Sialoglyco 2016
November 14 -17th
Santa Barbara, California (USA)

Organizers: Jamey Marth PhD & James C. Paulson PhD

Sialoglyco
We Thank the Following Sponsors for Their Generosity

[Logos of various sponsors]
Welcome to Sialoglyco 2016 at The Fess Parker Doubletree Resort in Santa Barbara, California.

The Sialoglyco symposium can be traced back to a meeting of the Japanese-German Sialic Acid Society in 1985, and is now held bi-annually to provide an international venue to explore the biology mediated by sialic acid containing glycans. Symposia to date have been held in Japan, Europe, and Australia. Despite a major presence of USA and Canadian scientists at these meetings from their inception, this is the first Sialoglyco symposium held in North America.

Sialoglyco 2016 is a three-day meeting of scientific sessions. The sessions will cover the biology and chemistry of sialic acids and their roles as ligands of glycan binding proteins that mediate host pathogen interactions, regulate of immune functions, and modulate disease processes. Among our goals in this meeting is to attract new investigators whose research paths have encountered sialic acids and who will both enrich the meeting through their presentations while meeting with members of the sialobiology research community.

It is with great pleasure that we host Sialoglyco 2016, and welcome nearly 150 attendees from around the world, including 38 speakers and 63 posters on display.

Sincerely,

Jamey Marth, Ph.D.  
James C. Paulson, Ph.D.

Jamey Marth, Ph. D., is the Director of the UCSB Center for Nanomedicine, Professor of MCD Biology and of Biomolecular Science and Engineering, and Professor of the SBP Medical Discovery Institute. He is the inaugural recipient of the John Carbon Endowed Chair of Biochemistry and Molecular Biology and recipient of the UCSB Duncan and Suzanne Mellichamp Endowed Chair of Systems Biology.

James C. Paulson, Ph. D., is a Professor in the departments of Cell and Molecular Biology, Chemical Physiology, and Immunology and Microbial Sciences at The Scripps Research Institute, in La Jolla, California. He is currently Cecil and Ida Green Professor and Chair of Cell and Molecular Biology.

Conference Coordinators

Anna Crie  
The Scripps Research Institute

Katelyn Jerlinga  
University of California—Santa Barbara
| Sialoglyco 2016 Scientific Advisory Board |
|-------------------------------|------------------|------------------|
| ▪ Andrew J Bennet             | ▪ Kay-Hooi Khoo  | ▪ James C Paulson |
|     Vancouver, Canada         |     Taipei, Taiwan |
| ▪ Nicolai Bovin              | ▪ Makoto Kiso    | ▪ Roland Schauer |
|     Moscow, Russia            |     Gifu, Japan  |     Kiel, Germany |
| ▪ Paul Crocker               | ▪ Ken Kitajima   | ▪ Sandro Sonnino |
|     Dundee, UK                |     Nagoya, Japan|     Milan, Italy |
| ▪ Philippe Delannoy          | ▪ Yuan C Lee     | ▪ Yasuo Suzuki   |
|     Lille, France             |     Baltimore, USA|     Kasugai City, Japan |
| ▪ Jukka Finne                | ▪ Yu-Teh Li      | ▪ Garry Taylor   |
|     Helsinki, Finland         |     New Orleans, USA|     St Andrews, UK |
| ▪ Rita Gerardy-Schahn        | ▪ Fu-Tong Liu    | ▪ Ajit Varki     |
|     Hannover, Germany         |     Taipei, Taiwan|     San Diego, USA |
| ▪ Mark von Itzstein          | ▪ Jamey Marth    | ▪ Shih-Hsiung Wu |
|     Gold Coast, Australia     |     Santa Barbara, USA |     Taipei, Taiwan |

![Sialoglyco 2016](image)
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monday, November 14, 2016</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 p.m. to 6:00</td>
<td>Registration</td>
</tr>
<tr>
<td>6:30 p.m. to 7:30</td>
<td>Welcome Reception</td>
</tr>
<tr>
<td><strong>Tuesday, November 15, 2016</strong></td>
<td></td>
</tr>
<tr>
<td>7:45 a.m. to 8:30</td>
<td>BREAKFAST</td>
</tr>
<tr>
<td>8:30 a.m. to 8:40</td>
<td>Opening Remarks</td>
</tr>
<tr>
<td><strong>8:40 a.m. to 12:00</strong></td>
<td>Session 1: Sialic Acids in Immunity</td>
</tr>
<tr>
<td>8:40 a.m. to 9:10</td>
<td>Sialic Acids, Siglecs and Molecular Mimicry at the Host-Pathogen Interface</td>
</tr>
<tr>
<td>9:15 a.m. to 9:35</td>
<td>The Mechanistic Role of Polysialylation in Autoimmunity</td>
</tr>
<tr>
<td>9:40 a.m. to 10:00</td>
<td>Expression of alpha 2,8-sialic Acid Glycoconjugates is Essential for Efficient CD4+ T Cell Activation</td>
</tr>
<tr>
<td>10:05 a.m. to 10:25</td>
<td>AM BREAK</td>
</tr>
<tr>
<td>10:25 a.m. to 10:45</td>
<td>Discovery of Novel Inflammation Amplification Loop by Ganglioside GM3 Molecular Species in Metabolic Syndrome</td>
</tr>
<tr>
<td>10:50 a.m. to 11:00</td>
<td>Targeting Polysialyltransferase as a Therapeutic Strategy for Cancer</td>
</tr>
<tr>
<td>11:15 a.m. to 11:35</td>
<td>Siglecs on B Cells and Dendritic Cells: Inhibiting Signalling and Preventing Autoimmunity</td>
</tr>
<tr>
<td><strong>11:40 a.m. to 12:00</strong></td>
<td>Poster 'Lightning Talks'</td>
</tr>
<tr>
<td>12:00 p.m. to 1:00</td>
<td>LUNCH</td>
</tr>
</tbody>
</table>
## Session 2: Sialic Acid Chemistry and Tools

Chair: Nicolai Bovin, Institute of Bioorganic Chemistry, Russian Academy of Science (Russia)

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Presenter, Institution (Country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:00 p.m. to 1:30</td>
<td><strong>Precision Glycocalyx Editing with Antibody-Enzyme Conjugates</strong></td>
<td>Plenary Lecture: Carolyn Bertozzi, HHMI, Stanford University (USA)</td>
</tr>
<tr>
<td>1:35 p.m. to 1:55</td>
<td><strong>A Liposome-assisted Strategy for Specific Labeling of Sialylation In Vivo</strong></td>
<td>Xing Chen, Peking University (China)</td>
</tr>
<tr>
<td>2:00 p.m. to 2:20</td>
<td><strong>Using Photocrosslinking Sugars to Discover Host Cell Receptors for Bacterial Toxins</strong></td>
<td>Jennifer Kohler, University of Texas Southwestern Medical Center (USA)</td>
</tr>
<tr>
<td>2:25 p.m. to 3:00</td>
<td><strong>PM BREAK</strong></td>
<td></td>
</tr>
<tr>
<td>3:00 p.m. to 3:20</td>
<td><strong>Glycoengineering - Towards Cell-based Assays for Dissection of Interactions with Glycans</strong></td>
<td>Henrik Clausen, University of Copenhagen (Denmark)</td>
</tr>
<tr>
<td>3:25 p.m. to 3:45</td>
<td><strong>Exploring the Sialic Acid/Siglec Axis for Immune Combination Therapy of Cancer</strong></td>
<td>Gosse Adema, Radboud University (the Netherlands)</td>
</tr>
<tr>
<td>3:50 p.m. to 4:10</td>
<td><strong>The Effect of Substrate Presentation and Activation on Neuraminidase NEU2 Specificity</strong></td>
<td>Robert J. Woods, University of Georgia (USA)</td>
</tr>
<tr>
<td>4:15 p.m. to 4:35</td>
<td><strong>Poster 'Lightning Talks'</strong></td>
<td></td>
</tr>
<tr>
<td>4:35 p.m. to 5:30</td>
<td><strong>Poster Session</strong></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>7:45 a.m.</td>
<td>BREAKFAST</td>
<td></td>
</tr>
<tr>
<td>8:30 a.m.</td>
<td><strong>Session 3: Sialic Acid Catabolism and Disease</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair: Taeko Miyagi, Miyagi Cancer Center Research Institute (Japan)</td>
<td></td>
</tr>
<tr>
<td>8:30 a.m.</td>
<td><strong>Challenges in Inhibitor Design: The 'Butterfly Effect'</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plenary Lecture: Mark von Itzstein, Griffith University (Australia)</td>
<td></td>
</tr>
<tr>
<td>9:05 a.m.</td>
<td><strong>The Multi-facets of the Lysosomal Sialidase NEU1 and Its Mode of Regulation in Human Diseases</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alessandra d’Azzo, St. Jude Children's Research Hospital (USA)</td>
<td></td>
</tr>
<tr>
<td>9:30 a.m.</td>
<td><strong>Neuraminidases 3 and 4 Direct Neuronal Development and Function by Reshaping the Composition of Brain Gangliosides</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alexey V. Pshezhetsky, University of Montreal (Canada)</td>
<td></td>
</tr>
<tr>
<td>9:55 a.m.</td>
<td>AM BREAK</td>
<td></td>
</tr>
<tr>
<td>10:25 a.m.</td>
<td><strong>Diversity in Sialoside Receptor Recognition by Influenza Hemagglutinins</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ian Wilson, The Scripps Research Institute (USA)</td>
<td></td>
</tr>
<tr>
<td>10:50 a.m.</td>
<td><strong>An Engineered Multivalent Sialic Acid Binding Biologic as a Preventative of Influenza</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Garry Taylor, University of St Andrews (United Kingdom)</td>
<td></td>
</tr>
<tr>
<td>11:15 a.m.</td>
<td><strong>Elusive Localization of the Insect CMP-sialic Acid Synthetases</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ken Kitajima, Nagoya University (Japan)</td>
<td></td>
</tr>
<tr>
<td>11:35 a.m.</td>
<td><strong>ST8Sia4-dependent Polysialylation is Part of the Developmental Program Required for Germ Layer Formation from Human Pluripotent Stem Cells</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Michael Pierce, University of Georgia (USA)</td>
<td></td>
</tr>
<tr>
<td>11:45 a.m.</td>
<td><strong>The Tumor-associated Sialyltransferase ST6Gal-I Confers a Cancer Stem Cell Phenotype and Promotes Resistance to Multiple Death-inducing Stimuli</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Susan L. Bellis, University of Alabama at Birmingham (USA)</td>
<td></td>
</tr>
<tr>
<td>11:55 a.m.</td>
<td><strong>Occurrence of Free Sialyl Oligosaccharides Related to N-glycans (sialyl FNGs) in Animal Sera</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tadashi Suzuki, RIKEN Global Research Cluster (Japan)</td>
<td></td>
</tr>
<tr>
<td>12:05 p.m.</td>
<td>LUNCH</td>
<td></td>
</tr>
<tr>
<td>1:00 p.m.</td>
<td><strong>Free Time / Winery Tour</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Advance registration)</td>
<td></td>
</tr>
</tbody>
</table>
Thursday, November 17, 2016

7:45 a.m. to 8:30 BREAKFAST

8:30 a.m. to 12:00 Session 4: Sialic Acid Receptors
Chair: Paul Crocker, University of Dundee (United Kingdom)

8:30 a.m. to 9:00 Sialyl Lewis X and E-selectin: The Drivers of Cell Therapeutics
Plenary Lecture: Robert Sackstein, Harvard Medical School (USA)

9:05 a.m. to 9:25 Soluble Siglec-14: a New Regulator of Pro-inflammatory Response
Takashi Angata, Academia Sinica Institute of Biological Chemistry (Taiwan)

9:30 a.m. to 9:50 Bacterial Sialidases & Vaginal Dysbiosis: Cellular and Secreted Sialoglycans Act as Room and Board for Unwelcome Guests
Amanda Lewis, Washington University - St. Louis (USA)

9:55 a.m. to 10:30 AM BREAK

10:30 a.m. to 10:50 Endogenous Siglec-8 Ligands in Human Airways
Ron Schnaar, Johns Hopkins School of Medicine (USA)

10:55 a.m. to 11:15 Sialylation of N-glycans on Autoantibodies Determines Pathogenicity in Rheumatoid Arthritis
Koichi Furukawa, Chubu University (Japan)

11:20 a.m. to 11:40 Haemophilus Influenzae has Evolved Preferential Use of the Human Specific Form of Sialic Acid as a Host Adaptation
Michael P. Jennings, Griffith University (Australia)

11:45 a.m. to 12:00 Poster 'Lightning Talks'

12:00 p.m. to 1:00 LUNCH

1:00 p.m. to 4:00 Session 5: Sialic Acids in Diseases and Therapies
Chair: Rita Gerardy-Schahn, Hannover Medical School (Germany)

1:00 p.m. to 1:30 Sialoptosis: Cell Death Induced by Aberrant Sialylation
Plenary Lecture: Thierry Hennet, University of Zurich (Switzerland)

1:35 p.m. to 1:55 Regulation of Quality and Quantity of Polysialic Acid by ST8SIA2/STX: Lessons from its SNPs Reported in Psychiatric Diseases
Chihiro Sato, Nagoya University (Japan)
2:00 p.m. to 2:20  Development of Siglec Targeting Nanoparticles for Abrogating Inflammation  
Christopher Scott, Queen's University Belfast (United Kingdom)

2:25 p.m. to 3:00  PM BREAK

3:00 p.m. to 3:20  Comparison of Eosinophil Siglec-F and Siglec-8 Biology, Signaling, Ligands and Therapeutic Targeting  
Bruce S. Bochner, Northwestern University Feinberg School of Medicine (USA)

3:25 p.m. to 3:45  Sialylation of GFRα1 (GDNF family receptor alpha 1) and Vasoerin by ST3Gal1 Promotes Tumor Growth and Angiogenesis  
Alice Yu, Chang Gung Memorial Hospital / Chang Gung University (Taiwan)

3:50 p.m. to 4:10  Three Different Glycomimetic Drug Candidates of Functional Sialylated Oligosaccharides in Clinical Trials for Inflammatory Disorders and Cancer  
John L. Magnani, GlycoMimetics (USA)

4:15 p.m. to 4:35  Poster 'Lightning Talks'

4:35 p.m. to 5:30  Poster Session

6:00 p.m. to 10:00  Banquet and Entertainment

---

Sialoglyco 2016
Session 1: Sialic Acids in Immunity

Title: Sialic Acids, Siglecs and Molecular Mimicry at the Host-Pathogen Interface
Authors: Victor Nizet
Institution(s): University of California – San Diego, CA (USA)

Sialic acid glycans (Sias) commonly decorate the termini of glycoproteins and glycolipids on mammalian cell surfaces. Sia-recognizing immunoglobulin superfamily lectins (Siglecs) are type I transmembrane proteins expressed on immune cells that recognize Sia motifs. Of note, many members of the rapidly evolving subset of CD33-related Siglecs have conserved cytoplasmic tyrosine-based inhibitory motifs (ITAMs) that recognize Sia “self-associated molecular patterns” (SAMPs) to dampen innate immune responses and prevent auto-reactivity. Conversely, the leading human neonatal bacterial pathogen group B Streptococcus (GBS) expresses a polysaccharide capsule virulence factor that contains terminally-linked Sia in molecular mimicry of the common host epitope. In this fashion, GBS can bind Siglecs and down-regulate leukocyte bactericidal capacity. Conversely, the macrophage receptor sialoadhesin binds the GBS capsule to promote effective phagocytosis and antigen presentation for the adaptive immune response. Interestingly, certain Siglecs can recognize non-Sia host proteins (e.g. HSP70) or glycosaminoglycan (e.g. hyaluronic acid) motifs as SAMPs, and different bacterial pathogens can evolve molecular mimicry of these non-canonical binding phenomena for Siglec-mediated immune evasion. More intrigue at this host-pathogen interface is provided by paired inhibitory/activating Siglec receptors (Siglecs 5/14, 11/16) that counterbalance one another to modulate leukocyte innate immune responses to pathogens, human polymorphisms that influence Siglec expression and infectious disease susceptibility, and the unexpected functional role of Siglecs on amniotic epithelium and platelets in immune regulation, and action of microbial glycosidases to perturb the tonic immune regulatory activity of Siglecs and the microbes capacity for molecular mimicry. As pathogen and host are both decorated with numerous sugar molecules that provide first points of contact while cloaking underlying structures, glycan-lectin interactions are key determinants of infectious disease pathogenesis.

Title: Roles of glycosylation during interstitial leukocyte trafficking
Authors: Michael Sixt
Institution(s): Institute of Science and Technology Austria

The G-protein coupled receptor (GPCR) CCR7 and its two chemokine ligands CCL19 and CCL21 essentially contribute to the homing of immune cells to secondary lymphoid organs. Although many GPCRs are glycosylated at their extracellular domain and most chemokines bind to sugar residues, the functional glycobiology of interstitial leukocyte trafficking is poorly understood. We show that CCR7 on dendritic cells (DCs) is posttranslationally polysialylated and that this modification is essential for recognition of CCL21 but dispensable for sensing CCL19. Structure-function analysis of chemokine-receptor interactions revealed an autoinhibitory site within CCL21, which, in the absence of polysialic acid, is blocked by the C-terminal extension of CCL21. Only polysialylated but not unmodified CCR7 releases this autoinhibition. Consequently, DC trafficking is abrogated in polysialyltransferase deficient mice, manifesting in disturbed lymph node homeostasis and severe autoimmunity. On the chemokine-side we demonstrate that immobilization of CCL21 to interstitial heparan sulfate residues is essential to shape gradients, which are optimally suited to guide the migrating cells towards their destination.
Title: Expression of $\alpha$2,8-sialic acid glycoconjugates is essential for efficient CD4+ T cell activation.

Authors: Villanueva T$^{1,2}$, Martínez-Duncker I$^1$

Institution(s): $^1$Laboratorio de Glicobiología Humana, Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa 62209, Cuernavaca, Morelos, México. $^2$Instituto de Biotecnología-Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa 62130, Cuernavaca, Morelos, México.

The CD4+ T helper lymphocytes (Th) orchestrate the immune response after their activation by antigen presenting cells. Previously, we have characterized through metabolic analogues and lectin binding the sialic acid dynamics in the cell surface of human naïve Th cells activated with anti-CD3/CD28 monoclonal antibodies (mAbs). The results of this characterization suggest that after activation there is an overall surface increase in $\alpha$2,8-sialylglycoconjugates, including gangliosides and glycoproteins. Further studies led us to identify that activation of human naïve Th cells causes an upregulation of $ST8SIA1$ (GD3 synthase) and $\beta4GALNT1$ (GM2/GD2 synthase). It was found that activation increases GD3 ganglioside expression and induces GD2 ganglioside neoexpression, the latter being clearly associated to TCR clustering. Additionally, it was found that shRNA silencing of the GM2/GD2 synthase reduces proliferation of human activated Th cells in contrast to reports in murine models.

The upregulation of $ST8SIA2$ (STX) and $ST8SIA4$ (PST) polysialytransferases during Th cell CD3/CD28 activation led us to search for proteins bearing polysialic acid (PSA) using anti-PSA mAb. The expression of surface PSA was identified by flow cytometry in resting and activated Th cells and several polysialylated glycoproteins were identified by western blotting and MS. The shRNA silencing of STX and PST in activated Th cells revealed a functional role of PSA-glycoproteins during activation of human naïve Th cells.

Title: Discovery of Novel Inflammation Amplification Loop by Ganglioside GM3 Molecular Species in Metabolic Syndrome

Authors: Jin-Ichi Inokuchi, Hirotaka Kanoh, Lucas Veillon, Shinji Go, and Masakazu Nagafuku

Institution(s): Institute of Molecular Biomembrane and Glycobiology, Tohoku Medical and Pharmaceutical University, Sendai, Miyagi 981-8558, Japan

Ganglioside GM3 has been known to participate in insulin signaling by regulating the association of the insulin receptor in caveolae microdomains (lipid rafts), which is essential for the execution of the complete insulin metabolic signaling in adipocytes. Macrophage-secreted factors including proinflammatory cytokines, TNF-$\alpha$ and IL1-$\beta$, in adipose tissues have been known to limit the local adipogenesis and inhibit insulin resistance, however, the interplay between adipocytes and macrophages upon regulation of GM3 expression is not clear. GM3 was virtually absent in primary adipocytes differentiated from macrophage-depleted mesenteric stromal vascular cells, which accompanies enhancement of insulin signaling and adipogenesis. We found that the expression of GM3 is governed by soluble factors including steady-state levels of proinflammatory cytokines secreted from resident macrophages. The direct involvement of GM3 in insulin signaling is demonstrated by the fact that embryonic fibroblasts obtained from GM3 synthase (GM3S) deficient mice have increased insulin signaling, when compared to wild type embryonic fibroblasts, which in turn leads to enhanced adipogenesis. In addition, GM3 expression in primary adipocytes is increased under proinflammatory conditions as well as in adipose tissue of diet-induced obese mice. Moreover, GM3S deficient mice fed high fat diets become obese but are resistant to the development of insulin resistance and chronic low-grade inflammatory states. Thus, GM3 functions as a physiological regulatory factor of the balance between homeostatic and pathological states in adipocytes by modulating insulin signaling in lipid rafts. Furthermore, we have identified the significant increases of GM3 molecular species possessing pro-inflammatory actions in human serum with metabolic syndrome. Collectively, we propose a novel inflammation amplification loop triggered by GM3 molecular species.
Title: Targeting polysialyltransferase as a therapeutic strategy for cancer
Authors: Robert A. Falconer, Goreti Ribeiro Morais, Xiaoxiao Guo, Anjana Patel, Mark Sutherland, Paul M. Loadman, Laurence H. Patterson, Steven D. Shnyder
Institution(s): Institute of Cancer Therapeutics, University of Bradford, U.K.

Polysialic acid (polySia) expressed on the surface of NCAM (neuronal cell adhesion molecule) of neuroendocrine tumors, notably neuroblastoma and small cell lung cancer, is strongly associated with poor prognosis and aggressive disease in patients in the clinic. PolySia modulates cell-cell and cell-matrix adhesion, migration, invasion and metastasis. For these reasons, and a growing body of evidence both in vitro and in vivo, interest in the polysialyltransferases (ST8SiaII and ST8SialV) responsible for polySia biosynthesis as potential therapeutic targets continues to gather pace.

Our efforts are focused on the development of novel human polysialyltransferase (polyST) inhibitors. We have established highly-sensitive HPLC-based assays (cell-free and cell-based) to assess polyST inhibition. Using isogenic cell lines (C6-ST8SiaII/C6-WT) and naturally polySia-expressing human neuroblastoma cells (SH-SY5Y/IMR-32), compounds were evaluated for their ability to modulate polySia expression, cell adhesion, migration and invasion in vitro. We have identified CMP-sialic acid precursors that reduce polySia expression and tumor cell migration by up to 70%. Specificity of agents for polySTs over other sialyltransferases was established via differential lectin probes. Agents have been shown to disturb the dynamics of focal adhesion kinase and to modulate ERK1/2, AKT and VEGFR3 signaling. Furthermore, we are utilizing computational chemistry on newly developed ST8Sia homology models, to identify compounds with more drug-like properties with the aim of identifying an agent for in vivo studies. To-date, we have synthesized >100 compounds from which we have identified agents with increased potency. We are currently utilizing a range of biophysical techniques to assess compound-target engagement.

We also report the first evidence that polySia expression is associated with tumor cell survival and migratory capacity under hypoxia, a condition of low oxygen tension found in poorly-vascularized tumour areas and a key source of chemoresistance. We have determined a potential role for HIF-1 and LDH-A in maintaining migratory capacity.

Title: Siglecs on B Cells and Dendritic Cells: Inhibiting Signalling and Preventing Autoimmunity
Authors: Lars Nitschke
Institution(s): Division of Genetics, University of Erlangen, Erlangen, Germany

CD22 (Siglec-2) and Siglec-G are two inhibitory receptors, which negatively regulate B-cell antigen receptor (BCR) signalling. Mice with mutated Siglec ligand binding domains showed that the association of the Siglec to the BCR regulates its inhibitory signalling function. Interestingly, loss of sialic acid binding in the CD22 protein led to stronger BCR signal inhibition, while the same mutation in Siglec-G led to a loss of the inhibitory function and stronger BCR signalling. We are addressing the regulation of the association of the Siglecs to the BCR by ligand-binding in more detail. Loss of the inhibitory receptors on B cells can lead to a higher susceptibility to a lupus-like autoimmune disease, as we could show in mouse models. Siglec-H is a Siglec with quite restricted expression on plasmacytoid dendritic cells (pDCs), a subpopulation of antigen-presenting dendritic cells. pDCs are known to produce most of the type1 interferon which is necessary for anti-viral responses. pDCs of Siglec-H/- mice produced higher levels of type1 interferon in vitro or after cytomegalovirus (CMV) infection in vivo. Several weeks after a CMV infection Siglec-H/- mice developed a strong lupus-like autoimmune disease, which was type1 interferon-dependent. These results show that Siglec-H is a receptor downregulating type1 interferon responses after virus infection. A defect in this pathway can cause the development of autoimmune diseases.
Successful tumors are able to evade the immune system, which is otherwise capable of killing transformed cells. Therapies that prevent this evasion have become revolutionary treatments for incurable cancers. This presentation will focus on our recent work targeting immune suppressive Siglec receptors and their sialylated glycan ligands, which are abundant within the cancer glycocalyx. We found that Siglec-ligand interactions can confer resistance to antibody-dependent cell cytotoxicity mediated by monoclonal antibody cancer drugs such as Herceptin. Based on this, we designed biotherapeutic molecules termed antibody-enzyme conjugates that selectively remove sialic acids from tumor cells and render them susceptible to immune cell killing. Editing the cancer cell glycocalyx with antibody-enzyme conjugates represents a new approach to cancer immune therapy.

**A Liposome-Assisted Strategy for Specific Labeling of Sialylation In Vivo**

**Authors:** Xing Chen

**Institution(s):** College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China

Specific labeling and visualization of sialoglycans, which is crucial for elucidating the biological functions of sialylation, represents a significant challenge. Herein, we present a liposome-assisted chemical reporter (LABOR) strategy for cell-selective and tissue-specific imaging of sialylation in vivo. The LABOR strategy exploits ligand-targeted liposomes to deliver sialic acid analogs bearing a bioorthogonal chemical reporter (e.g., the azide) to specific cell types or specific tissues. The azido sialic acids are metabolically incorporated into cellular sialoglycans and subsequently conjugated with imaging probes or affinity tags via click chemistry. This approach has enabled fluorescent imaging and glycoproteomic analysis of tumor-associated sialoglycans and brain sialylation in mice. The LABOR strategy has facilitated our studies on the dynamic changes of glycosylation in important physiological and pathological processes.

**Using Photocrosslinking Sugars to Discover Host Cell Receptors for Bacterial Toxins**

**Authors:** Amberlyn Wands, Akiko Fujita, Anirudh Sethi, Nicole Nischan, & Jennifer J. Kohler

**Institution(s):** Department of Biochemistry, UT Southwestern Medical Center

Many pathogenic bacteria secrete toxins that recognize glycosylated molecules on the surface of host cells. Evaluating the glycan-binding properties of these toxins can provide some insight into their host cell receptors, but cell-based experiments are required to identify which glycoconjugates are used as toxin receptors in a particular cell type. We reported photocrosslinking sugar analogs of sialic acid and N-acetylglucosamine (GlcNAc) that can be incorporated into mammalian glycoconjugates in place of the natural sugars. Cells metabolically labeled with photocrosslinking sugars are then incubated with bacterial toxins. Subsequent UV irradiation results in covalent crosslinking between the bacterial toxin and its glycoconjugate receptor(s). This strategy enables several lines of experimental inquiry including delineation of the class of glycoconjugates that are recognized by the toxin and identification of the protein portion of glycoprotein receptors. In this presentation, I will discuss how we used photocrosslinking sialic acid to show that cholera toxin subunit B (CTB) recognizes fucosylated glycoproteins on the surface of human colonic epithelial cells, and that these fucosylated molecules function in host cell intoxication. The results of these experiments have motivated new fucose-based strategies to competitively inhibit CTB binding to host cells and thereby block host cell intoxication. Motivated by our success in using photocrosslinking sialic acid to identify CTB receptors, we are now using similar approaches to define receptors for additional bacterial toxins. Preliminary data from these projects will be presented as well.
Glycoengineering - Towards Cell-based Assays for Dissection of Interactions with Glycans

Henrik Clausen

Center for Glycomics, Depart. of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Glycosylation is one of the most abundant and diverse posttranslational modifications of proteins, but arguably also one of the most difficult to study with respect to occurrence, structure, and biological functions in particular deciphering specific structure-function relationships. Heterogeneity in glycans on proteins and lipids is one of the major obstacles for analytic strategies, and this is also the major obstacle for exploiting specific biological functions of glycans in biotechnology. Protein glycosylation is a complex process involving many glycosyltransferases and even more accessory enzymes and transporters, and the non-template driven process is directed by multiple factors such as substrate specificities, kinetic properties, and topology. However, the general biosynthetic pathways and roles of most of these 500+ genes are fairly well outlined. We have therefore taken a genetic deconstruction & reconstruction approach to analysis and design of cellular glycosylation. We are using the gene editing tools (DNA nuclease “scissors” ZFNs, TALENs, CRISPR/Cas9) to simplify, dissect, and design glycosylation more or less at will. The strategy has enabled detailed mapping of different glycoproteomes and genetic dissection of glycosylation in cells enables us to explore molecular mechanisms of biological functions and diseases caused by specific glycans at specific sites in proteins. The resulting libraries of engineered isogenic cells with loss of distinct glycosylation features may also be used as a cell-based display platform for interrogation of interaction with glycans, and we are currently exploring the performance of such libraries in different assay formats.

References:

Exploring the Sialic Acid/Siglec Axis for Immune Combination Therapy of Cancer

Adema G.J.1, Büll C.1, Heise T.2, Wassink M.1, den Brok M.H.1, Boltje T.J.2

Department of Tumor Immunology, Radboud University Medical Center and Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, The Netherlands

Upon malignant transformation, tumor cells upregulate the synthesis of sialic acid sugar-carrying glycans (sialoglycans). A dense layer of sialoglycans confers resistance to apoptosis, promotes tumor cell adhesion and migration and mediates therapy resistance. More recent insights suggest a role for sialoglycans in protecting tumor cells from recognition and eradication by immune cells through interaction with immunosuppressive Siglec receptors. We set out to investigate the Sialic Acid/Siglec Axis in Oncoimmunology, applying Sialic Acid Blockade with rationally-designed glycomimetics, like 3Fax-Neu5Ac, and metabolic glycoengineering plus on-cell Siglec ligand synthesis.

Using multiple tumor models, our data show that sialic acid blockade is feasible in vivo, potently reduces tumor growth and can even result in regression of established tumors and inhibition of tumor metastasis. Blocking Sialic Acid expression in the tumor microenvironment leads to a dominant decrease in Sialic Acids on tumor cells, increased infiltration of effector immune cells (T cells, natural killer cells) and decreased numbers of immunosuppressive regulatory cells. Mechanistic studies on the mode of action of Sialic Acid blockade confirmed a minor role for NK cells and highlight a dominant role for CD8+ cytotoxic T cells. Results will be presented that are consistent with a dual effect of Sialic Acid Blockade on reprogramming the immunosuppressive tumor microenvironment and on the activation of antigen presenting dendritic cells and induction of potent CD8+ T cell responses. Subsequent metabolic glycoengineering studies combined with on cell synthesis of novel Sialic Acid Siglec ligands revealed an important role for immunosuppressive Siglec receptors in these processes.

In summary, the finding that interference with the Sialic Acid/Siglec Axis can boost anti-tumor immune responses provides a rationale for the development of sialic acid-focused immune combination therapies for cancer.
The Effect of Substrate Presentation and Activation on Neuraminidase NEU2 Specificity

Authors: Oliver C. Grant, Spandana Makeneni, Bethany L. Foley, Robert J. Woods*

Institution(s): Complex Carbohydrate Research Center and Department of Biochemistry, 315 Riverbend Road, University of Georgia, Athens, GA 30602, USA.

Human exo-α-sialidases/neuraminidases cleave terminal sialic acid from glycoconjugates and regulate fundamental cellular processes that are affected by sialic acid recognition, and the disrupted expression of sialidases has been causally linked to a variety of disease states. The activity of cytosolic neuraminidase 2 (NEU2) is not only affected by the linkage type between the terminal sialic acid residue (Neu5Ac in this case) and the penultimate residue in the glycoconjugate (α2-3/-6/-8) linkage), but also by the supramolecular context within which the sialoglycan is presented. It has been shown that NEU2 can cleave Neu5Ac from a mono-dispersed solution of sialoglycan GM1, but has undetectable activity against GM1 when it is present in a vesicle/micelle. The present study provides a structure-based rationale for the observed effect of supramolecular organization on NEU2 activity. Data from molecular dynamics simulations of NEU2-bound glycans illustrate that substrate distortion in the Michaelis complex is likely responsible for linkage specificity.

Wednesday, November 16, 2016

Session 3: Sialic Acid Catabolism and Disease

Challenge in Inhibitor Design: The 'Butterfly Effect'.

Authors: Mark von Itzstein

Institution(s): Institute for Glycomics, Griffith University, Gold Coast Campus

Many pathogens exquisitely exploit the host cell glycome through use of their own carbohydrate-recognising proteins to invade the host, facilitate their lifecycle and as a consequence cause disease. A significant socioeconomic impact on humanity is observed annually for viruses such as influenza virus, rotavirus and parainfluenza virus. All of these viruses have essential carbohydrate recognition processes in their life cycles that provide possible drug discovery targets.

Our interest in parainfluenza virus haemagglutinin-neuraminidase as a drug discovery target has led us to the identification of an active site rearrangement process that results from an induced opening of the HN 216-loop. The induced opening is a consequence of the accommodation of appropriately functionalised neuraminic acid-based inhibitors. This rearrangement causes an important ‘butterfly effect’ that has potential consequences in HN inhibitor design and defines criteria for the ideal substituent size inhibitors.
Title: The Multi-facets of the Lysosomal Sialidase NEU1 and Its Mode of Regulation in Human Diseases

Authors: Alessandra d’Azzo

Institution(s): St. Jude Children’s Research Hospital

NEU1, the most abundant and ubiquitous of the mammalian sialidases, is essential for cleaving terminal sialic acids (SIAs) from a broad range of sialo-glycoproteins. In this capacity, NEU1 contributes to the maintenance of cell and tissue homeostasis, as evidenced by the severe systemic effects of NEU1 deficiency in patients with the lysosomal disorder sialidosis. Besides its canonical degradative function, NEU1 can indirectly control basic physiological processes by altering the extent of sialylation of specific substrates. One of such process is lysosomal exocytosis, which NEU1 negatively regulates by processing the SIAs on the lysosomal membrane protein LAMP1. Deficiency or downregulation of NEU1 results in excessive exocytosis of lysosomal hydrolases and exosomes with deleterious consequences for cell-cell communication, plasma membrane composition and ECM integrity. We demonstrated that exacerbated lysosomal exocytosis is pathognomonic of the disease in Neu1–/– mice, a faithful model of sialidosis, and plays a major role in the expression and penetrance of individual phenotypes. These include EMH and splenomegaly, brain amyloidosis resembling AD, fibrosis and cancer. Thus, unrestrained lysosomal exocytosis downstream of NEU1 deficiency not only explains part of the symptomatology of sialidosis, but also potentially implicates this enzyme in common, adult conditions mainly associated with aging, such as Alzheimer’s disease, idiopathic fibrosis and cancer. In line with this idea, we have investigated the transcription regulation of NEU1. The observation that broad-spectrum HDAC inhibitors increased NEU1 mRNA expression and enzymatic activity in normal and sialidosis fibroblasts pointed to an epigenetic control of the NEU1 locus. We have identified the E-box (HLH) motif as the top scoring binding site in the promoter of NEU1 that is recognized by HDAC2-co-localizing members of the MITF family of transcription factors known to induce lysosomal biogenesis. These studies may provide new prognostic/diagnostic biomarkers and therapeutic targets for sialidosis and common diseases of aging (NIH GM60905, DK52025, GM104981, Assisi Foundation of Memphis, ALSAC).

Title: Neuraminidases 3 and 4 direct neuronal development and function by reshaping the composition of brain gangliosides

Authors: Xuefang Pan1, Camila De Britto Pará De Aragão1,2, Juan P. Velasco-Martin3, David A. Priestman4, Harry Y. Wu1, Kohta Takahashi3, Kazunori Yamaguchi5, Frances M. Platt4, Nathalie Lamarche-Vane5, Carlos R. Morales2, Taeko Miyag6 and *Alexey V. Pshezhetsky1,2

Institution(s): 1Sainte-Justine University Hospital Research Center, University of Montreal, Canada; 2Department of Anatomy and Cell Biology, McGill University, Montreal, Canada; 3Universidad Autónoma de Madrid, Spain; 4Department of Pharmacology, University of Oxford, Oxford UK; 5Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai, Japan; 6Miyagi Cancer Center Research Institute, Natori, Japan.

By regulating recognition, signaling and physiology of neurons gangliosides are essential to CNS function. The composition of gangliosides in the brain changes during development and aging being presumably regulated by neuraminidases, which cleave their terminal sialic acid residues. Two mammalian neuraminidases, Neu3 and Neu4 are major candidates for processing brain gangliosides, however, the corresponding knockout mice showed no changes in brain ganglioside metabolism or deficits in brain function.

To elucidate the physiological functions of Neu4 and Neu3 in the brain, we generated a gene-targeted mouse strain (neu3−/−;neu4−/−) devoid of both enzymes. At the age of 10 months the mice showed abnormal behaviour in Y-maze and Novel object recognition tests suggesting deficits in spatial and short-term memory, respectively. Pathological examination revealed a mild accumulation of lysosomal storage bodies in ~5% of neurones in the deep cortical layers, whereas the vast majority of microglia and vascular pericytes contained numerous vacuoles indicative of lysosomal storage of lipids. The number of astrocytes and activated microglia was increased in all studied brain areas, consistent with neuroinflammation. Neuraminidase activity against gangliosides in the brain tissues of neu3−/−;neu4−/− mice was below detection levels indicating that Neu3 and Neu4 are mainly responsible for desialylation of brain gangliosides. This was also consistent with progressive accumulation of GM3 in lysosomes of microglia and pericytes and decrease of plasma membrane associated GM1-ganglioside in neurones. Cultured hippocampal and cortical neurons also showed reduced levels of GM1 ganglioside in dendrites and impaired neuritogenesis.

Together our data for the first time provide in vivo evidence that Neu3 and Neu4 have unique roles in the regulation of brain ganglioside composition essential for CNS function: Neu4 generates the neuronal GM1 ganglioside necessary for axon growth and neuronal differentiation from GD1a whereas Neu3 preferentially catabolizes GM3 ganglioside to Lac-Cer in brain phagocytic cells.
Title: Diversity in sialoside receptor recognition by influenza hemagglutinins
Authors: Ian A. Wilson
Institution(s): The Scripps Research Institute

The influenza virus glycan receptor glycan terminates in sialic acid and can be connected to galactose through $\alpha_{2-6}$ (avian-type receptors) or $\alpha_{2-3}$ linkages (human-type receptors). Thus, avian influenza viruses have to change their receptor specificity to bind to human receptors and cross the species barrier. Nevertheless, different human subtypes recognize 2-6 sialosides with different combinations of residues in the receptor-binding site of influenza hemagglutinin (HA). H1 subtypes preferentially use HA 190 and 225 residues for receptor specificity, whereas H2 and H3 use 226 and 228. The sialic acid itself is buried in a highly conserved pocket in all subtypes. Thus, for H1-H3 of the 16 avian subtypes, only two mutations are needed to switch receptor specificity. However, for other subtypes, including H6, H5, H7 and H10 from emerging viruses, the mutations required to change specificity do not fall simply into these two sets of mutations, which may also explain why the viruses cannot so readily switch specificity and become transmissible in humans. We have determined crystal structures of the HAs from these subtypes that have infected humans to understand the structural basis of their individual receptor specificity and, with the Paulson lab, what is the fine specificity of the sialoside receptors in different subtypes. Such information is of value for monitoring of emerging viruses and even for seasonal virus such as H3 that are changing their receptor binding profiles. This information is also pertinent to the immune response as such diversity of residues in the receptor binding site in different subtypes makes it difficult to generate broadly neutralizing antibodies or therapeutic agents that are as broadly neutralizing as those to the highly conserved fusion domain in the HA stem. The structural work was performed by Netanel Tzarum, Peter S. Lee, Wenli Yu, and Xueyong Zhu in my lab and the receptor binding studies by Robert P. de Vries, Wenjie Peng, Andrew Thompson, Ryan McBride and James C. Paulson.

Title: An engineered multivalent sialic acid binding biologic as a preventative of influenza
Authors: Garry Taylor¹, Helen Connaris¹, Lei Yang¹, Margaret Taylor¹, Robert Webster², Elena Govorkova²
Institution(s): ¹University of St Andrews, St Andrews, Fife, UK. ²St Jude Children’s Hospital, Memphis, TN, USA.

Many sialidases possess sialic acid binding domains in addition to their catalytic domain which help target the enzymes and increase catalytic efficiency. Our structural studies on sialidases from Vibrio cholerae and Streptococcus pneumoniae showed that their carbohydrate-binding modules (CBMs) bind the terminal sialic acid of various sialoglycoconjugates with modest affinities (mM - µM). Multivalent forms of the CBMs were engineered and were shown to gain affinity through avidity, with sub-nanomolar affinities being achieved (JBC 284, 7339-7351, 2009).

The multivalent-CBMs were tested for their ability to bind to sialic acid receptors on various cell types and were shown to prevent cell entry of a panel of influenza viruses in vitro. Three candidates were tested in mice for their ability to protect against a lethal challenge with a mouse-adapted A/California/04/2009 (H1N1) strain. The best biologic, Sp2CBMTD, was further tested to explore the lowest dose and best dosing regimen. A single 1µg dose of Sp2CBMTD administered intranasally 7 days before lethal challenge with H1N1 gave complete protection, with repeat dosing at 0.1µg also affording complete protection (PNAS, 111, 6401-6406, 2014).

The lead biologic, Sp2CBMTD, was further tested in mice and showed protection against lethal challenges with both the A/Anhui/1/2013 (H7N9) virus and highly pathogenic A/Turkey/15/2006 (H5N1) virus (AAC, 59, 1495-1504, 2015). Sp2CBMTD induced the pulmonary expression of pro-inflammatory mediators and recruited neutrophils to the respiratory tract, which resulted in less pronounced inflammation and rapid virus clearance from mouse lungs. Sp2CBMTD administration did not affect the virus-specific adaptive immune response, which was sufficient to protect against reinfection with a higher viral dose.

Multivalent sialic acid-binding CBMs therefore show promise as biologics for the prevention of diseases caused by respiratory pathogens that utilize sialic acid receptors. The pro-inflammatory properties of these biologics suggest that they may also have application against pathogens that do not rely on sialic acid receptors.
Elusive localization of the insect CMP-sialic acid synthetases

Ken Kitajima1,2, Di Wu1,2, Akiko Fujita1, and Chihiro Sato1,2

1Biosci Biotech Center; 2Grad Sch Bioagr Sci, Nagoya Univ, Nagoya 464-8601, Japan

The occurrence and biological importance of sialic acid (Sia) and its metabolic enzymes in insect have been studied using Drosophila melanogaster. The most prominent feature of Drosophila CMP-Sia synthetase (DmCSS) is its Golgi-localization, compared with nuclear localization of vertebrate CSSs. However, it has remained unclear whether the Golgi-localization is common to other insect CSSs and why it happens. To answer these questions, Aedes aegypti (mosquito) CSS (AaCSS) and Tribolium castaneum (beetle) CSS (TcCSS) were characterized for their activity and subcellular localization. First, AaCSS and TcCSS were found to share the common overall structure with DmCSS in terms of evolutionarily conserved motifs and the absence of the C-terminal domain typical to mammalian CSSs. Second, like DmCSS, AaCSS and TcCSS showed in-cell and in vitro activities when expressed in mammalian and insect cells. When bacterially expressed, they did not show any activity until the N-terminal hydrophobic region was removed. Third, in Drosophila S2 cells, AaCSS and TcCSS were predominantly localized in ER, but not in Golgi. Surprisingly, DmCSS was mainly secreted into the medium, although partially detected in Golgi. Consistent with these results, it was shown that the N-terminal hydrophobic regions of AaCSS and TcCSS worked as signal peptide to make them soluble in ER, while that of DmCSS worked as membrane-spanning region of type II transmembrane protein whose cytosolic KLK sequence functioned as an ER export signal. Thus, the destination of insect CSSs is more complicated and diverse than previously recognized. The elusiveness of the insect CSS destination might tells some interesting feature of sialic acid evolution.

ST8Sia4-dependent polysialylation is part of the developmental program required for germ layer formation from human pluripotent stem cells.

Berger RP1,2, Sun YH1,2, Kulik M1,2, Lee JK1,3, Nairn AV1,3, Moremen KW1,3, Pierce M1,3, Dalton S1,2

1Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA
2Center for Molecular Medicine, University of Georgia, Athens, GA
3Complex Carbohydrate Research Center, University of Georgia, Athens, GA

Polysialic acid (PSA) is a carbohydrate polymer of repeating α-2,8 sialic acid residues expressed on a small set of glycoproteins, including the neural cell adhesion molecule (NCAM). PST (ST8Sia4) and STX (ST8Sia2) encode the two enzymes responsible for PSA modification of target proteins in mammalian cells, but despite widespread polysialylation at various stages of embryonic development, most studies have focused strictly on the role of PSA in neurogenesis. Using human pluripotent stem cells (hPSC), we investigated the developmental roles of PST and STX and show that although hPSC do not express PSA, early progenitors of all three embryonic germ layers are polysialylated on their cell surfaces. Changes in polysialylation result from lineage-specific expression of polysialyltransferase genes; PST expression is induced in endoderm and mesoderm, while STX is induced in ectoderm. In hPSC, PST and STX genes are epigenetically marked by overlapping domains of H3K27 and H3K4 trimethylation, indicating that they are held in a "developmentally-primed" state. Activation of PST transcription during early mesendoderm differentiation is under control of the T-Goosecoid transcription factor network, a key regulatory axis required for early cell fate decisions in the vertebrate embryo. This result establishes that polysialyltransferase gene transcription is required as part of the developmental program associated with germ layer establishment. Finally, we show by both shRNA knockdown and CRISPR-Cas9 genome editing that PST-dependent cell surface polysialylation is essential for endoderm specification. This is the first report to demonstrate an essential function for a glycosyltransferase in hPSC lineage specification. Stem Cells (2016) 34:1742-1752.
The tumor-associated sialyltransferase ST6Gal-I confers a cancer stem cell phenotype and promotes resistance to multiple death-inducing stimuli

Matthew J. Schultz, Andrew T. Holdbrooks, Asmi Chakraborty, Robert B. Jones, Colleen Britain, and Susan L. Bellis

University of Alabama at Birmingham

The α2-6 sialyltransferase ST6Gal-I has long been implicated in carcinogenesis, however its pro-tumorigenic function remains unclear. Immunohistochemical analyses from our group reveal ST6Gal-I upregulation in multiple cancers, and high levels correlate with metastasis and reduced patient survival. Contrarily, ST6Gal-I is low in normal epithelia, with the exception of strong expression in stem/progenitor compartments. Furthermore, ST6Gal-I is induced upon re-programming of differentiated cells into pluripotent stem cells. Given these findings, we hypothesized that ST6Gal-I bestows a Cancer Stem Cell (CSCs) phenotype. Through manipulating ST6Gal-I expression (overexpression/knockdown) in ovarian and pancreatic cancer cells, we find ST6Gal-I confers hallmark CSC characteristics including tumorspheroid growth, chemoresistance, and upregulation of stem-associated transcription factors (Sox9). Additionally, ovarian cancer patient cells sorted for high ST6Gal-I activity using SNA lectin grow as CSC spheroids whereas SNA-low cells are not viable. Using limiting dilution tumor-xenograft assays, ST6Gal-I was found to enhance tumor-initiating potential, and tumorigenesis is augmented in mice with conditional ST6Gal-I overexpression in a chemically-induced carcinogenesis model (AOM/DSS). ST6Gal-I activity also promotes hypoxia-induced CSC survival, evidenced by HIF1α stabilization, increased survival signaling (pAkt, pAMPK) and transcription of HIF-1α targets (VEGF, PDHK1). ST6Gal-I similarly improves viability of cells exposed to growth factor depletion. High ST6Gal-I expressors display increased activation of survival indicators (pAkt, cIAP2, pNFkB, survivin), but reduced cell death markers, under serum-depleted growth conditions. When paired with our prior work showing ST6Gal-I-dependent protection against death receptor (Fas, TNFR1) and galectin-dependent apoptosis, these results establish ST6Gal-I as a fundamental survival factor. Complementing ST6Gal-I overexpression/knockdown models, we observe consistent clonal selection for cells with high endogenous ST6Gal-I when cells are exposed to stressors including tumorspheroid culture, multiple chemotherapeutics, hypoxia, growth factor deprivation, and death receptor ligands (TNFα). Collectively these results point to a pervasive role for ST6Gal-I in driving tumor cell resistance to numerous death-inducing stimuli within the tumor microenvironment.

Occurrence of free sialyl oligosaccharides related to N-glycans (sialyl FNGs) in animal sera

Junichi Seino, Haruhiko Fujihira, Yuki Masahara-Negishi, Tadashi Suzuki

Glycometabolome Team, RIKEN Global Research Cluster

Free oligosaccharides that are structurally related to N-glycans (free N-glycans; FNGs) are widely distributed in the cytosol of animal cells (1). The diverse molecular mechanisms responsible for the formation of these FNGs have been well clarified. Previously, the occurrence of sialylated FNGs in human sera was demonstrated (2). The features of these extracellular FNGs are quite distinct from the cytosolic FNGs, as they are Gn2-type glycans, bearing an N,N'-diacetylchitobiose unit at their reducing termini, while the cytosolic FNGs are predominantly Gn1-type, with a single GlcNAc at their reducing termini. More recently, the structures of FNGs in various animal sera were compared (3). The major structures observed varied from species to species, and the structures of the FNGs appear to be correlated with the major sialyl N-glycans on serum glycoproteins, suggesting that the serum FNGs are produced by hepatocytes. Interestingly, glycan-profiles of the FNGs indicated that they are altered in a developmental stage-dependent manner. Sialyl FNGs in the sera may not only be of biological relevance, in that they might reflect the functionality of the liver, but also can be attractive sources for obtaining uniform sialyl FNGs in the chemoenzymatic synthesis of glycoproteins.

References:

The success of all cell-based therapeutics critically depends on the ability to deliver the relevant cells to the tissue(s) where they are needed. Injection of cells directly into affected tissue has limited applicability and can itself exacerbate parenchymal injury, prompting strategies to optimize vascular delivery of cells into target tissues. Under the influence of the “inflammatory” cytokines TNF and IL-1, endothelial cells within post-capillary venules express VCAM-1 and E-selectin. VCAM-1 is the ligand for integrin VLA-4, and E-selectin is a lectin that binds to the tetrasaccharide known as “sialyl Lewis X” (sLex; CD15s), a terminal type 2 (i.e., b(1,4)-linked) lactosamine unit that contains sialic acid a(2,3)-linked to galactose and fucose a(1,3)-linked to N-acetylglucosamine. Binding of E-selectin to sLex-bearing glycoconjugates on circulating cells initiates shear-resistant endothelial adherence, the key first step in recruitment of circulating cells to any target tissue; engagement of chemokine receptors then triggers activation of integrins such as VLA-4 (Step 2), resulting in firm adherence to VCAM-1 (Step 3) and subsequent transendothelial migration (Step 4). Among human cells, the most potent E-selectin ligand is a molecule known as "Hematopoietic Cell E-/L-selectin Ligand" (HCELL). HCELL is a specialized sLex-bearing glycovariant of the cell surface molecule called “CD44”. CD44 is a transmembrane glycoprotein that is expressed at high levels on essentially all mammalian cells, but, natively, the HCELL glycoform is uniquely expressed on human hematopoietic stem cells. We have developed a glycoengineering platform technology called "Glycosyltransferase-Programmed Stereosubstitution" (GPS) for custom-modifying CD44 glycans to enforce sLex expression, thereby creating HCELL on the surface of living cells. Preclinical studies have shown that systemic infusion of HCELL+ stem cells into mice with inflammatory conditions markedly augments cell infiltrates into affected tissues, with profound tissue-restoring effects. Notably, engagement of HCELL with vascular E-selectin triggers activation of integrin VLA-4 on the stem cell surface, resulting in firm adherence to VCAM-1 and ensuing extravasation via a chemokine-independent process. This "Step 2 Chemokine-bypass Pathway" is triggered by E-selectin-dependent ligation of sLex exclusively displayed on the CD44 scaffold, thereby both initiating and coordinating the multi-step cascade of events that engender transendothelial migration. Thus, this capacity of sLex engagement to mediate mechanosignalling is glycoconjugate-dependent, indicating that distinct sLex/E-selectin interactions can, in and of themselves, steer cell migration towards sites of tissue injury with consequent realization of therapeutic effect(s).
Title: Soluble Siglec-14: a new regulator of pro-inflammatory response?

Authors: Po-Chun (Jimmy) Huang, Iren Wang, Penk-Yeir Low, Shang-Te Danny Hsu and Takashi Angata

Institution(s): Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan

Rationale: Human Siglec-14 is a member of the Siglec family of sialic acid-binding proteins. Siglec-14 transduces activating signal and enhances pro-inflammatory responses of myeloid cells. Null polymorphism of Siglec-14 is known to influence the outcomes of some bacterially induced clinical conditions.

Although Siglec-14 is a type 1 transmembrane protein, a soluble form of Siglec-14 is also found in human serum. However, the function of soluble Siglec-14 and the mechanism of its generation remain unknown. We investigated potential function of the soluble Siglec-14, and the mechanism by which soluble Siglec-14 is generated.

Methods and Results: (1) RT-PCR revealed the presence of two major Siglec-14 splice variants that either retain or lack intron 5, which contains an in-frame stop codon. The splice variant retaining intron 5 yields soluble Siglec-14 by premature termination (as the exon 6 encodes transmembrane domain of Siglec-14), while the one without intron 5 yields the transmembrane form. ELISA using antibody against the unique C-terminal peptide segment of the soluble Siglec-14 confirmed that the soluble form in human serum is indeed generated by the splice variant we identified; (2) Structural analysis of a synthetic RNA, corresponding to the conserved guanine (G)-rich segment of intron 5, by circular dichroism and NMR revealed that the segment assumes a secondary structure called G-quadruplex, which is known to interact with proteins that regulate RNA splicing; (3) We analyzed the influence of recombinant soluble Siglec-14 on pro-inflammatory cytokine production by Siglec-14+ myeloid cells. NTHi stimulation of Siglec-14+ myeloid cells elicited the production of pro-inflammatory cytokines (as we reported previously), whereas soluble Siglec-14 suppressed this response in a dose-dependent manner.

Conclusion: Soluble Siglec-14 is generated through alternative splicing, which may be regulated by RNA G-quadruplex in the intron 5. Soluble Siglec-14 may regulate pro-inflammatory myeloid cell responses via competition against membrane-bound Siglec-14.

Acknowledgment: This work is supported by the Ministry of Science and Technology (MoST) of Taiwan (grant number: MOST 105-2321-B-001-051)

Title: Bacterial sialidases & vaginal dysbiosis: cellular and secreted sialoglycans act as room and board for unwelcome guests

Authors: Amanda Lewis

Institution(s): Washington University - St. Louis (USA)

Gardnerella vaginalis is a member of the phylum Actinobacteria; its overgrowth is associated with bacterial vaginosis, an imbalance for the vaginal microbiota linked with many adverse women’s health outcomes. We have found that a proportion of G. vaginalis isolates express sialidase activity and engage in sialic acid foraging behaviors. A mouse vaginal model of G. vaginalis infection replicates several of the features of BV, including elevated sialidase activity, evidence of sialic acid hydrolysis in the form of elevated levels of liberated sialic acid (Neu5Ac), and evidence of total sialic acid depletion. Virtually nothing is known about the mechanistic underpinnings of how BV develops as a polymicrobial condition. Here we describe the development of more complex models of BV that incorporate additional species of BV-associated bacteria. The goal is to develop a more mechanistic understanding of how sialidase-positive G. vaginalis enables infection by other bacteria during BV. Specifically, members of the Fusobacteria have been associated with vaginal colonization in women with BV and intrauterine infections in the context of preterm birth. Using experimental models incorporating human specimens, mouse models, and in vitro studies, we illustrate two new mechanisms by which a secondary pathogen benefits from a primary sialidase-producing strain of G. vaginalis.
Endogenous Siglec-8 Ligands in Human Airways

Most Siglecgs (sialic acid binding immunoglobulin-like lectins) are expressed on immune cells where ligation with sialoglycan ligands regulates immune responses. Siglec-8 is expressed on human allergic inflammatory cells: eosinophils, basophils and mast cells. Ligation of Siglec-8 with antibody or synthetic sialoglycans induces eosinophil apoptosis and inhibits mast cell degranulation. Knowledge of the endogenous sialoglycans responsible for Siglec-8 ligation may provide insights into allergic inflammation and aid in the design of new therapies for eosinophil-driven inflammatory diseases including asthma and chronic rhinosinusitis. Mice are limited as a model for studying Siglec-8 ligands, since mice lack Siglec-8. Instead, mouse eosinophils express Siglec-F, a functional paralog of Siglec-8 involved in the control of eosinophilic inflammation in the mouse. Tissue overlay of human and mouse airway sections with expressed tagged Siglec-F and Siglec-8 demonstrated that these two functionally similar siglecgs bind to sialoglycans with different tissue distributions and different molecular characteristics. Therefore, we focused on human lungs as the source of Siglec-8 ligands. Overlay of fixed human lung tissue sections with expressed tagged Siglec-8 and extraction of sialoglycans from fresh human lung tissues revealed that Siglec-8 binding is minimal in the lung parenchyma and enriched in the upper airways. Extraction of sialoglycans from human trachea followed by gel electrophoresis, blotting and overlay with tagged expressed Siglec-8 revealed three distinct sialoglycan ligands in the molecular weight range of 270-1000 KDa. Affinity purification of the 1000 KDa ligand, followed by proteomic mass spectrometry, revealed the proteoglycan aggrecan, which is known to carry sialylated keratan sulfate chains. Treatment of human airway tissues or airway sialoglycans with sialidase or keratanase eliminated Siglec-8 binding. These and other data are consistent with the conclusion that human airway Siglec-8 ligands include sialylated highly sulfated keratan sulfate chains carried on aggrecan. Supported by the US National Heart Lung and Blood Institute (P01HL107151, http://lidpeg.org).

Sialylation of N-glycans on autoantibodies determines pathogenicity in rheumatoid arthritis

Changes in glycosylation patterns of IgG from rheumatoid arthritis (RA) patients have been reported. But, implication of the glycosylation changes in the disease has not been clarified. We analyzed N-glycan structures on Fc portion of autoantibody, IgG (IgG-Fc) in RA patients, and autoantibodies generated in a couple of murine RA models. Then, we established knockout mice of alpha2,6-sialyltransferase 1 (ST6Gal1) in which sialylation of IgG was deleted in activated B cells. Finally, we modified N-glycans in monoclonal antibodies (mAbs) that can cause RA-like arthritis with genetic manipulation of beta1,4-galactosyltransferase and alpha2,6-sialyltransferase, and injected into mice together. Consequently, anti-citrullinated protein antibodies (ACPA) had reduced levels of sialic acid and galactose in N-glycans on IgG Fc from RA patients. Anti-collagen IgGs from sera of DR4 mice and DBA1 mice immunized by collagen type II also showed reduced sialylation. In ST6Gal1 knockout mice, exacerbation of collagen-induced arthritis (CIA) was observed, suggesting desialylation in IgG-Fc induced more pathogenic properties of anti-collagen IgG. Then, we injected sialylation-modified monoclonal antibodies, i.e. an ACPA antibody and an anti-collagen II antibody to cause collagen antibody-induced arthritis (CAIA). Sialylated antibodies induced no arthritis, while original form mAbs (almost desialylated) induced definite arthritis in both clinical scores and histochemical analysis. When these combined mAbs were injected during current CIA induction, sialylated mAbs suppressed pathogenicity of CIA, while non-sialylated mAbs rather enhanced frequency of arthritis. Notably, sialylated form of a non-relevant mAb showed no effects on the features of CIA. In conclusion, it was strongly suggested that sialylation in IgG-Fc of autoantibodies determines pathogenicity of RA, and antigen-specific antibody is important to affect the pathogenicity of RA based on the modification in N-glycans on IgG-Fc. Mechanisms for these differential function of IgGs remain to be investigated.

Title: *Haemophilus influenzae* has evolved preferential use of the human specific form of sialic acid as a host adaptation.

Authors: Preston S.K. Ng¹, Christopher J. Day¹, Lauren E. Hartley-Tassell¹, Linda E. Winter⁵, Tal Marshanski⁴, Vered Padler-Karavani⁴, Ajit Varki³, Stephen J. Barenkamp⁵ Michael A. Apicella² and Michael P. Jennings¹.

Institution(s): ¹Institute For Glycomics, Griffith University, Gold Coast, Queensland 4215, Australia. ²Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242-1109, USA. ³Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA 92093 Departments of Medicine. ⁴Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel. ⁵Department of Pediatrics, Saint Louis University School of Medicine, and the Pediatric Research Institute, Cardinal Glennon Children's Medical Center, Saint Louis, MO 63104, USA.

Non-typeable *Haemophilus influenzae* (NTHi) is a human adapted bacterial pathogen. NTHi expresses sialic acid acquired from the host as a terminal sugar on its outer membrane glycolipid, lipooligosaccharide (LOS). There are two major forms of mammalian sialic acid; *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). Humans exclusively express Neu5Ac, and Neu5Gc is recognized by humans as a foreign antigen. A recent study has hypothesized that dietary Neu5Gc is acquired by NTHi and presented on LOS as an antigen to induce human anti-Neu5Gc antibodies¹. Here we examined Neu5Gc uptake and presentation on NTHi LOS. We show that Neu5Gc and Neu5Ac are utilized equally well as a sole carbon source, but when equal amounts of Neu5Gc and Neu5Ac are provided in culture media there is significantly more Neu5Ac incorporated into LOS, suggesting a bias in some step in the pathway for LOS biosynthesis. NTHi CMP-Neu5Ac synthetase (SiaB) enzyme has a 4000-fold higher catalytic efficiency for Neu5Ac than Neu5Gc. Expression of Neu5Gc on NTHi LOS confers susceptibility to killing by human anti-Neu5Gc antibodies. The host adaptation of preferential utilization of the human form of sialic acid by NTHi is driven by immune evasion of the human immune response to Neu5Gc that it initiates.


Session 5: Sialic Acids in Diseases and Therapies

Title: Sialoptosis: cell death induced by aberrant sialylation

Authors: Marek W.J. Whitehead¹, Nikolay Khanzhin², Thierry Hennet¹

Institution(s): ¹Department of Physiology, University of Zurich, Switzerland; ²Glycom A/S, Kongens Lyngby, Denmark

A novel ligation technique has been developed to couple oligosaccharides to primary amines in aqueous solution and at neutral pH. This technique can be applied to ligate various oligosaccharides to proteins, phospholipids in solution or in the context of living cells. The decoration of animal cells with fucosylated oligosaccharides such as Fuc(α1-2)lactose and Fuc(α1-3)lactose increased the binding of Ulex europaeus lectin and Aleuria aurantia lectin without alteration of cell viability. The decoration of cells with the sialylated oligosaccharides Sia(α2-3)lactose and Sia(α2-6)lactose however induced the death of treated cells within 30 min. By comparison, the incubation of cells with soluble sialyllactose and the incorporation of sialyllactosylated phospholipids into cell membranes did not decrease cell viability. The rapid cell death induced by sialyllactose ligation was blocked by the pan-caspase inhibitor QVD-FMK and the caspase 1 inhibitor Z-WEHD-FMK, but not by the necroptosis inhibitor necrosulfonamide. To characterize the death pathway induced by sialylated oligosaccharide ligation, we have applied a gene inactivation screen using a CRISPR/Cas9 whole genome library. Gene disrupted cells surviving sialyllactose ligation were isolated and the targeted genes conferring resistance to cell death were identified by genomic sequencing of integrated guideRNA sequences. Genes encoding various effectors of the death signaling pathway induced by sialyllactose ligation of mammalian cells will be presented.
Polysialic acid (polySia, PSA) is a homopolymer of sialic acid with the degree of polymerization 8-400 Sia residues. PolySia mainly modifies neural cell adhesion molecules (NCAM) in embryonic brains. In adult brains, polySia expression is highly regulated in plastic areas of adult brains such as hippocampus, and olfactory systems. PolySia is a functionally important glycotope especially in vertebrate brains and is involved in learning, memory, circadian rhythm, and social behaviors. The molecular mechanism underlying these phenomena is considered to be related with not only the bulky and hydrated properties, but also reservoir functions as a scaffold for various neurological active molecules (1,2): the brain-derived neurotrophic factor (BDNF) (3-6), catecholamine neurotransmitters (7), and the fibroblast growth factor 2 (FGF2) (4,6,8). Accumulating data led us to hypothesize that the quantity and quality of polySia are highly regulated for normal brain functions. To confirm the hypothesis, we focused on the several SNPs on ST8SIA2/STX, one of two polysialyltransferase genes, reported to be associated with psychiatric diseases such as schizophrenia and bipolar disorder (1,2). We analyzed the effects of associated SNPs (cSNP, sSNP, iSNP and rSNP) on their products and found that all the SNPs of ST8SIA2/STX examined so far influenced on the structure and function of polySia (4,6,8). In addition, we analyzed effects of environmental factors on the polySia expression as well and found that polySia expression is highly sensitive to the environmental conditions. All these data indicate that normal condition of polySia expression is important for normal brain functioning.


Sepsis is the most common cause of death in hospitalised patients, with an annual incidence of 1 million cases and 200,000 deaths in the USA alone\(^1\). Moreover, approximately 25% of septic cases are complicated by the development of Acute Respiratory Distress Syndrome (ARDS), which also incurs a high mortality rate. Both sepsis and ARDS are characterised by excessive pro-inflammatory responses, for which there are currently no effective treatments. Instead, supportive therapy within the critical care setting forms the mainstay of treatment and so novel therapeutic strategies are urgently required.

Given the fundamental role of sialic acid-binding immunoglobulin-like lectins (Siglecs) in modulating immune responses, these receptors represent potential anti-inflammatory targets in sepsis and ARDS. In particular, previous studies demonstrated that engagement of murine Siglec-E negatively regulates Toll-like receptor (TLR)-mediated inflammation\(^2\). In this current work, a novel sialylated nanoparticle was developed to actively target Siglec receptors expressed on macrophages, with potential therapeutic utility in sepsis and ARDS.

Polylactic-co-glycolic acid (PLGA) nanoparticles were formulated via a salting-out approach and decorated with α2,8 N-acetylneuraminic acid targeting moieties (α2,8 NANA-NP). When tested in cultures of lipopolysaccharide (LPS)-stimulated murine macrophages, α2,8 NANA-NP potently inhibited the secretion of pro-inflammatory tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) cytokines in a Siglec-E dependent manner. Therapeutic efficacy was also confirmed in both systemic and pulmonary in vivo models of inflammation, where α2,8 NANA-NP enhanced survival rates, attenuated neutrophil infiltration, inhibited pro-inflammatory cytokine production and augmented anti-inflammatory IL-10 levels. Mechanistic studies uncovered that the functionality of α2,8 NANA-NP was both macrophage- and IL-10-dependent, given the loss of efficacy in clodronate-treated and IL-10 knockout mice, respectively.

Collectively, these findings demonstrate that the targeted cross-linking and activation of Siglecs can be exploited therapeutically in models of sepsis and ARDS. In addition to these syndromes, α2,8 NANA-NP may also find potential application in other conditions underpinned by aberrant pro-inflammatory signalling.

References: \(^1\) *Immunity* 2014, 40(4), 463-75. \(^2\) *J Immunol* 2009, 183(12), 7703-9
Eosinophils are major effector cells in type 2 diseases. Siglec-8 is selectively expressed on human eosinophils, and its cytoplasmic domain contains both an ITIM and an ITSM putatively responsible for signaling. Its closest functional paralog in the mouse is Siglec-F. Previous work has shown that engagement of Siglec-8 on human eosinophils induces apoptosis, especially in cytokine-primed cells. Murine experiments showed that Siglec-F regulates eosinophil accumulation and survival in vivo, but ligand-induced death is relatively weak compared to that with Siglec-8. These two receptors exhibit only modest homology, yet they both preferentially bind to the glycan 6'-sulfated-sialyl-Lewis X, and both the sialic acid and the sulfate are required for binding. However, Siglec-F, unlike Siglec-8, binds to additional branched α2,3-sialylated, non-sulfated glycans. Studies to identify natural tissue ligands for Siglec-8 and Siglec-F are ongoing, but for the latter, sialoglycans specifically expressed on the mucin Muc5b bind Siglec-F, whereas in humans MUC5B interacts with Siglec-9, not Siglec-8.

Following engagement, Siglec-F is internalized via a clathrin-independent pathway to the lysosome. In ongoing studies examining primary human eosinophils, Siglec-8 engagement led to its endocytosis, with about half being internalized within 90 minutes. Internalized Siglec-8, like Siglec-F, localizes to the lysosomal compartment. Using specific inhibitors, we determined that Siglec-8 endocytosis requires actin rearrangement, tyrosine kinase and PKC activities, and both clathrin and lipid rafts. Endocytosis in Siglec-8–transduced HEK293T cells required an intact ITIM. Leveraging Siglec-8 we determined that Siglec-8 endocytosis requires Src family kinases, Btk, PI3K, PKC and protein tyrosine phosphatases. In summary, there are overlapping but distinct pathways involving Siglec-8 and Siglec-F glycobiology, internalization and signaling. Regardless, the biology and dynamics of Siglec-8 surface expression appear suitable for treatment of eosinophil-associated diseases.

Title: Sialylation of GFRα1 (GDNF family receptor alpha 1) and Vasorin by ST3Gal1 promotes tumor growth and angiogenesis
Authors: Tan-chi Fan, Ming-Yi Ho, Wen-Der Lin, Huiling Yeo, Ko-Wa Chen, John Yu, Alice L. Yu
Institution(s): 1Institute of Stem Cell and Translational Cancer Research, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, 2Department of Biochemistry and Molecular Biology, Chang Gung University, Gueishan, Taoyuan, Taiwan, 3Chemical Biology and Molecular Biophysics Program, Taiwan International Graduate Program, Academia Sinica, Taipei, Taiwan, 4Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan, 5Genomics Research Center, Academia Sinica, Taipei, Taiwan, 6Department of Pediatrics/Hematology Oncology, University of California, San Diego, CA, USA

Downregulation of ST3Gal1 suppresses tumor growth but its substrates remain unknown. Here we identified glial cell line-derived neurotrophic factor (GDNF) family receptor alpha 1 (GFRα1) and vasorin (VASN) as protein targets of ST3Gal1 and demonstrated the importance of such sialylation for their functions. Binding of GDNF to GFRα1 induces trimerization with RET and promotes RET dimerization and subsequent phosphorylation, regulating neuronal cell proliferation and differentiation. GFRα1 and RET are overexpressed in estrogen receptor (ER)-positive breast cancers, and GDNF signaling leads to ER phosphorylation and estrogen-independent transcriptional activation of ER-dependent genes. We found that silencing of ST3Gal1 in breast cancer reduced GDNF-induced RET, AKT and ER phosphorylation, and GDNF-mediated cell proliferation. ST3Gal1 silencing greatly impaired the interaction between GFRα1 and RET and dampened their downstream signaling. Intriguingly, GDNF can stimulate the transcription of ST3Gal1, forming a positive feedback loop. Mechanistically, we showed the presence of Sp1 binding site on ST3Gal1 promoter by ChIP assay and that GDNF-induced Sp1 phosphorylation via PI3K/AKT pathway, leading to the upregulation of ST3Gal1.

Another target of ST3Gal1 we identified is Vasorin (VASN) which binds to TGF-β1 and inhibits TGFβ1-induced angiogenesis. MALDI-TOF analysis of secreted VASN revealed NeuNAcalpha2-3Galbeta1-3GalNAc as the major O-linked glycan, which is the glycan product of ST3Gal1. Conditional medium collected from ST3Gal1-silenced cells significantly reduced tube-formation ability of HUVEC, accompanied by downregulation of angiogenesis gene expression, including FGF1, FGF13, MMP, and VEGFA. Such suppression was abrogated by anti-VASN mAb, suggesting that VASN is important in angiogenesis. De-sialylation of VASN significantly enhanced its binding to TGFβ1. Our results revealed that the tumor promoting effects of ST3Gal1 could be attributed at least in part to the reciprocal feedback regulation of ST3Gal1 and GFRα1 signaling and heightened TGFβ1-mediated angiogenesis as a consequence of α2,3 sialylation of VASN by ST3Gal1.
Poster Abstracts

Key:
- Analytical (1 to 7)
- Biosynthesis Catabolism (8 to 21)
- Disease / Therapy (22 to 32)
- Pathogens (33 to 46)
- Receptors / Immunology (47 to 54)
- Synthesis / Tools (55 to 63)

Analytical

1 Glycosylation of HIV-1 Envelope Glycoprotein

Liwei Cao¹, Jolene Diedrich², Dan Kulp³, ⁴, Matthias Pauthner³, ⁴, Lin He³, Robin Park², Devin Sok³, ⁴, Chingyao Su³, Claire Delahunty², Raiees Andrabi³, Javier Guenaga³, Dennis Burton³, ⁴, William Schief³, ⁴, John Yates², James C. Paulson ¹, ², ³

Departments of ¹Cell and Molecular Biology, ²Chemical Physiology, and ³Immunology and Microbial Science, and ⁴the IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, California, USA

The dense N-glycan layer of the HIV-1 virus envelope glycoprotein gp120 creates a shield for the underlying polypeptides, and thus impedes the development of HIV-1 vaccine. Broadly neutralizing antibodies that can protect against virus challenge in animal models have N-glycans in formation of their epitopes. Nonetheless, the population of N-glycans at each glycosite of gp120 is extremely heterogeneous due to the variable glycan processing in host cells. To increase our understanding on the role of N-glycans in eliciting bNAbs response we developed a method that could rapidly assess the site occupancy and proportion of high mannose and complex type glycans at each glycosite of gp120. The method benefits from the powerful proteomics software to provide semi-quantitative site specific analysis of the three glycosylation states, and is helpful for the development of HIV-1 vaccine. (Supported by NIH R01AI113867 (JCP, JY, WRS); NIH UM1 AI100663 (DRB); the International AIDS Vaccine Initiative (WRS, DRB); and NIH P41 GM103533 (JY))

2 Dissecting three-dimensional structure of poly-α(2-8) N-acetyl neuraminic acid through structural studies of its oligomers

Hugo F. Azurmendi, Marcos D. Battistel and Darón I. Freedberg

Center for Biologics Evaluation and Research, USA

Neisseria meningitidis serogroup B and Escherichia coli K1 are encapsulated by polymeric α(2-8) N-acetyl neuraminic acid (PolySia). PolySia is also found on NCAM and oligomeric α(2-8) N-acetyl neuraminic acid (OligoSia's) are found in gangliosides and on cell surfaces. Understanding their structural properties is essential for delineating these molecules' diverse biological functions. We use OligoSia's of increasing length as structural models for PolySia. We will present our recent results on the three-dimensional structures of dimer, tetramer and polymers in the presence and absence of calcium. Recent in silico and NMR solution studies on OligoSia's predict previously unreported hydrogen bonding patterns in these molecules and therefore shed new light on the unique properties of OligoSia's. An unusual structural feature arises from the fact that, in contrast to on cell PolySia, free PolySia in solution is found mostly (~97%) with the reducing end in the β instead of α configuration. This configuration favors a hydrogen bond between residues 1 and 2 of the dimer and consequently stabilizes the dimer. This has distinct structural consequences for Oligo- and PolySia near the reducing end. We demonstrate the existence of this hydrogen bond and show that the net result is a stable conformation with well-defined hydration and charge patterns that determine the structure and dynamics for OligoSia's, for at least the first two residues of the oligomer. Additionally the studies indicate that this effect is observed in higher order OligoSia's. We also show strong evidence for hydrogen bonding in a tetramer of sialic acid, which leads to a stable helix at -10 °C. Site-specific calcium binding significantly perturbs the tetramer's structure. Similar effects are seen in cryogenic electron microscopy where PolySia is on the surface of E. coli.
Mapping the occurrence and carriers of sulfo-sialoglycans on the B and T cells of mice and man

Jian-You Chen¹, Merrina Anugraham², Takashi Angata¹,² and Kay-Hooi Khoo¹,²

¹Institute of Biochemical Sciences, National Taiwan University, and ²Institute of Biological Chemistry, Academia Sinica, Taiwan

It is now well appreciated that a GlcNAc-6-O-sulfated version of \( \alpha_2-6 \)-sialyl LacNAc constitutes a higher affinity ligand of CD22 and may have important functional consequences since it is down regulated upon naive B cell activation and migration into germinal centre of lymph nodes. Curiously, such a sulfation was not detected at comparable level in murine B cells. Instead, murine B cells exhibit a preferential switch from \( \alpha_2-6 \)-NeuGc to NeuAc-based sialyl LacNAc and thus similarly exhibit a lower binding affinity to mCD22. It is unclear at present if such unmasking by replacing tight cis-binding with less than optimum binding would confer any functional advantage, particularly in rendering it more receptive to trans-binding. We have previously provided the first mass spectrometry-based evidence for the occurrence of 6-sulfo-\( \alpha_2-6 \)-sialyl LacNAc on human B cells and have since developed additional complementary analytical approaches to more definitively map the occurrence and relative level of sulfated glycans on human B-lymphoma cells, particularly B-CLL. We further show that unlike B cells, both human and murine T cells carry significant level of sulfated glycans. Moreover, upon activation, different murine T cell subsets including Th1, Th2, Th17 and Treg exhibit distinctive changes in sulfation and/or NeuGc/NeuAc \( \alpha_2-6/2-3 \)-sialylation, thus potentially attenuates to different extent any possible interaction with CD22-bearing cells. On a separate front, we have initiated glycoproteomics that aims to identify as many protein carriers of the target sulfated glycotopes in conjunction with global sialylglycopeptides enrichment versus CD22-proximity labelling capture at the protein level. We emphasize in this communication the advances we made in the development of enabling analytical techniques and where cutting edge sulfoglycomics and sulfoglycoproteomics now stand.

Occurrence of free sialyl oligosaccharides related to N-glycans (sialyl FNGs) in animal sera

Junichi Seino, Haruhiko Fujihira, Yuki Masahara-Negishi, Tadashi Suzuki

Glycometabolome Team, RIKEN Global Research Cluster, Japan

Free oligosaccharides that are structurally related to N-glycans (free N-glycans; FNGs) are widely distributed in the cytosol of animal cells (1). The diverse molecular mechanisms responsible for the formation of these FNGs have been well clarified. Previously, the occurrence of sialylated FNGs in human sera was demonstrated (2). The features of these extracellular FNGs are quite distinct from the cytosolic FNGs, as they are Gn2-type glycans, bearing an N,N'-diacetylchitobiose unit at their reducing termini, while the cytosolic FNGs are predominantly Gn1-type, with a single GlcNAc at their reducing termini. More recently, the structures of FNGs in various animal sera were compared (3). The major structures observed varied from species to species, and the structures of the FNGs appear to be correlated with the major sialyl N-glycans on serum glycoproteins, suggesting that the serum FNGs are produced by hepatocytes. Interestingly, glycan-profiles of the FNGs indicated that they are altered in a developmental stage-dependent manner. Sialyl FNGs in the sera may not only be of biological relevance, in that they might reflect the functionality of the liver, but also can be attractive sources for obtaining uniform sialyl FNGs in the chemoenzymatic synthesis of glycoproteins.

References:

Differences detected in glycosylation critical quality attributes of epoetin alpha biosimilars

Rebecca I. Thomson1, Richard A. Gardner2, Katja Strohfeldt1, Daryl L. Fernandes2, Graham P. Stafford3, Daniel I.R. Spencer2 and Helen M.I. Osborn1

1Reading School of Pharmacy, University of Reading, Reading, Berkshire, United Kingdom; 2Ludger Ltd., Culham Science Centre, Abingdon, Oxfordshire, United Kingdom; 3Integrated BioSciences, School of Clinical Dentistry, University of Sheffield, United Kingdom

Erythropoietin, a sialoglycoprotein hormone, is responsible for regulating the level of erythrocytes in the bloodstream and is the main treatment for anaemia. After expiration of the patent for the originator erythropoiesis stimulating agent (ESA) Eprex, so-called biosimilar products, such as Binocrit, entered the market. Although the amino acid core is retained between biosimilars, the posttranslational glycosylation pattern, including sialic acid (SA) content, can differ. These differences must be monitored to ensure the quality, safety and efficacy of a biotherapeutic. In the presented study, 1,2-diamino-4,5-methylenedioxybenzene (DMB) labelled SA derivatives and procainamide (PROC) labelled N-glycans (figure below) were analysed by liquid chromatography-mass spectrometry (LC-MS) and LC. Furthermore, samples were digested by a sialate-O-acetylemesterase to confirm the presence of O-acetyl groups. Eprex was found to contain the greatest relative abundance of O-acetylated SA derivatives; Binocrit expressed the least N-glycolyl-neuraminic acid (Neu5Gc, an immunogenic SA); and CIGB-EPO (a development ESA) showed the greatest variety of high-mannose-phosphate structures. Given the extent of these differences and their possible impact on quality, safety and efficacy, it is recommended that ESAs under development be subjected to the same level of analysis.

![Fluorescence graph](image-url)
Developmental Changes in the Level of Free and Conjugated Sialic Acids, Neu5Ac, Neu5Gc and KDN in Different Organs of Pig: A LC-MS/MS Quantitative Analyses

Suna Ji, Fang Wang, Yue Chen, Changwei Yang, Panwang Zhang, Xuebing Zhang, Frederic A. Troy II, and Bing Wang

School of Medicine, Xiamen University, Xiamen City 361005 P.R. China; Department of Biochemistry and Molecular Medicine, University of California School of Medicine, Davis, CA, USA; School of Animal & Veterinary Sciences, Charles Sturt University, Wagga, Australia

Background/Aim: Recent studies have shown a relationship between the level of the sialic acid (Sia), N-glycolylneuraminic acid (Neu5Gc) in red meat and its risk in cancer, cardiovascular and inflammatory diseases. Pork is one of the major sources of dietary red meat protein for humans. Unresolved is the concentration of the Sias in different organs of pigs during development. Our aim was to determine the level of free and conjugated forms of Neu5Gc, N-acetylneuraminic acid (Neu5Ac) and ketodeoxynonulsonic acid (KDN) in fresh and cooked tissues from spleen, kidney, lung, heart, liver, and skeletal muscle, and to determine the potential impact of the Sias on health.

Methods: The concentration of Sias in fresh and cooked tissues from spleen, kidney, lung, heart, liver, and skeletal muscle from 3, 38 and 180-day-old (adult) pigs was analyzed by LC-MS/MS.

Results: Our new findings show: (1) Lung tissue from 3 days-old piglets contained the highest level of total Sia (14.6 µmol/g protein) compared with other organs and age groups; (2) Unexpectedly, Neu5Gc was the major Sia in spleen (67-79%) and adult lung (36-49%), while free KDN was the major Sia in skeletal muscle. Conjugated Neu5Ac was the highest Sia in other organs (61-84%); (3) Skeletal muscle contained the lowest concentration of Neu5Gc in fresh and cooked tissues; (4) KDN accounted for <5% of the total Sia in most organs; (5) During development, the total Sia concentration showed a 44-79% decrease in all organs; (6) In adult piglets, the high to low rank order of total Sia was lung, heart, spleen, kidney, liver and skeletal muscle.

Conclusion: The high level of Neu5Gc in all organs compared to skeletal muscle is a potential risk factor suggesting that dietary consumption of organ meats should be discouraged in favor of muscle to protect against cancer, cardiovascular and other inflammatory diseases.

Funding sources: This study was supported by a research grant from the School of Medicine, Xiamen University.


Characterization of Glycoproteins using Light Scattering technology

Jeffrey A. Ahlgren and Michelle H. Chen

Wyatt Technology Corporation, Santa Barbara, California, USA

Multi-angle static light scattering (MALS) can be used to determine the absolute molar mass of macromolecules such as glycoproteins in solution without the usual requirement for molar mass standards traditionally used with size-exclusion column chromatography (SEC) for calibration. When MALS is combined with both a differential refractive index detector (dRI) and a UV absorbance spectrophotometer during SEC, the combination of these 3 detection methods permits determination of the molar mass of the complex as well as the degree of modification of the protein by carbohydrate (and other) moieties, and additionally, the molar mass of the protein backbone and carbohydrate modifications can be measured. This multi-detector SEC-MALS approach was used to explain why the same glycoprotein expressed by two different expression systems (one mammalian, one insect) demonstrated different SEC mobility when fractionated under the same experimental fractionation conditions. A careful analysis of the MALS, dRI and UV signals demonstrated that the protein backbone was correctly produced in vitro by these two different expression systems and had identical molar mass from both sources. What was shown to be different by this analysis is that the mammalian expression system produced a higher degree of glycosylation than was observed in the insect expression system, resulting in a lower overall molar mass of the glycoprotein produced by the insect system compared to the glycoprotein product made by the mammalian expression system. The theory of how this analytical tool is used to calculate the degree of protein modification will be presented.
Sialic acid is crucial to maintain fetal-maternal immune homeostasis

Markus Abeln, Samanta Cajic, Erdmann Rapp, Anja Münster-Kühnel, Rita Gerardy-Schahn, Birgit Weinhold
Institute for Cellular Chemistry, Hannover Medical School; Max-Planck Institute for Dynamics of Complex Technical Systems, Magdeburg

Mammalian pregnancy represents an immunologically challenging situation, as due to their paternal components, the developing fetus and its extraembryonic tissues are a semi-allograft to the mother. Hence, the maternal decidua has to provide an immunologically specialised environment, in which the developing fetus is protected from pathogens and excessive maternal immune activation at the same time. We could show that the fetal-maternal interface, which is established by fetal trophoblast cells, is heavily α2,3 sialylated in control mice and abrogation of sialylation in CMP-sialic acid synthase deficient (Cmas−/−) mice results in extensive infiltration of maternal neutrophils. In addition, trophoblasts of Cmas−/− animals exhibit increased deposit of the complement component C3 at day 8.5 of development (E8.5). Concomitantly with inflammation of the fetal-maternal interface, Cmas−/− mice revealed loss of trophoblast organisation and severe placentation deficits. Moreover did Cmas−/− embryos suffer from intrauterine growth restriction (IUGR), which was presumably caused by increased thickness of the outermost fetal basement membrane, the Reichert’s membrane, due to augmented deposit of extracellular matrix material. To eliminate maternal influences on embryonic development, we also analysed Cmas−/− murine embryonic stem cells and by xCGE-LIF analysis could show that Cmas−/− N-glycans were entirely asialo. Using embryoid body differentiation and subsequent transcriptome analysis we could further show that loss of CMAS activity did not impair in vitro embryonic development until the post-streak stage. Taken together we here show that activation of sialic acid by CMAS has no redundancy in mice and Cmas−/− animals fail to maintain fetal-maternal immune homeostasis, leading to embryonic lethality around E8.5. Inflammation related IUGR and placentation deficits have previously been reported in mouse models of recurrent fetal loss and human pregnancy complications such as preeclampsia, which might indicate a potential role of sialoglycoconjugates in the progression of these pathologies.

The tumor-associated sialyltransferase ST6Gal-I confers a cancer stem cell phenotype and promotes resistance to multiple death-inducing stimuli

Matthew J. Schultz, Andrew T. Holdbrooks, Asmi Chakraborty, Robert B. Jones, Colleen Britain, and Susan L. Bellis
University of Alabama at Birmingham, USA

The α2-6 sialyltransferase ST6Gal-I has long been implicated in carcinogenesis, however its pro-tumorigenic function remains unclear. Immunohistochemical analyses from our group reveal ST6Gal-I upregulation in multiple cancers, and high levels correlate with metastasis and reduced patient survival. Contrarily, ST6Gal-I is low in normal epithelia, with the exception of strong expression in stem/progenitor compartments. Furthermore, ST6Gal-I is induced upon re-programming of differentiated cells into pluripotent stem cells. Given these findings, we hypothesized that ST6Gal-I bestows a Cancer Stem Cell (CSCs) phenotype. Through manipulating ST6Gal-I expression (overexpression/knockdown) in ovarian and pancreatic cancer cells, we find ST6Gal-I confers hallmark CSC characteristics including tumorspheroid growth, chemoresistance, and upregulation of stem-associated transcription factors (Sox9). Additionally, ovarian cancer patient cells sorted for high ST6Gal-I activity using SNA lectin grow as CSC spheroids whereas SNA-low cells are not viable. Using limiting dilution tumor-xenograft assays, ST6Gal-I was found to enhance tumor-initiating potential, and tumorigenesis is augmented in mice with conditional ST6Gal-I overexpression in a chemically-induced carcinogenesis model (AOM/DSS). ST6Gal-I activity also promotes hypoxia-induced CSC survival, evidenced by HIF1α stabilization, increased survival signaling (pAkt, pAMPK) and transcription of HIF-1α targets (VEGF, PDHK1). ST6Gal-I similarly improves viability of cells exposed to growth factor depletion. High ST6Gal-I expressors display increased activation of survival indicators (pAkt, cIAP2, pNFkB, survivin), but reduced cell death markers, under serum-depleted growth conditions. When paired with our prior work showing ST6Gal-I-dependent protection against death receptor (Fas, TNFR1) and galectin-dependent apoptosis, these results establish ST6Gal-I as a fundamental survival factor. Complementing ST6Gal-I overexpression/knockdown models, we observe consistent clonal selection for cells with high endogenous ST6Gal-I when cells are exposed to stressors including tumorspheroid culture, multiple chemotherapeutics, hypoxia, growth factor deprivation, and death receptor ligands (TNFα). Collectively these results point to a pervasive role for ST6Gal-I in driving tumor cell resistance to numerous death-inducing stimuli within the tumor microenvironment.
The human neuraminidase enzymes (NEU) are rapidly emerging as important mediators of physiological pathways. Members of the NEU family have been implicated in tumorigenesis, cancer metastasis, inflammation, cell adhesion, and insulin signaling. There are four known isoenzymes in the NEU family, which vary both in their subcellular location and their preference for glycolipid (NEU3, NEU4) or glycoprotein (NEU1, NEU2, NEU4) substrates. In addition to developing an understanding of the active site topologies among the NEU isoenzymes, our group has been exploring the role of these enzymes in cell adhesion and cell migration. Using in vitro cell migration assays, we have identified a role for NEU in regulation of b1 integrin-mediated migration. Enzyme activity is usually pro-migratory; therefore, chemical inhibitors of the enzymes could act as antimetastatics. Investigations of the mechanism of b1 integrin regulation have implicated a role for changes in both the glycan of integrins and glycolipid composition. Biophysical investigations confirm that integrin receptors involved in cell migration have altered interactions with the cytoskeleton. We will present current cell migration results with iso-enzyme selective inhibitors developed in our group. In total, our studies confirm an important role for NEU in the regulation of cell migration, and suggest new avenues for the design of antimetastatic therapeutics.

Although glycosphingolipids are known to show structural diversity in their glycan and ceramide portions, little has been elucidated about the biological significance of their structural diversity. Recently we found a novel phenomenon that both glycan and ceramide structures of GM3 changed during myoblast differentiation of mouse satellite-derived C2C12 cell: Sialic acid (Sia) residue of the GM3 glycan shifted from Neu5Ac to Neu5Gc, while the chain length of acyl group of the ceramide part changed from C24 to C16. In this study, to elucidate molecular mechanisms for these structural changes of GM3, we examined the expression levels of the gene for CMP-Neu5Ac hydroxylase (CMAH), which catalyzes conversion of CMP-Neu5Ac to CMP-Neu5Gc, and those for ceramide synthetases (CerS) subtype 1 to 6, which synthesize different ceramide species from sphingosines and fatty acids of different chain length in a subtype-specific manner, during myoblast differentiation. Based on the real-time PCR results, the expression level of CMAH was found to increase rapidly during differentiation, while that of CerS2, which is a long acyl chain-specific isozyme, dramatically decreased. These results are consistent with the observed structural changes of GM3 are regulated at least by transcription levels of CMAH and CerS. We also examined effects of exogenously added Neu5Gc or GM3 with a short acyl chain on myoblast differentiation. Based on the morphology and the expression of differentiation marker proteins, exogenous Neu5Gc induced changes in the shape of myotubes, while ceramides with a short acyl chain appeared to affect the efficiency of differentiation. These results suggest that the structural regulation of GM3 is crucial for myoblast differentiation.

The human neuraminidase enzymes (NEU1, NEU2, NEU3, and NEU4) are a class of enzymes implicated in pathologies including cancer and diabetes. Several reports have linked neuraminidase activity to the regulation of cell migration. The β1 integrin complexes, such as α4β1 and α5β1, are the most likely candidate receptors involved in this process. Using an in vitro cell migration assay, we investigated the role of these enzymes in the migration of four different cell lines. We have observed that human breast cancer cells (MDA-MB-231) and prostate cancer cells (PC-3) showed significant retardation of cell migration when treated with inhibitors of NEU3 and NEU4. Interestingly inhibitors which target different isoenzymes show distinct activities.

We investigated the mechanism of action of the inhibitors using multiple biochemical assays. We found that the compounds were nontoxic, and did not have a significant effect on total cell surface sialic acid content. Analysis of the sialylation of the α4β1 integrin complex in NEU3-treated cells suggests that changes to glycosylation of the β1 chain, but the cδ5 chain may not be involved. We found evidence that selective targeting of NEU3 and NEU4 was more effective than the use of non-specific inhibitors, such as DANA. Thus, inhibition of cancer cell migration using isoenzyme-specific inhibitors of human neuraminidase enzymes has significant potential in the development of anti-adhesion therapies. We will report our ongoing studies of the influence of neuraminidase activity on integrin endocytosis.
ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3 (ST8SIA3) functions as a signal coordinator in the striatum

Chien-Yu Lin¹, Hsing-Lin Lai¹, Hui-Mei Chen¹, Jian-Jing Siew¹, Ching-Pang Chang¹, Kay-Hooi Khoo², Yijuang Chern¹

¹Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; ²Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3 (ST8SIA3) belongs to the type II membrane protein and exists in the Golgi apparatus. It is responsible for transferring one or multiple sialic acids to the terminal galactose of the N-linked or O-linked glycan chains of glycoproteins. ST8SIA3 is highly expressed in the striatum, the brain area that controls motor coordination in vivo. To investigate the pathophysiological function of ST8SIA3, we generated a St8sia3-knockout (KO) mouse model using the CRISPR/Cas9-mediated genome engineering technology. No apparent abnormality was observed in the gross development and fertility of St8sia3-KO mice. Glycomic analyses demonstrated a significant reduction of the disialylated terminal glycotopes in the striatum of St8sia3-KO mice. Magnetic resonance imaging (MRI) analysis revealed that St8sia3-KO mice have a smaller striatum, but not other brain areas tested, than their littermate controls. To identify the protein substrates of ST8SIA3, we evaluated the expression of several striatum-enriched proteins in St8sia3-KO mice. Western blot analyses suggested that the A₂A adenosine receptor (A₂AR), the type V adenylyl cyclase (AC5) and the D₂ dopamine receptor (D₂R) might be potential substrates of ST8SIA3, because these proteins harvested from the striatum of St8sia3-KO mice traveled faster in SDS-PAGE gels than those in their littermate controls. Treatment with sialidase, which released all sialic residues from sialylated glycans, eliminated the difference in protein sizes of A₂AR, AC5, and D₂R between WT and St8sia3-KO mice, further suggesting that these three proteins might be modified by the ST8SIA3-mediated sialylation. Consistently, the motor responses to antagonists of A₂AR and D₂R were also altered in St8sia3-KO mice. Collectively, ST8SIA3 plays a critical role in the striatum by mediating sialylation of several important striatal proteins and subsequently modulate striatal functions.

St8sia3-KO mice have a smaller striatum, but not other brain areas tested, than their littermate controls. To identify the protein substrates of ST8SIA3, we evaluated the expression of several striatum-enriched proteins in St8sia3-KO mice. Western blot analyses suggested that the A₂A adenosine receptor (A₂AR), the type V adenylyl cyclase (AC5) and the D₂ dopamine receptor (D₂R) might be potential substrates of ST8SIA3, because these proteins harvested from the striatum of St8sia3-KO mice traveled faster in SDS-PAGE gels than those in their littermate controls. Treatment with sialidase, which released all sialic residues from sialylated glycans, eliminated the difference in protein sizes of A₂AR, AC5, and D₂R between WT and St8sia3-KO mice, further suggesting that these three proteins might be modified by the ST8SIA3-mediated sialylation. Consistently, the motor responses to antagonists of A₂AR and D₂R were also altered in St8sia3-KO mice. Collectively, ST8SIA3 plays a critical role in the striatum by mediating sialylation of several important striatal proteins and subsequently modulate striatal functions.

Establishment of a new method for evaluating the repulsive function of polySia-NCAM

Airi Mori¹,², Yuki Niimi¹,², Masaya Hane¹,², Ken Kitajima¹,² and Chihiro Sato¹,²


Polysialic acid (polySia), which mainly occurs on the neural cell adhesion molecule (NCAM), is observed in whole embryonic brain as well as in restricted areas of adult brain including hippocampus and olfactory systems. PolySia shows not only an anti-adhesive effect on NCAM-involved cell-cell interactions due to its bulky and hydrated properties, but also a reservoir function for neurologically active molecules (1). Although the anti-adhesive effect of polySia is conceptually interest, very few studies have so far evaluated the polySia effect experimentally. Our objective is, thus, to establish an in vitro method for evaluating the effect of polySia on the NCAM-mediated homophilic interactions. Firstly, we prepared polySia-NCAM-Fc, oligoSia-NCAM-Fc, and asialo-NCAM-Fc and subjected to surface plasmon resonance-based method to monitor their mutual binding. The sensor gram for the homophilic interactions of polySia-NCAM showed negative RU values in a concentration-dependent manner, which is considered to stand for repulsive interactions. Furthermore, using polySia-NCAM-Fc synthesized by one of the following polysialyltransferases: ST8sia2 (wild-type), ST8sia4 (wild-type) and ST8sia2 (E141K, schizophrenia-type), the repulsive interaction of the polySia synthesized by each enzyme was evaluated. We found that polySia synthesized by ST8sia2 (schizophrenia-type) showed weaker repulsive interaction than that synthesized by ST8sia2 (wild-type). All these data suggest that polySia has a different role depending on the responsible enzyme for a polySia biosynthesis.

Evolution of a KDNase by modification of a bacterial sialidase

Saeideh Shamsi Kazem Abadi, Andrew J. Bennet

1Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada;
2Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

Sialic acids are often found at the terminal positions on the glycan chains that adorn all vertebrate cells and glycoproteins. This prominent position confers an essential role to sialic acid residues in biology, evolution and disease propagation. Glycoside hydrolase family 33 (GH33) contains exo-sialidases (E.C. 3.2.1.18, neuraminidases), from both eukaryotes and prokaryotes, that catalyze the hydrolysis of sialic acid from glycoconjugates. Interestingly, subtle differences exist in both the structure of the particular sialic acid and its position of attachment to glycoconjugate chains between humans and other mammals. These differences are indicators of the unique aspects of human evolution, and are relevant to understanding an array of human conditions. We will be exploring two routes to further unravel the importance of sialic acids.

We are developing tools to probe for various sialic acid structures such as Kdn, a sialic acid family member that is less well understood. To this end, we have constructed a random mutant library of the sialidase from the soil bacterium Micromonospora viridifaciens (Mv) and identified a number of recurring mutations in the sialidase gene which lead to a more efficient hydrolysis of synthetic natural substrate analogues such as 8FMU α-Kdn-(2→6)-β-D-Galp (1). We have also used the three dimensional structure of MvS bound to the inhibitor Neu2en5Ac (2) to identify amino acids involved in recognition and binding to various functional groups in sialic acid. Using this information we have generated genetic libraries that we will use to identify clones with modified catalytic activities.

We have created MvS mutant libraries containing saturation mutations of the above identified amino acids. By monitoring the hydrolytic activity of mutant enzymes in the presence of 8FMU α-KDN-(2→6)-β-D-Galp and 8FMU α-Neu5Ac-(2→6)-β-D-Galp we will identify clones with specific catalytic activity.

Despite their documented role of sialic acid in numerous diseases, our knowledge of the functions and metabolism of certain sialoglycans, linkage specific attachment or cleavage of sialic acids to cellular glycans are scant and require further in depth research to delineate the role of various sialic acid structures.
16 Expression of neural cell adhesion molecule and polysialic acid in human bone marrow-derived mesenchymal stromal cells

Maria S. Skog¹, Johanna Nystedt², Matti Korhonen², Heidi Anderson¹, Timo A. Lehti¹, Maria I. Pajunen¹, Jukka Finne³

¹Department of Biosciences, University of Helsinki, Helsinki, Finland; ²Finnish Red Cross Blood Service, Helsinki, Finland

Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) are attractive candidates for cellular therapy and regenerative medicine. They are adult progenitor cells that hold potential for secretion of immunomodulatory factors as well as multilineage differentiation. For the development of novel clinical applications and understanding of therapeutic mechanisms detailed molecular characterization of hBM-MSCs is required. Neural cell adhesion molecule (NCAM, CD56) is a transmembrane glycoprotein modulating cell–cell and cell–matrix interactions. A post-translational modification of NCAM results from the addition of polysialic acid (polySia), which maintains developmental plasticity and cell migration in tissues by influencing cellular interactions. Due to its background, NCAM is often considered a marker of neural lineage commitment, and it is generally held that hBM-MSCs do not express NCAM. We have studied NCAM and polySia expression in five donor-specific hBM-MSC lines at the mRNA and protein level. Our results show that all five known NCAM isoforms are expressed in the cells at the mRNA level and the three main isoforms are present at the protein level. Both polysialyltransferases, ST8SIA2 and ST8SIA4 that are generally responsible for NCAM polysialylation, are expressed at the mRNA level. However, only very few cells express polySia at the cell surface. Our results differ greatly from the previous reports and thus underline the need for a careful control of methods and conditions in the characterization of MSCs. Despite some promising clinical results related to refractory graft-versus-host disease, the biological properties of MSCs remain largely unknown and clinical MSC applications are lacking good markers that would reflect the clinical efficacy of the cells. NCAM and polySia represent new candidate molecules for predicting functional potency and influencing MSC interactions.

17 Sialylation and Desialylation Dynamics of Monocytes upon Differentiation and Polarization to Macrophages

Dan Wang, Evgeny Ozhegov, Aimin Zhou, Huan Nie, Yu Li, Xue-Long Sun

Department of Chemistry, Chemical and Biomedical Engineering and Center for Gene Regulation in Health and Disease (GRHD), Cleveland State University, Cleveland, Ohio, USA

Sialic acids (SAs) often exist as the terminal sugars of glycan structures of cell surface glycoproteins and glycolipids. The level and linkages of cell surface SAs, which are controlled by both sialylation and desialylation processes and environment cues, can dramatically impact cell properties and represent different cellular statuses. Many evidences have hinted to a potential role of cell-surface SAs in the status and functions of macrophages. In this report, we systematically examined the sialylation and desialylation profiles of THP-1 monocytes after differentiation to M0 macrophages, and polarization to M1 and M2 macrophages by the combination of LC-MS/MS, flow cytometry and confocal microscopy. Interestingly, both α2,3- and α2,6-linked SAs on the cell surface were found to be decreased after monocytes were differentiated to macrophages, which was in accordance with the increased level of free SA in the cell culture medium and the elevated activity of endogenous Neu1sialidase. Meanwhile, the siaoglycoconjugates inside the cells increased as confirmed by confocal microscopy and the total SA inside the cells increased as determined by LC-MS/MS. Western blot analysis showed higher expression levels of sialyltransferases, including ST3Gal-I, ST3Gal-V, ST6Gal-I and ST6GalNAc-II. Further, upon polarization, the cell surface sialylation levels of M1 and M2 macrophages remained the same as M0 macrophages, while a slight decrease of cellular SAs in the M1 macrophages but increase in the M2 macrophages were confirmed by LC-MS/MS. Overall, profiling macrophage cell surface sialylation and desialylation status will contribute to delineate macrophage diversity and define their phenotypes and functions.
A possible mechanism of enhanced EGFR signaling by sialidase NEU3

Koji Yamamoto1,2, Kohta Takahashi3, Kazuhiro Shiozaki4, Kazunori Yamaguchi5, Hiroshi Shima2, and Taeko Miyagi2


We previously demonstrated that sialidase NEU3, a key glycosidase for ganglioside degradation, is up-regulated in various human cancers, leading to increased cell invasion, motility and survival of cancer cells through activation of EGF signaling (1). Its up-regulation is also important for promotion stage of tumorigenesis in vivo and in vitro. Our recent studies exhibited that NEU3 potentiates EGFR-mediated tumorigenesis through the stimulation of EGFR phosphorylation and Src activity (2). To address the regulation mechanism of the activation of EGF signaling by the sialidase, we focused on NEU3-mediated enhanced EGFR phosphorylation. Increased tyrosine-phosphorylation of EGFR by NEU3 has been reported to occur by recruitment of NEU3 to EGFR, as assessed by co-immunoprecipitation, followed by EGFR activation through ganglioside modulation, dependent of the sialidase activity (2).

In the present study, using human lung cancer cells, we observed that lung cancer cells with EGFR mutations, H1650 and H1975 cells, showed higher endogenous sialidase activity towards gangliosides as compared to wild type A549 cells, and then found NEU3 to undergo EGF-induced tyrosine-phosphorylation and to get activated. The phosphorylation was, however, abrogated by the inhibitors for EGFR or/and Src and was hardly detectable in the NEU3 mutant of the tyrosine residues (Y370C). The phosphorylation level seemed to be positively correlated to the sialidase activity. These results suggest that up-regulation of NEU3 would enhance EGFR phosphorylation and get activated by EGFR in a positive feedback manner, leading to potentiation of tumorigenesis and then acceleration of malignant phenotype of the cancer cells.

References: (1) Wada et al. Oncogene 2007; (2) Yamamoto et al. PLOS One 2015

Discovery and analyses of hyper-diversity of oligo/polysialyltransferase genes in echinoderm sea urchin

Atsushi Yoshimura1,2, Naofumi Hagiwara1,2, Sayaka Toki1,2, Shinji Miyata1,2, Masato Kiyomoto3, Chihiro Sato1,2, Ken Kitajima1,2


Oligo/polysialic acids (oligo/polySia) are homo-oligo/polymeric structure of sialic acids. It is also known that oligo/polySia structures with three different intersialyl linkages (α2,5Oglycolyl, α2,8, and α2,9) exist in echinoderm sea urchin. Functionally, the α2,5Oglycolyl-linked polySia, α2,8-linked oligoSia, and α2,9-linked polySia are suggested to be involved in regulation of intracellular pH of sperm, sperm binding to egg vitelline layer, and regulation of intracellular Ca ion of sperm, respectively. On the other hand, for the biosynthetic mechanism of different linkages of oligo/polySia, no gene has ever been identified other than 7 kinds of α2,8-sialyltransferase gene in eukaryotes. In this study, we thus tried to identify various oligo/polysialyltransferases with different linkage specificity in sea urchin.

First, 33 genes were found in the purple sea urchin (Strongylocentrotus purpuratus) database by searching for homologous genes to known vertebrate α2,8-sialyltransferases. On phylogenetic analyses, those 33 genes were classified into two gene clusters that are similar and dissimilar to mammalian oligo/polysialyltransferases, respectively. Second, quantitative analyses of mRNA expression revealed that at least 2 genes whose expression was detected in a closely related sea urchin species (Hemicentrotus pulcherrimus) showed the same expression profile as flagellasialin, a carrier protein of α2,9-polySia (1). So far, we have successfully cloned 3 including the above two, of 33 genes. Their deduced amino acid sequences contained those similar to mammalian α2,8-polysialyltransferases as well as those unique to sea urchin enzymes, suggesting that these 3 genes may be α2,9-oligo/polysialyltransferase genes.

Identification, regulation and possible functions of newly identified polysialic acid carriers in microglia and macrophages

Sebastian Werneburg 1, Hauke Thiesler 1, Falk F.R. Buettner 1, Herta Steinkellner 2, Harald Neumann 2, Martina Mühlenhoff 1, Herbert Hildebrandt 1

1Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany; 2Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria

Polysialic acid (polySia) has been implicated in modulating the activity of microglia, the innate immune cells of the brain. During brain development, polySia is mainly presented by the neural cell adhesion molecule NCAM on the surface of neurons. Recently, however, we discovered a pool of polySia in the Golgi compartment of NCAM-negative microglia and demonstrated that neuropilin-2 is one, but not the only polySia carrier in these cells (NRP2; Werneburg et al. 2015, Glia 63:1240). We therefore performed an unbiased glycoproteomic analysis and identified the E-selectin ligand-1 (ESL-1) as novel polySia protein carrier residing in the Golgi of stem cell-derived microglia and human THP-1 macrophages (Werneburg et al. 2016, Glia 64:1314). Comparing microglia from mice negative for either of the two the polysialyltransferases, ST8SIA2 and ST8SIA4, discloses that ESL-1 and NRP2 are polysialylated by ST8SIA4. As shown in organotypic brain slice cultures, Golgi-localized polySia appears during injury-induced microglia activation and is lost in response to inflammatory stimulation by lipopolysaccharides (LPS). In cultured microglia and THP-1 macrophages, LPS causes cell surface translocation and a rapid release of the two polysialylated proteins. Addition of metalloproteinase inhibitors prevents polySia depletion, indicating that the release is mediated by protein ectodomain shedding. Furthermore, we demonstrate elevated levels of LPS-induced activation in ST8SIA4-negative microglia as well as the inhibition of nitric oxide and inflammatory cytokines by soluble bacterial polySia (colominic acid) or by polySia produced on an NCAM fragment in glycoengineered plants (Kallolimath et al, PNAS early edition, doi:10.1073/pnas.1604371113). Together these data demonstrate that polySia inhibits microglia activation independent of its protein carrier, and indicate that ectodomain shedding of polySia-ESL-1 and polySia-NRP2 constitutes a cell-intrinsic mechanism for a negative feedback regulation of LPS-induced activation of microglia and possibly macrophages. The potentially distinct mechanisms for shedding and responding to the two microglial polySia carriers are currently under investigation.

Efficient myelination, myelin repair and motor recovery after demyelination require Ncam1 and St8sia2

Sebastian Werneburg 1, Iris Röckle 1, Hannelore Burkhardt 1, Iris Albers 1, Viktoria Gudi 2, Thomas Skripuletz 2, Martin Stangel 2, Herbert Hildebrandt 1

1Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany; 2Clinical Neuroimmunology and Neurochemistry, Department of Neurology, Hannover Medical School, Hannover, Germany

Multiple sclerosis is a demyelinating disease of the central nervous system. Remyelination can occur spontaneously but often fails or is incomplete, most likely because of impaired differentiation of oligodendrocyte precursors into remyelinating oligodendrocytes. Polysialic acid (polySia) is a prominent posttranslational modification of mainly the neural cell adhesion molecule NCAM, which generates repulsive forces that are implicated in the control of myelination and myelin repair. PolySia is produced by two polysialyltransferases, ST8SIA2 and ST8SIA4, with overlapping but distinct cell-type expression patterns and protein acceptor specificities. Here we used the cuprizone model to study the impact of NCAM and ST8SIA2 on de- and remyelination. 8-week-old Ncam1-/- or St8sia2-/- mice and respective wildtype controls received cuprizone for 5 weeks. Despite a mild developmental delay in both, Ncam1-/- and St8sia2-/- mice, myelination at the age of 8 weeks and also the loss of myelin during the five weeks of cuprizone treatment were indistinguishable between the wildtype and knockout lines. Densities of oligodendrocyte precursors, astrocytes and microglia were unaffected. However, remyelination and restoration of oligodendrocyte densities after cessation of the cuprizone diet were severely impaired in Ncam1-/- and, to the same extent, also in St8sia2-/- mice. This was reflected by a significant delay in the recovery of motor performance in the rotarod test. Together, these results demonstrate that NCAM and ST8SIA2 are indispensable for timely developmental myelination, efficient remyelination and motor recovery. The same negative impact of Ncam1 or St8sia2 deficiency indicates that remyelination requires polysialylation of NCAM by ST8SIA2. This is in stark contrast to the previously observed acceleration of remyelination in St8sia4-/- mice (Koutsoudaki et al. 2010 Neuroscience 171:235) revealing opposing roles of the two polysialyltransferases in remyelination and suggesting differential targeting of the two enzymes as a therapeutic strategy to improve myelin repair.
**Disease / Therapy**

**22 Sialylation-deficient podocytes – a model for glomerular disease**

Kristina Borst¹, Linda Blume¹, Henri Wedekind¹, Mario Schiffer², Birgit Weinhold¹, Rita Gerardy-Schahn¹ and Anja Münster-Kühnel¹

¹Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany; ²Clinic for Renal Diseases and Hypertension, Hannover Medical School, Hannover, Germany

The role of sialic acid (Sia) in kidney development and function is still not fully understood. A prerequisite for the synthesis of sialoglycans is the activation of Sia to CMP-Sia, catalyzed by the nuclear enzyme CMP-Sia synthetase (CMAS). An overall reduction of the CMAS expression level in Cmas⁻/⁻ mice results in kidney failure within three days after birth (Weinhold et al. 2012, JASN). The lethality could be attributed to pathological changes in podocytes forming the visceral layer of the glomerular filtration barrier. Mice with a podocyte-specific deletion of Cmas (P-Cmas⁻/⁻) mimic nephropathies such as Focal Segmental Glomerulosclerosis (FSGS) and die within two months after birth. Electron microscopy revealed that in knockout animals the podocyte morphology was drastically disorganized with effaced podocyte foot processes and a loss of slit diaphragms. To investigate the role of Sia for podocyte morphology and function on the cellular level, we first generated an immortalized wildtype podocyte cell line. These cells are able to proliferate in culture and can be differentiated into an in vivo-like state. Subsequently, we generated Cmas knockout cell lines using the CRISPR/Cas system. The knockout cells are viable, show wildtype morphology and can also be differentiated in culture like the wildtype. The biochemical characterization of these cells and the impact of sialylation for podocyte proteins like podocalyxin and nephrin, the major structural component of the slit diaphragm, are currently under investigation.

**23 Targeting Aberrant Sialylation in Cancer Cells Prevents Metastasis**

Christian Büll¹, Torben Heise², Melissa Wassink¹, Martijn H. den Brok¹, Thomas J. Boltje², Gosse J. Adema¹

¹Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands; ²Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands

Cancer cells present a significantly different glycosylation pattern relative to their normal counterparts, and several cancer-specific glycans have been identified that promote tumor growth and progression. The glycosylation changes reported in cancer, aberrant high expression of sialoglycans is commonly found. Although the molecular mechanisms underlying aberrant sialylation in cancer cells are not fully understood, overexpression of sialyltransferases is likely to result in increased sialylation and the deposition of cancer-specific sialoglycans on the cell surface. Due to hypersialylation, cancer cells acquire distinct characteristics including enhanced migratory properties and the ability to metastasize. Therefore, approaches to counteract aberrant sialic acid expression in cancer cells could be of high therapeutic value. We have found that a fluorinated sialic acid mimic (3F₅α,Neu5Ac) that was developed by Rillahan and co-workers as sialyltransferase inhibitor potently blocks sialic acid expression in cancer cells. Treatment of cancer cells with 3F₅α,Neu5Ac had no effect on cell viability, but reduced cancer cell adhesion and migration. Moreover, melanoma cells with blocked sialylation failed to form metastases in a mouse lung metastasis model. To deliver the sialic acid mimic specifically to melanoma cells, we have encapsulated it into poly(lactic-co-glycolic acid)-based nanoparticles that were decorated with melanoma antigen-recognizing antibodies. Using these tumor-targeting nanoparticles we were able to deliver 3F₅α,Neu5Ac specifically to melanoma cells. Due to the slow release of the sialic acid mimic from the nanoparticles, sialylation of melanoma cells was inhibited for multiple days. Finally, targeted delivery of 3F₅α,Neu5Ac to melanoma cells in the blood stream largely prevented their metastatic spread. These findings stress the importance of sialoglycans in metastasis and advocate that sialyltransferase inhibitors could help to effectively prevent cancer metastasis.
Sialyl-glycoconjugates and sialyltransferases regulate pre-B ALL leukemia cell growth and survival under hypoxia
Chih-Hsing Chou1,2, John Groffen1,2,3,4 and Nora Heisterkamp1,2,3,4

1Section of Molecular Carcinogenesis, Department of Pediatrics, The Saban Research Institute of Children's Hospital Los Angeles, CA, USA, 2Division of Hematology/Oncology and Bone Marrow Transplantation, 3Leukemia and Lymphoma Program, Norris Comprehensive Cancer Center, University of Southern California, CA, USA, 4Departments of Pediatrics and Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

On cancer cells, sialic acids are involved in many important cellular functions such as cell-tumor microenvironment interactions, cell signaling, immune cell attack and cancer cell survival. Aberrant expression of sialic acids is also important as tumor-associated carbohydrates that participate in cancer development and metastasis. Meta-analysis of a large gene expression data set of precursor B-lineage acute lymphoblastic leukemias (pre-B ALL), a leukemia of immature B-lineage hematopoietic, showed up-regulation of the sialyltransferases ST3Gal1 and ST6Gal1 in leukemic bone marrow compared to healthy samples. In this study, we investigated if these sialyltransferases also regulate important characteristics of pre-B ALL cells. As expected, overexpression of ST3Gal1 and ST6Gal1 in pre-B ALL US7 cells by lentiviral transduction increased sialyl-glycoconjugates on the cell surface compared to control cells. Increased ST3Gal1 and ST6Gal1 also enhanced cell growth. We also treated these cells with vincristine, one component of first-line chemotherapy for pre-B ALL. Interestingly, at 2.5 and 5 nM vincristine treatment, ST3Gal1, but not ST6Gal1 overexpression, enhanced cell viability. The bone marrow, the niche for pre-B ALL development, is a low-oxygen microenvironment. Under 3% low-oxygen conditions, ST3Gal1 overexpressing ALL cells showed increased cell viability with more cells in the S phase of the cell cycle compared to control cells. ALL cells with increased ST3Gal1 kept at hypoxia or treated with vincristine also showed increased Src kinase family phosphorylation, suggesting a connection between key cell surface 2,3-sialoglycoconjugates, signal transduction, and ALL cells growth/survival under conditions relevant to ALL bone marrow relapse. Further study is required to identify the sialyl-glycoproteins that are synthesized by ST3Gal1 in pre-B ALL cells in vitro, and to further investigate how they contribute to ALL chemoresistance and growth under hypoxia. Identifying ST3Gal1 regulated sialyl-glycoproteins may also lead to the identification of targets for diagnosis and therapeutic strategies in pre-B ALL.

Targeting polysialyltransferase as a therapeutic strategy for cancer
Robert A. Falconer, Goreti Ribeiro Morais, Xiaoxiao Guo, Anjana Patel, Mark Sutherland, Paul M. Loadman, Laurence H. Patterson, Steven D. Shnyder

Institute of Cancer Therapeutics, University of Bradford, U.K.

Polysialic acid (polySia) expressed on the surface of NCAM (neuronal cell adhesion molecule) of neuroendocrine tumors, notably neuroblastoma and small cell lung cancer, is strongly associated with poor prognosis and aggressive disease in patients in the clinic. PolySia modulates cell-cell and cell-matrix adhesion, migration, invasion and metastasis. For these reasons, and a growing body of evidence both in vitro and in vivo, interest in the polysialyltransferases (ST8SiaII and ST8SiaIV) responsible for polySia biosynthesis as potential therapeutic targets continues to gather pace. Our efforts are focused on the development of novel human polysialyltransferase (polyST) inhibitors. We have established highly-sensitive HPLC-based assays (cell-free and cell-based) to assess polyST inhibition. Using isogenic cell lines (C6-ST8Sialii/C6-WT) and naturally polySia-expressing human neuroblastoma cells (SH-SY5Y/IMR-32), compounds were evaluated for their ability to modulate polySia expression, cell adhesion, migration and invasion in vitro. We have identified CMP-sialic acid precursors that reduce polySia expression and tumor cell migration by up to 70%. Specificity of agents for polySTs over other sialyltransferases was established via differential lectin probes. Agents have been shown to disturb the dynamics of focal adhesion kinase and to modulate ERK1/2, AKT and VEGFR3 signaling. Furthermore, we are utilizing computational chemistry on newly developed ST8Sia homology models, to identify compounds with more drug-like properties with the aim of identifying an agent for in vivo studies. To-date, we have synthesized >100 compounds from which we have identified agents with increased potency. We are currently utilizing a range of biophysical techniques to assess compound-target engagement.

We also report the first evidence that polySia expression is associated with tumor cell survival and migratory capacity under hypoxia, a condition of low oxygen tension found in poorly-vascularized tumour areas and a key source of chemoresistance. We have determined a potential role for HIF-1 and LDH-A in maintaining migratory capacity.
Low Level Pancreatic Beta Cell Sialylation in the Onset of Autoimmune Diabetes

Douglas M. Heithoff, Won Ho Yang, Peter V. Aziz, and Jamey D. Marth

Center for Nanomedicine, Sanford-Burnham-Prebys Medical Discovery Institute, University of California-Santa Barbara, Santa Barbara, California, USA

The pathogenesis of tissue-specific autoimmune disease reflects innate or acquired defects in immunological tolerance but remains poorly understood. In Type 1 diabetes (T1D), a cell type-specific defect in immunological tolerance results in the destruction of pancreatic beta cells. In the past decade, multiple studies have linked the post-translational modification of proteins by sialyltransferases with mechanisms of immunological tolerance. Sialyltransferases generate sialic acid linkages on most mammalian cell surfaces modulating autoimmunity and immunological tolerance. We have noticed that normal pancreatic beta cells have relatively low levels of sialic acid linkages among cell surface glycans. This intrinsic low level of sialic acids may be advantageous in normal physiological contexts but disadvantageous in the presence of a dysfunctional immune system. To investigate this hypothesis, we chose to initially study the Non-Obese Diabetic Shll/J (NOD) mouse because of its well-defined disease signs that include spontaneous insulitis progressing to beta cell destruction, and because of the large body of immunological work that has been achieved using this animal model of autoimmune diabetes. We have generated and analyzed multiple transgenic NOD mice bearing increased expression of sialic acids on pancreatic beta cell proteins. Our findings reveal that augmentation of sialic acid linkages protects from insulitis, hyperglycemia, and the immunological destruction of pancreatic beta cells.

Rational drug design of anti-human parainfluenza virus compounds using a novel in silico approach combining quantum chemistry and bioinformatics

Naoya Matsuo1, Sundaram Arulmozhiraja1,2, Shogo Nakano3, Sohei Ito4, Tadanobu Takahashi4, Takashi Suzuki5, Kiyoshi Ikeda5, Mark von Itzstein6, Hiroaki Tokiwa1,2

1Department of Chemistry, Rikkyo University, Japan; 2Research Center for Smart Molecules, Rikkyo University, Japan; 3Department of Food and Nutritional Sciences, University of Shizuoka, Japan; 4Department of Biochemistry, University of Shizuoka, Japan; 5School of Pharmaceutical Sciences, Hiroshima International University, Japan; 6Institute for Glycomics, Griffith University, Australia

Global warming increases risk of virus infectious diseases (VIDs), so that developing effective anti-viral drugs has become the most important issue for chemical biologists and medicinal chemists. Among various VID, human parainfluenza viruses (hPIVs), type 1 – 4, are serious human pathogenic ones that cause upper and lower respiratory tract diseases in infants and young children, and also impact the elderly and immunocompromised patients. Neither effective clinical vaccines nor antiviral drugs are developed to treat the parainfluenza virus infections. Hemagglutinin-neuraminidase (HN), which is one of the surface envelope glycoproteins of the hPIVs, is a multifunctional protein for the initial glycan-mediated binding of the virus to the host cell and for the release of the virus progeny from the infected host cell (Sialidase). Recently, three different groups, including us, simultaneously found that N-acetyl-2,3-didehydro-2-deoxy-neuraminic acid (Neu5Ac2en) derivatives have potential inhibitory activities against the sialidase of hPIVs. Nevertheless, these derivatives yet are applied for the clinical treatments presumably due to the lack of sufficient antiviral potency. To rationally design and develop high potential inhibitory candidates, we used a new approach combining the first-principals calculations based fragment molecular orbital (FMO) method, which correctly evaluates not only electrostatic but also van der Waals dispersion interactions, with bioinformatics based multiple sequence alignment analysis. The new guidelines for theoretical drug design without checking drug resistance against all hPIV sub-types are established in the present study by performing dynamic induced-fit analysis of viral protein using FMO method, Molecular Dynamics (MD) simulation, and by predicting conservative regions around the sialidase active site in sequences of the viral protein. Modeling the appropriate candidates based on these results is in progress.
Design of a Influenza A virus-glycan interaction map (glycointeractome)

Juliane Mayr1, Jimmy C. Lai2, John Nicholls3, Mark von Itzstein1 and Thomas Haselhorst1

1Institute for Glycomics, Griffith University, Gold Coast Campus, Australia; 2Dept. of Pathology, The University of Hong Kong, China

Influenza A virus is the most common influenza type and can cause serious disease in humans and animals. Human infections with the pandemic H1N1 (‘swine flu’), the highly pathogenic avian H5N1 (‘bird flu’), and the more recent avian H7N9 have caused widespread public concern. Especially the avian H5N1 strain has shown high mortality rate in human but has not yet adopted an efficient human-to-human transmission that potentially could lead to the next deadly pandemic. The growing resistance to the commonly used anti-influenza drug oseltamivir carboxylate (Tamiflu®) emphasises the need for the development of novel antivirals. To accomplish this goal it is essential to gain structural knowledge of the mechanism by which influenza viruses attach and enter the host cell.

We have developed a unique protocol using Nuclear magnetic resonance (NMR) spectroscopy that allows us to directly investigate the interaction of whole influenza virus particles with host cell carbohydrate receptors (glycans) at an atomic level. In combination with virus neutralisation assays our approach has successfully been used to characterize the interactions of the viral surface glycoprotein hemagglutinin with cell surface glycans.

A virus-glycan interaction map (glycointeractome) will be presented that not only advances our understanding of virus specificity and host cell tropism but is also of crucial importance to guide structure-assisted design of the next generation of broad-spectrum anti-influenza drugs.

Sialic acid is crucial for kidney function

Linda Blume1, Kristina Borst1, Elina Kats1, Iris Albers1, Mario Schiffer2, Stephanie Groos3, Rita Gerardy-Schahn1, Birgit Weinhold1 and Anja Münster-Kühnel1

1Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany; 2Clinic for Renal Diseases and Hypertension, Hannover Medical School, Hannover, Germany; 3Institute for Cellular Biology, Hannover Medical School, Hannover, Germany

The biosynthesis of sialylated glycoconjugates essentially depends on the activation of sialic acid (Sia) to CMP-Sia, catalyzed by the nuclear enzyme CMP-Sia synthetase (CMAS). A shortage of CMP-Sia due to an overall reduction of the CMAS expression level in CmasΔk mice results in kidney failure within 3 days after birth [1]. The lethality could be attributed to maturation defects in podocytes forming the visceral layer of the glomerular filtration barrier. This barrier is composed of the fenestrated endothelium, the glomerular basement membrane and the slit diaphragm, a protein complex on the surface of podocyte foot processes. To address the significance of Sia for podocyte function, we generated a podocyte-specific Cmas knockout mouse model (P-Cmas−/−). These mice mimic nephropathies such as Focal Segmental Glomerulosclerosis (FSGS) and die within two month after birth. The progressive loss of sialylation as demonstrated for nephrin and podocalyxin, two major sialoglycoproteins of the podocyte, correlated with the development of proteinuria around 4 weeks after birth. Initial analyses did not indicate alterations of nephrin and podocalyxin interaction with their respective partners. However, a progressive loss of nephrin at the cell surface was observed and might contribute to the phenotype. Strikingly, in P-Cmas−/− mice the mesenchymal markers N-cadherin, fibronectin and FSP1 were upregulated in the outermost cellular layer of the glomerular tuft, while the epithelial marker ZO-1 was decreased, suggesting that asialo-podocytes might undergo an epithelial to mesenchymal transition. To study the molecular mechanism underlying the alterations in Sia-deficient podocytes, primary podocytes have been isolated from P-Cmas−/− mice. Unexpectedly, all podocytes showed CMAS expression indicating incomplete CMAS depletion. The presence of intact podocytes explains in all likelihood the extended life span of P-Cmas−/− mice. Moreover, the failure to isolate CMAS-negative podocytes suggests that these cells might have a disadvantage to grow or survive in cell culture.

Deficiency of a sulfotransferase for sialic acid-modified keratan sulfate in microglia mitigates Alzheimer's pathology

Zui Zhang¹, Yoshiko Takeda-Uchimura¹, Tahmina Foyez¹, Shiori Ohtake-Niimi¹, Narentuya¹, Tony Wyss-Coray², Kenji Kadomatsu¹ & Kenji Uchimura¹

¹Department of Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan; ²Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Keratan sulfate (KS) is an extracellular sulfated glycan expressed in the central nervous tissues. We previously showed that microglial KS was induced in amyotrophic lateral sclerosis (1). However, the functional roles of the glycan and its synthetic enzyme in neurodegenerative diseases, such as Alzheimer's disease (AD), a progressive disorder, are unclear. In our study, KS modified with sialic acids having a molecular mass of 125-220 kDa and N-acetylglucosamine-6-O-sulfotransferase 1 (GlcNAc6ST1), previously known as a sulfotransferase for ligands of L-selectin (2), were upregulated in the brains of two transgenic mouse models (J20 and Tg2576) and the brains of patients with AD. GlcNAc6ST1-deficient J20 (J20/GlcNAc6ST1⁻/⁻) mice demonstrated a complete absence of the microglial sialylated KS. J20/GlcNAc6ST1⁻/⁻ primary microglia showed an increased level of amyloid b phagocytosis and were hyper-responsive to interleukin-4, a potent anti-inflammatory cytokine. Moreover, J20/GlcNAc6ST1⁻/⁻ mice manifested reduced cerebral amyloid b deposition and attenuation of spontaneous alternation deficits. GlcNAc6ST1-synthesizing sialylated KS thus modulates AD pathology. Inhibition of KS synthesis by targeting GlcNAc6ST1 may therefore be beneficial for controlling AD pathogenesis.

References:

Multi-Action Antibody-Sialidase Conjugates for Cancer Immunotherapy

Han Xiao, Elliot C. Woods, Carolyn R. Bertozzi

Department of Chemistry and Howard Hughes Medical Institute, Stanford University, USA

Cell surface sialosides constitute a central axis of immune modulation that is exploited by tumors to evade immune destruction. Therapeutic strategies that target tumor-associated sialosides may therefore potentiate anti-tumor immunity. Here, we report the development of antibody-sialidase conjugates that enhance tumor cell susceptibility to antibody-dependent cell-mediated cytotoxicity (ADCC) by selective desialylation of the tumor cell glycocalyx. The antibody-sialidase conjugate desialylated tumor cells in a HER2-dependent manner, reduced binding by natural killer (NK) cell inhibitory Siglec receptors, and enhanced binding to the NK cell activating receptor NKG2D. Precision glycocalyx editing with antibody-enzyme conjugates is a promising avenue for cancer immune therapy.
33 Accelerated Aging and Turnover of Host Anti-Inflammatory Enzymes Contributes to the Pathogenesis of Gram-negative Sepsis.

Won Ho Yang, Douglas M. Heithoff, Peter V. Aziz, Michael J. Mahan, and Jamey D. Marth
Center for Nanomedicine, Sanford-Burnham-Prebys Medical Discovery Institute; University of California-Santa Barbara, Santa Barbara, California, USA.

Recent discoveries by this laboratory identified a mechanism of secreted protein aging and turnover that is composed of glycosidase–mediated N-glycan remodeling (Yang et al., 2015). We now find that this mechanism is modulated in mouse models of Gram-negative sepsis caused by infection with the human bacterial pathogens Salmonella enterica serovar Typhimurium and Escherichia coli. During the onset of sepsis, we have measured an increased rate of N-glycan remodeling with ensuing endocytic lectin ligand formation among secreted proteins in the blood plasma, resulting in rapid decreases in the abundance and function of key host anti-inflammatory enzymes, namely tissue non-specific and intestinal alkaline phosphatases (TNAP and IAP). Our findings demonstrate that both of these secreted anti-inflammatory protein enzymes are thereby post-translationally regulated as a means of determining their concentrations and activities in the blood. Our data further reveal that the endogenous sialyltransferase ST3Gal-VI is responsible for TNAP and IAP sialylation, without which both alkaline phosphatase isozymes are rapidly cleared from circulation by the Ashwell-Morell receptor (AMR). This accelerated remodeling of TNAP and IAP in sepsis is due to the induction of neuraminidase activity in the blood. This induction includes Neu1 and Neu3 and can be recapitulated by the lipopolysaccharide component of Gram-negative bacteria, thereby accelerating the aging and turnover of the anti-inflammatory enzymes TNAP and IAP. We further show that the induction of Neu activity with the subsequent reduction of alkaline phosphatase activity are responsible for increased LPS-phosphate levels linked with inflammation and reduced survival of Gram-negative sepsis. These findings demonstrate unexpected features of pathogen and host interactions during sepsis that target an intrinsic host mechanism of secreted protein aging and turnover. The resulting rapid changes in protein abundance and function among the secreted proteome have significant impacts on the pathogenesis and survival of sepsis.

34 Diversity of Ligand Recognition by the Siglec-like Adhesins of Oral Streptococci: Implications for Infective Endocarditis

Barbara A. Bensing¹, Dayoung Park², Carlito B. Lebrilla² and Paul M. Sullam⁷
¹Department of Medicine, San Francisco Veterans Affairs Medical Center and University of California, San Francisco
²Department of Chemistry, University of California, Davis

Infective endocarditis (IE) is a life-threatening infection of the cardiovascular system. The viridans group streptococci, which include S. gordonii, S. sanguinis and S. mitis, are a leading cause of IE. These organisms express a family of “Siglec-like” adhesins that mediate binding to alpha 2-3 linked sialic acids on human glycoproteins. One known receptor for the Siglec-like adhesins is the human salivary mucin MG2/MUC7, which likely contributes to streptococcal colonization of the oropharynx. However, upon entry of streptococci into the bloodstream, the Siglec-like adhesins can mediate attachment to the same or similar sialylated glycans on platelet GPIb, which may target bacteria to damaged cardiac valves, thus contributing to IE. We previously identified specific glycan ligands for more than a dozen of the Siglec-like adhesins, and found a range of preferences. GspB is a Siglec-like adhesin expressed by S. gordonii that is highly selective for sialyl-T antigen (sTa). Replacement of an essential arginine residue in GspB resulted in the loss of S. gordonii binding to human platelets in vitro, and attenuated virulence in an animal model of IE. Suggestive evidence indicates that streptococci that bind 3’sialyllactosamine (3’SLn) instead of sTa may be less virulent, despite the ability to bind GPIb. Thus, it is unclear whether there are sialylated glycoprotein targets for GspB beyond GPIb that may contribute to this disease. In order to determine how binding to sTa versus 3’SLn may impact the ability of streptococci to infect the endocardium, we have used the recombinant Siglec-like adhesins as probes in far-western blotting experiments, and to capture and identify additional sialylated glycoprotein targets in plasma and cardiac valve tissue. Results obtained thus far indicate that the sTa- versus 3’Sln-selective adhesins recognize different glycoforms of certain plasma and endocardial proteins, and suggest that these interactions have a significant impact on the propensity of streptococci to establish endocardial infections.
35 Towards antiviral sialic acid conjugates for the prevention or treatment of acute hemorrhagic conjunctivitis

Rémi Caraballo, Emil Johansson, Weixing Qian, Georg Zocher*, Nitesh Mistry**, David Andersson, Niklas Arnberg**, Thilo Stehle*, Mikael Elofsson

Department of Chemistry, Umeå University, SE90187 Umeå, Sweden. *Interfaculty Institute of Biochemistry, University of Tuebingen, 72076 Tuebingen, Germany. **Department of Clinical Microbiology, Division of Virology Umeå University, SE90185 Umeå, Sweden.

Acute hemorrhagic conjunctivitis (AHC) is a severe ocular infection with epidemic potential. Although the infection is self-limited and resolves within 1-2 weeks, patients suffer from various symptoms ranging from eye pain and subconjunctival hemorrhages to even neurological impairments. Despite the occurrence of outbreaks and pandemics, there is no vaccine or antiviral drug available for the prevention or treatment of AHC. Coxsackievirus A24 variant (CVA24v) has been identified as the main causative agent of AHC infection during the last decades. The virus uses sialic acid-based receptors to facilitate host cell attachment and subsequent infection (PLoS Pathog, 2014, 10(10):e1004401). These recent findings open up for the development of antiviral sialic acid conjugates targeting cell entry of CVA24v virions, a strategy that was successfully applied to ocular virus Adenovirus type 37 (OBC, 2015, 13(35):9134-9205). The sialic acid recognition sites, located at solvent exposed protruding regions of the CVA24v capsid, are clustered in a pentameric geometry. With support of molecular modelling, we designed and synthesized a first generation of starfish-like sialic acid conjugates. The compounds inhibit attachment to and infection of human corneal epithelial cells by CVA24v (IC_{50} = 0.12-1.39 mM). In binding inhibition assays, the pentavalent conjugates are three orders of magnitude more potent than sialic acid. Crystal structures of the compounds in complex with CVA24v virions confirmed a pentavalent binding mode. Current efforts are focusing on the optimization of these antiviral sialic acid conjugates by variation of the pentavalent core, linker lengths and the sialic acid residues.

36 Molecular mechanism of TNF-induced sialyl-LewisX expression in human bronchial mucosa and effect on bacterial adhesion.

Florent Colomb1, Marie-Ange Krzewinski-Recchi1, Agata Steenackers1, Audrey Vincent2, Marie Bobowski1, Anne Harduin-Lepers1, Sophie Groux-Degroote1 & Philippe Delannoy1

1Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F-59000 Lille, France; 2Univ. Lille, Inserm, CHU Lille, UMR-S 1172 - JPARC - Jean-Pierre Aubert Research Center, F-59000 Lille, France

Bronchial mucins from severely infected patients suffering from lung diseases such as chronic bronchitis or cystic fibrosis (CF) exhibit increased amounts of sialyl-Lewis^x (NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc-R, sLe^x) glycan structures. In CF, sLe^x and its sulfated form 6-sulfo-sialyl-Lewis^x (NeuAcα2-3Galβ1-4[Fucα1-3](HO3S-6)GlcNAc-R, 6-sulfo-sLex) serve as receptors for Pseudomonas aeruginosa and are involved in the chronicity of airway infection. Number of studies previously focused on the mechanisms responsible for increased sialylation and sLe^x expression in airways from CF patients and we have shown that pro-inflammatory cytokine tumor necrosis factor (TNF) is involved in sLe^x over-expression on mucin-type glycoproteins in human bronchial explants and cell lines. We also indentified ST3Gal IV as the main sialyltransferase involved in sLe^x biosynthesis in human bronchial mucosa (Colomb et al., 2012, Biochimie, 94, 2045-53).

We investigated the signaling pathways involved in TNF-induced sialyltransferase gene ST3GAL4 over-expression in A549 human lung epithelial cells and in human bronchial explants. Our results show that TNF increased the expression of ST3GAL4 transcript isoform BX and sLe^x on glycoproteins through p38/ERK and downstream MSK1/2 pathways. Moreover, the FliD/sLe^x dependent adhesion of P. aeruginosa was increased after TNF treatment, demonstrating the relationship between ST3GAL4 over-expression and bacterial adhesion (Colomb et al., 2014, Biochem J., 457, 79-87).

Finally, we deciphered the molecular mechanism involved in TNF-induced ST3GAL4 gene BX transcript over-expression in the bronchial mucosa and showed the involvement of an intronic ATF2-responsive element located downstream BX transcript transcription start site as a new mechanism of transcriptional regulation for sialyltransferases (Colomb et al., 2016, in revision). The role of ATF2 in the regulation of inflammatory genes makes it a new and attractive target to treat inflammation in lung diseases such as CF or chronic bronchitis.
Visualization and inhibition of Sialic acid transfer in NTHi

Torben Heise¹, Jeroen Langereis², Christian Büll³, Emiel Rossing¹, Gosse J. Adema³, Thomas J. Boltje²

¹Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, AJ Nijmegen, The Netherlands; ²University Medical Center, GA Nijmegen, The Netherlands, Pediatric Infectious Diseases and Immunology, Department of Pediatrics, Radboudumc, Nijmegen, The Netherlands; ³Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud Medical Center, GA Nijmegen, The Netherlands

Sialic acids are a diverse glycan family, highly expressed on every human cell. Among other functions, sialic acids regulate immune cell activation and function therefore serving as safe-signal on human cells. Some pathogens have evolved to abuse this mechanism by either synthesizing or stealing human sialic acids. Non-typeable Haemophilus influenzae (NTHi) is an example for such a pathogen colonizing the respiratory tract causing otitis media in children and COPD in adults. Uptake of Sialic acids leads to complement resistance of NTHi and therefore complicates the etiopathology. Here we report on a Sialic acid transfer-assay using endothelial cells to show the transfer of labeled Sialic acids from human cells to NTHi, including concentration dependent gain of complement resistance. Finally, we were able to design an inhibitor, specific for bacteria to prevent Sialic acid uptake and therefore reduce complement resistance in NTHi.

Identifying the cell-surface glycans that mediate binding of Pertussis toxin

Nicole Nischan, Jennifer J. Kohler

University of Texas Southwestern Medical Center, Dallas, TX, USA

INTRODUCTION. Bordetella pertussis, or whooping cough, is a contagious bacterial disease without available treatment that infects about 16 million people annually worldwide with a mortality of 2% in infants younger than one year. US infections are rising to 1950s levels (41,880 cases in 2012) despite population-wide vaccination, rendering the development of treatment necessary. Reasons for increased infection rates include B. pertussis expressing increased levels of its key virulence factor, pertussis toxin (PT). PT is an AB5-type toxin, that binds to cell surface glycans followed by cellular uptake and host cell intoxication, leading eventually to holding off the host immune response. This work seeks to identify the glycan epitope necessary for cellular binding and uptake of PT, which will allow to develop a PT-neutralizing reagent.

RESULTS. Here, I present my findings on the impact of cell-surface glycans on PT-binding. I modified the cell-surface glycans of bronchial epithelial cells with inhibitors of ganglioside synthesis, the maturation of N-linked glycans, the synthesis of O-linked glycans, as well as fucosylation and sialylation. Subsequently, I assessed the impact on PT-binding with Western Blot, on-cell-ELISA and Flow cytometry. Inhibiting the synthesis of gangliosides or O-linked glycans had no effect on PT-binding. The inhibition of both maturation of N-linked glycans and sialylation leads to a significant reduction of PT binding; these effects are cumulative. Intriguingly, the inhibition of fucosylation leads to a 50% increase of PT binding.

CONCLUSIONS. N-linked glycans and sialic acid are involved in PT-binding. Fucosylation may play a protective role against PT-binding. Additional ongoing efforts focus on probing the impact of cell-surface glycans on cellular uptake and intracellular activity of PT. Fucosidases and neuraminidases need to be used to elucidate further detail about the composition of PT-binding glycans. The identification of PTs binding partners is expected to enable the development of therapeutic agents for perturbing cell intoxication by PT.
Bacterial and mammalian sialic acid analogs attenuate gonococcal infection in mice – design of novel therapeutics based on the CMP-sialic acid scaffold

Ian C. Schoenhofen1, Sunita Gulati2 and Sanjay Ram2

1Human Health Therapeutics Portfolio, National Research Council of Canada, Ottawa, ON, Canada; 2Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA

Neisseria gonorrhoeae (Ng) is the causative agent of the sexually transmitted infection gonorrhea – where multidrug-resistant isolates are posing a global public health problem. Ng deploys a unique immune evasion strategy wherein the lacto-N-neotetraose (LNnT) structure of lipooligosaccharide (LOS) is capped via its sialyltransferase Lst, using host derived CMP-Neu5Ac or CMP-sialic acid. This sialic acid capping allows evasion of complement mediated killing by recruiting factor H, an inhibitor of the alternative complement pathway, and by limiting classical pathway activation resulting in serum resistance. Previously, we have shown that the CMP-activated bacterial sialic acid analog CMP-Leg5,7Ac2 (variations in the C7-C9 exocyclic moiety relative to Neu5Ac) serves as a substrate for Ng Lst, where Leg5,7Ac2 LOS incorporation results in Ng being fully serum-sensitive. Importantly, administration of CMP-Leg5,7Ac2 intravaginally significantly reduced the duration of infection and bacterial burden in the BALB/c mouse vaginal colonization model, even when testing multidrug-resistant isolates. In contrast, the CMP-activated mammalian sialic acid analog CMP-Neu5Gc (variation at C5 relative to Neu5Ac), while also serving as a gonococcal Lst substrate, results in high level serum resistance and Ng survival similar to the ‘protection’ afforded by CMP-Neu5Ac. Surprisingly, we have now identified a CMP-sialic acid C5 variant, CMP-KDN or CMP-3-deoxy-D-glycero-D-galacto-nonulosonic acid, that not only serves as a substrate for Ng Lst, but leaves Ng fully serum sensitive. KDN is found in organisms from bacteria to vertebrates with trace amounts present in mammals/humans. Similar to results obtained for CMP-Leg5,7Ac2 using the BALB/c mouse model, administration of CMP-KDN significantly reduced Ng clearance times and infection burden. It is not yet clear why the acetamido (Neu5Ac) and hydroxyacetamido (Neu5Gc) structural variations at C5 afford protection to Ng, whereas the hydroxyl variant (KDN) does not. The implications of these findings will be elaborated upon in the context of a therapeutic/preventative strategy to control antimicrobial resistant Ng.

Asn347-Sialoglycans of Corticosteroid-Binding Globulin Fine-tune the Human Immune Response by Modulating Proteolysis by Host and Pathogen Elastase

Zeynep Sumer-Bayraktar1, Jodie L. Abrahams1, Vignesh Venkatakrishnan1, Oliver C. Grant2, Robert J. Woods2, Nicolle H. Packer1, Morten Thaysen-Andersen1

1Macquarie University, Sydney, Australia, 2Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

Corticosteroid-binding globulin (CBG) is a NeuAc-rich serum glycoprotein which delivers anti-inflammatory cortisol to inflamed tissues upon elastase-based proteolysis of the exposed reactive center loop (RCL). However, the molecular mechanisms that regulate the RCL proteolysis by coexisting host and bacterial elastases in inflamed/infected tissues remain unknown. LC-MS/MS-based site-specific characterization demonstrated that the RCL-localized Asn347-sialoglycans of human CBG are highly branched heterogeneous structures with terminal α2,3- and α2,6-sialylation. We here document that the Asn347-sialoglycans fine-tune the RCL cleavage rate by human neutrophil elastase (NE) and Pseudomonas aeruginosa elastase (PAE) by different mechanisms (Sumer-Bayraktar et al., J Biol Chem, 291(34):17727, 2016). NE- and PAE-generated fragments of native and exoglycosidase-treated blood-derived CBG of healthy individuals were monitored by gel electrophoresis and LC-MS/MS to determine the cleavage site(s) and Asn347-glycosylation as a function of digestion time. The site-specific (Val344-Thr345) and rapid (seconds-to-minutes) NE-based RCL proteolysis was significantly antagonized by several volume enhancing Asn347-glycan features (i.e. occupancy, triantennary GlcNAc branching, and α1,6-fucosylation) and augmented by Asn347 sialylation (all p < 0.05). In contrast, the inefficient (minutes-to-hours) PAE-based RCL cleavage, which occurred equally well at Thr345-Leu346 and Asn347-Leu348, was abolished by the presence of Asn347-glycosylation but was enhanced by sialoglycans on neighboring CBG N-sites. Molecular dynamics simulations of various Asn347-sialoglycoforms of uncleaved CBG indicated that multiple Asn347-glycan features are modulating the RCL digestion efficiencies by NE/PAE. Interestingly, high concentrations of cortisol showed bacteriostatic effects toward virulent P. aeruginosa, which may explain the low RCL potency of the abundantly secreted PAE during host infection. Finally, structural examination of the RCL localized sialoglycans in the homologous rat CBG revealed significant NeuAc O-acetylation indicating an addition level of molecular regulation in rats. In conclusion, we show that site-specific CBG N-sialoglycans regulate the bioavailability of cortisol in inflamed environments by fine-tuning the RCL proteolysis by endogenous and exogenous elastases.
41 Sialic acid biosynthesis in microalgae and a potential role in viral infection

Ben A. Wagstaff¹, Gill Malin² and Robert A. Field¹

¹John Innes Centre, Norwich Research Park, Norwich, UK; ²University of East Anglia, Norwich Research Park, Norwich, UK

Prymnesium parvum is a toxin-producing Haptophyte that causes harmful algal blooms globally, leading to large scale fish kills that have severe ecological and economic implications. The biosynthesis and subsequent release of these toxins remains a mystery, but emerging evidence within other clades of microalgae suggests viral lysis of algal cells may play a crucial role in toxin release. For the model organism of the Haptophytes, Emiliania huxleyi, it has been shown that cell surface sialic acids are fundamental in regulating these virus-alga interactions¹, but little work has been done looking at viruses of P. parvum, or the biosynthesis of sialic acids in microalgae. This work reports the isolation of a lytic virus of P. parvum from natural water samples, as well as the discovery and characterization of a biosynthetic pathway from P. parvum leading to the CMP-activated sialic acid, 2-keto-3-deoxy-d-glycero-d-galacto-nononic acid (KDN). Follow up phylogenetic analysis suggests sialic acid biosynthesis is much more widespread in microalgae than previously believed.


42 Distribution of O-acetylated sialic acids in influenza host respiratory tissues

Brian R Wasik, Karen N Barnard, Colin R Parrish

Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA

Sialic acids (Sias) posses a unique chemistry allowing for vast modified forms being found in nature. The relative display and distribution of modified Sias in varied organisms is fairly unknown due to limited molecular tools. We have developed a robust toolkit of recombinant 'virolectin' Sia probes (viral glycoprotein fused to the Fc region of human IgG1) for detection of specific Sia forms. Nidovirus HEs are specific for O-acetyl Sia modifications: MHV-S for 4-O-Ac, BCoV-Mebus for 7,9-O-Ac, PToV-P4 for 9-O-Ac. In particular, we are interested in the role of O-acetylated Sia forms on influenza viruses. Some modified Sias have been identified as infection inhibitors (horse or guinea pig serum, Neu4,5Ac) or as negative regulators on NA efficiency (Neu5,9Ac). Influenza C and D strains utilize 9-O-Ac modified Sia as primary receptors. Therefore, modified Sias may contribute to evolved Hemagglutinin (HA) and Neuraminidase (NA) specificities seen between viral strains and different hosts. We used these virolectin probes to perform an in situ histological survey of modified Sias in respiratory tissues of multiple influenza natural and laboratory hosts, including duck, human, pig, horse, dog, guinea pig, and ferret. Our survey confirms the presence of O-acetyl modified Sias in host respiratory tissues with varied patterns of distribution. The 4-O-Ac Sia modification is significantly enriched in horse and guinea pig tissues, but can be detected in other hosts, including avian (duck). The 7,9- and 9-O-Ac Sias are more broadly distributed across host species and tissues, including in human respiratory tissues. Confirming the presence of modified Sias in host respiratory tissues justifies the need for experimental systems to understand their effects on influenza. Having identified modified Sias in major cell culture lines used for influenza studies, we are currently developing 'glyco-engineered' cell lines for the direct investigation of modified Sias and influenza infection.
**High-efficiency capture of drug resistant-influenza virus by live imaging of sialidase activity**

Yuuki Kurebayashi¹, Tadanobu Takahashi¹, Chihiro Tamoto¹, Keiji Sahara², Tadamune Otsubo³, Tatsuya Yokozawa³, Nona Shibahara¹, Hirohisa Wada⁴, Akira Minami¹, Kiyoshi Ikeda⁴, Takashi Suzuki¹

¹Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka-shi, Shizuoka, Japan; ²Shizuoka Institute of Environment and Hygiene, Shizuoka-shi, Shizuoka, Japan; ³Department of Organic Chemistry, School of Pharmaceutical Sciences, Hiroshima International University, Kure-shi, Hiroshima, Japan; ⁴Shizuoka City Institute of Environmental Sciences and Public Health, Shizuoka-shi, Shizuoka, Japan

Influenza A and B viruses possess a neuraminidase protein that shows sialidase activity. Influenza virus-specific neuraminidase inhibitors (NAIs) are commonly used for clinical treatment of influenza. However, some influenza A and B viruses that are resistant to NAIs have emerged in nature. NAI-resistant viruses have been monitored in public hygiene surveys and the mechanism underlying the resistance has been studied. Here, we describe a new assay for selective detection and isolation of an NAI-resistant virus in a speedy and easy manner by live fluorescence imaging of viral sialidase activity, which we previously developed (1, 2), in order to achieve high-efficiency capture of an NAI-resistant virus. An NAI-resistant virus maintains sialidase activity even at a concentration of NAI that leads to complete deactivation of the virus. Infected cells and focuses (infected cell populations) of an oseltamivir-resistant virus were selectively visualized by live fluorescence sialidase imaging in the presence of oseltamivir, resulting in high-efficiency isolation of the resistant viruses. The use of a combination of other NAIs (zanamivir, peramivir, and laninamivir) in the imaging showed that the oseltamivir-resistant virus isolated in 2008 was sensitive to zanamivir and laninamivir but resistant to peramivir. Fluorescence imaging in the presence of zanamivir also succeeded in selective live-cell visualization of cells that expressed zanamivir-resistant NA (3). Fluorescence imaging of NAI-resistant sialidase activity will be a powerful method for study of the NAI resistance mechanism, for public monitoring of NAI-resistant viruses, and for development of a new NAI that shows an effect on various NAI-resistant mutations.

References:

**Cell Surface Glycoprotein Aging and Turnover Modulates a Constitutive Anti-Inflammatory Mechanism of Host Protection that is Progressively Disabled by a Foodborne Pathogen**

Won Ho Yang, Douglas M. Heithoff, Peter V. Aziz, Markus Sperandio, Victor Nizet, Michael J. Mahan, and Jamey D. Marth

Center for Nanomedicine, Sanford-Burnham-Prebys Medical Discovery Institute, University of California-Santa Barbara, Santa Barbara, California, USA

Intestinal inflammation is the central pathological feature of colitis and the inflammatory bowel diseases. These syndromes arise primarily from unidentified environmental factors. We have discovered that sub-lethal oral infection of mice with *Salmonella enterica* Typhimurium (*ST*), a major pathogen source of human food poisoning, caused a progressive inflammation of the intestinal tract that persisted following pathogen clearance and escalated with recurrent infections. In this model of human food poisoning, *ST* infection disabled a previously unknown protective mechanism in the host that maintains intestinal alkaline phosphatase (IAP) function. The lipopolysaccharide endotoxin present in Gram-negative bacterium such as *ST* recapitulated the induction of host Neu3 neuraminidase by *ST*, and thereby accelerated the molecular aging and turnover of nascent IAP at the enterocyte cell surface. Uninfected mice genetically deficient in the ST3Gal-VI sialyltransferase and resulting IAP sialylation exhibited similar inflammatory disease signs. Oral administration of IAP or the neuraminidase inhibitor Zanamivir was therapeutic with reduction and elimination of disease signs of intestinal inflammation and tissue damage. These discoveries reveal that an environmental pathogen disrupts a constitutive host anti-inflammatory mechanism linked to the regulation of cell surface IAP glycoprotein aging and turnover.
Amino acid substitutions contributing to α2,6-sialic acid linkage binding specificity of human parainfluenza virus type 3 hemagglutinin-neuraminidase

Keijo Fukushima¹, Tadanobu Takahashi¹, Hiroo Ueyama¹, Masahiro Takaguchi¹, Seigo Ito¹, Kenta Oishi¹, Akira Minami¹, Erika Ishitsubo², Hiroaki Tokiwa², Toru Takimoto³, Takashi Suzuki¹

¹Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 4228526, Japan; ²Department of Chemistry, Faculty of Science, Rikkyo University, Tokyo 1718501, Japan; ³Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, USA

Human parainfluenza virus type 3 (hPIV3) and type 1 (hPIV1) are respiratory pathogens that cause laryngotracheobronchitis and bronchiolitis in infants and young children. hPIV1 and hPIV3, which belong to the genus Respirovirus in the family Paramyxoviridae, have two envelope glycoproteins, hemagglutinin-neuraminidase (HN) glycoprotein and fusion glycoprotein. Both hPIVs bind to terminal sialic acids of glycoconjugates on the host cell surface through the HN glycoprotein to initiate infection.

Our previous studies showed that hPIV1 and hPIV3 have different recognition specificities for terminal sialyl linkages: hPIV1 and the hPIV1 HN (HN1) glycoprotein preferentially recognize α2,3-linked sialic acids, whereas hPIV3 and the hPIV3 HN (HN3) glycoprotein recognize both α2,3- and α2,6-linked sialic acids on the host cell surface (1,2). Elucidation of the mechanism of sialyl linkage recognition is important for understanding cell and tissue tropism of hPIVs. To identify amino acid residues that confer α2,6-linked sialic acid recognition of hPIV3, amino acid residues in or neighboring the sialic acid binding pocket of the HN3 glycoprotein were substituted for the corresponding residues of hPIV1 HN. Hemadsorption assay with sialyl linkage-modified red blood cells indicated that amino acid residues at positions 275, 277, 372, and 426 contribute to α2,6-linked sialic acid recognition of the HN3 glycoprotein (3).

References:

Synthesis of Lipooligosaccharide of C. jejuni Associated with Gullain-Barré Syndrome

Hiroki Yoshinaka¹, Akihiro Imamura¹, Hiromune Ando¹, Hideharu Ishida¹, Makoto Kiso¹

¹Dept. of Applied Bioorganic Chemistry, Gifu University, Japan; ²iCeMS, Kyoto University, Japan

Gullain-Barré syndrome (GBS), which can frequently cause acute neuromuscular paralysis, is one of autoimmune diseases. Pathogenesis of GBS is thought to be associated with intestinal infection with the Gram-negative bacterium Campylobacter jejuni since one of C. jejuni strains, Penner’s serotype 19 (PEN 19), which has been very frequently isolated from patients with GBS. It has been suggested that molecular mimicry of the lipopolysaccharide (LPS) of C. jejuni (PEN 19) by the myelin ganglioside GM1/GD1a would play a crucial role for pathogenesis of the disease. Significantly, the carbohydrate structure in its LPS is identical to the non-reducing end oligosaccharide sequence of GM1/GD1a. To clarify the structural relationship between the LPS of PEN 19 and autoantibody production against the peripheral nerves ganglioside GM1/GD1a, detailed structural analysis of the carbohydrate epitope would be imperative. For the purpose, enough amounts of pure carbohydrate epitope are strongly desired. We report here the synthesis of lipooligosaccharide (LOS) as part of the LPS by chemical approach based on the convergent synthetic strategy. The target oligosaccharide was disconnected as three major fragments: 1) GD1a-core pentasaccharide, 2) L-glycero-d-manno-heptose-containing trisaccharide, 3) 3-deoxy-d-manno-2-octulosonic acid (KDO). These fragments were in turn assembled, affording the framework of the target oligosaccharide.
47  **High Affinity Siglec Ligand Expressing Cells (HASLECs) Redirect the Immune Response**

Christian Büll¹,⁴, Torben Heise²,⁴, Niek van Hilten³, Johan Pijnenborg⁶, Victor Bloemendal⁶, Lotte Gerrits⁷, Esther D. Kers-Rebel¹, Tina Ritschel³, Martijn H. den Brok¹, Gosse J. Adema¹,⁵ & Thomas J. Boltje²,⁵

¹Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Geert Grootplein 28, 6525 GA Nijmegen, The Netherlands
²Cluster for Molecular Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
³Computational Discovery and Design Group, Centre for Molecular and Biomolecular Informatics, Radboud University Medical Center, 6500 HB Nijmegen, The Netherlands.
⁴,⁵These authors contributed equally

Sialic acids are cell surface glycans and are recognized by immunoregulatory receptors called sialic acid-binding immunoglobulin-like lectins (Siglec). This recognition is essential to maintain immune homeostasis and aberrations in Siglec and/or sialic acid expression have been linked to pathological conditions such as cancer and autoimmunity. SiglecS are therefore interesting therapeutic targets. Herein we report a simple and fast method to create High Affinity Siglec Ligand Expressing Cells (HASLECs). Clickable alkyne or azide sialic acids were metabolically incorporated into glycans of living cells and reacted with a library of azide or alkyne ligands. The thereby modified cells showed dramatically increased binding towards the different Siglec family members. Rational design was used to reduce cross-reactivity and led to new, selective Siglec-5 ligands. Using this approach, HASLECs optimized to bind Siglec-3 (CD33) were able to dampen the activation of Siglec-3+ monocyte cells by LPS, thereby revealing the therapeutic potential.

48  **Induction of Siglec-1 by Endotoxin Tolerance Suppresses the Innate Immune Response by Promoting TGF-β1 Production**

Yin Wu, Chao Lan, Dongren Ren, Guo-Yun Chen

Children's Foundation Research Institute, Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN 38103

Sepsis is one of the leading causes of death worldwide. Although the prevailing theory for the sepsis syndrome is a condition of uncontrolled inflammation in response to infection, sepsis is increasingly being recognized as an immunosuppressive state known as endotoxin tolerance. We found sialylation of cell surface was significantly increased on LPS-induced tolerant cells; knockdown of Neu1 in macrophage cell line RAW 264.7 cells resulted in enhanced LPS-induced tolerance, whereas overexpression of Neu1 or treatment with sialidase abrogated LPS-induced tolerance, as defined by measuring TNF-α levels in the culture supernatants. We also found that the expression of Siglec-1 (a member of sialic acid-binding Ig (I)-like lectin family members, the predominant sialic acid binding proteins on cell surface) was specifically up-regulated in endotoxin tolerant cells and the induction of Siglec-1 suppresses the innate immune response by promoting TGF-β1 production. The enhanced TGF-β1 production by Siglec-1 was significantly attenuated by spleen tyrosine kinase (Syk) inhibitor. Knockdown of siglec-1 in RAW 264.7 cells resulted in inhibiting the production of TGF-β1 by ubiquitin-dependent degradation of Syk. Mechanistically, Siglec-1 associates with adaptor protein DNAX-activation protein of 12kDa (DAP12) and transduces a signal to Syk to control the production of TGF-β1 in endotoxin tolerance. Thus, Siglec-1 plays an important role in the development of endotoxin tolerance and targeted manipulation of this process could lead to a new therapeutic opportunity for patients with sepsis.
49  Siglec-8 engagement causes death of human eosinophils via mechanisms that differ depending on priming status but involve phosphatase activity and mitochondrial ROS production.  

Daniela Janevksa, Jeremy A. O’Sullivan, Yun Cao, Bruce S. Bochner.  
Division of Allergy-Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, USA

Eosinophils are major effector cells in allergic and other related inflammatory diseases. Siglecs are type I transmembrane proteins expressed primarily on leukocytes. Among them is Siglec-8, a CD33 subfamily member that is selectively expressed on the surface of human eosinophils. Siglec-8 has an intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM), putatively responsible for signal transduction, but their specific contributions to signaling remain unclear. In cytokine-primed eosinophils, Siglec-8 binding causes apoptosis with increased ROS production, but how priming and Siglec-8 engagement influences ROS generation and cell death are unknown. Using a monoclonal antibody (2C4) against Siglec-8, we first examine potential differences in Siglec-8 signaling in the presence or absence of IL-5 priming.

Cytokine priming of eosinophils involves the recruitment of SHP-2, c-Abl, and mitochondrial ROS production. After Siglec-8 cross-linking of IL-5 primed eosinophils with 2C4 showed increased phosphorylation of c-Abl that was observed over 2 h in IL-5 primed cells exposed to 2C4 compared to a more transient phosphorylation in eosinophils exposed to 2C4 after being cultured overnight without IL-5, suggesting that IL-5 priming recruits additional molecules that contribute to Siglec-8 signaling in eosinophils. Additional total phosphotyrosine western blot analysis following Siglec-8 cross-linking of IL-5 primed eosinophils with 2C4 showed increased phosphorylation of c-Abl that was detectable within 60 min.

Coimmunoprecipitation data showed that Siglec-8 associates with SHP-2, a protein tyrosine phosphatase, but not SHP-1 or SHIP, and 2C4-induced eosinophil apoptosis was inhibited by pretreatment with sodium orthovanadate (a protein phosphatase inhibitor) or mitoTempo (a specific mitochondrial ROS scavenger) with IC50’s of 22 pM and 1.3 mM respectively, suggesting that SHP-2 is functionally involved in signaling and that the source of ROS production following Siglec-8 engagement is the mitochondria. These data suggest that Siglec-8-mediated apoptosis in cytokine primed eosinophils involves the recruitment of SHP-2, c-Abl, and mitochondrial ROS production.

50  Loss of CMAH During Human Evolution Primed the Monocyte-Macrophage Lineage Towards a More Inflammatory and Phagocytic State

Jonathan Okerblom1,2, Flavio Schwarz1,2, Josh Olson3, William Fletes1,4, Syed Raza Ali1,3, Paul T. Martin5, Chris Glass1,2, Victor Nizet1,3, Ajit Varki1,2

1Glycobiology Research and Training Center, 2Departments of Medicine and Cellular and Molecular Medicine, 3Department of Pediatrics and Skaggs School of Pharmacy and Pharmaceutical Sciences, 4Initiative for Maximizing Student Development (IMSD) Program, University of California, San Diego, CA, USA 5Departments of Pediatrics, Physiology and Cell Biology, Ohio State University College of Medicine, Columbus, OH, USA and Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA

Humans and chimpanzees are more sensitive to endotoxin than mice or monkeys, but any underlying differences in inflammatory physiology have not been fully described or understood. We studied innate immune responses in Cmah+/+ mice, emulating human loss of the gene encoding production of Neu5Gc, a major cell surface sialic acid. CMAH loss occurred ~2-3 million years ago, after the common ancestor of humans and chimpanzees, perhaps contributing to speciation of the genus Homo. Cmah+/+ mice manifested a human-like decreased survival in endotoxemia following bacterial lipopolysaccharide (LPS) injection. Macrophages from Cmah+/+ mice secreted more inflammatory cytokines with LPS-stimulation and showed more phagocytic activity. Macrophages and whole blood from Cmah+/+ mice also killed bacteria more effectively. Metabolic re-introduction of Neu5Gc into Cmah+/+ macrophages suppressed these differences. Cmah+/+ mice also showed enhanced bacterial clearance during sub-lethal lung infection. Although monocytes and monocyte-derived macrophages from humans and chimpanzees exhibited marginal differences in LPS responses, human monocyte-derived macrophages killed E. coli and ingested E. coli bioparticles better. Metabolic re-introduction of Neu5Gc into human macrophages suppressed these differences. While multiple mechanisms are involved, one cause is altered expression of C/EBPβ, a transcription factor affecting macrophage function. Loss of Neu5Gc in Homo likely had complex effects on immunity, providing greater capabilities to clear sub-lethal bacterial challenges, at the cost of endotoxic shock risk. This trade-off may have provided a selective advantage when Homo transitioned to butchery using stone tools. The findings may also explain why the Cmah null state alters severity in mouse models of human disease.
51 Characterization of a new transgenic mouse strain in which human Siglec-8 is engineered to be selectively expressed in the eosinophil compartment

Jeremy A. O'Sullivan¹, Daniela Janevsk¹, Liliana Moreno-Vinasco¹, Yun Cao¹, Yadong Wei², Fengrui Zhang³, James. J. Lee³, Zhou Zhu³, and Bruce S. Bochner¹

¹Division of Allergy-Immunology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; ²Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, USA; ³Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ, USA

Sialic acid-binding immunoglobulin-like lectin (Siglec)-8 is a human cell surface protein expressed on eosinophils, mast cells, and basophils that, when engaged, induces eosinophil apoptosis and inhibits mast cell mediator release. This makes Siglec-8 a promising target to treat diseases involving these cell types. However, in vivo studies of the effects of Siglec-8 targeting are lacking due to the fact that this protein is only found in humans and chimpanzees. To rectify this, we have developed a new mouse strain in which human Siglec-8 is selectively expressed in the eosinophil compartment. The SIGLEC8 gene and an upstream loxP-flanked STOP cassette were introduced by homologous recombination into the ROSA26 locus. When crossed with mice expressing Cre recombinase driven by the eosinophil peroxidase promoter (EPX-Cre), the STOP cassette is removed and Siglec-8 is expressed in these cells. To initially characterize this new strain, we examined Siglec-8 expression, endocytosis, and signaling. By flow cytometry, all eosinophils examined (from peripheral blood, spleen, peritoneal lavage, bone marrow, and gut lamina propria) and only eosinophils expressed Siglec-8 in these mice. Siglec-8 was found to be expressed on the surface of bone marrow-derived eosinophils shortly after Siglec-F and well before the late eosinophil developmental marker CCR3, consistent with the timing of eosinophil peroxidase expression during eosinophil development. In response to antibody ligation, Siglec-8 was internalized more rapidly than Siglec-F, and this ligation was found to alter the phosphotyrosine profile of these cells, indicative of ligand-induced signaling. No changes in baseline blood or tissue eosinophil numbers were evident compared to littermate controls. Thus, this mouse strain should be a suitable tool to investigate Siglec-8 targeting in vivo in a variety of eosinophilic disease settings. By enabling more facile manipulation compared to human eosinophils, this mouse strain may also shed new light on other aspects of Siglec-8 biology.

52 Induction of Antigen-Specific B Cell Tolerance with Antigenic Nanoparticles

Lijuan Pang, Matthew S. Macauley, Britni Arlian Cruz, Corwin Nycholat, James C. Paulson

The Scripps Research Institute, La Jolla, CA, USA

Undesired immune responses to protein antigens are responsible for numerous medical conditions, and have a profound impact on human health. Current therapeutic options for the treatment and control of pathological immunogenic responses largely rely on immunosuppressive drugs that can compromise immunity to opportunistic infections, lead to reactivation of latent pathogens, or development of tumors [1]. Targeting antigen-reactive B cells offers a direct approach for systematic induction of humoral tolerance to the desired antigens. Siglec-engaging tolerance-inducing antigenic liposomes (STALs) have been developed and optimized to force the co-localization of the inhibitory receptors CD22 with BCR and to induce B cell tolerance by inducing apoptosis of the antigen specific B cells [2]. While STALs can induce antigen-specific B cell tolerance, addressing the antigen-specific T cells is expected to lead to more profound tolerance and potentially enable STALs to be efficacious in sensitized mice. To strengthen the inhibition potency of STALs, an immunomodulator, rapamycin, was encapsulated into STALs. Numerous formulations of STALs encapsulated with rapamycin have been tested in mice for their ability to induce immunological tolerance. Here the results have shown that STAL formulated with rapamycin can lead to more robust tolerance, and such formulations are a promising platform for the induction of antigen-specific B cell tolerance and the treatment of undesired immune responses.

Supported by SNF Fellowship Programme P2BSP3_151887 (LP) and NIH Grants R01 AI099141 & R01 AI050143 (JCP)

Targeting of Hematopoietic Cells With Glycan Ligands of Siglecs
Amrita Srivastava and James C Paulson
The Scripps Research Institute, La Jolla, CA, USA

Sialic acids, a group of sugars with nine carbon backbone, are present ubiquitously as a terminal sugars on the glycan chains of glycoconjugates on the cell surface. Due to their unique location, they can mediate diverse physiological processes. Siglecs, sialic acid –binding immunoglobulin-type lectins, recognize sialic acid containing glycans as ligands and modulate the activity of cell signaling receptors. There are fourteen human and nine murine Siglecs, which have been identified so far, most being expressed in cells of the immune system. In an effort to understand their natural role, various high affinity glycan ligands specific for individual members of the Siglec family have been developed in our laboratory. Targeted drug delivery to various Siglec expressing cells can be efficiently achieved by using nanoparticles decorated with these ligands. For example, we have successfully delivered doxorubicin to B lymphoma cells using CD22-targeted liposomal nanoparticles, and developed antigenic nanoparticles that induce tolerance to specific antigens in vivo. Despite of their various advantages, these liposomal nanoparticles have several limitations such as large-scale manufacturing for human use and encapsulation of chemotherapeutics other than doxorubicin.

In order to overcome these limitations, we have developed, biodegradable and biocompatible Siglec targeted PLGA and PLGA lipid hybrid nanoparticles as an alternative nanoparticle platform. PLGA lipid hybrid nanoparticles consisting of a polymeric core and a lipid shell possess the characteristics of both liposomes and nanoparticles. Targeted antigenic PEG-PLGA and hybrid nanoparticles with glycan ligands Siglec (e.g. CD22) have been formulated, and optimized for binding to cell lines expressing the respective targeted Siglec. These antigenic PLGA and hybrid nanoparticles bind to human Daudi B cells, inhibit Ca++ flux in HEL specific murine B cells. Details of their preparation, characterization and in vitro analysis will be presented (NIH grants AI050143, AI099141).

References:

Comprehensive study of α2,8-sialylation role during activation of human naïve CD4+ T cells
Villanueva-Cabello T1,2, Martínez-Duncker I1
1Laboratorio de Glicobiología Humana, Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, México; 2Instituto de Biotecnología-Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México.

Human CD4+ T cells organize the adaptive immune response after being activated by antigen presenting cells. Activation of these cells is characterized by specific cytokine secretion but also by glycosylation changes that affect their functions and homeostasis [1]. During activation, downregulation of ST3Gal I and ST6Gal I sialyltransferases that transfer sialic acid (Sia) in α2,3 and α2,6 positions to galactose of glycoconjugates results in an asialophenotype defined by reduced SNA (α2,6-Sia) and MAL II (α2,3-Sia) lectin binding with increased binding of PNA lectin (asialo Core-1-O-glycans) [2,3]. We previously reported by metabolic labeling using ManNAz that cell surface SiaNAz de novo incorporation in human naïve CD4+ T cells was increased during activation in presence of anti-CD3/anti-CD28 monoclonal antibodies [4]. This finding shows a possible upregulation of surface α2,8-Sia glycoconjugates during naïve CD4+ T cell activation. Analysis of the six human α2,8-sialyltransferases by RT-PCR showed overexpression of ST8Sia 1, ST8Sia 2 and ST8Sia 4 during CD4+ T cell activation. The ST8Sia 1 is accountable for ganglioside α2,8-sialylation whereas ST8Sia 2 and ST8Sia 4 polysialyltransferases would allow glycoprotein polysialylation that has not been reported in these cells. In this work we describe the expression of α2,8-polysialylated glycoconjugates by using anti-polysialic acid antibody during CD4+ T cell activation. In addition we demonstrate that the shRNA silencing of ST8Sia 2 and ST8Sia 4 promotes a robust cytokine production revealing the important role of 2,8-sialylation during CD4+ T cells activation.
55  **9-Azido analogs of three sialic acid forms for metabolic remodeling of cell-surface sialoglycans**

*Bo Cheng, Lu Dong, Yuntao Zhu, Rongbing Huang, Yuting Sun, Xing Chen*

*College of Chemistry and Molecular Engineering, Peking University, Beijing, China*

Neu5Ac, Neu5Gc and KDN are three basic forms of sialic acids in vertebrates. We reported the synthesis and metabolic incorporation of the 9-azido analogs of three sialic acid forms in mammalian cells. The sialic acid probes installed with azido tag were used to remodel cell-surface glycoconjugates through metabolic glycan incorporation and evaluated for glycoproteomics study. Click chemistry assisted flow cytometry and quantitative HPLC analysis showed that 9AzNeu5Ac and 9AzNeu5Gc are significantly incorporated into cellular glycoconjugates, while 9AzKDN was a poor but definitely metabolizable sialic acid probe for metabolic glycan labeling in mammalian system. Ours results demonstrated mammalian cells incorporate 9-azido sialic acid probes bearing varying C-5 substitution with different efficiency, indicating the implication of C-5 position in unnatural sialic acid metabolism.

56  **Liposome assisted in vivo metabolic labeling of sialoglycans in the mouse brain.**

*Yifei Du, Ran Xie, Lu Dong, Yuntao Zhu, Rui Hua, Chen Zhang, and Xing Chen*

*Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China*

Sialoglycans are highly enriched in mammalian brains, which tightly involves in brain development and disease progression. However, it is still challenging for in vivo labeling and visualization of sialoglycans in the mouse brain due to the blood-brain barrier. Here we report a liposome-assisted bioorthogonal reporter (LABOR) strategy to shuttle a sialic acid reporter, 9-azido sialic acid (9AzSia), into the mouse brain for metabolic labeling of sialoglyco-conjugates, including sialylated glycoproteins and glycolipids. Subsequent bioorthogonal ligation of fluorescent probes to the incorporated 9AzSia enabled visualization of sialoglycans both in brain slices and in living animals. This method enabled us to monitor the distribution of newly synthesized sialoglycans, which was found to be widely distributed on neuronal cell surfaces and in particular at synaptic parts. A large-scale proteomic profiling which identified 140 brain sialylated glycoproteins also identified a wealth of synapse-associated proteins. Furthermore, by taking advantage of metabolic labeling, the dynamic sialylation of diverse brain regions could be visualized by performing a pulse-chase experiment. The dynamic sialylation was found to be spatially regulated, and that turnover of sialoglycans in the hippocampus is significantly slower than that in other brain regions. The LABOR strategy provides a way to directly visualize and monitor the sialoglycan biosynthesis in the mouse brain and will facilitate elucidating the functional role of brain sialylation.
Human breast milk is a rich and complex medium considered the gold standard in infant nutrition. It contains a large number of oligosaccharides (~200 structures), approximately ten times more than bovine milk. Other than lactose, human milk oligosaccharides (HMOs) are not metabolised by the new born gut, but are reported to exert beneficial effects on the development of the intestinal microbiota and the mucosal immune system. HMOs are produced in the mammary gland and regulated by the expression of glycosyltransferases. The acidic fraction of HMOs contains sialylated oligosaccharides. The large amount of sialic acid in human compared to bovine milk is believed to be an important factor in infant brain development. Sialyltransferases (SIATs) are glycosyltransferases that transfer sialic acid to a Gal, GalNAc or Sia sugar acceptor on glycoproteins, glycolipids or oligosaccharides. Four families of SIATs share a common ancestral origin where gene duplication and mutation have resulted in specificity for different sugar acceptor substrates. This project investigates the synthesis from lactose of the two main sialylated HMOs, 3' - and 6'-sialyllactose, using sialyltransferases ST3Gal and ST6Gal respectively. Because the biochemical properties of enzymes are affected by their environments, ST3Gal and ST6Gal were sourced and cloned from various species encompassing the metazoan tree of life. Stable CHO cell lines were engineered to express these novel sequences. Expression of SIATs in transfected CHO cells was confirmed by qPCR, lectin staining and western blotting. Affinity purified enzymes show strong activity in a phosphate-linked sialyltransferase assay. Sequencing identified both silent and missense mutations in SIAT genes isolated from some species and especially those isolated from human cell lines. Enzymes show temperature optimal activities based on the environmental origins. HPLC confirmed synthesis of the sialyllactoses.

A novel method for the synthesis of α-sialosides using a newly developed C5-ureido-modified sialyl donor is reported. The donor was found to be useful for α-selective sialidation with various glycosyl acceptors, forming α(2,6)Glc, α(2,6)Gal, and α(2,3)Gal linkages in excellent yield and with stereoselectivity. It was also found that the IBr-AgOTf and p-NO2PhSCI-AgOTf promoter systems were appropriate to activate the donor. Furthermore, C5-ureido functionality-specific 1,5-lactamization enabled specific isolation of the α-sialoside from the reaction mixture after sialidation. Successful application of the C5-ureido sialyl donor to the synthesis of a sialoside confirmed the usefulness of the present method.

Almost all membrane proteins are post-translationally modified with glycans. It is difficult to analyze the function of each glycan because of its structure diversity and heterogeneity. In this study, we displayed the synthetic glycan on living cell surface using HaloTag technology [1] and analyzed its function. HaloTag is the tag protein that can smoothly form covalent bond with a halo alkane (HaloTag ligand).

As the target, we chose Asn linked glycan (N-glycan). We planned to analyze the dynamics of the membrane proteins modified with N-glycans. For this study, we synthesized the HaloTag ligand with fluorescence unit and N-glycan. The HaloTag ligand was then successfully introduced to the HaloTag to display the membrane protein model modified with N-glycan on cells. The behavior of this glycan modified membrane protein are now investigating by fluorescence recovery after photobleaching (FRAP) system with confocal microscopy.

We also planned to display a glycolipid model to analyze the interaction between glycolipids and various pathogens. We synthesized the HaloTag ligand possessing glycan part of Gb3 and introduced to the HaloTag expressed cells. The affinity and cytotoxicity of Shiga toxin was evaluated using the cells displaying Gb3 glycolipid model. In order to improve the affinity to Shiga toxin by using multivalency effect, we synthesize the Gb3 glycodendrimer using dendrimeric polylysine core.

Lectin-based protein microarray analysis of differences in glycosylation of various prospective colorectal cancer biomarkers in different types of samples

Martina Zámorová1, Alena Holazová1, Goran Miljušc, Dragana Robajacć, Miloš Šunderićć, Vesna Malenković3, Blagoe Đukanović1, Peter Gemeiner1, Olgica Nedić2, Jaroslav Katrlík1

1Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia; 2Institute for the Application of Nuclear Energy (INEP), University of Belgrade, Serbia; 3Clinical-Medical-Center „Bežanijska kosa“, Belgrade, Serbia

Glycomic phenotype offers a general overview of the glycosylation status of glycoconjugates and it may also indicate the trend in changes of glycan composition which occur due to aging, life style or disease. Many studies suggested that altered glycosylation of proteins is one of the cancer-related markers and information on glycosylation status can significantly increase the informative value of protein biomarkers. We have studied the differences in glycan composition of prospective colorectal cancer (CRC) biomarkers in various sample types using lectin-based protein microarray platform employing glycan – lectin recognition. The samples included native ones as sera (from healthy persons and patients with CRC), cytosol/membrane proteins (from non-tumor and tumor colon tissue), isolated glycoproteins (alpha-2-macroglobulin, α2M and insulin-like growth factor-binding protein 3, IGFBP-3) and isolated membrane receptors for the insulin-like growth factors (IGF1R and IGF2R). The samples were spotted into arrays on microarray slide, incubated with a panel of biotinylated lectins and fluorescent conjugate of streptavidin. The signal intensities were detected using microarray scanner. Statistically significant differences in signal intensities were found for the combination of some samples and lectins implying changes in glycosylation, e.g. in the content of α2,6 sialic acid, α2,3 sialic acid, N-acetylglucosamine, mannose residues and fucose. However, the found differences between samples of CRC patients and healthy individuals (and between samples from non-tumor and tumor colon tissue of the same person) somewhat differ for different types of samples which needs further investigation. The used method is applicable for fast and high-throughput glyco-recognition analysis of differences in glycosylation pattern in various types of samples containing glycoconjugates.

Acknowledgement: This work was supported by the bilateral cooperation grants APVV SK-SRB-2013-0028 and 451-03-545/2015-09/01 and by national grants VEGA 2/0162/14 and APVV-14-0753 (Slovakia), and grant no. 173042 (Serbia).

Ganglioside—lipid raft interaction unveiled by novel ganglioside probes

Naoko Komura1, 2, Kenichi Suzuki2, Hiromune Ando1, 2, Akihiro Imamura1, Hideharu Ishida1, Akihiro Kusumi2, Makoto Kiso1, 2

1Department of Applied Bioorganic Chemistry, Gifu University, Japan; 2Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Japan

Gangliosides have significant roles in signal transductions mediated by lipid rafts. In this study, to elucidate the behaviors of gangliosides in lipid rafts by single molecule imaging, we intended to develop novel ganglioside analogs, in which glycan parts are site-specifically labeled with fluorescent dyes.

We designed the replacement of the C9 hydroxyl of Neu of gangliosides (GM3, GM2 and GM1) with amino groups to selectively introduce dyes by amide formation. The glycans having a trifluoroacetamide at the C9 position were glycosidated with the Glc-Cer, yielding the skeletons of gangliosides, which were finally conjugated with dyes. Next, fluorescent gangliosides were subjected to biophysical evaluations (eg. DRM analysis), and their results demonstrated that these analogs behaved much like native gangliosides. By single molecule imaging of the fluorescent gangliosides on the cell membrane, we have observed specific colocalizations with CD59 clusters (GPI-anchored protein) for the first time (N. Komura, et al., Nat. Chem. Biol., 2016, 12, 402-410.). Furthermore, single molecule imaging have revealed that gangliosides frequently form homodimers at the resting state.
62 Synthesis of fluorescent disialyl ganglioside probes useful for single molecule imaging

Miku Konishi¹, ², Kenichi G.N. Suzuki², Akihiro Imamura¹, Hiromune Ando¹, ², Hideharu Ishida¹, Akihiro Kusumi², Makoto Kiso¹, ²

¹Department of Applied Bioorganic Chemistry, Gifu University, Japan; ²Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Japan

Lipid raft is a functional mesoscale domain in the cell membrane and closely related to cell signaling, viral infection and cell adhesion. Recently, we have developed fluorescent ganglioside probes (GM3, GM2 and GM1) to detail the interactions between gangliosides and lipid raft domain by single molecule tracking technique. In this study, we addressed on the synthesis of fluorescently labeled analogs of disialyl gangliosides GD3 and GQ1b, which are specifically expressed in the central nervous system.

The common non-reducing end moiety (Neu-Neu-Gal) of the target molecules was efficiently synthesized by the coupling of Neu donor with 1,5-lactamized Neu-Gal acceptor, which was then converted into the suitably protected donor with a trifluoroacetamide at the terminal C9 position. The stepwise conjugation and global deprotection afforded amino-terminated gangliosides GD3 and GQ1b, respectively. Finally, the fluorescent dye was attached to each molecules through an amide linkage to produce fluorescent GD3 and GQ1b, respectively.

63 Synthetic Study on Novel Bioactive Ganglioside SJG-2

Hiroyuki Mano¹, Ryutaro Ohyama¹, Kouichi Sakamoto¹, Hiromune Ando¹, ², Akihiro Imamura¹, Hideharu Ishida¹, Makoto Kiso¹, ²

¹Department of Applied Bioorganic Chemistry, Gifu University, Japan; ²Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Japan

Recently, novel ganglioside SJG-2 has been found in the seacucumber Stichopus japonicas by Higuchi and co-workers in Kyusyu Univ[1]. SJG-2 exhibited the most potent neuritogenic activity toward PC12 cells among gangliosides ever evaluated. In this study, we aimed to synthesize ganglioside SJG-2 for elucidating the molecular basis underlying the neurite outgrowth enhanced by exogenous ganglioside.

Retrosynthetic analysis of the target molecule provided three building units; Neu5Acα(2,3)[Neu5Acα(2,4)]Gal donor (as the outer unit), Neu5Acα(2,3)GalNAcβ(1,3)Gal acceptor (as the inner unit) and glucosylceramide unit. First, to excute disialylation of the vicinal hydroxyl groups at the C3 and C4 positions of Gal, we have developed a 1,5-lactamized Neuα(2,3)Gal acceptor. As expected, the sialylation of the C4 hydroxyl group of the acceptor provided high yield of disialyl Gal. Next, the disialyl Gal was successfully combined with the inner core unit at the C8 hydroxyl group of Neu residue to give a hexasaccharide. Currently, the hexasaccharide derivative is undergoing the conversion into a glycosyl donor for the final coupling with a glucosylceramide unit.

Sialoglyco 2016
November 14 -17th
Santa Barbara, California (USA)

Attendees

Abeln, Markus
Hannover Medical School
e-mail: abeln.markus@mh-hannover.de

Adema, Gosse
Radboud University
e-mail: gosse.adema@radboudumc.nl

Ahlgren, Jeff
Wyatt Technology Corporation
e-mail: jahlgren@wyatt.com

Angata, Takashi
Academia Sinica, Institute of Biological Chemistry
e-mail: angata@gate.sinica.edu.tw

Aziz, Peter
University of California – Santa Barbara
e-mail: peter.aziz@lifesci.ucsb.edu

Ballet, Romain
Stanford University
e-mail: ballet.r@gmail.com

Bellis, Susan
University of Alabama at Birmingham
e-mail: bellis@uab.edu

Bennet, Andrew
Simon Fraser University
e-mail: bennet@sfu.ca

Bensing, Barbara
University of California – San Francisco
e-mail: barbara.bensing@ucsf.edu

Bertozzi, Carolyn
Stanford University
e-mail: bertozzi@stanford.edu

Bochner, Bruce
Northwestern University Feinberg
e-mail: bruce.bochner@northwestern.edu

Boltje, Thomas
Radboud University
e-mail: t.boltje@science.ru.nl

Borst, Kristina
Hannover Medical School
e-mail: borst.kristina@mh-hannover.de

Bovin, Nicolai
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS
e-mail: professorbovin@yandex.ru

Bristow, Leila
TCI - Tokyo Chemical Industry
e-mail: Leila.Bristow@tcichemicals.com

Broderick, James
Palleon Pharma, Inc.
e-mail: jwb@palleonpharma.com

Büll, Christian
Radboud University
e-mail: christian.buell@radboudumc.nl

Cairo, Chris
University of Alberta
e-mail: ccairo@ualberta.ca

Caraballo, Rémi
Umeå University
e-mail: remi.caraballo@umu.se

Chen, Guoyun
University of Tennessee Health Science Center
e-mail: gchen14@uthsc.edu

Chen, Jian-You
National Taiwan University
e-mail: d00b46011@ntu.edu.tw

Chen, Xing
Peking University
e-mail: xingchen@pku.edu.cn

Cheng, Bo
Peking University
e-mail: pkuchengbo@pku.edu.cn

Chern, Yijuan
Academia Sinica, Institute of Biomedical Sciences
e-mail: bmychem@ibms.sinica.edu.tw

Chou, Chih-Hsing
Children's Hospital Los Angeles
e-mail: chchou@chla.usc.edu

Chow, Jesse
Palleon Pharma, Inc.
e-mail: jchow@palleonpharma.com

Clausen, Henrik
University of Copenhagen
e-mail: hclau@sund.ku.dk

Crocker, Paul
University of Dundee
e-mail: p.r.crocker@dundee.ac.uk

d’Azzo, Alessandra
St. Jude Children's Research Hospital
e-mail: Sandra.dazzo@stjude.org

Darvish, Daniel
HIBM Research Group
e-mail: ddarvish@hibm.org

Derecki, Noel
Janssen Pharmaceutical Co.
e-mail: nderecki@ITS.JNJ.com

Diaz, Ed
e-mail: sl Diaz@ucsd.edu

Diaz, Sandra
University California – San Diego
e-mail: sl Diaz@ucsd.edu

Du, Yifei
Peking University
e-mail: dododyf@pku.edu.cn

Falconer, Robert
University of Bradford
e-mail: r.a.falconer1@bradford.ac.uk

Field, Rob
John Innes Centre
e-mail: rob.field@jic.ac.uk

Freedberg, Daron
Food and Drug Administration
e-mail: Daron.Freedberg@fda.hhs.gov

Fukushima, Tatsuya
Amano Enzyme Inc.
e-mail: tatusya.fukushima@amanoenzyme.com

updated on November 13, 2016
Furukawa, Koichi
Chubu University
e-mail: koichi@isc.chubu.ac.jp

Imamura, Akihiro
Gifu University
e-mail: aimamura@gifu-u.ac.jp

Kohler, Jennifer
University of Texas Southwestern
e-mail: Jennifer.Kohler@UTSouthwestern.edu

Gerardy-Schahn, Rita
Hannover Medical School
e-mail: Gerardy-Schahn.Rita@mh-hannover.de

Inamura, Noriaki
Seikagaku Corporation
e-mail: ina0810@seikagaku.co.jp

Komura, Naoko
Gifu University
e-mail: komura@gifu-u.ac.jp

Go, Shiori
Nagoya University
e-mail: go.shiori@i.mbox.nagoya-u.ac.jp

Inokuchi, Hiroko
e-mail: jin@tohoku-mpu.ac.jp

Konishi, Miku
Gifu University
e-mail: konishi@gifu-u.ac.jp

Gray, Melissa
Stanford University
e-mail: grayma@stanford.edu

Inokuchi, Jin-ichi
Tohoku Medical and Pharmaceutical University
e-mail: jin@tohoku-mpu.ac.jp

Lamphier, Marc
Eisai Research Institute
e-mail: marc_lamphier@eisai.com

Grosveld, Gerard
St. Jude Children's Research Hospital
e-mail: gerard.grosveld@stjude.org

Ishida, Hideki
TCI - Tokyo Chemical Industry
e-mail: Hideki.Ishida@tcichemicals.com

Lauricella, Richard
GlycoSyn
e-mail: R.Lauricella@Glycosyn.com

Haslund-Gourley, Ben
University of California - Santa Barbara
e-mail: benhaslund@gmail.com

Jennevska, Daniela
Northwestern University
e-mail: Daniela.Jennevska2012@u.northwestern.edu

Lehti, Timo
University of Helsinki
e-mail: timo.lehti@helsinki.fi

Hawkins, Lynn
Eisai Research Institute
e-mail: Lynn.Hawkins@eisai.com

Jennings, Michael
Griffith University
e-mail: m.jennings@griffith.edu.au

Lewis, Amanda
Washington University in St. Louis
e-mail: allewis@wustl.edu

Heise, Torben
Radboud University
e-mail: t.heise@science.ru.nl

Jiang, Weiping
BioLegend, Inc.
e-mail: wjiang@biolegend.com

Lin, Chien-Yu
Academia Sinica
e-mail: jerry.sinica@gmail.com

Heisterkamp, Nora
Children's Hospital Los Angeles, USC
e-mail: heisterk@usc.edu

Juge, Nathalie
Institute of Food Research
e-mail: nathalie.juge@ifr.ac.uk

Magnani, John L.
GlycoMimetics, Inc.
e-mail: jmagnani@glycomimetics.com

Heithoff, Douglas
University of California - Santa Barbara
e-mail: heithoff@lifesci.ucsb.edu

Kabayama, Kazuya
Osaka University
e-mail: kaba@chem.sci.osaka-u.ac.jp

Mahan, Michael
University of California – Santa Barbara
e-mail: michael.mahan@lifesci.ucsb.edu

Hennet, Thierry
University of Zurich
e-mail: thierry.hennet@uzh.ch

Katrik, Jaroslav
Slovak Academy of Sciences
e-mail: jaroslav.katrik@savba.sk

Malaker, Stacy
Stanford University
e-mail: smalaker@stanford.edu

Higuchi, Koji
Seikagaku Corporation
e-mail: koji.higuchi@seikagaku.co.jp

Katrikova, Eva
e-mail: katrik@yahoocom

Mane, Ulla
University of Copenhagen
e-mail: ulma@sund.ku.dk

Hildebrandt, Herbert
Hannover Medical School
e-mail: hildebrandt.herbert@mh-hannover.de

Khoo, Kay-Hooi
Academia Sinica
e-mail: kkho0@gate.sinica.edu.tw

Mano, Hiroyuki
Gifu University
e-mail: u8121047@edu.gifu-u.ac.jp

Houeix, Benoit
National University of Ireland
e-mail: benoithoueix@nuigalway.ie

Kitajima, Ken
Nagoya University
e-mail: kitajima@agr.nagoya-u.ac.jp

Marth, Jamey
University of California – Santa Barbara
e-mail: jmarth@sbspdiscovery.org

Howlader, Amran
University of Alberta
e-mail: howlader@ualberta.ca

Kitos, Theresa
e-mail: bennet@sfu.ca

Martinez-Duncker, Ivan
Universidad Autónoma del Estado de Morelos
e-mail: duncker@uaem.mx

updated on November 13, 2016
Mast, Steven
ProZyme, Inc.
e-mail: smast@prozyme.com

Peng, Li
Palleon Pharma
e-mail: lpeng@palleonpharma.com

Skog, Maria
University of Helsinki
e-mail: maria.skog@helsinki.fi

Matsuo, Naoya
Rikkyo University
e-mail: n_matsuo@rikkyo.ac.jp

Pierce, Michael
University of Georgia, CCRC
e-mail: hawkeye@uga.edu

Smith, Benjamin
University of Stanford
e-mail: bahsmith@stanford.edu

Mayr, Juliane
Griffith University
e-mail: juliane.h.mayr@gmail.com

Pierce, Stephanie
e-mail: hawkeye@uga.edu

Srivastava, Amrita
The Scripps Research Institute
e-mail: amrisriv@scripps.edu

Miyagi, Taeko
Miyagi Cancer Cntr Research Institute
e-mail: miyagi-ta@nifty.com

Pshezhetsky, Alexey
University of Montreal
e-mail: Alexei.pchejetski@umontreal.ca

Sun, Xue-Long
Cleveland State University
e-mail: x.sun55@csuohio.edu

Mori, Airi
Nagoya University
e-mail: i.r67m@gmail.com

Sackstein, Beth
e-mail: rsackstein@partners.org.

Suzuki, Reiko
e-mail: suzuki@u-shizuoka-ken.ac.jp

Müenster-Küehnel, Anja
Hannover Medical School
e-mail: Muenster.Anja@mh-hannover.de

Sackstein, Robert
Harvard Medical School
e-mail: rsackstein@partners.org.

Suzuki, Tadashi
RIKEN Institute
e-mail: tsuzuki_gm@riken.jp

Nischan, Nicole
University of Texas Southwestern
e-mail: Nicole.Nischan@utsouthwestern.edu

Sato, Chihiro
Nagoya University
e-mail: chi@agr.nagoya-u.ac.jp

Suzuki, Takashi
University of Shizuoka
e-mail: suzuki@u-shizuoka-ken.ac.jp

Nitschke, Lars
University of Erlangen
e-mail: lars.nitschke@fau.de

Schnaar, Cindy
e-mail: schnaar@jhu.edu

Taylor, Garry
University of St Andrews
e-mail: gtl2@st-andrews.ac.uk

Nizet, Victor
University of California – San Diego
e-mail: vnizet@ucsd.edu

Schnaar, Ronald
Johns Hopkins School of Medicine
e-mail: schnaar@jhu.edu

Terme, Mickael
OGD2 Pharma Co.
e-mail: mickael.terme@hotmail.fr

Normington, Karl
Palleon Pharma
e-mail: knormington@palleonpharma.com

Schoenhofen, Ian C.
National Research Council of Canada
e-mail: ian.schoenhofen@nrc-cnrc.gc.ca

Thaysen-Andersen, Morten
Macquarie University
e-mail: morten.andersen@mq.edu.au

O’Sullivan, Jeremy A.
Northwestern University
e-mail: jeremy.osullivan@northwestern.edu

Scott, Chris
Queen’s University Belfast
e-mail: c.scott@qub.ac.uk

Thibault, Romain
e-mail: R.I.Thomson@pgr.reading.ac.uk

Okerblom, Jonathan
University California – San Diego
e-mail: jokerbl@ucsd.edu

Shamsi Kazem Abadi, Saeideh
Simon Fraser University
e-mail: ssahamsi@sfu.ca

Thomson, Rebecca
University of Reading
e-mail: R.I.Thomson@pgr.reading.ac.uk

Pang, Lijuan
The Scripps Research Institute
e-mail: lpang@scripps.edu

Shnyder, Steve
University of Bradford
e-mail: s.d.shnyder@bradford.ac.uk

Tokiwa, Hiroaki
Rikkyo University
e-mail: tokiwa@rikkyo.ac.jp

Paulson, James C.
The Scripps Research Institute
e-mail: jpaulson@scripps.edu

Singh, Ranjit
University of Western Ontario
e-mail: ranjit1987genetics@gmail.com

Uchimura, Kenji
Nagoya University
e-mail: arumihcu@med.nagoya-u.ac.jp

Pedram, Kayvon
Stanford University
e-mail: kypedram@stanford.edu

Sixt, Michael
IST Austria
e-mail: michael.sixt@ist.ac.at

Uehara, Keiji
Kyowa Hakko Kirin Co., Ltd.
e-mail: keiji.uehara@kyowa-kirin.co.jp

updated on November 13, 2016
Valles, Yadira
HIBM Research Group
e-mail: yadira@hibm.org

van Helden, Mary
Aduro Biotech Europe
e-mail: MvanHelden@aduro.com

Vann, Willie F.
NIH/FDA
e-mail: wvann@mail.nih.gov

Varki, Ajit
University California – San Diego
e-mail: a1varki@ucsd.edu

Varki, Nissi
University California – San Diego
e-mail: nvarki@ucsd.edu

Villanueva-Cabello, Tania
Universidad Autónoma del Estado de Morelos
e-mail: tamavic@gmail.com

von Itzstein, Mark
Griffith University
e-mail: m.vonitzstein@griffith.edu.au

Wagstaff, Ben
John Innes Centre
e-mail: ben.wagstaff@jic.ac.uk

Wang, Bing
Charles Sturt University
e-mail: biwang@csu.edu.au

Wasik, Brian
Cornell University
e-mail: brw72@cornell.edu

Wetter, Michael
LimmaTech Biologics AG
e-mail: Michael.wetter@limbio.com

Whitfield, Dennis
Sussex Research, NRC
e-mail: dwhitfield@sussex-research.com

Willand-Charnley, Rachel
Stanford University
e-mail: rawc@stanford.edu

Williams, Karla Chinnery
University of Western Ontario
e-mail: kchinnery@gmail.com

Wilson, Ian
The Scripps Research Institute
e-mail: wilson@scripps.edu

Woods, Elliot C.
Stanford University
e-mail: elliotcwoods@gmail.com

Woods, Robert J.
University of Georgia, CCRC
e-mail: rwoods@ccrc.uga.edu

Wu, Peng
The Scripps Research Institute
e-mail: pengwu@scripps.edu

Xiao, Han
Stanford University
e-mail: xiaoken@stanford.edu

Yang, Won Ho
University of California – Santa Barbara
e-mail: wyang@sbpdiscovery.org

Yoshimura, Atsushi
Nagoya University
e-mail: yoshimura.atsushi@j.mbox.nagoya-u.ac.jp

Yoshinaka, Hiroki
Gifu University
e-mail: u8121052@edu.gifu-u.ac.jp

Yu, Alice
Chang Gung Memorial Hospital / Chang Gung University
e-mail: aliceyu@cgmh.org.tw

Yuasa, Noriyuki
TCI America
e-mail: Noriyuki.Yuasa@tcichemicals.com

Zhang, Jian
Z Biotech, LLC
e-mail: jzhang@zbiotech.com

Anna Crie
The Scripps Research Institute
e-mail: annacrie@scripps.edu

Katelyn Jerlinga
University of California – Santa Barbara
e-mail: kjerlinga@sbpdiscovery.org
CANADIAN GLYCOMICS SYMPOSIUM
MAY 9-11, 2018
SIALOGLYCO
May 10-13, 2018
BANFF, ALBERTA