Glycobiology (31) 931-946.
[5] C.Pett and U. Westerlind (2014), Chemistry (20) 7287–7299.
[6] V. G. Correia et al (2021), Microbiol. Spectr. 9 e01826-21.

Mucin-Microbe Interactions

The gut commensal *Blautia* maintains colonic mucus function under low fiber consumption through short-chain fatty acid-mediated activation of Ffar2

Sandra M. Holmberg[1,2]*, Rachel H. Feeney[1,2]*, Vishnu Prasoodanan P.K.[1,2]*, Fabiola Puértolas-Balint[1,2], Dhirendra K. Singh[1,2], Supapit Wongkuna[1,2], Lotte Zandbergen[1,2], Hans Hauner[3,4], Beate Brandl[5], Anni I. Nieminen[6], Thomas Skurk5, Bjoern O. Schroeder[1,2]

1. Department of Molecular Biology, Umeå University, Sweden
2. Laboratory for Molecular Infection Medicine Sweden (MIMS) and Umeå Center for Microbial Research (UCMR), Umeå University, Sweden
3. Institute in Nutritional Medicine, TU Munich, Germany
4. TU Munich, School of Medicine, Germany
5. ZIEL Institute for Food and Health, TU Munich, Germany
6. Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland
* Equal contribution

Beneficial gut bacteria are indispensable for developing colonic mucus and fully establishing its protective function against intestinal microorganisms. Low-fiber diet consumption alters the gut bacterial configuration and disturbs this microbe-mucus interaction, but the specific bacteria and microbial metabolites responsible for maintaining mucus function remain poorly understood. By using human-to-mouse microbiota transplantation and ex vivo analysis of colonic mucus function, our proof-of-concept study demonstrates that individuals who increase their daily dietary fiber intake can improve the capacity of their gut microbiota to prevent diet-mediated mucus defects. Mucus growth, a critical feature of intact colonic mucus, correlated with the abundance of the gut commensal Blautia, and supplementation of Blautia coccoides to mice confirmed its mucus-stimulating capacity. Mechanistically, B. coccoides stimulated mucus growth through the production of the short-chain fatty acids propionate and acetate via activation of the short-chain fatty acid receptor Ffar2, which could serve as a new target to restore mucus growth during mucus-associated lifestyle diseases.

Mucin-Microbe Interactions

Phage-bacteria-eukaryotic cells interplay in a mucosal environment

Daniel Patricio[1], Sophie Gholadze[1], Gabriel Almeida[2], Lotta-Riina Sundberg[2]

1. Department of Biological and Environment Science, University of Jyväskylä, Jyväskylä, Finland.
2. Marine Bioprospecting group of the CANS-UiT, The Arctic University of Norway, Tromso, Norway.

Phages are the most abundant biological entities on Earth and can be found in every environment, from deep-sea vents to human microbiome. The impact of phage infections can be seen in important processes like biogeochemical cycles, trophic chains, and bacterial evolution. Recent evidence points out that the impact of phages on life goes beyond the direct infections with their bacterial hosts, reaching a trans-domain evolutionary axis mediated by mucosal interactions and transcytosis through eukaryotic cells. Interactions between phages and bacteria often occur on mucosal surfaces of a eucaryotic host in a complex environment that has significant implications for homeostasis. The mucus in these tissues segregates phage and bacterial populations, promoting a balance in their co-existence. Beyond physical barrier, mucus is also able to increase bacterial virulence factor expression as well as increasing susceptibility to phage infections. This phenomenon suggests that environmental factors might be crucial for development of new phage therapy approaches. The eukaryotic cells and phage interactions might represent important but overlooked biological processes that likely affect homeostasis, and dysbiosis control and are crucial for the proper use of phages as antibacterial. Phage therapy is an alternative method to antibiotic treatment that is expanding as a solution to the increasing multidrug-resistant bacteria crisis. P. aeruginosa is an aerobic gram-negative motile bacterium which is known to be part of healthy human microbiota. Considered an opportunistic bacterium, P. aeruginosa is responsible for serious illnesses such as pneumonia and sepsis syndromes, frequently acquiring multidrug-resistant mechanisms. The main goal of this project is to understand the role of the mucosal environment on phages and eukaryotic cells for homeostatic balance and control of bacteria-induced dysbiosis by using P. aeruginosa as a pathogenic bacteria model. Preliminary results showed that A549 cells are more sensitive to P. aeruginosa PA14 compared with CN573 strain. In addition, porcine gastric mucin (PGM) treatment affected cell viability in a bacteria-dependent manner. PGM also enhance the proinflammatory gene expression in A549 cells triggered by CN573 strain. GEC_PNG14 phage infects and replicates in both P. aeruginosa strains, CN573 being more sensitive to phage infection than PA14. Interestingly, PGM does not affect P. aeruginosa CN573 or PA14 growth alone but increases the growth of phage-resistant bacteria. Further experiments are required to elucidate the effects of PGM on the tripartite organisms.

Mucin-Microbe Interactions

Bacteroides carbohydrate binding modules 32 specifically recognizes *O*-glycan epitopes in colonic mucins

Naba Salman[1], Ana Luis[1,2]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg. Gothenburg, Sweden
2. Department of Microbiology and Immunology, University of Michigan. Ann Arbor, USA
3. SciLifeLab, Gothenburg, Sweden

The human microbiota has a huge impact in health and disease. In the colon, the microbiota colonizes the mucus layer that act as a barrier between the gut microbes and the intestinal epithelium preventing the close contact and inflammation. The major component of the mucus layer are mucins, glycoproteins extensively decorated with hundreds of O-glycans. The glycosylation of mucins is complex, variable between species and along the gastrointestinal tract.

The glycosylation of mucins has an impact on the microbiota community. We hypothesized that the interactions between microbiota and mucin O-glycans have a key role on gut colonization with a healthy community. However, it remains unclear how commensal bacteria recognize and bind to mucin O-glycans.

Bacteroides encode all the required proteins to recognize and utilize a specific glycan in Polysaccharide Utilization Loci (PULs). Previous studies have shown that Bacteroides fragilis PUL bf3579-83 encodes the commensal colonization factor (ccf) required during gut colonization and horizontal transmission. This protein (BF3579) contains a carbohydrate-binding domain family 32 (CBM32). However, despite its key role on colonization, the binding specificity of this protein remains unclear. Bacteroides thetaiotaomicron (B. theta) BT0865 is a homolog of B. fragilis ccf. This protein is encoded in a PUL upregulated during growth on O-glycans and in vivo in presence of mucins. Here we show that B. theta CBM32 protein binds to human mucins in vitro and in human colonic tissue sections. BT0865_CBM32 binding specificity is dependent of the H antigen epitope. Further structural and biochemical studies reveal that this protein is a dimer and the binding is dependent of the increased avidity. Indeed, the dimerization of BF3579 was required to promote the binding of this protein. Overall, these results suggest that gut bacteria encode binding proteins that specifically recognize O-glycan epitopes. The over-expression of these binding proteins at the bacteria cell surface increases the avidity and the binding to the target epitope. Therefore, we suggest that the control of the expression of binding proteins has a key role in driving microbiota-glycan interactions that determine the gut colonization with a healthy microbiota.

Mucin-Microbe Interactions

From structure to function: exploring the structural determinants of mucin in inhibiting influenza virus

Cosmin Butnarasu[1], Jolly Thomas[1], Robyn Diehn[1], Fabian Nußhardt[1], Daniel Lauster[1]

1. Institute of Pharmacy, Freie Universität Berlin, Germany

Mucins are large polymeric glycoproteins (200 kDa – 200 MDa) forming the dynamic framework of mucus that coats all the wet surfaces of the human body. They play a pivotal role in engaging several virus species through glycan-mediated interactions during the initial stages of viral infection. However, the precise structural characteristics orchestrating this interaction such as mucin size, role and density of individual glycans remain still largely unexplored.
In this study, we employed horizontal and vertical cleavage techniques on mucin from bovine origin (BSM) to elucidate the chemical and structural determinants governing the interaction between mucin and influenza virus. Enzymatic methods were utilized for selective cleavage of sialic acid and N-linked glycans, while oxidation followed by β-elimination was employed to remove both O- and N-linked glycans. Additionally, the modulation of mucin size was achieved through the use of enzymes sourced from bacterial, animal, and plant origins, targeting the aminoacidic core of the protein. The inhibition efficiency of the mucin samples against influenza virus was monitored using hemagglutination inhibition assay.
Our findings confirm the critical role of sialic acid in regulating the anti-influenza virus activity. Likewise, O-linked glycans are pivotal players, as their removal drastically decreased inhibition efficiency by more than two orders of magnitude. Interestingly, the removal of N-linked glycans had negligible impact on the inhibition efficiency of mucin, suggesting a minor role in the virus-mucin interaction. Furthermore, a size-activity relationship was observed, with a minimum antiviral activity threshold detected at approximately 200 kDa.
These insights underscore the significance of sialic acid, O-linked glycans, and mucin size in mediating interactions with the influenza virus. They could provide valuable guidelines for the design and development of mucin-inspired biomaterials targeting influenza virus infections.

Mucin-Microbe Interactions

History of repeated antibiotic usage leads to microbiota-dependent mucus defects

Kertu Liis Krigul[1*], Rachel H Feeney[2,3,4*], Supapit Wongkuna[2,3,4], Oliver Aasmets[1], Sandra M Holmberg[2,3,4], Reidar Andreson[1,5], Fabiola Puértolas Balint[2,3,4], Kateryna Pantiukh[1], Linda Sootak[1,5], Tõnis Org[1,5], Tanel Tenson[6], Elin Org[1#], Björn Schröder[2,3,4#]

1. Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia
2. Department of Molecular Biology, Umeå University, Umeå Sweden
3. Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Umeå, Sweden
4. Umeå Centre for Microbial Research (UCMR), Umeå University, Umeå, Sweden
5. Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia
6. Institute of Technology, University of Tartu, Tartu, Estonia
*These authors contributed equally
#These authors contributed equally

Recent evidence indicates that repeated antibiotic usage lowers microbial diversity and lastingly changes the gut microbiota community. However, the physiological effects of repeated – but not recent – antibiotic usage on microbiota-mediated mucosal barrier function are largely unknown.

By selecting human individuals from the deeply-phenotyped Estonian Microbiome Cohort (EstMB) we here utilized human-to-mouse faecal microbiota transplantation to explore long-term impacts of repeated antibiotic use on intestinal mucus function. While a healthy mucus layer protects the intestinal epithelium against infection and inflammation, using ex-vivo mucus function analyses of viable colonic tissue explants, we show that microbiota from humans with a history of repeated antibiotic use cause reduced mucus growth rate and increased mucus penetrability compared to healthy controls in the transplanted mice. Moreover, shotgun metagenomic sequencing identified a significantly altered microbiota composition in the antibiotic-shaped microbial community, with known mucus-utilizing bacteria, including Akkermansia muciniphila and Bacteroides fragilis, dominating in the gut. The altered microbiota composition was further characterized by a distinct metabolite profile, that may be caused by differential mucus degradation capacity.

Consequently, our findings suggest that long-term antibiotic use in humans results in an altered microbial community that has reduced capacity to maintain proper mucus function in the gut.

Mucin-Microbe Interactions

Sialidases from *Akkermansia muciniphila*, including a member of a novel family, mediate the removal of all sialic acid mucin caps and their sharing with the mucus associated community

Michael Pichler[1], Bashar Shuoker[1,2], Chunsheng Jin[3], Hiroka Sakanaka[1], Haiyang Wu[4], Ana Martínez Gascueña[4], Jining Liu[5], Tine Sofie Nielsen[1], Jan Holgersson[5], Eva Nordberg Karlsson[2], Nathalie Juge[4], Sebastian Meier[6], Jens Preben Morth[1,8], Niclas G. Karlsson[3], Maher Abou Hachem[1]

1. Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, 2800, Denmark
2. Department of Chemistry, Lund University, Lund, Sweden.
3. Proteomics Core Facility at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
4. Quadram Institute Bioscience, Norwich, United Kingdom.
5. Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
6. Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark.

Mucin O-glycans, which display high structural complexity and diversity, are capped by sulphated, fucosylated and sialylated terminal epitopes. These caps confers resistance to microbial attack and provides adhesion site for mucus-adapted bacteria of human gut microbiota, thereby playing a key role in host-microbiome symbiotic and pathogenic interactions.
Akkermansia muciniphila is a prevalent mucin-degrading specialist strongly associated with host metabolic health. A. muciniphila encodes an array of decapping enzymes including sialidases, but the specificities of these decapping enzymes toward mucin O-glycans have remained unexplored.
We have recently investigated the specificities of A. muciniphila sialidases for mucin-conjugated and free O-glycans. Employing mucins with 160 assigned O-glycan structures, we showed that A. muciniphila decapping enzymes possess diverse selectivities. Strikingly, while some enzymes are mono-specific towards a single glycan motif, others are highly promiscuous, which is modulated by subtle modifications in active size loops.
Two A. muciniphila GH33 sialidases, have a complex modular architecture and display overlapping and complementary preferences for sialo-motifs. Interestingly we report the discovery and characterization of a novel sialidase family, which is strictly targeting the sialyl-T antigen amongst all other sialo-O-glycans from mucin. This founding member of the new CAZy sialidase family GH181, exhibits unique structural features consistent with its strict specificity. Collectively, the sialidase arsenal confers the decapping of all mucin sialyl epitopes. Our findings bring novel insights into the initiation of mucin O-glycan degradation by A. muciniphila sialidases and demonstrate how the decapping enzymes mediate cross-feeding to mucus associated gut microbiota groups.

Mucin-Microbe Interactions

Structural signatures of enzymes targeting *O*-conjugates from the mucin-degrading symbiont *Akkermansia muciniphila*

Mathias Jensen[1], Michael Jacob Pichler[1], Jens Preben Morth[1], Maher Abou Hachem[1]

1. Section of Protein Chemistry & Enzyme Technology, Department of Biotechnology & Biomedicine, DTU, 2800 Kongens Lyngby, Denmark.

Akkermansia muciniphila is prevalent member of the human gut microbiota (HGM). The abundance of A. muciniphila is positively correlated with lean body mass, lower inflammation and lower insulin resistance1,2 in humans. This bacterium, which colonizes the outer mucus layer, harnesses mucin, the main structural scaffold of the gut mucosa as a sole carbon and nitrogen source. Additionally, it has been proposed that A. muciniphila plays a role in the turnover of mucus, by stimulating mucin secretion from Goblet cells. Recently, we have been investigating the enzymatic machinery used by A. muciniphila to deconstruct mucin O-glycans. We have reported enzymes, which confer removal of fucosyl- and sialyl caps that decorate terminal epitopes in mucin, showing that this decapping is crucial to initiate mucin deconstruction3. To advance our understanding of the evolutionary adaptation of A. muciniphila to mucin breakdown, we sought to dissect the structural element that underpin the A. muciniphila enzymes efficiency on human glyco-conjugates.
The presence of appended carbohydrate binding modules (CBMs) on several A. muciniphila enzymes may increase enzyme affinity to their O-glycoconjugate substrates. The catalytic domains of these enzymes appear also to possess accessible active site topologies, effectuated by shortened surface loops, compared to characterized homologues from other bacteria. The catalytic sites of the A. muciniphila enzymes share a common electrostatic footprint, being flanked by rings of positively charged surface patches, which is reflected by isoelectric points, typically >8. These features appear to be an important adaptation that confers both steric and electrostatic compatibility to access conjugated human O-glycans decorated with negatively charged sialic acid and/or sulfate caps. Our analyses sheds light on the molecular adaptations of enzymes from A. muciniphila to efficiently target human mucin glyco-conjugates, allowing the bacterium to thrive in the outer mucus layer of the gut.

1. Derrien, M., Collado, M. C., Ben-Amor, K., Salminen, S. & De Vos, W. M. The mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. Appl. Environ. Microbiol. 74, 1646–1648 (2008).
2. Cani, P. D., Depommier, C., Derrien, M., Everard, A. & de Vos, W. M. Akkermansia muciniphila: paradigm for next-generation beneficial microorganisms. Nat. Rev. Gastroenterol. Hepatol. 19, 625–637 (2022).
3. Shuoker, B. et al. Sialidases and fucosidases of Akkermansia muciniphila are crucial for growth on mucin and nutrient sharing with mucus-associated gut bacteria. Nat. Commun. 14, 1833 (2023).

Mucin-Microbe Interactions

Utilization of human colonic mucin by gut bacteroides

Grete Raba[1,2], Ana S. Luis[2,3]

1. Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia
2. Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden
3. SciLifeLab, University of Gothenburg, Gothenburg, Sweden

The gut microbiota colonizes the intestinal mucus layer which creates a protective barrier between the bacteria and the epithelium. The major component of this layer is Mucin-2 (MUC2), a secreted mucin with over a hundred different O-glycan structures attached to its protein core. Some of the gut bacteria can degrade and utilize these complex glycans. The microbial foraging on mucin has been linked to disruptions of the colonic mucus layer and inflammation, leading to diseases such as inflammatory bowel disease (IBD). However, the mechanisms of colonic mucin utilization by the microbiota remain unclear.
Bacteroides are one of the most common bacteria found in human gut. Several of these species are known to be able to utilize mucin O-glycans1. In Bacteroides the enzymes required to degrade specific glycans are encoded in specialized Polysaccharide Utilization Loci (PULs). To utilize O-glycans Bacteroides upregulate multiple PULs encoding dozens of enzymes2. Bacteroides thetaiotaomicron (B. theta) sulfatases have been previously identified as key enzymes for the utilization of porcine colonic mucins1. Due to the complexity of mucin O-glycans structures, we hypothesize that additional enzymes targeting terminal O-glycans epitopes can also have a key role in initiating mucin utilization by B. theta. Moreover, mucin glycosylation is variable between species and along the gastrointestinal tract. Previous microbiota studies have relied on the utilization of porcine mucins. Here we aimed to identify the PULs and key enzymes needed by the bacteria grown on human colonic mucins and to define the basic interaction mechanisms between the microbiota and human colonic mucins.
We have collected and purified mucins from the colons of human transplant donors. A selection of gut commensal bacteria has been screened for their potential to utilize these human colonic mucins O-glycans (HcMO). Three Bacteroides species have been identified as HcMO utilizers. These Bacteroides spp. show different growth profiles when cultivated on HcMO, suggesting alternative O-glycans utilization mechanisms. Identifying the key enzymes required for the utilization of human colonic mucin O-glycans by commensal bacteria will offer potential drug targets to inhibit mucin degradation by the microbiota in diseases such as IBD.

1. Luis, A. S., et al., A single sulfatase is required to access colonic mucin by a gut bacterium. Nature, 2021. 598(7880):332-337.
2. Martens, E. C., et al., Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host & Microbe, 2008. 4(5):447-457.

Mucins in Cancer

MUC1 roles on lung cancer cells properties and cisplatin chemoresistance.

Marine Goujon[1], Emma Bourdon[1], Michaël Perrais[1]

1. Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277 – CANTHER – Cancer Heterogeneity Plasticity and Resistance to Therapies, Equipe « Mucines, cancer et résistance aux drogues » F-59000 Lille, France.

Introduction : Despite advances in cancer diagnosis and treatment, lung cancer remains the leading cause of cancer death worldwide, killing 1.8 million patients each year. Its aggressiveness is such that its 5-year survival rate doesn't exceed 20%. Represented at 85% by the non-small cell subtype (NSCLC), lung cancer is often diagnosed at locally advanced stages. When surgery is not possible, treatment is mainly based on systemic therapies, such as platinum-based chemotherapy (cisplatin), in combination with other treatments : immunotherapies which target the "immune checkpoints" or targeted therapies. Nevertheless, a large number of patients has a primary or secondary resistance to these drugs.
MUC1 is a large transmembrane O-glycoprotein expressed at the apical pole of epithelial cells and it main function is to protect the underlying epithelia. Nevertheless, MUC1 is over-expressed in 2/3 of cancers, including lung cancer, especially in 40-60% of adenocarcinoma NSCLC subtype. Although MUC1 plays a role in protection in physiological conditions, in cancer situation, is known to play a role in tumor progression inducing proliferation, migration and invasion and to be associated with signatures of chemoresistance.
In this context, the project aims to better understand MUC1 roles on lung cancer cells properties and chemoresitance to cisplatin.

Methods : In our project, two adenocarcinoma cell lines are used: H1975 invalidated for MUC1 expression by Crispr/Cas9 technology or knock-down by ASO (antisens oligonucleotides) as well PC9 stably overexpressing MUC1 (PC9). MTS, proliferation assays, migration/ invasion assays, immunofluorescence, western blot, comet assays and qPCR are used.

Results : Our results show that MUC1 expression (i) is associated with increased cell survival, proliferation, migration and invasion (ii) leads to cisplatin chemoresistance, (iii) increases the expression of ABC family efflux pumps, and (iv) protects cells treated with cisplatin against DNA damages, a proliferation stop and apoptosis.

Conclusion/ discussion : In conclusion, in NSCLC, our results show that MUC1 is an actor of tumor progression, by promoting cell survival, proliferation, migration and invasion and is involved in cisplatin chemoresistance. ASO strategy can reverse these properties. Overall our data suggest that MUC1 may represent a novel therapeutic approach to limit NSCLC progression and improve drug sensitivity.

Keywords : NSCLC, lung cancer, chemoresistance, cisplatin, chemotherapies

Mucins in Cancer

The role of MUC13 in gastric cancer cell death inhibition and dysbiosis

Baptiste Oosterlinck[1], Wout Arras[1], Joris G. De Man[1], Benedicte Y. De Winter[1], Annemieke Smet[1]

1. Laboratory of Experimental Medicine and Paediatrics, Faculty of Medicine and Health

BACKGROUND: One of the hallmark features of gastric adenocarcinomas is aberrant mucin expression which drives tumorigenesis by influencing cellular growth and survival and has been linked to initiation, progression and poor prognosis. In gastric cancer, MUC13 is overexpressed in 64.9% of cases and has been associated with worse patient survival and enrichment of oral pathogens. However, how MUC13 affects cell death signaling in the gastric carcinogenesis process remains unknown.

AIMS: Here, we aimed to identify whether MUC13 is involved in tumour cell death resistance in gastric cancer and unravel the signalling pathways involved.

METHODS: MKN-7 gastric cancer cells, incubated at 37°C and 5% CO2 in RPMI-1640 medium till 70% confluency, were transfected with Silencer Select MUC13 and Negative Control siRNA (control siRNA, Invitrogen) using RNAiMAX lipofectamine reagent (Invitrogen) according to manufacturer's instructions. Forty-eight hours post-transfection, cells were stimulated with either TNF-a or IL-1B at 0, 60 or 80 ng/mL. Twenty-four hours post-treatment, an MTT-cell survival assay was performed to assess cell survival through measurement of optical density and RNA extracted for bulk RNA sequencing (150bp paired end) to analyse differential gene expression and gene set enrichment.

RESULTS: In the absence of cytokine treatment, knock down of MUC13 expression resulted in significant increased cell survival compared to control siRNA transfected cells (N= 64, P<10-5, t-test). When treated with 60 ng/mL cytokine, no significant difference in cell survival was observed between MUC13 knock-down and control cells for IL-1β while for TNF-α treated MUC13 knock-down cells survival was significantly increased (N=32, P= 0.01). In case of 80 ng/mL cytokine treatment, control cells have a significantly increased cell survival compared to MUC13 knock-down cells with a greater effect for TNF-α (N=32, P=0.0046) compared to IL-1β (N=32, P=0.047). These results are being confirmed in an in vivo gastric cancer mouse model in which female MUC13-/- or wild type litter mates were orally infected with Helicobacter pylori (SS1), H. felis (CS1) or given trypticase broth as control. After a two-week recovery period, infected animals received drinking water supplemented with 240 ppm N-Methyl-N-nitrosourea (MNU) on alternating weeks for 5 weeks. Twenty-six weeks after MNU treatment the mice were sacrificed, and the stomach (corpus & antrum) sampled for transcriptome analysis in addition to microscopic analysis of inflammation through immunohistochemistry. Transcriptome analysis is currently being performed on siRNA transfected MKN7 cells and gastric tissue samples from the Helicobacter/MNU-treated gastric cancer mouse model. Analysis of the RNA sequencing data is currently ongoing.

CONCLUSIONS: Our results emphasize a key role of MUC13 in promoting tumour cell survival in gastric cancer.

Mucins in Cancer

Structural elucidation and prognostic relevance of 297-11A-sulfated glycans in ovarian carcinoma

Daisuke Inoue[1,2], Hitomi Hoshino[1], Ya-Ying Chen[3], Makoto Yamamoto[2], Akiya Kogami[1], Mana Fukushima[1], Kay-Hooi Khoo[3,4], Tomoya O. Akama[5], Yoshio Yoshida2, Motohiro Kobayashi[1]

1. Department of Tumor Pathology, Faculty of Medical Sciences, University of Fukui, Eiheiji, Japan
2. Department of Obstetrics and Gynecology, Faculty of Medical Sciences, University of Fukui, Eiheiji, Japan
3. Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan
4. Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan
5. Department of Pharmacology, Kansai Medical University, Hirakata, Japan

Ovarian carcinoma is usually diagnosed at an advanced stage with peritoneal dissemination and/or lymph node metastasis, and the prognosis for such advanced carcinoma is very poor. Therefore, new biomarkers to predict patient prognosis are needed. Miyamoto et al. previously showed that keratan sulfate (KS) detected by the 5D4 monoclonal antibody was expressed in ovarian carcinoma. However, the detailed structure of such KS was not determined, and the biological significance of this finding remained to be clarified. We previously generated the 297-11A monoclonal antibody, which recognizes galactose (Gal)-6-O-sulfated N-acetyllactosamine (LacNAc) located at the non-reducing terminus. Since the 297-11A epitope overlaps with that of 5D4, here we chose to use the 297-11A antibody as a tool to analyze KS and related structures. We conducted immunohistochemical analysis of 98 ovarian carcinoma cases with 297-11A antibody combined with a series of glycosidases and performed mass spectrometry analysis of the human serous ovarian carcinoma cell line OVCAR-3 to deduce the glycan structure of 297-11A-sulfated glycans. We also performed western blot analysis to assess a potential association of 297-11A-sulfated glycans with MUC16 (CA125) mucin core proteins. Finally, we examined the relationship between 297-11A expression and patient prognosis. Consequently, 297-11A-sulfated glycans were primarily expressed in serous and endometrioid carcinomas and poorly expressed in mucinous and clear cell carcinomas. We revealed the structure of 297-11A-sulfated glycans expressed in ovarian carcinoma to be O-glycans carrying partially sialylated, Gal-6-O-sulfated LacNAc, and these glycans were displayed on MUC16 mucin core proteins. Of clinical importance is that expression of 297-11A-sulfated glycans correlated with shorter progression-free survival in patients. Thus, 297-11A-sulfated glycans may serve as a predictor of ovarian carcinoma recurrence.

Mucins in Cancer

Characterization of 2′-5′-oligoadenylate synthetase (OAS) genes as MUC4-associated new biomarkers in pancreatic adenocarcinoma

Nicolas Jonckheere[1], Diane Ghesquiere[1], Julie Auwercx[1], Lucie Coppin[1], and Isabelle Van Seuningen[1]

Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277-CANTHER-Cancer Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France.

Pancreatic ductal adenocarcinoma (PDAC) is the major form of pancreatic cancer and is characterized by a short survival curve. The Cancer Genome Atlas (TCGA) in order to provide comprehensive mapping of the key genomic changes occuring during carcinogenesis. Notably, the pancreatic adenocarcinoma TCGA dataset (TCGA-PAAD) can be used to propose new prognostic biomarkers associated with molecular alterations. MUC4 is considered as a pro-tumorigenic biomarker since its expression is associated with PDAC progression and aggressiveness. We used different bioinformatic tools such as Linkedomics, GEPIA, Gene Expression Omnibus (GEO), R studio, kmplot, PROGgeneV2 in pancreatic cancer datasets. We investigated genes that are correlated with MUC4 in PAAD-TCGA dataset and performed Gene Set Enrichment Analysis (GSEA). We also characterized their expression in pancreatic cancer and their impact on patient survival. We performed an unsupervised hierarchical clustering analysis to identify a gene signature composed by OAS1/2/3/L, IRF1/3/7/9 and MyD88. 10556 genes were deregulated. The most enriched GO terms corresponded to skin (347 genes) and epidermis (387 genes) development (GO:0043588 and GO:0008544). Moreover, 85 deregulated genes belonged to response to type I interferon GO term (GO:0034340) including genes associated with 2'-5'-oligoadenylate synthesis (OAS1, OAS2, OAS3, OASL), innate immune signal transduction adaptor MyD88, signal transducer and activator of transcription 1 (STAT1) or interferon regulatory factor (IRF1, IRF2, IRF3, IRF6, IRF7 and IRF9). Receiver Operating Characteristic (ROC) curve analyses showed a good performance of OAS1, OAS2, OAS3, OASL (AUROC = 82.27-87.9) suggesting that OAS1/2/3/L are that new genes of interest are associated with patient survival and could be potential biomarkers. We are currently investigating whether MUC4 directly regulate the expression of these genes.

Mucins in Cancer

The cancer mucin barrier provides multifactorial physical defense against immune cell attack

Justin H. Paek[1], Audrey Struzyk[1], Sangwoo Park[2], Matthew J. Paszek[1,2,3]

1. Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University
2. Field of Biophysics, Cornell University
3. Robert Frederick Smith School of Chemical and Biomolecular Engineering

Cancer is often associated with the aberrant expression of cell-surface mucins, resulting in a thick cellular glycocalyx that coats the cancer cell membrane and governs its interactions with surveilling immune cells. Aside from presenting inhibitory ligands to biochemically suppress immune cell activity, densely packed mucin biopolymers on the cell surface can also assemble into a nanoscale barrier that physically protects cells from immune cell-mediated lysis. However, the precise mechanisms by which the mucin barrier acts to physically disrupt the many dynamic events leading up to cell-mediated cytotoxicity are not known. Using a cellular model with inducible expression of the cancer-associated mucin Muc1, we examine the effect of the mucin barrier on the dynamics of interactions with Natural Killer (NK) cells. Live-cell imaging of co-cultures reveals that target cells with a thin mucin barrier allow for a high proportion of stable contacts with NK cells, resulting in rapid lysis of the target within minutes. Meanwhile, target cells with a thick mucin barrier are significantly more likely to resist stable NK cell adhesion, activation, and killing. Strikingly, these target cells are still able to resist continuous engagement and attack for several hours. Using live-cell reporters of lysis by pore formation, we find that high mucin-expressing target cells require a much longer duration of contact in order to be killed by an NK cell, and that this death is characterized by the appearance of apoptotic blebs rather than by lytic pore formation. At high enough densities, mucins also cause dramatic bending of the plasma membrane, resulting in exotic membrane shapes that surveilling immune cells must form synapses with. Analysis of the NK-target contact interface further implicates mucin-induced plasma membrane curvature as an additional defensive mechanism that impedes the formation of a productive immune synapse. Taken together, these results suggest that the mucin barrier acts along multiple levels to provide physical resistance to NK cell attack, by (i) hindering stable contact formation, (ii) preventing effector cell activation and polarization, (iii) resisting pore-mediated lysis and (iv) generating dramatic membrane curvature that discourages productive synapse formation.

Mucins in Cancer

The potential of targeting the mucin MUC4 in Pancreatic Adenocarcinoma as a partner of RTKs

Tristan Girgin[1], Nicolas Stoup[1], Elsa Faillon[1], Nicolas Lebègue[2], Isabelle Van Seuningen[1]

1. Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277-CANTHER-Cancer Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France.
2. Univ. Lille, Inserm, CHU Lille, UMR-S1172 - LiNC - Lille Neuroscience & Cognition, F-59000 Lille, France

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disorder, for which neither efficient diagnosis nor effective treatment exist, associated with high tumor resistance to treatments. It is thus crucial for this devastating cancer to find new therapeutic alternatives. In this aim, we work on the interaction of the mucin MUC4 with receptor tyrosine kinases (RTK), and the targeting of MUC4 using small inhibitory molecules. Neo- and over-expressed as early as the pancreatic pre neoplastic stage, MUC4 is associated with poor prognosis of the tumor, an increase of proliferation, migration, as well as apoptosis and resistance to therapies, resulting in poor overall survival of patients. Recently, we have discovered that MUC4 EGF domains interact with the receptor ErbB2/HER2 and that this interaction mediates tumor progression. These results indicate that MUC4 is a potential therapeutic target in PDAC, and as such we have identified for the first time an inhibitory peptide of this interaction with therapeutic value under evaluation.
Widening our studies we found that MUC4 interacts with and activates, at the cell membrane surface, other RTK such as ErbB/HER1-3-4, C-Met, Axl, EpCAM, IGF-1R. We thus hypothesize that MUC4 could interact with these RTK, in a similar manner as with ErbB2, making MUC4 a RTK platform at the surface of PDAC cells modulating PDAC tumor properties.
To decipher the molecular mecanism underlying this, we studied these interactions using Co-Immunoprecipitation, GST pull-down, Proximity Ligation Assay, and MicroScale Thermophoresis.
Our results show that MUC4 interacts directly with ErbB1,3,4, as well as C-Met, Axl, IGF-1R and EpCAM. We confirmed that this interaction was possible after siRNA-mediated ErbB2 downregulation. Finally, our results tend to show that these interaction are capable of activating these RTK, and generate signaling pathways, even after ErbB2 downregulation, to maintain proliferation and migration capacities of pancreatic cancer cells.
Taken together, these results provide a new perspective on the mechanisms of action of RTKs at the surface of pancreatic cancer cells, and reinforce the potential of targeting MUC4 in PDAC as well as in other MUC4-overexpressing cancers.

Mucins in Cancer

Inhibition of *O*-glycosylation by peracetyl N-thioglycolyl-D-galactosamine (Ac5GalNTGc) puts brakes on in vivo melanoma growth and metastasis

Abhiraj Rajasekharan[1], Tanya Jain[1], Jyoti Rautela[1], Santiswarup Singha[2], Perumal Nagarajan[3], and Srinivasa-Gopalan Sampathkumar[1]*

1. Laboratory of chemical glycobiology
2. Nanobiotechnology Laboratory
3. Experimental Animal Facility

The mammalian immune system is capable of eliminating cancerous growth at a very early stage. However, complications due to aging and exhaustion lead to the suppression of immune response and promote tumor growth. Tumor microenvironments are immunosuppressive due to interactions between immune checkpoint molecules (e. g. PD1/CD279-PDL1/CD274; CTLA4/CD152-B7-1/CD80). Antibody blockade of PD1-PDL1 interaction is a proven concept in cancer immunotherapy. Recent research has shown that the Siglecs-sialoglycan axis provides an additional checkpoint for immunosuppression. Siglecs (e. g. CD22 and CD33) carry ITIM (immuno-tyrosine inhibitory motif) in their cytoplasmic domain and activate inhibitory signaling. It has been shown that conjugates of antibodies and sialidase are effective in releasing the Siglec-sialic acid immune checkpoint and enhance tumor clearance (Ref: Stanczak, M. A. et al. Sci. Transl. Med. (2022)).

In this context, we hypothesized that small molecules capable of inducing hypo-sialylation would abrogate the Siglec-sialic acid interactions and facilitate tumor clearance. It is known that in the case of tumor cells, the expression of mucin and mucin-domain glycoproteins is increased multi-fold. The mucin-hydrogel matrix around cancer cells acts as a sialic acid depot to engage the immunosuppressive Siglecs, and protects from drugs and endogenous tumor-clearing immune cells. Mucins carry 50-70 % by weight of mucin-type O-glycans (MTOG) on their polypeptide backbone. MTOG biosynthesis is initiated by the addition of N-acetyl-D-galactosamine (GalNAc) to Ser/Thr residues in mucins and decorated further by the addition of D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-neuraminic acid, and L-fucose through complex glycosylation machinery. Our laboratory has shown that the peracetyl N-thioglycolyl-D-galactosamine (Ac5GalNTGc) is an efficient inhibitor of O-glycosylation both in vitro and in vivo (Ref: Agarwal, K. et al J. Am. Chem. Soc (2013); Wang, S. S. et al. Cell Chem. Biol. (2021)). We hypothesized that treatment of tumor cells with Ac5GalNTGc would result in the inhibition of MTOG on mucins and consequently disrupt Siglec-binding to tumor cells and release the immune checkpoint. Using the B16F10-Luc2 cells, constitutively expressing luciferase, we studied the effect of a panel of GalNAc analogues both in vitro and in vivo in syngeneic immunocompetent C57BL/6J mice. Our results showed that treatment with Ac5GalNTGc effectively decreased the growth of subcutaneously implanted B16F10-Luc2 cells, their metastasis to the lung tissue, and enhanced the survival rate in mice. In vitro results from Siglec binding, migration and invasion assays, and 3D-spheroids supported the unique ability of Ac5GalNTGc to retard melanoma growth and metastasis. Our results show that targeting biosynthesis of MTOG using pharmacological agents provides novel opportunities for cancer therapy, in combination with other modalities.

Mucins in Cancer

Engaging undergraduate students in research through course-based undergraduate research experiences

Teresa Brooks[1], Cory Brooks[1]

1. California State University Fresno, Department of Chemistry and Biochemistry

An undergraduate course-based research experience (CURE) is a high impact practice which provides genuine research experiences to a large and diverse student population. At primarily undergraduate institutions such as Fresno State, they also provide a means to increase research productivity while carrying a heavy teaching load. Here is presented an example of a CURE module included within an upper division biochemistry lab course. Our research has centered around understanding the nature of the cancer-specific CA125 epitope found on human MUC16 using cancer-specific monoclonal antibodies. One antibody in particular, AR9.6, has shown great promise as a therapeutic for ovarian and pancreatic cancer due to its high specificity to ability to reduce tumor burden and oncogenic signaling in various models of pancreatic duct adenocarcinoma (PDAC). AR9.6 binds a conserved epitope found in the SEA (Sea urchin sperm, Enterokinase, Agrin) domain of the MUC16 tandem repeat region. Previous work has established that AR9.6 affinity for the SEA domain is enhanced by glycosylation. However, the mechanism by which antibody affinity is altered and which glycosylation sites play a role in this affect are unaddressed questions. The goal of this project was to engage in undergraduate researchers in a lab course environment to address these research questions. Students employed structural analysis of an AR9.6-SEA domain crystal structure in order to identify potential glycosylation sites near the antibody-antigen interface. We then created expression vectors to recombinantly express variants of the same SEA domain in Chinese Hamster Ovary (CHO cells) with relevant serine, threonine and asparagine amino acids mutated to block glycosylation. Once expressed and purified these proteins will be used in surface plasmon resonance studies to determine antibody affinity for the variants. This project will provide tools and preliminary data for future research, conducted within our lab. Students participating in the course have learned valuable skills in scientific literacy, experimental design, trouble-shooting and communicating data. Our institution has been incorporating CUREs in many STEM classes ranging from the freshman (1-year) to senior (4th year) level since 2018 and proven a valuable way or bridging undergraduate education and hypothesis driven faculty research projects.

Mucins in Cancer

Defining the role of mucus barrier dysfunction in microbiota-dependent initiation of colorectal cancer

Karl Hansson[1], Jozefina Dzanan[1], Clothilde Wiel[1], Kevin Ali[1], Volkan Sayin[1], Eva Angenete[1], George Birchenough[1]

1. University of Gothenburg

Colorectal cancer (CRC) has surged globally, particularly in industrialized nations, driven mainly by negative lifestyle factors, notably the Western style diet (WSD) markedly lacking in complex fibres. Prolonged WSD exposure is implicated in oncogenic alterations within the colonic microbiota, synergizing with dysregulated immune responses to promote tumour formation. However, early steps in this process remain poorly understood.
The colonic mucus barrier plays a pivotal role in host-microbiota interactions. Mouse studies suggest that WSD exposure disrupts mucus barrier function and that deletion of the key mucus gel-forming mucin Muc2 results in spontaneous CRC. While the relevance of mucus barrier dysfunction in CRC patients is unclear, our state-of-the-art ex vivo analysis of biopsies from healthy individuals and CRC patients undergoing routine screening and resection, respectively, reveals compromised mucus barrier function in CRC patients, suggesting its role in CRC-initiation.
To model microbiota-dependent CRC initiation in vivo, we generated Muc2+/-Apcmin/+ mice, combining reduced Muc2 expression with a predisposition to spontaneous intestinal adenoma formation. Compared to Muc2+/+Apcmin/+ littermates, these mice show increased tumour load in both small intestine and colon. Strikingly, broad-spectrum antibiotic intervention inhibits increased tumour burden in Muc2+/-Apcmin/+ mice, while having little effect on Muc2+/+Apcmin/+ mice. Demonstrating that enhanced tumorigenesis in this model is microbiota-dependent, highlighting its potential use for the study of microbiota-induced CRC initiation in the context of a weakened mucus barrier.
Combined, our data indicates the necessity of a functional mucus barrier in preventing CRC and provides new tools for elucidating the mechanisms behind mucus barrier dysfunction during CRC progression.

Mucins in Cancer

Establishing the role of secreted mucus matrix in appendiceal mucinous neoplasms and pseudomyxoma peritonei

Milly McAllister[1], David Thornton[1], Bipasha Chakrabarty[2], Omer Aziz[2], Sarah O'Dwyer[2], and Patrick Caswell1

1. University of Manchester
2. The Christie NHSFT

Pseudomyxoma Peritonei (PMP) is a poorly understood neoplastic condition, commonly originating from appendiceal mucinous neoplasms (AMN), and is characterised by significant accumulation of intraperitoneal mucus and peritoneal metastases (PM). To date, there is a lack of basic research and pre-clinical models, resulting in limited treatments and therapeutic advancements. These PMs are unique in that they frequently secrete a mucus matrix with suspended tumour cells and result in poor outcomes due to mucinous bowel obstruction. Upregulated mucin production and aberrant glycosylation have been identified in numerous cancers, however their roles in PMP remain undefined. Here, we explore the composition of the mucus matrix within PMP and glycan modifications to explore their potential as druggable targets to modify invasion and metastasis.
Proteomic analysis using the Orbitrap Exploris mass spectrometer on intraperitoneal mucus revealed the presence of MUC2, MUC5AC, and MUC5B along with multiple matrisome proteins including extracellular matrix structural proteins (COL1A1, COL2A1 and COL11A1), matrix remodelling proteins (MMP2, 3, and 12), and core matrisome proteins (LAMA1, VTNC, and FN1). Furthermore, following picrosirius red staining on PMP mucus, we found intact collagen fibrils, suggesting a structural support system for disseminating tumour cells. Interestingly, following caesium chloride purification of mucins (predominantly MUC2 and MUC5AC) from intraperitoneal mucus, we identified differentially sialylated, glycan modified mucins secreted from patients with low-grade PMs (α2-3 linked sialic acid and α-linked N-acetylgalactosamine (GalNAc; the Tn antigen)). When biochemically purified mucins were supplemented into PMP organoids, a significant increase in organoid growth was observed over 7 days when compared to untreated organoids. Furthermore, when assessing PMP cell invasion (N14A cell line) in a 3D in vitro model, mucin addition promoted significant invasion into collagen based hydrogels over 24 hours when compared to the untreated control.
Our data suggests intraperitoneal mucus could provide both structural support for dissemination of AMN and proliferation of PMs along with important signalling cues via differentially sialylated mucins, playing crucial roles in neoplastic growth and progression of PMP. Through this, we have identified key pathways for mechanistic investigation and potential novel therapeutic targets.

Mucins in Infection and Inflammation

Antibiotics damage the colonic mucus barrier in a microbiota-independent manner

Shai Bel[1]

1. Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel.

Integrity of the mucus layer which separates the host intestinal epithelium from the luminal microbiota is crucial for gut health. Breakdown of this mucus barrier is a hallmark of inflammatory bowel diseases (IBD). Antibiotic use is a risk factor for development of IBD, yet how antibiotics affect the mucus barrier is not clear. Here, we systematically determined the effects of different antibiotics on mucus layer penetrability. We found that oral antibiotic treatment led to breakdown of the mucus barrier and penetration of bacteria into the mucus layer. Using fecal microbiota transplant, RNA sequencing followed by machine learning, germ-free mice and ex vivo mucus secretion measurements, we found that vancomycin and neomycin inhibit mucus secretion in a microbiota-independent manner. This inhibition leads to penetration of bacteria into the colonic mucus layer and translocation of microbial antigens into circulation. Thus, antibiotics use might predispose to development of intestinal inflammation by impeding mucus production.

Mucins in Infection and Inflammation

Interferon-induced genes and down-regulation of oxidative phosphorylation dominate the response of mouse gastric surface mucus cells to acute *H. pylori* infection in vivo

Mattias, Erhardsson[1], Licínia Santos[1], Sinan Sharba[1], Kaisa Thorell[2], Sara Lindén[1]

1. University of Gothenburg, Sahlgrenska Academy, Institute for Biomedicine, Department of Medical Biochemistry and Cell Biology
2. University of Gothenburg, Faculty of Science, Department of Chemistry & Molecular Biology

The bacterium Helicobacter pylori (H. pylori) is the main risk-factor for gastric cancer. H. pylori easily develops antibiotic resistance and utilizes several methods to circumvent host defences. The first layer of defence is the gastric surface mucus-producing epithelial cells. Deepened understanding of H. pylori pathogenesis could enable improved treatment and prevention.
We used experimentally infected male C57BL/6 mice to study the effects of H. pylori on mouse gastric surface mucus-producing cell gene expression. These mucus-cells from 10 mice, 5 infected and 5 non-infected, were extracted with laser microdissection and the gene expression determined with RNA sequencing. The reads were processed through a standardised data pipeline and then analysed in R and Cytoscape. The experimental infection was verified histologically.
The results showed that genes required for oxidative phosphorylation were down-regulated in acutely H. pylori-infected mice, and that this reduction might be caused by stimulation of several interferons such as interferon γ. A sharp down-regulation of Nkx6-3 could also be seen in the infected mice, which was indicative of the gastric pre-cancerous cascade having been initiated.
The use of RNA sequencing of cells captured with laser microdissection showcased this as a viable method to study host-pathogen interactions in vivo. A detailed protocol is available to facilitate more widespread use of this method.

Mucins in Infection and Inflammation

Mucin isoform signatures as potential novel biomarkers to evaluate disease status in patients with Inflammatory Bowel Diseases

Wout Arras[1], Baptiste Oosterlinck[1], Aranzazu Jauregui-Amezaga[2], Michaël Somers[2], Elisabeth Macken[2], Joris De Man[1], Bendicte Y. De Winter[1,2], Annemieke Smet[1]

1. Laboratory of Experimental Medicine and Pediatrics, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium
2. Department of Gastroenterology and Hepatology, University Hospital Antwerp, Belgium

Introduction
Mucosal barrier dysfunction and aberrant mucin expression are major hallmarks in the pathophysiology of IBD. Mucins are highly polymorphic, and the presence of genetic differences can alter gene expression, resulting in several mRNA isoforms via alternative splicing. While most isoforms encode similar biological functions, others alter protein function, potentially resulting in progression towards disease. Currently, little attention has been given to the importance of mucin mRNA isoforms in IBD.
Aim
The aim of our study is to investigate the potential of mucin mRNA isoforms as novel biomarkers for the evaluation of IBD activity and subtypes.
Methods
To obtain this goal, RNA was extracted from colonic and terminal ileal biopsies of IBD patients and controls that underwent an endoscopy. Library preparation was performed with the PacBio Iso-Seq multiplex protocol adapted for targeted transcriptome sequencing. Targeted capture was accomplished by using a custom-designed pool of probes, developed for the capture of all mucin gene transcripts. In total 106 biopsies were sequenced on the PacBio platform. The resulting intestinal mucin transcriptome was merged with the human reference transcriptome. On this combined mucin transcriptome Illumina bulk RNA sequencing data from over 2000 intestinal biopsies (GEO dataset GSE193677) were mapped to determine mucin isoform expression. An external dataset (GEO dataset GSE165512) was used for additional validation. A classification random forest was trained on this data to distinguish inflamed IBD from non-inflamed control patients based on the mucin isoform expression alone.
Results
In total 208 different mucin isoforms were found of which a large portion was found to be novel. Especially for MUC2, MUC3A, MUC4, MUC12, MUC13 and MUC17 up to 38 different isoforms were found with our targeted approach. Random forest model trained on mucin isoform expression data performed well on train and test datasets with AUC between 89.1% and 93.3% but decreased in the external validation to values between 53.9% and 76.8%. Dividing the samples based on disease phenotype greatly increases performance on the external validation dataset (AUC[IBD] 53.9%, AUC[CD] 59.2% and AUC[UC] 76.8%). When only training on ileal biopsies, the model proved to be excellent in distinguishing Crohn's disease patients from controls with an AUC of 91.1%, 89.0% and 74.5% for the train, test and external validation dataset, respectively. Classification of inflamed ulcerative colitis from control patients based on only the biopsies from the distal colon was similar to the latter.
Conclusions
The intestinal mucin mRNA isoform landscape is diverse and contains a large amount of MUC2, MUC3A, MUC4, MUC12, MUC13 and MUC17 isoforms. Our machine learning model was able to distinguish Crohn's disease from control patients and ulcerative colitis from control patients based on mucin mRNA isoform expression.

Mucins in Infection and Inflammation

Stress-induced Mucin 13 reductions drive intestinal microbiome shifts and despair behaviors

Courtney Rivet-Noor[1,2,3], Andrea Merchak[1,2,3]. Caroline Render[4], Naudia Gay[1,2,3], Rebecca Beiter [1,2,3], Ryan Brown[1,2,3], Austin Keeler[5], G. Brett Moreau[6], Sihan Li[2,7], Deniz Olgun[8], Alexandra Steigmeyer[10], Rachel Ofer[11], Tobey Mihn Huu Phan[9], Kiranmayi Vemuri[11], Lei Chen[12], Keira Mahoney[10], Jung-Bum Shin[2], Stacy Malaker[10], Chris Deppmann[5], Michael Verzi[11], John Rawls [13], Alban Gaultier[2]

1. Center for Brain Immunology and Glia, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.
2. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.
3. Graduate Program in Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.
4. Undergraduate Department of Global Studies, University of Virginia College of Arts and Sciences, Charlottesville, VA, 22904, USA.
5. Department of Biology, University of Virginia College of Arts and Sciences, Charlottesville, VA, 22904, USA.
6. Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia, USA.
7. Graduate Program in Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.
8. Undergraduate Department of Computer Science, University of Virginia School of Engineering and Applied Science, Charlottesville, VA 22904, USA.
9. Undergraduate Department of Neuroscience Studies, University of Virginia College of Arts and Sciences, Charlottesville, VA, 22904, USA.
10. Department of Chemistry, Yale University, New Haven, CT 06511, USA.
11. Department of Genetics, Human Genetics Institute of New Jersey, Rutgers Cancer Institute of New Jersey, Rutgers Center for Lipid Research, Division of Environmental & Population Health Biosciences, EOHSI, New Brunswick, New Jersey, 08901, USA.
12. School of Life Science and Technology, Key Laboratory of Developmental Genes and Human Disease, Southeast University, Nanjing, China.
13. Department of Molecular Genetics and Microbiology, Duke University, Durham, NC 27710.

Microbiome dysbiosis is observed in numerous pathological conditions, ranging from irritable bowel syndrome to mental health disorders. While the underlying mechanisms of these diseases vary, stress is a common triggering event. However, the routes through which stress mediates microbiome dysbiosis are still unknown. Additionally, treating microbiome dysbiosis remains difficult due to the complexities of the human microbiome, technical colonization limitations, and poor understanding of the intricate microbial community structures within the gut. Thus, identification of upstream mediators of stress-induced microbiome dysbiosis is critical for new therapeutic avenues. Here, we demonstrate that stress-induced microbiome dysbiosis is initiated through alteration of transmembrane mucin 13. We begin by demonstrating that exposure to a model of unpredictable chronic mild restraint stress (UCMRS) induces changes in behavior, changes in stress hormones, and induces microbiome dysbiosis. UCMRS also induces a unique and significant reduction in the transmembrane mucin, mucin 13. This reduction is not driven by changes in the microbiome as germ free mice exposed to stressed or naïve microbes show no changes in mucin 13 expression. Mechanistically, we demonstrate that this stress-induced loss of mucin 13 is driven by a reduction in the transcription factor hepatocyte nuclear factor 4 alpha (HNF4a) as loss of this critical transcription factor significantly reduced mucin 13 expression and protein levels. Finally, utilizing a mucin 13 knockout line, we demonstrate that loss of mucin 13 is sufficient to induce microbiome dysbiosis that parallels stress-induced microbial changes and induces depressive- and anxiety-like behaviors in mice. Taken together, our data identifies a mechanistic regulator of transmembrane mucin 13 and highlights a novel role of transmembrane mucins in microbiome dysbiosis in the context of stress. This work underscores the importance of the glycocalyx in regulating intestinal homeostasis and brings to light new therapeutic targets for microbiome dysbiosis. Future directions for this work are ongoing and include understanding the role of transmembrane mucins in infection and inflammatory states. In addition, we are actively working to understand the transcriptional regulators of transmembrane mucins.

Mucins in Infection and Inflammation

Immunomodulatory Mucin 1 expression is significantly altered in both airway epithelium and immune cell populations following influenza virus infection

Leila Abdelhamid[1], Shrestha Mathur[1], and Margaret Scull[1]

1 Department of Cell Biology & Molecular Genetics, Maryland Pathogen Research Institute, University of Maryland, College Park, Maryland, USA.

Cell-associated mucin 1 (MUC1) is a component of the mucus barrier in the respiratory tract that acts as a physical barrier and signaling molecule. Our prior work demonstrated increased MUC1 expression during influenza virus (IAV) infection in human airway epithelial (HAE) cultures in vitro. Here, we provide a comprehensive analysis of MUC1 expression patterns and report that IAV-induced cytokines, including type I interferons (IFNs), trigger the expression of a low-molecular-weight (LMW) form of MUC1 which localizes to the cytoplasm and nucleus and is expressed in different epithelial cell types, particularly in basal cells. Employing Iso-Seq analysis, we have identified distinct MUC1 isoforms in HAE. Further, following IAV infection in vivo, we confirmed elevated MUC1 expression in EPCAM + airway epithelium and also revealed altered MUC1 levels in lung immune cells. Upon IAV infection, MUC1 is significantly upregulated and expressed on the surface of CD4 + , and CD8 + T cells and their CD44 + CD62L - effector populations. Notably, we observed more significant changes in CD4 + compared to CD8 + T cells with a differential expression of MUC1 in the early activated CD69 + T cells, suggesting that MUC1 may have different impacts on the responses of specific T cell subsets to IAV. MUC1 was significantly downregulated in alveolar macrophages (AMΦs) flowing IAV infection. Concurrently, we found a negative correlation between the frequency of MUC1+ AMΦs and the expansion of effector T-cell populations, supporting the putative immunoregulatory roles of MUC1. Together, our data reveals that MUC1 expression is not confined to the airway epithelium and displays significant changes in T-cells that potentially impact their responses to IAV infection.

Mucins in Infection and Inflammation

Defining a new role for Charcot-Leyden crystals in Type 2 immunity and airway obstruction

Helena Aegerter[1], Ines Heyndrickx[1], Jasmijn Burgelman[1], Alysia Wayenberg[1], Ursula Smole[1], Kenneth Verstraete[2], Melia Magnen[3,4,5], Wendy Toussaint[1], Wilfred Raymond[4,5], Mark Looney[3,4,5], John Fahy[4,5], Bas van der Woning[6], Savvas Savvides[2], Bart Lambrecht[1,7]

1. Immunoregulation Unit, VIB Center for Inflammation Research, Ghent, Belgium; Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium.
2. Unit for Structural Biology, VIB Center for Inflammation Research, Ghent, Belgium; Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium.
3. ImmunoX Initiative, University of California San Francisco, San Francisco, CA, USA.
4. Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of California San Francisco, San Francisco, CA, USA.
5. Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA, USA.
6. arGEN-X, Ghent, Belgium
7. Department of Pulmonary Medicine, ErasmusMC, Rotterdam, Netherlands.

Protein crystallization is an important feature of type 2 inflammation, as evidenced by the convergent evolution of Galectin-10 crystals in humans, and Ym1/2 crystals in mice. Despite the prevalence of crystals as a principal feature of pathogenic mucus in human diseases such as asthma, chronic rhinosinusitis and allergic bronchopulmonary aspergillosis, their biological importance has not been explored, due to a substantial lack of tools.
We have undertaken several novel approaches to investigate these crystals in an in vivo setting. We have developed several mouse lines to understand the role of Galectin-10 (normally absent in mice) in contributing to various features of allergic diseases and inflammation. Transgenic mice that endogenously express Galectin-10 demonstrate increased inflammation during house dust mite-induced asthma, as well as a striking phenotype of mucus production and increased bronchial hyperreactivity; phenotypes which resemble clinical symptoms of severe asthmatics. Furthermore, we have developed a novel and tune-able system by which we administer synthetic mucus containing crystals to the lungs of mice, creating plugs and airway obstruction. Here, we demonstrate extensive interactions of neutrophils with Galectin-10 crystals, which act to drive a hyper-activated type 2 niche around airways containing crystals, contributing to airway plugging and persistent inflammation. This new understanding, together with translational (proof-of concept) studies using human patient samples, provide a unique platform to investigate Galectin-10 crystals as a therapeutic target for an impenetrable aspect of airway disease.

Mucins in Infection and Inflammation

The *Salmonella* adhesin Rck mediates entry through the epidermal growth factor receptor in a MUC13-dependent manner

Shaofang Xu[1], Daphne A.C. Stapels[1], Karin Strijbis[1]

1. Utrecht University

In the intestine, epithelial cells are separated from commensal and pathogenic bacteria by a protective layer of secreted and transmembrane mucins. Traditionally, transmembrane mucins are thought as the protective role in the intestine. However, some transmembrane mucins are used by bacteria as receptors for invasion. For example, the enteropathogen Salmonella enterica requires MUC1 to trigger its invasion into enterocytes. Recently, Salmonella was reported to also invade cells via a zipper mechanism, in which the outer membrane protein Rck binds to the epidermal growth factor receptor (EGFR), and initiates receptor-mediated endocytosis. Multiple transmembrane mucins, including MUC1 and MUC13, have EGF-like domains and/or have been shown to interact with signaling receptors of the EGFR family. Therefore, we want to investigate the role of MUC1 and MUC13 in Rck/EGFR-mediated invasion.
To study Rck-mediated invasion, we expressed full-length Rck, or truncated Rck that cannot mediate invasion, in Escherichia coli and Salmonella enteritidis. Gentamicin protection assays were performed to quantify the number of intracellular bacteria in the human colon cell lines HT29-MTX and HRT18 and their MUC1 knockout (KO) and MUC13 KO derivates. These showed that Rck-mediated invasion was similar in MUC1 KO cells compared to WT cells. However, the invasion was dramatically decreased in MUC13 KO cells. In addition, we inhibited EGFR kinase activity with gefitinib before infection to confirm the role of EGFR in Rck-mediated invasion. Rck-mediated invasion was decreased dramatically in gefitinib-treated WT cells. Furthermore, the gentamicin protection assay on MUC13 KO HRT18 cells complemented with MUC13 showed that bacterial invasion levels were restored to the WT level. These data demonstrate that MUC13, and not MUC1, promotes Rck-mediated invasion of Salmonella.

Mucins in Infection and Inflammation

Ulcerative colitis patients have unique mucin signatures which correspond with inflammation

Alyssa Gutierrez[1], Anna Tingler[1], Amy Engevik[1], Melinda A. Engevik[1,2]

1Department of Regenerative Medicine & Cell Biology
2Department of Microbiology & Immunology

Background: In the human colon, goblet cells synthesize and secrete the mucin proteins MUC2 and MUC5B. In addition to goblet cells, all epithelial cells harbor mucins anchored to their apical membrane. These secreted and adherent mucins create a barrier to maintain gut homeostasis. Dysfunction of mucins can lead to increased susceptibility to infection, inflammation, and contribute to the pathology of inflammatory bowel disease (IBD). Previous studies have indicated that IBD patients, particularly those with ulcerative colitis, have reduced numbers of goblet cells, altered mucin glycosylation and more permeable mucus compared to healthy individuals. We set out to generate a comprehensive analysis of mucins and glycosyltransferases in IBD in the context of inflammation. Methods & Results: We analyzed bulk RNAseq data from rectal mucosal biopsies from 206 control non-IBD individuals and new-onset patients with ulcerative colitis (GSE109142). Ulcerative colitis patients were classified based on the level of inflammation as determined by calprotectin levels and histological severity score during diagnostic colonoscopy. RNAseq revealed that ulcerative colitis patients had increased levels of adherent mucins MUC1, MUC4, and MUC13 and these mucins were elevated with increasing histological severity. We also found that ulcerative colitis patients had decreasing levels of MUC3A and MUC20 with increasing levels of inflammation. No changes were observed in other adherent mucins such as MUC12, MUC16, MUC17 or MUC19. In terms of secreted mucins, we found that histological severity scores of 2 and 3 were associated with increased MUC2, MUC5B and MUC5AC. We confirmed the levels of MUC5B and MUC5AC protein by immunostaining colonic tissue from healthy individuals and ulcerative colitis patients with varying levels of inflammation. To determine if host-derived cytokines could shift the mucin profiles, we examined the mucin profiles of intestinal organoids derived from healthy individuals and ulcerative colitis patients after passaging. Interestingly, only MUC4 was significantly elevated in ulcerative colitis organoids; suggesting that other components absent in the organoid cultures were responsible for elevating mucins. To identify which cytokines could be involved, we examined mucin expression in organoids after incubation with INFy, IL-17A, IL-22 and TNF. We found that only IL-22 shifted the mucin profile and increased MUC1, MUC4, MUC13 and MUC5B in treated organoids. Finally, we found decreased levels of glycosyltransferases GALNT3, GALNT5, GALNT7, GALNT12, B4GALT4, B4GALT5, B3GNT2, B3GNT3, B3GALT5, B3GNT7, B3GNT8, and ST6GALNAC1, and increased levels of ST3GAL1 and ST3GAL2 in ulcerative colitis patients; suggesting that the mucins are not properly glycosylated. Conclusions: These data point to a causative link between inflammatory cytokines and mucin profiles in IBD patients.

Mucins in Infection and Inflammation

MUC1-mediated apical invasion of *Salmonella* into enterocytes is dependent on α2-3 linked sialic acids

Koen C.A.P. Giesbers[1], Weiwei Peng Joost Snijder, Joost Snijder[2] and Karin Strijbis[1]

1. Department of Biomolecular Health Sciences, Division of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
2. Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

The intestinal tract is covered by a mucosal surface that consists of highly glycosylated secreted and transmembrane (TM) mucins. TM mucins are a group of highly diverse large glycoproteins involved in cell signaling, inflammation and barrier functions. The enteric pathogen Salmonella Enteridites is able to circumvent and even utilize the mucosal barrier for invasion. Salmonella expresses a giant adhesin SiiE that can interact with the TM mucin MUC1,and mediates invasion at the apical surface of intestinal epithelial cells. The SiiE-MUC1 interaction is depended on the terminal sialic acids on the O-glycan structures on MUC1. In this study, we further investigated the expression of SiiE and the nature of the SiiE-MUC1 interaction using the HT29-MTX intestinal epithelial cell line. We found that expression and secretion of SiiE is highly regulated and reaches highest levels in late logarithmic growth phase and can be stimulated by oxygen shock. We demonstrated by confocal microscopy that HT29-MTX cells express both α2,3- and α2,6-linked sialic acids. Invasion studies with HT29-MTX WT and ΔMUC1 monolayers treated with specific sialidases suggested that α2,3-linked sialic acids on MUC1 are essential for SiiE-mediated invasion. Blocking of α2,3-linked sialic acids with MAL-II lectin but not α2,6-linked with SNA lectin also reduced invasion through the SiiE-MUC1-SiiE pathway. To be able to perform efficient MUC1 immunoprecipitation experiments, we reverse engineered the anti-MUC1 antibody 139H2 and produced it recombinantly. We are currently in the process of performing MUC1 purification to allow O-glycan analysis of MUC1 in HT29-MTX cultures by mass spectrometry. Our findings show that in a human intestinal cell model, the Salmonella SiiE adhesin preferentially binds to α2,3-linked sialic acids on MUC1. These results open up new research lines to investigate targeting of specific intestinal cell types and/or hosts by this zoonotic pathogen.

Mucins in Infection and Inflammation

Mucin-targeted gene therapy for asthma

Sahana Kumar[1], Maria Rife[1], Margaret A. Scull[3], Gregg A. Duncan[1,2]

1. Biological Sciences Program, , University of Maryland, College Park, MD 20740
2. Fischell Department of Bioengineering, , University of Maryland, College Park, MD 20740
3. Deparment of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20740

Asthma is a globally burdensome respiratory disease, affecting millions worldwide. It is characterized by chronic airway inflammation, airway remodeling, and impaired mucociliary clearance (MCC). Mucus comprises gel-forming mucin proteins mucin 5B (MUC5B) and mucin 5AC (MUC5AC). Healthy airway mucus is predominantly composed of MUC5B, with reduced MUC5AC. However, in individuals with asthma, biochemical analysis of their mucus revealed a shift from MUC5B to MUC5AC as the predominantly secreted mucin. These changes to mucus composition have been shown to impair MCC and obstruct airways due to increased mucus plugs in asthma patients. Given the overproduction of MUC5AC in the asthmatic airway, we hypothesized delivery of MUC5AC siRNA to the airway epithelium, could improve MCC, reduce airway hyperreactivity, and improve asthma symptoms in vitro and in vivo.
For in vitro studies to validate MUC5AC as a relevant therapeutic target in asthma, immortalized basal human airway epithelial (HAE) cells were differentiated into secretory and ciliated cells by culturing at air-liquid interface (ALI). To establish an asthma-like phenotype, ALI cultures were stimulated with cytokine interleukin-13 (IL-13). We utilized adeno-associated virus serotype 6 (AAV6), which shows tissue tropism for lungs, as the viral vector in these experiments. To determine if AA6 could penetrate the mucus barrier in asthmatic airways, multiple particle tracking (MPT) analysis was used to compare AAV6 diffusion in mucus from unstimulated and IL-13 stimulated ALI cultures. To characterize AAV6 transduction in HAE cells, AAV6 expressing enhanced green fluorescent protein was used to assess transduction efficacy. The efficacy of AAV6 carrying MUC5AC-siRNA (AAV6-MUC5AC-siRNA) to silence MUC5AC was evaluated using immunofluorescence and quantitative real-time polymerase chain reaction (qRT-PCR).
MPT analysis of AAV6 in HAE mucus with and without IL-13 stimulation revealed that the mean squared displacement of AAV6 was similar in unstimulated and IL-13 stimulated mucus, indicating AAV6 can diffuse through asthma–like mucus barrier enriched with MUC5AC. MPT analysis further revealed changes in mucus microstructure in IL-13 stimulated mucus, indicated by increased pore sizes. AAV6 transduced unstimulated and IL-13 stimulated HAE cells at multiplicities of infection 10^4 and 10^5. Initial studies of AAV6-MUC5AC-siRNA showed promise, with expected trends of elevated MUC5AC messenger RNA expression in IL-13-stimulated mucus and reduced expression in the AAV6-MUC5AC-siRNA treatment group. Future and ongoing work include evaluating MCC in MUC5AC-siRNA treated HAE cultures and proof-of-concept in vivo studies using a mouse model of allergic asthma. If successful, this gene delivery approach could open avenues for mucin-targeted therapies aimed at asthma and other related chronic lung diseases.

Mucins in Infection and Inflammation

Mucosal protection against *C. rodentium* infection is coordinated by regional goblet cell (GC) subpopulations and GC plasticity

Mahadevan Venkitasubramani[1], Lars Vereecke[2], and George Birchenough[1,3]

1. Mucin biology groups, Dept. Medical Biochemistry, Inst. Biomedicine, University of Gothenburg, Sweden
2. Host-Microbiota Interaction lab (HMI), VIB Center for Inflammation Research, Department of Rheumatology, University of Ghent, Belgium
3. Wallenberg Centre for Molecular and Translational Medicine (WCMTM), University of Gothenburg

A functional inner mucus layer (IML) physically isolates the intestinal microflora to maintain epithelial sterility (Fig. 1). The primary structural component of the IML - mucin2 (Muc2), is secreted by intestinal GCs; Upon secretion, monomers of Muc2 are crosslinked by transglutaminase 3 (Tgm3) that catalyze intermolecular protein cross linkages to enhance barrier protection. Moreover, intercrypt GCs (icGCs) generate a supportive substructure of the IML referred to as intercrypt mucus. Nonetheless, pathogens that breach the IML may threaten crypt residing stem cells. Sentinel GCs (senGCs) have been proposed to initiate compound Muc2 secretion through activation of Nlrp6 inflammasome complex, forming the secondary GC- intrinsic colonic defence. However, the relative contributions of heterogenous GCs against in vivo bacterial infections have not been characterized. To reveal the role of GC intrinsic primary and secondary protection, we infected mice lacking Tgm3, icGCs or functional senGCs with C. rodentium (a murine pathogen that circumvents intestinal protection) and evaluated their defensive function. While Tgm3 was dispensable, mice lacking icGCs displayed accelerated disease severity with delayed pathogen clearance; SenGCs contributed to increased protection during acute C. rodentium infection. Combining mRNA sequencing with in situ hybridization, we identified a novel deep crypt Nlrp6+ GC subpopulation during infection. Our state-of-the-art ex vivo mucus growth kinetics indicate a potential secretory function for these novel GCs. Together, our study recapitulates the defensive contributions of GC subtypes against bacterial infections and key gene expression changes that drive GC mediated protection.

Mucins in Infection and Inflammation

Mucus: The first line of defence against infection

Carmen Phoon[1], Grant Burgess[1]

1. Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom 1

As multidrug-resistant bacterial diseases are increasingly threatening the health of human globally, innovative approaches to combat bacterial infections are crucial. Secretion of a surface mucosal layer as a first line defence mechanism is a strategy widely adopted by many living organisms to prevent invasion of microbes. Cnidarians and ctenophores are the first living organisms found to be evolving, secreting and synthesising surface mucosal layer. One way in which corals resist infection is by secreting a surface mucus layer (SML). The SML is a multi-functional hydrogel interface between the underlying epithelium and the external environment. It acts as a chemical and physical barrier and is considered a first line defence mechanism against colonisation by potential pathogens. Mucus isolated from jellyfish such as Aurelia aurita, Chrysaora melanogaster and Rhopilema esculenta contain a qniumucin gene that has similar structure to mucin found in human lungs and stomach (MUC5AC). Furthermore, amino acid content in mucins isolated from blue blubber jellyfish, Catostylus mosaicus was found to have characteristic similar to bovine mucins. The similarities of the structures of mucus secreting cells and the composition of mucus in cnidarians and humans were also observed. Therefore, research in the secreted mucins evolved in metazoans is not just a valuable study for the degrading coral reefs population but also useful for higher mammals in tackling bacterial infections. However, the pathogenesis of infectious diseases affecting corals and human remains poorly understood despite its apparent importance, except for the gel-forming mucin MUC2 in human. In this study, the virulence mechanisms of coral pathogens including 7 Vibrio spp. and a single species of Photobacterium were evaluated, using a range of physicochemical methods. A novel in vitro mucus permeation assay was developed, using porcine small intestinal mucus (PSIM) as a model system. The assay was used to quantitatively assess the ability of the pathogens to penetrate through and degrade the mucus layer. Motility was also investigated to understand whether this was a factor influencing mucus penetration. Results obtained indicated that coral pathogens appeared to be able to penetrate the PSIM layer. Additionally, rheological and biochemical examination of mucus exposed to Vibrio coralliilyticus (CC007) has been demonstrated and showed that coral pathogen is able to considerably alter the rheology of mucus through production of potent mucolytic enzymes. These results provide important preliminary insights into the possible mechanism of disease pathogenesis of the species studied, which could be used to inform and develop innovative strategies to tackle infection in corals and potentially higher mammals.

Keywords: Coral, Mucus, Infectious Disease, Pathogens, Vibrio spp., Penetration, Motility

Mucins in Infection and Inflammation

Shared mucosal inflammatory signatures in pelvic radiation disease and inflammatory bowel disease

S. Devarakonda[1], K. Bergmark[1,2], H. Fagman[3], J. Fransson[4], P. Hedenström[5], Y. Li[6], A. Thorsel[7], A. Toft[1], E. Villablanca[4], C. Bull[1]

1. Division of Clinical Cancer Epidemiology, Department of Oncology, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
2. Pelvic Cancer Rehabilitation, Sahlgrenska University Hospital, Gothenburg, Sweden
3. Department of Laboratory Medicine, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg and Department of Clinical Patology, Sahlgrenska University Hospital, Gothenburg, Sweden
4. Immunology and Allergy Unit, Department of Medicine, Solna, Karolinska Institute and University Hospital, Stockholm, Sweden
5. Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, and Department of Gastroenterology and Hepatology, Sahlgrenska University Hospital
6.National Bioinformatics Infrastructure, Uppsala, Sweden
7. Proteomics Core Facility, The Sahlgrenska Academy, University of Gothenburg

Background
Radiation damage to the intestine following pelvic radiotherapy can result in severe and chronic symptoms that limit the ability to lead a fulfilling life. Moreover, the risk of permanent damage to the non-cancerous tissue prevents the use of radiotherapy to its full potential. The problem is significant; globally, approximately one million people undergo pelvic radiotherapy every year and the majority are believed to experience persisting changes in their bowel habits. Yet only a handful of studies exist on the long-lasting pathophysiological processes that occur in the intestinal mucosa of the irradiated pelvic cancer survivor. In the biopsy study BIOSURV (BIOpsies in cancer SURVivors), we are mapping these processes 2-20 years after pelvic radiotherapy. We recently demonstrated that pelvic radiotherapy causes a chronic low-grade inflammation in the colorectal mucosa, likely driven by the penetration of bacteria over a permanently damaged mucus barrier. Here, we compare changes in mRNA-and protein expression in high-dose exposed biopsies versus low-dose exposed biopsies, and compare these expression patterns with those of Crohn's disease and ulcerative colitis, two inflammatory bowel diseases with approved treatments and multiple new treatments in the pipeline. The purpose is to identify possible overlapping mechanisms that might give rise to novel treatment targets and opportunities.
Methods Biopsies of mucosa from 24 irradiated cancer survivors were analyzed using RNA sequencing and tandem mass tag (TMT) mass spectrometry. Mucosa exposed to high radiation doses was compared to mucosa exposed to low radiation doses in the same patient. The results from Gene Set Enrichment Analysis (GSEA) of altered mRNA and protein expression between low-dose and high-dose biopsies were compared to analyses of mRNA data from Crohn's disease and ulcerative colitis retrieved from public databases.
Results In contrast to inflammation in IBD, we found little to no support for activity through IFN-γ in the irradiated tissue, and only weak support for JAK-STAT activation. However, we found strong support for chronic TNF-α signaling in the irradiated tissues, and possibly also IL-17 (GSEA() and gseKEGG(), p.adjust <= 0.1 and 0.001, setSize >= 10).
Conclusion Our preliminary findings suggest that chronic inflammation in the mucosa after radiation therapy appears to have several similarities with inflammation in IBD but also clear differences. The disclosure of a chronic TNFalpha-signalling in the irradiated mucosa could ultimately have implications for the development of new strategies to preserve or restore intestinal health in those treated with pelvic radiotherapy.

Mucins in Infection and Inflammation

Investigating the goblet cell marker landscape during *Trichuris muris* infection

Erin-Claire Pallott[1], Catherine Sharpe[1], Matthew Hepworth[1], David Thornton[1], Richard Grencis[1]

1. Faculty of Biology, Medicine and Health, The University of Manchester

Intestinal goblet cells and the mucins they produce play critical roles in resolving gastrointestinal helminth infection. Previous work in our lab showed that immune driven goblet cell hyperplasia and changes in both mucus quantity and quality are indispensable for resistance against the mouse parasite Trichuris muris following acute infection in C57BL/6 mice. Recent work across goblet cell biology has suggested the existence of varied and distinct functional profiles of goblet cells. Together, these data demonstrate a need to re-evaluate goblet cell responses during infection, with more in depth analyses of their functional capacity during T. muris infection. Moreover, we have now characterised the goblet cell response during chronic T. muris infection in C57BL/6 mice, uncovering an unexpected phenotype. An increase in the number of goblet cells per crypt was observed, associated with crypt hyperplasia, despite chronic infection to T. muris being driven by Type 1 immunity. Immunohistochemistry and in situ hybridisation techniques were employed to investigate the spatial and temporal aspects of goblet cell functionality in the T. muris infection landscape. I will be presenting our current data of fluorescent in situ hybridisation investigations, which showed altered localisation and dynamic expression of goblet cell genes between acute and chronic infection in C57BL/6. Utilising knockout mouse strains, we have also begun to investigate the involvement of the cytokines IL-13 and IL-22 in controlling the goblet cell responses. In chronic infection, both cytokines contributed to the phenotype, demonstrating active type 2 and type 3 immune signatures despite the type 1-dominated immune environment. Our work begins to define the complexity of goblet cell functions during infection compared to previous immunological studies that often treat goblet cells as a homogenous cell type.

Mucins in Infection and Inflammation

Loss of colonic mucus barrier function during early life results in premature induction of regulatory T cells

Jonas Ambjörnsson[1], Molly Söderlund Garsveden[1], Sofia Jäverfelt[1], Jenny Gustafsson[1]

1. Department of Physiology, University of Gothenburg

During early life, goblet cells regulate colonic immunity by delivering luminal antigens to the immune system for induction of tolerance, and by secreting the protective mucus layer. Loss of mucus barrier integrity is known to trigger an inflammatory response directed towards the microbiota, but whether this process breaks tolerance is unknown. To address this, we used mice with a compromised mucus barrier (C1galt1-/-) and studied T cell differentiation, and regulatory T cell (Treg) induction in the distal colon during early life (2-, 3- and 4-week-old mice). C1galt1-/- mice lack the core1-synthase in intestinal epithelial cells resulting in incomplete O-glycosylation of the mucus, rendering it susceptible to bacterial degradation. In wild type colon, T effector (Teff) cells (CD62LlowCD44high) were rare in 2-week-old mice, but increased in numbers by week 3 and 4 of life as part of the normal development of the colonic immune system. In the C1galt1-/- colon, increased numbers of Teff cells were observed in 2-week-old mice, and the Teff cell population continued to expand until week 3 of life, after which it decreased in size but remained elevated in 4-week-old C1galt1-/- mice, as compared to controls. When analyzing the composition of the Teff cells, we observed that the majority of the cells were FoxP3+ Tregs at all three ages, and the percentage of Tregs were similar C1glat1-/- and controls. Combined these results suggest that the colonic mucus barrier regulates both the timing and magnitude of T cells responses during early life. However, loss of mucus barrier integrity does not inhibit Treg induction, which may help restore homeostasis following periods of colitis.

Mucins in Infection and Inflammation

Stimulating mucin production aids the removal of *H. pylori* from murine gastric niche

Licínia Santos[1], Sinan Sharba[1], Stefany Loibman[1], Macarena P. Quintana-Hayashi[1], and Sara K. Lindén[1]

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg

Helicobacter pylori is the most common gastric pathogen and infected individuals are prone to develop gastritis, peptic ulcers, and gastric adenocarcinoma. The increasing resistance of H. pylori to antibiotics is problematic, urging the need for alternative approaches.
In the stomach, Helicobacter species can be found attached to gastric epithelial cells and residing in the mucus layer. The infection impairs the mucus barrier by decreasing the binding ability of the mucins, decreasing the growth-limiting activity of mucins, and decreasing mucin production creating a more stable environment.
It has been described that mucins can regulate pathogens' virulence and growth. Additionally, mucins can also bind and remove them. Hence, we aim to displace H. pylori from the gastric niche by stimulating mucin production.
Experimentally infected mice were treated with mucus-inducing compounds and the effects of the compounds in restoring mucus production and its effect on H. pylori density were evaluated. We noted that the compounds stimulated mucin production and there was a reduction in H. pylori density in mice stomachs when compared to those infected non-treated mice. To confirm that the decrease of H. pylori density observed in mice treated with the mucus-inducing compounds was a result of the stimulation of mucus production we checked that the compounds did not have a cytotoxic effect or alter the expression of virulence factors of the bacteria. However, the treatment with the compounds did not affect the serum anti-H. pylori antibody levels neither at 10 nor 14 days post-infection (dpi).
Thus, we combined some of the compounds previously tested with an immunization. The immunization consisted of H. pylori lysate plus an adjuvant: cholera toxin (CT) or multiple mutant cholera toxin (mmCT). The mice immunized 4 to 5 days before infection, presented a higher anti-H. pylori antibody level at both 10 and 14 dpi then the infected non-immunized mice. When combining the immunization with the treatment with mucus-inducing compounds, there was an increase in mucin production and lower H. pylori density than in infected non-treated mice.
Our results suggest that modulating mucosal defenses by treating mice with compounds and/or combining those compounds with immunization strategies stimulates mucin production leading to the removal of H. pylori from the gastric niche. This approach could be considered as an antibiotic-free alternative; however, further investigation is needed to identify more potent treatments and regimes.

Mucins in Infection and Inflammation

BMP signaling impairment in telocytes-Foxl1+ cells induces alterations in colonic mucin glycosylation influencing susceptibility to infectious colitis in mice

Vilcy Reyes Nicolas[1], Dianne Pupo[1], Gisela Marrero[2], Elena Verdu[3], Marco Constante[3], Alfredo Menéndez[2], Nathalie Perreault[1]

1. Dépt d'Immunologie et de Biologie Cellulaire, Université de Sherbrooke.
2. Dépt de Microbiologie et Infectiologie, Université de Sherbrooke.
3. McMaster University

Telocytes-Foxl1+ (TCFoxl1+) are mesenchymal cells forming a 3D network directly underlying epithelial cells which serve as key players in the colonic stem cells niche. The disturbance of bone morphogenetic proteins (BMP) signaling, vital for epithelial-mesenchymal communication, is implicated in various gut disorders. Our previous findings demonstrated that targeted deletion of BMP-signaling in TCFoxl1+ leads to a dysfunctional colonic mucus layer with impaired goblet cell glycobiology and increased susceptibility to DSS-induced colitis. Yet, the impact of this mucus dysfunction on mucosal homeostasis during bacterial infection remains unexplored.

To Investigate the molecular mechanisms underlying mucus layer dysfunction in Bmpr1aΔFoxl1+ mice and assess its impact on susceptibility to infectious colitis.

To further characterize the impact of BMP signaling in TCFoxl1+ cells on intestinal homeostasis, we performed a qualitative study of mucin glycoconjugates was performed using a panel of lectins. We performed a compositional analysis of the cecal microbiota from P90 control and BmpR1aΔFoxL1+ mice using 16S rRNA gene sequencing. In addition, fecal content of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) was studied in these mice. P250-day-old BmpR1aΔFoxL1+ mice, exhibiting a stronger phenotype, were orally infected along with control littermates with C. rodentium. Stool bacterial burdens were monitored daily, and histopathological damage was evaluated at day 8 post-infection (dpi).

The mucin analysis of Bmpr1aΔFoxl1+ mice revealed alterations in fucose and sialic acid content, while galactose residues were only detectable post-desulfation and enzymatic digestion. Bmpr1aΔFoxl1+ mice exhibited a distinct reduction in T antigen, indicating a potential disruption in the early stages of the mucin glycosylation pathway. No significant differences in alpha or beta diversity in the cecal microbiota study were found. Interestingly, a significant increase of SCFAs and BCFAs concentrations was observed in BmpR1aΔFoxL1+ mice compared to controls. Deregulated expression of colonic butyryl CoA synthetase (Ascm3) was detected in BmpR1aΔFoxL1+ mice, as well as in several host defence factors such as Retlnb (Relmβ) and Reg3g (RegIIIγ). A significant increase in C. rodentium burden was observed in P250 BmpR1aΔFoxL1+ mice, accompanied by extended tissue damage, inflammation, and depletion of goblet cell mucin content.

These results indicate that BmpR1aΔFoxL1+ mice face functional challenges in dealing with enteric infections, emphasizing the critical role of BMP signaling in TCFoxl1+ for maintaining mucosal homeostasis.

Mucins in Infection and Inflammation

GlaPep8 is a safe and effective drug candidate for treatment of mucus accumulation in lung disease

Anna Ermund[1], Christian V. Recktenwald[1], Sergio Trillo-Muyo[1], Gunnar C. Hansson[1]

1. Department of Medical Biochemistry and Cell Biology, University of Gothenburg, SE-405 30, Gothenburg, Sweden

With the exception of CFTR modulators for cystic fibrosis treatment, no new pharmacological treatments for chronic lung disease have been developed since inhaled corticosteroids in the 1970s. Common for muco-obstructive diseases such as cystic fibrosis, chronic obstructive lung disease as well as acute bronchitis is accumulation of mucus, airway obstruction and cough. Because mucus is part of the innate immune system and as such needed to protect from inhaled particles and microbes, we targeted mucus attachment instead of production when outlining our drug candidate. Using our knowledge about the function of bicarbonate in normalizing mucus properties, we designed a calcium ion binding peptide consisting of eight gamma-carboxylglutamic acid residues. This modification of glutamic acid occurs naturally in several coagulation factors, allowing them to bind calcium. Systems for rapid elimination of the drug candidate are thus available, preventing prolonged and off-target effects. To test the hypothesis that the drug candidate, GlaPep8, effectively removes attached airway mucus, we induced mucus accumulation and plugging with the inflammatory mediator IL-13 in mice and administered GlaPep8 at different doses either as an aerosol or intranasally as a solution. The effect was evaluated with morphometry in Carnoy-fixed paraffin sections stained with Alcian blue and Periodic acid Schiff´s reagent (Ab-PAS). The aim was to evaluate potency and efficacy as well as potential adverse effects to determine the therapeutic window. GlaPep8 decreased mucus attachment and plugging to levels of naïve mice, without causing any damage to the epithelium or pathological changes to the lung parenchyma. Nebulization resulted in a more even distribution than intranasal administration. Mucus plugging was normalized at lower doses than mucus attachment. We conclude that GlaPep8 is a safe and effective mucus active drug candidate for treatment of mucus accumulation in lung disease.

Mucins in Infection and Inflammation

The glycocalyx-forming membrane mucin MUC17 prevents entry of cholera toxin in human enterocytes

Sofia Jäverfelt[1] , Andrew Boucher[2] , Ulf Yrlid[2] , Thaher Pelaseyed[1]*

1. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Box 440, 405 30 Gothenburg, Sweden.
2. Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, 405 30 Gothenburg, Sweden

The intestinal epithelium is protected against bacteria by the glycocalyx-forming membrane mucin MUC17 that coats the apical brush border of intestinal enterocytes. However, the protective role of membrane mucins against bacterial toxins during enteropathogenic infections is currently unknown. Here, we focused on cholera toxin (Ctx) from enteropathogenic Vibrio Cholerae and hypothesized that MUC17 in the apical glycocalyx prevents binding and entry of cholera toxin in enterocytes. Using differentiated enterocyte-like Caco-2 cell monolayers, we show that the O-glycosylated mucin domain of MUC17 blocks Ctx-induced surge in intracellular cAMP levels, a critical host cell response to cholera infection. Furthermore, we demonstrated that MUC17 prevents GM-1-dependent and -independent binding of the CTB subunit of Ctx to Caco-2 cells. Lastly, we translated our findings to human jejunal organotypic 2-dimentional enteroid cultures where induction of MUC17 expression prevents attachment of CTB to enterocytes. Together, we show that MUC17 acts as a line of defense against Ctx from V. Cholerae by preventing binding of the CTB subunit to the surface epithelium.

Mucins in Infection and Inflammation

Extracellular activity of the cysteine protease Cathepsin S drives altered mucus proteolysis in the onset of colitis

Moa Smith[1], Vahap Canbay[2], Kostas Kalogeropoulos[2], Elisabeth Nyström[1]

1. Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden
2. Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark.

Non-communicable mucosal diseases, such as inflammatory bowel disease and colorectal cancer are increasingly prevalent due to modern lifestyle changes. Common to these diseases are alterations in the mucus layer protecting the mucosal epithelium, demonstrating the critical importance of proper mucus function in the maintenance of health and prevention of disease.
Proteolysis is known to be critical for remodelling glycoprotein polymeric networks throughout the human body. As the colonic mucus is largely a polymeric network of the mucin-2 glycoprotein, we hypothesised that proteolysis might also shape the mucus layer, and we have previously shown that the metalloprotease Calcium-activated chloride channel regulator 1 (CLCA1) is important for regulating baseline mucus properties. However, the involvement of mucus proteolysis in inflammatory bowel disease remains unexplored.
By combining *ex vivo* mucus investigations of mouse colonic mucus upon dextran sodium sulphate-induced colitis, with *in vitro* proteolysis assays and mass-spectrometry based proteolysis profiling, we found proteolysis driven mucus alterations in the early phases of colitis development. These effects were largely driven by induced secretion of the cysteine protease Cathepsin-S into the mucus, which acted on the mucus both directly and *via* Clca1. Thus, the data indicate a potential role of Cathepsin-S as a key regulator of colonic mucus properties in the onset of inflammation, opening up for potential targeting of the mucus defects in inflammatory bowel disease.

Mucins in Infection and Inflammation

Understanding the contribution of the bladder mucus layer to protection against infection

Laura Ramirez Finn[1,2], Maryse Moya-Nilgues[3], Molly A. Ingersoll[1,2]

1. Université Paris Cité, Institut Cochin, INSERM U1016, Paris, France
2. Department of Immunology, Institut Pasteur, Paris, France
3. Institut Pasteur Ultrastructural Bio Imaging, UTechS, Université Paris Cité, Paris, France

Mucus layers are well-studied in mucosal organs in contact with a microbiome, pathogens, and the external environment, such as the gastrointestinal, respiratory, and reproductive tracts. The bladder, however, is a notable exception, with few studies of its mucus layer. This is remarkable given the prevalence of cystitis, or infection of the bladder. Approximately 50% of women will have a urinary tract infection in her lifetime, and many of these infections will recur in these individuals. The mucus layer of the bladder is the first passive host defense mechanism encountered by the bacteria. Supporting that this layer is protective against infection, early studies demonstrated that treating bladders with acid or pentachlorophenol to disrupt the mucus layer results in higher bacterial colonization in rat infection models. Human and rabbit bladders have a thin layer of hyaluronic acid identified as 'mucopolysaccharides', and overall, the bladder mucus layer is reported to be thinner than that of the small or large intestine.

Although it is frequently cited that a bladder mucus layer protects organisms against bacterial infection, understanding of the biology of this layer is lacking. Using scanning electron microscopy (SEM) with ruthenium red to stain sugars, we identified a mucus layer on the luminal-facing surface of naïve mouse bladders. This layer is disrupted following infection with uropathogenic Escherichia coli, the main causative agent of urinary tract infection. This is due, in part, to the exfoliation of the top layer of urothelial cells. Staining cross-sections of bladders with periodic acid Schiff and Alcian blue revealed a layer of variable thickness and with a fluffy appearance coating the uppermost urothelial cell layer, supporting our SEM findings. Using RNA sequencing, we identified numerous mucins expressed at varying levels in naïve mice and during the progression of infection. These mucins included both transmembrane and mucus-forming mucins, suggesting the bladder has a glycocalyx of transmembrane mucins and a gel-like mucus on top. Proteomic analysis are ongoing to better understand the composition of the mucus in homeostasis and infection, to identify components of the mucus layer involved in tissue protection and to assess how this layer is re-established after infection. Better understanding of the mucus layer in the bladder may identify targets of these pathways to better protect individuals at risk of recurrent urinary tract infection.

Mucins in Infection and Inflammation

Subversion of the mucus barrier by atypical enteropathogenic Escherichia coli (aEPEC) strains.

Juan Josue Puño Sarmiento[1], Pedro Henrique[1], Soares Nunes[1], Liana, De Oliveira Trovão[1], Cecilia Mari[1], Abe[2], Daniela, Santoro Rosa[1], Fernando, Navarro García[3], Mônica Aparecida[1], Midolli Vieira[1], Tânia Aparecida[1], Tardelli Gomes[1]

1. Federal University Of São Paulo (Unifesp), Departament Of Microbiology, Immunology And Parasitology, São Paulo, Brazil.
2. Butantan Institute (Ib), Laboratory Of Bacteriology, São Paulo, Brazil.
3. Center For Research And Advanced Studies Of The National Polithecnic Institute (Cinvestav), Departament Of Celular Biology, Mexico City, Mexico.

The mucus layer acts as a barrier to prevent harmful enteric pathogens from entering the intestinal epithelium. Mucins are the main component of this barrier and can provide a survival matrix for successful bacterial colonization. This study aimed to examine whether atypical enteropathogenic Escherichia coli (aEPEC) strains can induce excessive mucus production in rabbit ileum and goblet-like cell culture. The study aimed to evaluate the ability of bacterial cultures to interact with, degrade, and utilize mucin, as well as investigate the potential link between mucus hypersecretion and pro-inflammatory cytokines production. Thirteen bacterial cultures were incubated with LS174T cell monolayers for 5 hours. Commercial kits were used to quantify cytokine production from collected supernatants. Mucins were revealed by staining preparations with Alcian Blue and Periodic Acid-Schiff. Image J was used to analyze and identify differences in color intensity from the obtained images. MUC2 and MUC5AC mucin types were identified on non-permeabilized cells by immunofluorescence using monoclonal antibodies and confocal microscopy analysis. Actin staining and electron microscopy (EM) analyses were used to evaluate the bacterial ability to form Attaching and Effacing (A/E) lesions. Lastly, 9 aEPEC strains were tested in the in vivo rabbit ileal loop model. For the mucinolytic activity assessment, an adhesion test was performed in 1% agar plates, with and without 5% porcine stomach mucin. To assess their ability to utilize mucin as a carbon source, growth kinetics were measured in an M9 medium supplemented with 0.3% mucin at 2, 4, and 6 hours. Transwells (8 µm pores) coated with 10% mucin and DMEM were mounted in 24-well plates to determine their potential to penetrate after 3 h of incubation. Four of the 13 strains induced mucus hypersecretion in vitro and in the in vivo model, three of which belonged to a close phylogenetic cluster. There was no direct relationship between mucus hypersecretion and increased production of pro-inflammatory cytokines in aEPEC infection in vitro. The confocal images confirmed the hypersecretion of MUC2 and MUC5AC and revealed A/E lesion formation, also confirmed by the EM images. All strains, except MA3456, utilized mucin as the sole carbon source. Out of 10 strains that penetrated the mucus layer extensively, only 4 were highly adherent to mucin agar plates. The findings of this study suggest that mucus hypersecretion, along with binding, utilization, and degradation of mucin associated with A/E lesion formation on goblet cells, should improve aEPEC colonization in vivo. This approach brought new insights into the pathogenicity mechanisms of aEPEC.

Mucins in Infection and Inflammation

Investigations into the barrier and receptor functions of mucins during viral and bacterial respiratory infections

Dave Lifka[1], Maitrayee Chatterjee[1], Liane Z. X. Huang[1], Karin Strijbis[1]

1. Department of Biomolecular Health Sciences, Division Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

Respiratory infections are among the most common diseases in humans and animals and are associated with high mortality and morbidity rates. Mucins play an essential role in protecting the respiratory tract against microbial infections, but can also serve as receptors for some pathogens. The heavily O-glycosylated soluble mucins MUC5AC and MUC5B eliminate pathogens by mucociliary clearance. Transmembrane mucins MUC1, MUC4, and MUC16 can restrict microbial invasion at the apical surface of the epithelium. We previously demonstrated that enzymatic removal of extracellular mucin domains, but not individual sialic acid or fucose sugars, enhances SARS-CoV-2 infection. Removal of glycosylated mucin domains from the cellular surface increased binding of purified spike protein to the cellular surface. This might indicate that extracellular mucin domains shield viral receptors thereby preventing viral access to the receptor. There is also evidence that some viral and bacterial pathogens benefit from mucins and mucin glycans to infect the human lung. Pseudomonas aeruginosa is an important bacterial respiratory pathogen that is capable of forming biofilms on the respiratory surface. In this study, we aim to determine the role of MUC1 during Pseudomonas aeruginosa adhesion and invasion to the respiratory epithelium. Using CRISPR/Cas9, we generated ΔMUC1 cells in the respiratory cell line A549. WT and ΔMUC1 A549 cells were differentiated under air-liquid interface (ALI) conditions on Transwell inserts. Under ALI conditions, MUC1 expression was increased compared to liquid-liquid interface (LLI) conditions. The transepithelial electrical resistance (TEER) was increased in ALI cultures compared to LLI, but comparable between WT and ΔMUC1 cells. Also no differences in translocation of 40 and 70 kDa FITC-Dextran tracers across the WT and ΔMUC1 monolayers was observed. Immunofluorescence microscopy and adhesion/invasion experiments with Pseudomonas aeruginosa showed a trend of increased bacterial adhesion to MUC1 expressing A549 cells compared to ΔMUC1 cells. We want to further investigate the molecular interactions of Pseudomonas aeruginosa and other respiratory bacterial pathogens with specific mucins and mucin glycans to understand the protective and facilitating roles of mucins in respiratory infections.

Mucins in Infection and Inflammation

Antibiotics damage the colonic mucus barrier in a microbiota-independent manner

Jasmin Sawaed[1]*, Lilach Zelik[1]*, Yehonatan Levin[2], Rachel Feeney[3,4], Maria Naama[1], Ateret Gordon[1], Mor Zigdon[1], Elad Rubin[1], Shahar Telpaz[1], Sonia Modilevsky[1], Shira Ben-Simon[1], Aya Awad[1], Sarina Harshuk-Shabso[1], Meital Nuriel-Ohayon[1], Michal Werbner[1], Bjoern O Schroeder[3,4], Amir Erez[2] and Shai Bel[1]

1. Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel.
2. Racah Institute of Physics, The Hebrew University, Jerusalem, Israel.
3. Department of Molecular Biology, Umeå University, Umeå, Sweden.
4. Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå, Sweden

Antibiotic use is a risk factor for development of inflammatory bowel diseases (IBDs). IBDs are characterized by a damaged mucus layer, which does not properly separate the host intestinal epithelium from the microbiota. We hypothesized that antibiotics might affect the integrity of the mucus barrier. By systematically determining the effects of different antibiotics on mucus layer penetrability we found that oral antibiotic treatment led to breakdown of the mucus barrier and penetration of bacteria into the mucus layer. Using fecal microbiota transplant, RNA sequencing followed by machine learning and ex vivo mucus secretion measurements, we determined that antibiotic treatment induces ER stress and inhibits colonic mucus secretion in a microbiota-independent manner. This mucus secretion flaw led to penetration of bacteria into the colonic mucus layer, translocation of microbial antigens into circulation and exacerbation of ulcerations in a mouse model of IBD. Thus, antibiotic use might predispose to development of intestinal inflammation by impeding mucus production.

Mucins in Infection and Inflammation

The role of altered mucus glycosylation in the pathogenesis of colitis

Molly Söderlund Garsveden[1] and Jenny Gustafsson[1]

1. Department of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden.

The colonic mucus layer plays an important role in regulating the colonic barrier by separating the microbiota and the epithelium. Previous studies have shown that the colonic mucin 2 (muc2) is heavily glycosylated by core 1 and 3 O-glycans. Loss of core 1 O-glycosylation of muc2 makes it susceptible to bacterial degradation, resulting in development of spontaneous colitis in mice. In addition to secreting mucus, goblet cells form goblet cell-associated antigen passages (GAPs) that deliver luminal antigens to the immune system. The aim of this study was to characterize the inflammatory response in mice lacking a functional mucus barrier the C1galt1fl/flVillinCre/+ (C1galt1-/-) model of colitis and investigate whether the GAP function of goblet cells, contribute to disease onset. C1galt1-/- and littermate controls were injected with EdU to assess epithelial proliferation, while exposure to intraluminal dextran was used to quantify GAP formation. Changes in O-glycosylation was assessed by the lectins UEA1 that binds fucose and Jacalin that binds GalNAc. Mucus barrier integrity was evaluated by using a general bacteria FISH probe EUB338. The result showed that C1galt1-/- mice loose fucosylation in the distal half of the colon, resulting in increased proliferation and crypt length starting at 3-weeks of age. Analysis of mucus barrier integrity revealed an intact mucus barrier in 2-week-old C1galt1-/- mice, that was lost at 3 weeks and restored in 4-week-old C1galt1-/- mice. GAP formation was similar in C1glat1-/- mice and littermate controls at 3 and 4 weeks. In conclusion, our results show that disease onset coincides with loss of mucus barrier function. Furthermore, our results show that GAPs are present in C1galt1-/- at disease onset, supporting our hypothesis that GAPs can contribute to driving the disease.

Mucins in Infection and Inflammation

The presence of interactions between goblet cells and lamina propria antigen presenting cells in Ulcerative colitis patients

Penn Ljungholm[1], Jenny, Gustafsson[1]

1. Department of Physiology at the University of Gothenburg Sweden

It has previously been shown that in mice, mucus-producing goblet cells (GC) play an important role in regulating the colonic immune system by forming goblet cell-associated antigen passages (GAPs), which deliver substances from the lumen to lamina propria antigen presenting cells (LP-APCs) for induction of tolerance to the luminal content. This project has focused on the knowledge gap regarding whether or not human colonic goblet cells interact with LP-APCs and whether interactions between GCs and LP-APCs are altered in patients with Ulcerative colitis (UC) one of the inflammatory bowel diseases. The pathogenesis of UC is unknown, but it is known that the inflammation is directed towards the microbiota, and studies of how the epithelium interacts with the immune system can improve our understanding of the pathophysiology of the disease.

Colonic biopsies from the ascending and sigmoid colon of control patients and ulcerative colitis (UC) patients were evaluated for GC – LP-APC interactions using immuno-histochemistry and confocal microscopy. LP-APCs were identified by HLA-DR, while RNAse1 was used to identify GCs.

Our results demonstrate more frequent interactions between GCs and HLA-DR+ LP-APCs in the ascending colon as compared to sigmoid colon in both control and UC patients. In conclusion, our results confirm that human colonic GCs interact with lamina propria antigen presenting cells and we will now proceed to further characterize the subsets of antigen presenting cells that interact with GCs.

Appendix 4

First name	Last name	Email	Institution	Country
Maher	Abou Hachem	maha@bio.dtu.dk	Technical University of Denmark	Denmark
Helena	Aegerter	helenaa@irc.vib-ugent.be	VIB-Ghent University	Belgium
Carol	Aherne	carol.aherne@ucd.ie	University College Dublin	Ireland
Wout	Arras	wout.arras@uantwerpen.be	University of Antwerp	Belgium
Ori	Avinoam	ori.avinoam@weizmann.ac.il	Weizmann Institute of Science	Israel
Shai	Bel	shai.bel@biu.ac.il	Bar-Ilan University	Israel
John	Benktander	john.benktander@gu.se	University of Gothenburg	Sweden
Alex	Bennett	alex.bennett@gu.se	University of Gothenburg	Sweden
Serena	Berberolli	mmsbe@leeds.ac.uk	University of Leeds	UK
Maryse	Berkhout	maryse.berkhout@wur.nl	Wageningen University	Netherlands
Carolyn R.	Bertozzi	bertozzi@stanford.edu	Stanford University	USA
George	Birchenough	george.birchenough@gu.se	University of Gothenburg	Sweden
Marilena	Bohley	marilena.bohley@pharma.ethz.ch	ETH Zurich	Switzerland
Luke	Bonser	luke.bonser@astrazeneca.com	AstraZeneca	UK
Francois	Boudreau	francois.boudreau@usherbrooke.ca	Université de Sherbrooke	Canada
Emma	Bourdon	emma.bourdon.etu@univ-lille.fr	University of Lille	France
Cory	Brooks	cbrooks@csufresno.edu	California State University Fresno	USA
Teresa	Brooks	tebrooks@mail.fresnostate.edu	California State University Fresno	USA
Cecilia	Bull	cecilia.bull@gu.se	University of Gothenburg	Sweden
Mary	Burns	mary.burns@utsouthwestern.edu	UT Southwestern Medical Center	USA
Cosmin	Butnarasu	cosmin.butnarasu@fu-berlin.de	Freie Universität Berlin	Germany
Laura	Campbell	Laura.Campbell-2@manchester.ac.uk	University of Manchester	UK
Gerard	Cantero Recasens	gerard.cantero@vhir.org	Vall d'Hebron Research Institute	Spain
Kris	Chadee	kchadee@ucalgary.ca	Cumming School of Medicine	USA
Maitrayee	Chatterjee	maitrayee.chatterjee@thetimcompany.com	The TIM company	Netherlands
Erik	Chow	ec829@cornell.edu	Cornell University	USA
María del Mar	Collado González	mdmcg1@um.es	University of Murcia	Spain
Viviana	Correia	vivianacorreia@campus.fct.unl.pt	UCIBIO, FCT/NOVA	Portugal
Thomas	Crouzier	tc@cirqle.bio	DTU, HealthTech	Denmark
Scott	Danielsen	scott.danielsen@mse.gatech.edu	Georgia Institute of Technology	USA
Carol	de Ram	carol.deram@wur.nl	Wageningen University	Netherlands
Burton	Dickey	bdickey@mdanderson.org	MD Anderson Cancer Center	USA
John	Dickinson	jdickins@unmc.edu	University of Nebraska Medical Center	USA
Robyn	Diehn	r.diehn@fu-berlin.de	Freie Universität Berlin	Germany
Brendan	Dolan	brendan.dolan@medkem.gu.se	University of Gothenburg	Sweden
Gregg	Duncan	gaduncan@umd.edu	University of Maryland	USA
Melinda (Mindy)	Engevik	engevik@musc.edu	Medical University of South Carolina	USA
Elizabeth	Engle	eengle@umd.edu	University of Maryland - College Park	USA
Mattias	Erhardsson	mattias.erhardsson@gu.se	University of Gothenburg	Sweden
Anna	Ermund	anna.ermund@medkem.gu.se	University of Gothenburg	Sweden
Christopher	Evans	christopher.evans@cuanschutz.edu	University of Colorado School of Medicine	USA
Deborah	Fass	deborah.fass@weizmann.ac.il	Weizmann Institute of Science	USA
Rachel	Feeney	rachel.feeney@umu.se	Umeå University	Sweden
Elena	Fekete	elena.fekete@gu.se	University of Gothenburg	Sweden
Giorgio	Fois	giorgio.fois@uni-ulm.de	Universität Ulm	Germany
Manfred	Frick	manfred.frick@uni-ulm.de	Universität Ulm	Germany
Douglas	Fudge	fudge@chapman.edu	Chapman University	USA
Sandra	Gendler	gendler.sandra@mayo.edu	Mayo Clinic Medical and Graduate School	USA
Koen	Giesbers	c.a.p.giesbers@uu.nl	Utrecht University	Netherlands
Tristan	Girgin	tristan.girgin.etu@univ-lille.fr	University of Lille	France
Hayley	Gorman	hayley.gorman1@ucalgary.ca	University of Calgary	Canada
Marine	Goujon	marine.goujon.etu@univ-lille.fr	University of Lille	France
Francisco Martin	Goycoolea Valencia	fmartin.goycoolea@um.es	University of Murcia	Spain
Marcelo	Guerin	mrccri@ibmb.csic.es	Institute of Molecular Biology of Barcelona	Spain
Phaedra	Guilbert	Phaedra.Guilbert@irc.vib-ugent.be	VIB-Ghent University	Belgium
Jenny	Gustafsson	jenny.gustafsson@gu.se	University of Gothenburg	Sweden
Meital	Haberman	meital.haberman@weizmann.ac.il	Weizmann Institute of Science	USA
Gunnar C.	Hansson	gunnar.hansson@medkem.gu.se	University of Gothenburg	Sweden
Karl	Hansson	karl.hansson@medkem.gu.se	University of Gothenburg	Sweden
Bianca	Hazt	mmbh@leeds.ac.uk	University of Leeds	UK
Sandra	Holmberg	sandra.holmberg@umu.se	Umeå University	UK
Jessica	Holmén Larsson	jessica.holmen.larsson@gmail.com	AstraZeneca	Sweden
Hitomi	Hoshino	hhoshino@u-fukui.ac.jp	University of Fukui	Japan

Sophie	Janssens	sophie.janssens@irc.vib-ugent.be	VIB-Ghent University	Belgium
Ana Maria	Jaramillo	anamaria.jaramillohernandez@cuanschutz.e	University of Colorado School of Medicine	USA
Sofia	Jäverfelt	sofia.javerfelt@gu.se	University of Gothenburg	Sweden
Mathias	Jensen	matjen@dtu.dk	Technical University of Denmark	Denmark
Chunsheng	Jin	chunsheng.jin@gu.se	University of Gothenburg	Sweden
Malin	Johansson	malin.johansson@medkem.gu.se	University of Gothenburg	Sweden
Kristina	Johansson	kristina.bc.johansson@gu.se	University of Gothenburg	Sweden
Nicolas	Jonckheere	nicolas.jonckheere@inserm.fr	University of Lille	France
Nathalie	Juge	nathalie.juge@quadram.ac.uk	Quadram Institute Bioscience	UK
Sofia	Kallin	matjen@dtu.dk	Technical University of Denmark	Denmark
Niclas	Karlsson	niclaska@oslomet.no	Oslo Metropolitan University	Norway
Roxana	Khatib Shahidi	roxana.khatib.shahidi@gu.se	University of Gothenburg	Sweden
Hisanao	Kishimoto	kisimoto@toyaku.ac.jp	Tokyo University of Pharmacy and Life Sciences	Japan
Motohiro	Kobayashi	motokoba@u-fukui.ac.jp	University of Fukui	Japan
Melissa	Kordahi	melissa.kordahi@inserm.fr	Inserm U1016	France
Aicha	Kriaa	aicha.kriaa@umu.se	Umeå University	Sweden
Sahana	Kumar	skumar97@umd.edu	University of Maryland	USA
Bart	Lambrecht	Bart.Lambrecht@ugent.be	Ghent University	Belgium
Daniel	Lauster	daniel.lauster@fu-berlin.de	Freie Universität Berlin	Germany
Fredrik	Leo	fredrik.leo@mau.se	Malmö University	Sweden
Maude	Liegeois	Maude.liegeois@ucsf.edu	University of California, San Francisco	USA
Dave	Lifka	d.lifka@uu.nl	Utrecht University	Netherlands
Sara	Lindén	sara.linden@gu.se	University of Gothenburg	Sweden
Penny	Ljungholm	guspenlj@student.gu.se	University of Gothenburg	Sweden
Ruben	Lopez Sandoval	ruben.lopez_sandoval@roche.com	Institute of Human Biology	Switzerland
Ana	Luis	ana.luis@medkem.gu.se	University of Gothenburg	Sweden
Stacy	Malaker	stacymalaker@gmail.com	Yale University	USA
Filipa	Marcelo	filipa.marcelo@fct.unl.pt	NOVA School of Science and Technology	Portugal
Milly	McAllister	milly.mcallister@manchester.ac.uk	University of Manchester	UK
Benjamin	Medoff	bmedoff@mgh.harvard.edu	Massachusetts General Hospital, Harvard University	USA
France	Moreau	fmoreau@ucalgary.ca	Cumming School of Medicine	Canada
Iwona	Myszor	iwona.myszor@medkem.gu.se	University of Gothenburg	Sweden
Maria	Na'ama	nama.maria95@gmail.com	Bar-Ilan University	Israel
Yoshiki	Narimatsu	yoshiki@sund.ku.dk	Copenhagen University	Denmark
Jonas	Nilsson	jonas.gm.nilsson@gu.se	University of Gothenburg	Sweden
Rickard	Nordén	rickard.norden@microbio.gu.se	University of Gothenburg	Sweden
Geraldine	Nouailles	geraldine.nouailles@charite.de	Charité - Universitätsmedizin Berlin	Germany
Elisabeth	Nyström	elisabeth.nystrom@gu.se	University of Gothenburg	Sweden
Baptiste	Oosterlinck	baptiste.oosterlinck@uantwerpen.be	University of Antwerpen	Belgium
Justin	Paek	jhp265@cornell.edu	Cornell University	USA
Petar	Pajic	petarpaj@buffalo.edu	University at Buffalo	USA
Erin-Claire	Pallott	erin-claire.pallott@manchester.ac.uk	University of Manchester	UK
Eleonora	Pastremoli	eleonora.pastremoli@inrae.fr	INRAE MaIAGE	France
Matthew	Paszek	paszek@cornell.edu	Cornell University	USA
Daniel	Patricio	daniel.o.patricio@gmail.com	University of Jyväskylä	Finland
Thaher	Pelaseyed	thaher.pelaseyed@medkem.gu.se	University of Gothenburg	Sweden
Nathalie	Perreault	nathalie.perreault@usherbrooke.ca	Université de Sherbrooke	Canada
Andrea	Persson	andrea.persson@genovis.com	Genovis AB	Sweden
Victor	Pettersson	victor.pettersson@gu.se	University of Gothenburg	Sweden
Frederik	Petursson Madsen	fm@cirqle.bio	Cirqle Biomedical	Denmark
Carmen	Phoon	c.k.m.phoon@ncl.ac.uk	Newcastle University	UK
Michael	Pichler	mijap@dtu.dk	Technical University of Denmark	Denmark
Elizabeth	Plender	plendere@uw.edu	University of Washington	USA
Juan Josue	Puño Sarmiento	juan.josue@unifesp.br	Federal University of São Paulo (UNIFESP)	Brazl
Grete	Raba	grete.raba@gu.se	University of Gothenburg	Sweden
Inas	Rahou	inas.rahou@uantwerpen.be	University of Antwerp	Belgium
Abhiraj	Rajasekharan	abhirajr2019@nii.ac.in	National Institute of Immunology	India
Yesheswini	Rajendran	yesheswini.rajendran@rcb.res.in	Regional Centre for Biotechnology	India
Laura	Ramirez Finn	laura.ramirez-finn@pasteur.fr	Institut Pasteur	France
Christian	Recktenwald	christian.recktenwald@medkem.gu.se	University of Gothenburg	Sweden
Katharina	Ribbeck	ribbeck@mit.edu	Massachusetts Institute of Technology	USA
Courtney	Rivet-Noor	courtney.rivetnoor@duke.edu	Duke University	USA
Monet	Roberts	monetr@vt.edu	Virginia Tech	USA
Naba	Salman	nabadawoodsalman@gmail.com	University of Gothenburg	Sweden

Nadine	Samara	nadine.samara@nih.gov	National Institutes of Health	USA
Licínia	Santos	licinia.santos@gu.se	University of Gothenburg	Sweden
David	Schwartz	David.schwartz@cuanschutz.edu	University of Colorado School of Medicine	USA
Margaret	Scull	scull@umd.edu	University of Maryland	USA
Mikael	Sellin	mikael.sellin@imbim.uu.se	Uppsala University	Sweden
Rebecka	Simre	rebecka.simre00@gmail.com	University of Gothenburg	Sweden
Akshi	Singla	akshi.singla@gu.se	University of Gothenburg	Sweden
Annemieke	Smet	annemieke.smet@uantwerpen.be	University of Antwerp	Belgium
Molly	Söderlund Garsveden	molly@morrisarkiv.com	University of Gothenburg	Sweden
Alexandra	Steigmeyer	alexandra.steigmeyer@yale.edu	Yale University	USA
Corey	Stevens	coreyste@mit.edu	Massachusetts Institute of Technology	USA
Hannah	Straeussnigg	hannah.straeussnigg@gmail.com	University of Gothenburg	Sweden
Karin	Strijbis	K.Strijbis@uu.nl	Utrecht University	Netherlands
Jinyi	Su	j.su@uu.nl	Utrecht University	Netherlands
Lotta-Riina	Sundberg	lotta-riina.sundberg@jyu.fi	University of Jyväskylä	Finland
Kelly	Ten Hagen	Kelly.Tenhagen@nih.gov	NIDCR, National Institutes of Health	USA
Gabby Sara	Tender	gtender@stanford.edu	Stanford University	USA
Susann	Teneberg	Susann.Teneberg@medkem.gu.se	University of Gothenburg	Sweden
Alexandra	Thiran	alexandra.thiran@irc.vib-ugent.be	VIB-Ghent University	Belgium
Dave	Thornton	dave.thornton@manchester.ac.uk	University of Manchester	UK
Marthe	Tofthagen	matof0720@oslomet.no	Oslo Metropolitan University	Norway
Sergio	Trillo-Muyo	sergio.muyo@medkem.gu.se	University of Gothenburg	Sweden
Michael	Tuvim	mtuvim@mdanderson.org	UT MD Anderson Cancer Center	USA
Raphael	Valdivia	raphael.valdivia@duke.edu	Duke University	USA
Fleur	van de Koolwijk	fleurvandekoolwijk@hotmail.com	University of Gothenburg	Sweden
Carlos	Vazquez	cvazquez3@ucmerced.edu	University of California Merced	USA
Mahadevan	Venkita Subramani	mahadevan.venkita.subramani@medkem.gu	University of Gothenburg	Sweden
Lars	Vereecke	lars.vereecke@irc.vib-ugent.be	VIB-Ghent University	Belgium
Sonja	Visentin	sonja.visentin@unito.it	University of Turin	Italy
Joana	Volk	joana.volk@medkem.gu.se	University of Gothenburg	Sweden
Amanda	Wood	amanda.wood@utah.edu	University of Utah	USA
Lindsay	Wray	lwray@kaphabio.com	Kapha Bio	USA
Shiyi	Xi	xi.s@wehi.edu.au	The Walter and Eliza Hall Institute of Medical Biology	Australia
Shaofang	Xu	s.xu1@uu.nl	Utrecht University	Netherlands
Noa	Yeshaya	noa.yeshaya@weizmann.ac.il	Weizmann Institute of Science	Israel
Dani	Zalem	dani.zalem@gu.se	University of Gothenburg	Sweden
Birgitte	Zeuner	bzeu@dtu.dk	DTU Bioengineering, Technical University of Denmark	Denmark