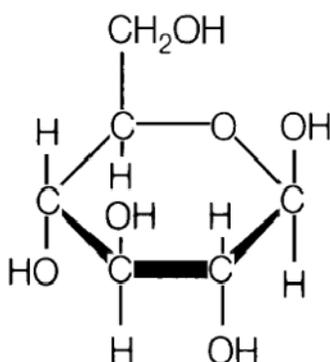


NIH & FDA GLYCOSCIENCES RESEARCH DAY

May 28th, 2009
Natcher Conference Center
Bethesda, Maryland



Dr. John Cipollo, FDA, Chair
Dr. Kelly Ten Hagen, NIDCR, Co-Chair

Sponsored by
The NIH Glycobiology Scientific Interest Group
The NIH Office of Intramural Research
The Food & Drug Administration
The National Institute of Dental & Craniofacial Research
The National Institute of General Medical Science
The Foundation for NIH

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NIH & FDA Glycosciences Research Day
Balconies B&C
Natcher Conference Center

Dr. John Cipollo, FDA, Chair
Dr. Kelly Ten Hagen, NIDCR, Co-Chair

Morning Plenary Session

8:25am - 12:30p.m.,

8:25 a.m., **Dr. John Cipollo**, Principal Investigator, DBPAP, OVRR, CBER, FDA, *"Welcome"*

8:30 a.m., **Dr. John Cipollo**, Principal Investigator, DBPAP, OVRR, CBER, FDA, *"Research in the Glycosciences at the FDA"*

8:40 a.m., **Dr. Kelly Ten Hagen**, Chief, Developmental Glycobiology Unit, LCDB, NIDCR, NIH, *"Research in the Glycosciences at the NIH"*

8:50 a.m., **Dr. Pamela Marino**, Program Director, NIGMS, NIH, *"Extramural Resources for Researchers in the Glycosciences"*

9:00 a.m., **Dr. Mike Pierce**, Professor & Director, University of Georgia Cancer Center, *"How Sweet It Is: Applying New Glycomics Technologies to Identify Stem Cell and Cancer Markers"*

9:30a.m., **Dr. Gary Nabel**, Director, Vaccine Research Center, NIAID, NIH, *"The Relevance of Glycobiology to Vaccine Design."*

10:00 a.m., **Dr. Craig J. Thomas**, Group Leader, Chemistry, Chemical Genomics Center, NIH, *"The NIH Chemical Genomics Center: Expanding the Role of Small Molecules in the Glycosciences."*

10:30 a.m.-11:00a.m., Break, *Refreshments provided by NIDCR*

NIH & FDA Glycosciences Research Day
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Natcher Conference Center

Plenary Session (cont.)

11:00 a.m., **Dr. Cathy Costello**, Director, Mass Spectrometry Resource, Boston University School of Medicine, *"Glycobiology at the Cutting Edge of Mass Spectrometry."*

11:30 a.m., **Dr. Jeffery K. Taubenberger**, Investigator, Laboratory of Infectious Diseases, NIAID, NIH, *"The Role of Receptor Binding in the Virulence of the 1918 Influenza Virus."*

12:00 p.m., **Dr. Ronald Schnaar**, Professor of Pharmacology and Professor of Neuroscience, Johns Hopkins University School of Medicine, *"Brain Sialoglycan Biosynthesis and Function"*

12:30 p.m., **Dr. Donna Krasnewich**, NIGMS, *Poster Awards Announced*

Mentoring Sessions – Working Lunch

12:35p.m., Lunch, a working lunch is being provided for all meeting registrants by the Foundation for NIH

12:35-1:15p.m., Postdoctoral Mentoring Luncheon for those who pre-registered

Poster Session

12:35p.m. – 1:30p.m., Session I, Atrium
Posters open for viewing

NIH & FDA Glycosciences Research Day

Mini Symposia I & II

1:30 p.m. - 2:45 p.m.

Mini Symposia I

Balcony B, Natcher

Glycoconjugates in Vaccine Development

Session Chair, Dr. **Joanna Kubler-Kielb**, Research Fellow, Section on Bacterial Disease, Pathogenesis & Immunity, NICHD, NIH

1:30 p.m., Dr. **Rachel Schneerson**, Senior Investigator, Section on Bacterial Disease, Pathogenesis & Immunity, NICHD, NIH, "*Lipopolysaccharide-Based Vaccines*"

1:50 p.m., Dr. **Paul Kovac**, Senior Investigator, Laboratory of Bioorganic Chemistry, NIDDK, NIH, "*Development of Conjugate Vaccines from Synthetic Carbohydrates*"

2:10 p.m., Dr. **Dennis Klinman**, Head, Immune Modulation Group, Laboratory of Experimental Immunology, NCI, NIH, "*CpG Oligonucleotides as Vaccine Adjuvants*"

Short Talks

2:30 p.m., "**Targeted Analysis of N-Linked Glycopeptides from H5N1 Influenza Hemagglutinin by Liquid Chromatography–Tandem Mass Spectrometry.**" Thomas A. Blake, Tracie L. Williams, James L. Pirkle, and John R. Barr Biological Mass Spectrometry Laboratory, Emergency Response and Air Toxicants Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA

2:35 p.m., "**Immunomodulatory Effect of Neisseria meningitidis type C Polysaccharide (MCPS) on the BAFF System.**" Sunita Kanswal, Nora Katsenelson & Mustafa Akkoyunlu Laboratory of Bacterial Polysaccharides, Division of Bacterial Parasitic & Allergenic Products, US Food and Drug Administration, Rockville, MD 20852

2:40 p.m., "**Expression, Glycoform Characterization, and Antigenicity of a HIV-1 V3 Domain Fused to Human IgG1Fc.**" Qiang Yang, Cishan Li, Wei Huang, Yadong Wei, Lai-Xi Wang Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD21201

2:45p.m., Break, *Refreshments provided by NIDCR*

Poster Session

2:45 – 3:30p.m., Session II, Atrium

NIH & FDA Glycosciences Research Day

Mini Symposia I & II

1:30 p.m. - 2:45 p.m.

Mini Symposia II

Balcony C, Natcher

Lectins and Adhesins in Disease

Session Chair, **Dr. Carole Bewley**, Senior Investigator, Laboratory of Bioorganic Chemistry, NIDDK, NIH

1:30p.m., **Dr. John Cisar**, Chief, Microbial Receptors Section, Oral Infection and Immunity Branch, NIDCR, NIH, *"Streptococcal Receptor Polysaccharides: Recognition Molecules for Oral Biofilm Development"*

1:50p.m., **Dr. Konstantinos Konstantopoulos**, Professor and Chair, Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, *"Cancer Cells in Transit: the Vascular Interactions of Tumor Cells"*

2:10p.m., **Dr. Barry O'Keefe**, Group Leader, Protein Chemistry and Molecular Biology, Molecular Targets Development Program, NCI, NIH, *"The Broad Spectrum Antiviral Lectin Griffithsin"*

Short Talks

2:30p.m., **"Maturation of Myeloid Precursor Cells into Mature Dendritic Cells is Associated with Changes in Expression of Polysialic Acid"** Dr. Nicholas Stamatos, Institute of Human Virology, Department of Medicine, University of Maryland Medical Center

2:35p.m., **"Development of an SPR-based Assay to Facilitate the Development of Immunoglobulin Products for Prophylaxis and Treatment of Avian Influenza"** Dr. Tracy Kamikawa, Division of Hematology, Center for Biologics Evaluation and Research, FDA

2:40p.m., **"Comparative Structural, Functional and Molecular Characterization of Closely Related Streptococcus oralis Coaggregation Receptor Polysaccharides and S. pneumoniae Capsular Polysaccharides"** Dr. Allen Bush, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County

2:45p.m., Break, *Refreshments provided by NIDCR*

Poster Session

2:45 – 3:30p.m., Session II, Atrium

NIH & FDA Glycosciences Research Day

Mini Symposia III & IV

3:30 p.m. - 4:45 p.m.

Mini Symposia III

Balcony B, Natcher

The Role of Glycans in Development and Disease

Session Chair, **Dr. Kelly Ten Hagen**, Chief, Developmental Glycobiology Unit, LCDB, NIDCR, NIH

3:30 p.m., **Dr. Gerardo Vasta**, Professor, University of Maryland Biotechnology Institute, "*Roles of Galectins in Infection: The First Barrier (and the Trojan Horse?)*"

3:50 p.m., **Dr. Michael Collins**, Chief, Skeletal Clinical Studies Unit, NIDCR, NIH, "*GALNT3 in Human Disease*"

4:10 p.m., **Dr. Dona Love**, Laboratory of Cell Biochemistry and Biology, NIDDK, NIH, "*Genetic Models of O-GlcNAc Cycling*"

Short Talks

4:30 p.m., "**Mutation in Fukutin related protein helps to elucidate the disease mechanism of dystroglycanopathies**" Akanchha Kesari¹, Susan E. Sparks^{1,2} and Eric P. Hoffman¹

¹Research of center for Genetic Medicine, Children's National Medical Center, Washington DC, ²Department of Pediatrics, Levine Children's Hospital at Carolinas Medical Center, Charlotte, NC.

4:35 p.m., "**Chondroitin-4-sulfation regulates axonal guidance and growth**" Hiro Katagiri, Wang Hang, Aviva Symes*, Herbert Geller, Developmental Neurobiology Section, NHLBI, NIH, Bethesda, MD; *Department of Pharmacology, USUHS, Bethesda, MD

4:40 p.m., "**A glycosyltransferase is required for proper cell adhesion during Drosophila development**" Liping Zhang and Kelly G. Ten Hagen Developmental Glycobiology Unit, National Institutes of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892

Poster Session

4:45-5:30p.m., Session III, Atrium

NIH & FDA Glycosciences Research Day

Mini Symposia III & IV

3:30 p.m. - 4:45 p.m.

Mini Symposia IV

Balcony C, Natcher

Glycosylation & Glycan Structure & Function

Session Chair, **Dr. Joseph Barchi**, Senior Scientist, Laboratory of Medicinal Chemistry, NCI, NIH

3:30 p.m., **Dr. Phil Deshong**, Professor of Chemistry, University of Maryland College Park,
"Carbohydrate Functionalized Surfactant Vesicles: From Physical Chemistry to Lipid Rafts to Vaccines"

3:50 p.m., **Dr. Lai Xi Wang**, Associate Professor, Institute for Human Virology, Baltimore,
"Chemoenzymatic Synthesis of Glycoproteins"

4:10 p.m., **Dr. Anu Puri**, Staff Scientist, Center for Cancer Biology Nanobiology Program, NCI,
"Glycosphingolipid-mediated Restriction of Lateral Mobility of HIV-1 Receptors: Implications on HIV-1 Entry"

Short Talks

4:30 p.m., **"Cellular Expression and Localization of the Key Enzymes in Sialic Acid Biosynthesis"** Katherine Patzel¹, Melis Anahtar¹, Carla Ciccone¹, Heidi Dorward¹, Irini Manoli¹, Terry D Butters², William A. Gahl¹, and Marjan Huizing¹. ¹ Medical Genetics Branch, NHGRI, NIH; Bethesda, MD, ² Glycobiology Institute, Department of Biochemistry, Oxford University, UK.

4:35 p.m., **"Exploring Stem Cell Biology and Tissue Engineering with Sugar Analogues"** Jian Du, Pao-Lin Che, Elaine Tan, Zhi-Yun Wang, Kevin J. Yarema* Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD, 21218

4:40 p.m., **"Galectin-1 is a Novel Functional Receptor for Tissue Plasminogen Activator in Pancreatic Cancer"** Oriol Roda^{1,2}, Elena Ortiz-Zapater^{1,2}, Neus Martínez-Bosch¹, Ricardo Gutiérrez-Gallego^{2,3}, Miquel Vila-Perelló², Coral Ampurdanés¹, Hans-Joachim Gabius⁴, Sabine André⁴, David Andreu², Francisco X. Real^{1,2,5} & Pilar Navarro¹. ¹ Cancer Research Programme, Municipal Institute of Medical Research, Barcelona, Spain. ² Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain. ³ Neuropsychopharmacology Program, Municipal Institute of Medical Research, Barcelona, Spain ⁴ Institut für Physiologische Chemie, Tierärztliche Fakultät, Ludwig-Maximilians-Universität, Veterinärstr. München, Germany ⁵ Molecular Pathology Programme, Spanish National Cancer Research Center, Madrid, Spain * O.R. and E.O.Z. have made equivalent contributions and share first authorship

Poster Session

4:45 – 5:30p.m., Session III

NIH & FDA Glycosciences Research Day
Atrium
Natcher Conference Center

Poster Sessions

8:00a.m., Posters open for viewing

Dr. Krasnewich, Program Director, NIGMS, Session Chair &

Dr. Hanover, Chief, Laboratory of Cell Biochemistry and Biology NIDDK, Session Co-Chair

Poster Program:

All Posters to go up 7:30a.m. & are to remain up until 5:30p.m.

8:00a.m.-11:00a.m., Silent Judging

12:30 p.m., **Dr. Donna Krasnewich**, NIGMS, *Poster Awards Announced*

12:35p.m.-1:30p.m., **Poster Session I**

Posters open for viewing

2:45p.m.-3:30p.m., **Poster Session II**

Presenters with odd numbered posters available at posters for discussion

4:45p.m.-5:30 p.m., **Poster Session III**

Presenters with even numbered posters available at posters for discussion

The NIH Glycobiology Interest Group, 2008-09 Activities

The NIH Glycobiology Scientific Interest Group brings together researchers from laboratories across the intramural NIH, and FDA with interests in the glycosciences including those pursuing studies of: glycan structure; synthesis; metabolism; function; and lectin biology.

The activities of the Glycobiology SIG are organized by a Steering Committee of volunteers from multiple institutes, and highlight the ongoing research efforts in the glycosciences taking place across the NIH campus.

These include:

- A “Special Topics in the Glycosciences” seminar series given by senior intramural staff;
- Invited seminars from outstanding extramural researchers;
- An annual NIH-FDA Glycosciences Research Day, where investigators from the intramural NIH, the FDA, and extramural investigators working in the glycosciences share their latest findings.
- A website <http://sigs.nih.gov/GBIG/Pages/default.aspx> and discussion list for dissemination of information

The Glycobiology SIG cross posts activities with the NIH glyco-immunology SIG, the proteomics SIG, the Vaccine Research Center Seminar list, and the Chemistry SIG.

The NIH Glycobiology SIG Steering Committee

Members

Dr. Barchi, NCI
Dr. Blithe, NICHD
Dr. Cipollo, FDA
Dr. Hanover, NIDDK
Dr. Huizing, NHGRI
Dr. Krasnewich, NIGMS
Dr. Krueger, NCI
Dr. Kubler-Kielb, NICHD
Dr. Marino, NIGMS
Dr. Qasba, NCI
Dr. Sheeley, NCRR
Dr Srinivas, NHLBI
Dr. Sarkar, NHLBI
Dr. Taylor, NIAID
Dr. Ten Hagen, NIDCR

Join US!

Membership in this SIG and on its steering committee is open to all who have interest in the field and time to devote to SIG activities!

History of the Glycobiology Interest Group & Glycoscience at NIH

By Diana Blithe and John Hanover

What's wrong with this picture? DNA → RNA → Protein → → → CELL?

The sequence ignores at least two important classes of macromolecules: Glycans and Lipids. These molecules suffer from a phenomenon known as the "Rodney Dangerfield Syndrome" (they get no respect).

In the early days, investigators studying glycosylated proteins were considered "poor biochemists" because they seemed to have difficulty purifying the sugars away from the proteins. As befitted their biochemical skills, the sessions on glycosylated proteins were scheduled last at major meetings (usually Saturday afternoon).

Analytical technology was a problem and methods for quantifying glycans lagged behind those for proteins and nucleic acids. The inherent structural diversity of complex carbohydrates, the difficulty in determining their sequence, and the absence of a chromophore contributed to the lack of sensitive analytical methods. Furthermore, biosynthesis could not be directly predicted from a template. The development of new alkaline pH HPLC technology for improved analysis of sugar composition expanded the analytical capability of investigators.

In 1987, the NIH Carbohydrate Interest Group was established through the efforts of Drs. Gilbert Ashwell and Vincent Hascall. Activities of the group were further catalyzed by Dr. Mike Bedford. Mike had sold one of the first alkaline pH HPLC units to Drs. John Hanover and Gilbert Ashwell, and he suggested gathering other researchers for a group meeting to share experiences with the technology. Gil and Vince took the lead in organizing the first meeting of what became the NIH Carbohydrate Interest group. The name of the group reflected that of the national association, the Society for Complex Carbohydrates (often confused with advocates for cereal and donuts). Monthly Meetings were held at 4 PM at the FAES Building – adjacent to the NIH campus. The location was chosen because the building allowed wine to be served. Enthusiasm over new analytical alkaline pH HPLC technology brought people together. In addition to the wine, cheese, fruit and crackers were served. Collaborations abounded!

Our colleagues Drs. Y.C. Lee and Saul Roseman had formed a similar Interest Group at Johns Hopkins University, in Baltimore. Upon his return to Hopkins, Dr. Gerald Hart spearheaded the efforts in the Hopkins Medical Campus. In addition to monthly meetings, the two groups (NIH and Johns Hopkins) began to hold annual joint meetings. The first annual meeting entitled "Glycoday" was held May 30th, 1995 in Annapolis, MD. Over 100 attendees were present at the meeting, which was reported on in the NIH Catalyst <http://www.nih.gov/catalyst/back/95.07/toc.july95.html>

From Carbohydrates to Glycobiology

"The term "Glycobiology" was first coined in 1988 by Rademacher, Parekh, and Dwek to recognize the coming together of the traditional disciplines of carbohydrate chemistry and biochemistry with modern understanding of the cellular and molecular biology of glycans" from *Essentials in Glycobiology*. Wikipedia, the free encyclopedia, says: "Defined in the broadest sense, glycobiology is the study of the structure, biosynthesis, and biology of saccharides (sugar

chains or glycans) that are widely distributed in nature. Sugars or saccharides are essential components of all living things and aspects of the various different roles they play in biology are researched in various different medical, biochemical and biotechnological fields.”

The journal, *Glycobiology* began in September, 1990. The analytical methods bottle neck that had held back the field was eased as more sensitive carbohydrate analysis continued to be developed, and at least two companies (Dionex, and Oxford Glycosystems) sought to improve technologies for carbohydrate analysis. In 1993, the Society for Complex Carbohydrates changed its name to the Society for Glycobiology. Following their lead, the NIH Carbohydrate Interest Group (CIG) changed its name to the Trans NIH Glycobiology Interest Group (GIG) On September 13-15, 1993, the group organized a major meeting entitled: “Glycobiology: New Perspectives on Human Disease,” held at the National Institutes of Health in Bethesda, Maryland. The conference organizers were: Drs. G. Ashwell, D.L. Blithe, J.A. Hanover, G.W. Hart, V.C. Hascall, G.D. Holt, D.M. Krasnewich and Y.C. Lee. Principal topics included Glycobiology as it relates to:

- Infectious diseases —pathogen adhesion, evasion of immune system recognition, suppression of immune system activation.
- Human development —tissue organization and outgrowth, differentiation markers, fertilization, glycoprotein hormones.
- Cell adhesion, inflammation, and metastasis —metastatic cell adhesion, leukocyte homing, selectins and the inflammatory response, angiogenesis.
- Therapeutics —carbohydrates as drugs, chemotherapeutic potential of processing inhibitors, engineering oligosaccharide structures, drug targeting.
- Diagnosis—tumorigenesis, markers of metastatic potential, rheumatoid arthritis, metabolic disorders and storage diseases.

GLYCODAY, the annual Washington-Baltimore Glyco-Symposia continued until 1997 (revived by the Baltimore group in 2001). With the advent of numerous other NIH interest groups (7 major and 122 “minor” IGs), activities of the NIH GIG became less frequent and less regular, however, a core of NIH scientists remained active. Seminars, meetings, and announcements for the GIG continued on an *ad hoc* basis under the leadership of Dr. Diana Blithe. The Baltimore group has remained active as well. In the fall of 2006, Drs. Blithe, Hanover, and Manzoni made a concerted effort to re-establish a regular seminar series for the GIG. The inaugural lecture for the newly established Series was given by Dr. Pamela Stanley. This led to a spirited revival and reorganization of the NIH GIG, with the establishment of a Steering Committee composed of both intra and extramural scientists (Drs. Manzoni, Barchi, Ten Hagen, Hanover, Blithe, Qasba, Srinivas, Sheeley, Sarkar, Huizing, Marino) who proceeded to chart a course for the GIG that included: a seminar series highlighting the second edition of *Essentials of Glycobiology*; a new version of Glycoday, entitled **Glycosciences Research Day**; and a website to accompany an expanded email list. The GIG Steering Committee has continued to expand and hopes now to establish a core group of laboratories working in the glycosciences to serve as a nucleus for an intramural glycosciences training program.

The 2008- 2009 GB-SIG Special Topics in the GlycoSciences Seminar Series

Date: Thursday November 20th, 2008

Title: Molecular Features of a Novel, Glycan-Dependent, Signal Transduction Cascade

Speaker: Dr. John Hanover, Chief, LCBB, NIDDK, NIH

http://intramural.niddk.nih.gov/research/faculty.asp?People_ID=1608

Date: Wednesday, December 3, 2008

Title: Sialic Acids and Bacterial Polysaccharides

Speaker: Dr. Willie Vann, Principal Investigator, OVR / DBPAP / LBP, FDA

<http://www.fda.gov/Cber/research/vann.htm>

Date: Thursday, December 18, 2008

Title: Clinical Glycobiology

Speaker: Dr. Donna Krasnewich, Deputy Clinical Director, NHGRI, NIH

<http://www.genome.gov/10000429>

Date: Thursday, January 15, 2009

Title: Proteoglycans and Principles of Structural Analysis

Dr. Yasuhiro Katagiri, Staff Scientist, Developmental Neurobiology Laboratory, NHLBI, NIH

<http://dir.nhlbi.nih.gov/labs/ldn/labmembers.asp>

Date: Thursday, February 26, 2009

Title: Heparan Sulfate Proteoglycans Mediate Developmental Cell Signaling by Multiple Mechanisms

Speaker: Dr. Kenneth Kramer, Principle Investigator, Genetics and Developmental Biology Center , NHLBI, NIH

<http://dir.nhlbi.nih.gov/labs/ldb/pg/>

Date: Thursday, March 12, 2009

Title: Proteins That Recognize Glycans

Dr. Gerardo Vasta, Professor, University of Maryland Biotech Institute, Baltimore, MD

<http://www.umbi.umd.edu/comb/faculty-directory/vasta/index.php>

Date: Thursday, March 26, 2009

Title: O-Glycosylation During Eukaryotic Development; Mucin-Type O-Linked Glycosylation

Dr. Kelly Ten Hagen. Principle Investigator Developmental Glycobiology Unit, LCDB, NIDCR, NIH

<http://www.nidcr.nih.gov/Research/NIDCRLaboratories/CellDevelopmental/Hagen.htm>

Date: Thursday, April 30, 2009

Title: Glycobiology in Biotechnology and Medicine: Glycan Arrays & Vaccine Development

Dr. Jeff Gildersleeve , Head, Chemical Biology Section , LCM, NCI, Frederick, MD

<http://ccr.cancer.gov/staff/staff.asp?profileid=7853>

Date: Thursday, May 14, 2009

Title: What's new in innate immunity: the role of sialic acid modulation in inflammation

Dr. Alan Cross

<http://medschool.umaryland.edu/InfectiousDisease/Cross.asp>

2009- 2010 GB-SIG Special Topics in the GlycoSciences Seminar Series

Begins this September

Date: September

Title: Mass Spectrometry of Carbohydrates as a Tool for Characterization of Bacterial Vaccines and Pathogens

Dr. John Cipollo, Principle Investigator, OVR / DBPAP, LBP, FDA

<http://www.fda.gov/Cber/research/cipollo.htm>

If you would like to present in this series, please post marinop@nigms.nih.gov

Training at NIH in the Glycosciences

Interested in coming to NIH to train in the glycosciences? Laboratories you may wish to consider/contact include:

NCGC

Craig J. Thomas, Ph.D

Chemical Genomics Center

Expanding the role of small molecules in the glycosciences: small molecule tools directed at elucidating glycosylation pathways

<http://www.ncgc.nih.gov/about/craigt.html>

NCI

Joseph J. Barchi Jr., Ph.D.

Laboratory of Medicinal Chemistry

Multivalent presentation of tumor-associated carbohydrate antigens (TACA) and TACA-peptide conjugates as modulators of tumor cell adhesion and novel immunogens.

<http://ccr.cancer.gov/staff/staff.asp?profileid=6281>

Jeffrey C. Gildersleeve, Ph.D.

Laboratory of Medicinal Chemistry

Carbohydrate chemistry and glycobiology: carbohydrate-based cancer vaccines; diagnostic agents; therapeutic agents; and identification of cancer biomarkers.

<http://ccr.cancer.gov/staff/staff.asp?profileid=7853>

Barry O'Keefe, Ph.D.

Molecular Targets Development Program

Studies of broad spectrum antiviral lectins

<http://ccr.cancer.gov/staff/staff.asp?profileid=7138>

Dennis Klinman, M.D., Ph.D.

Laboratory of Experimental Immunology

CpG Oligonucleotides as Vaccine Adjuvants

<http://ccr.cancer.gov/staff/staff.asp?profileid=12379>

Pradman K. Qasba, Ph.D.

CCR Nanobiology Program

Protein-Carbohydrate Interactions: Structure/function and design of novel glycosyltransferases for the development of targeted drug deliver systems

<http://ccr.cancer.gov/staff/staff.asp?profileid=5752>

David D. Roberts, Ph.D.

Laboratory of Pathology

Heparin sulfate proteoglycans, angiogenesis, immunology, extracellular matrix

<http://ccr.cancer.gov/Staff/staff.asp?profileid=5850>

Alexander Wlodawer, Ph.D.

Protein Structure Section

Investigating lectins with antiviral activity; a variety of other proteases, ribonucleases, and kinases; and a number of cytokines and cytokine-receptor complexes

<http://mcl1.ncifcrf.gov/wlodawer.html>

NHGRI

Marjan Huizing, Ph.D.

Cell Biology of Metabolic Disorders Unit

Sialic acid metabolism and human disorders of glycosylation

<http://www.genome.gov/11007099>

NHLBI

Herbert M. Geller, Ph.D.

Developmental Neurobiology Section

Proteoglycans in the nervous system

<http://dir.nhlbi.nih.gov/labs/ldn/hmg.asp>

Kenneth Kramer, Ph.D.

Developmental Glycobiology Section

Heparan sulfate proteoglycans mediation of developmental cell signaling by multiple mechanisms

<http://dir.nhlbi.nih.gov/labs/ldb/pg/>

NIAID

James Arthos, Ph.D.

Immunopathogenesis Section

HIV-1 Envelope Interactions with Dendritic Cell Receptors

<http://www3.niaid.nih.gov/labs/aboutlabs/lir/immunopathogenesisSection/fauci.htm>

David N. Garboczi, Ph.D.

Structural Biology Section

T cell receptor recognition of antigens and their activation in an immune response.

<http://www3.niaid.nih.gov/labs/aboutlabs/lig/structuralBiologySection/>

Peter Sun, Ph.D

Structural Immunology Section

Recognition Of The Dendritic Cell Surface Receptor Dc-sign

<http://sis.niaid.nih.gov/>

Jeffery K. Taubenberger, M.D., Ph.D

Laboratory of Infectious Disease

Studies of Viral Hemagglutinin Binding"

<http://www3.niaid.nih.gov/labs/aboutlabs/lid/respiratoryVirusesSection/Taubenberger.htm>

NICHD

Joanna Kubler-Kielb, Ph.D.

Section on Bacterial Disease, Pathogenesis & Immunity

Carbohydrate based vaccine development

http://www.nichd.nih.gov/about/staff/bio.cfm?nih_id=0011073016

Vince Pozsgay, Ph.D.

Section on Bacterial Disease, Pathogenesis & Immunity

Synthetic oligosaccharide-based vaccines

http://www.nichd.nih.gov/about/staff/bio.cfm?nih_id=0010119885

Rachel Schneerson, M.D.

Section on Bacterial Disease, Pathogenesis & Immunity

Polysaccharide/oligosaccharide-protein conjugates; Immunogenicity of polysaccharides and vaccine development

http://www.nichd.nih.gov/about/staff/bio.cfm?NIH_ID=0010049869&renderforprint=1

Shousun Szu

Section on Bacterial Disease, Pathogenesis & Immunity

Protein and polysaccharide conjugate vaccines

http://www.nichd.nih.gov/about/staff/bio.cfm?nih_id=0010151052

NIDCR

John Cisar, Ph.D.

Microbial Receptors Section

Streptococcal Receptors and Adhesins

<http://www.nidcr.nih.gov/Research/NIDCRLaboratories/OralImmunity/JohnCisar.htm>

Kelly Ten Hagen, Ph.D.

Developmental Glycobiology Unit

Role of O-glycosylation during eukaryotic development; mucin-type O-linked glycosylation; O-glycans in Drosophila development;

Cell adhesion, signaling and migration; and identification of in vivo substrates destined to be glycosylated

<http://www.nidcr.gov/Research/NIDCRLaboratories/CellDevelopmental/Hagen.htm>

Mathew Hoffman, D.D.S., Ph.D.

Matrix and Morphogenesis Unit

Heparan sulfate and its role in salivary gland development

<http://www.nidcr.gov/Research/NIDCRLaboratories/CellDevelopmental/Hoffman.htm>

NIDDK

Carole Bewley, Ph.D.

Laboratory of Bioorganic Chemistry

Specificity and recognition of novel carbohydrate binding proteins.

https://ugsp.nih.gov/scholars_mentors/mentors_d.asp?m=07&id=1427

John A. Hanover, Ph.D.
Laboratory of Cell Biochemistry & Biology
Molecular features of a novel, glycan-dependent, signal transduction cascade.
<http://www2.niddk.nih.gov/NIDDKLabs/IntramuralFaculty/HanoverJohn.htm>

Paul Kovac, Ph.D.
Carbohydrates Section
Development of conjugate vaccines from synthetic carbohydrate antigens
<http://www2.niddk.nih.gov/NIDDKLabs/IntramuralFaculty/KovacPaul.htm>

Lawrence A. Tabak D.D.S./Ph.D.
Office of the Director
Structure, biosynthesis & function of complex glycoconjugates; novel glycosyltransferases
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Laboratory of Bacterial Polysaccharides
Mass spectrometry of carbohydrates, as a tool for characterization of bacterial vaccines and pathogens.
<http://www.fda.gov/cber/research/cipollo.htm>

Daron I. Freedberg, PhD
Laboratory of Bacterial Polysaccharides
New NMR spectroscopy and light scattering technologies to characterize vaccines and blood products.
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Willie F. Vann, PhD
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Understanding the synthesis and interactions of bacterial carbohydrates and glycosyltransferases; evaluation and characterization of vaccines.
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NIH & FDA Glycosciences Research Day
May 28th, 2009
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Meeting Abstracts

NIH & FDA Glycosciences Research Day Meeting Abstracts

The 1918 Influenza Pandemic, receptor binding, and pathogenesis

Jeffery K. Taubenberger, M.D., Ph.D., Chief, Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, NIAID, NIH

Background: The 1918 influenza pandemic caused more than 40 million deaths and likely resulted from the introduction and adaptation of a novel avian-like virus. Influenza A virus hemagglutinins are important in host switching and virulence. Avian-adapted influenza virus hemagglutinins bind sialic acid receptors linked via alpha2-3 glycosidic bonds, while human-adapted hemagglutinins bind alpha2-6 receptors. Sequence analysis of 1918 isolates showed hemagglutinin genes with alpha2-6 or mixed alpha2-6/alpha2-3 binding. **Methods:** To characterize the role of the sialic acid binding specificity of the 1918 hemagglutinin, we evaluated in mice chimeric influenza viruses expressing wild-type and mutant hemagglutinin genes from avian and 1918 strains with differing receptor specificities. **Results:** Viruses expressing 1918 hemagglutinin possessing either alpha2-6, alpha2-3, or alpha2-3/alpha2-6 sialic acid specificity were fatal to mice, with similar pathology and cellular tropism. Changing alpha2-3 to alpha2-6 binding specificity did not increase the lethality of an avian-adapted hemagglutinin. **Conclusion:** The 1918 hemagglutinin contains murine virulence determinants independent of receptor binding specificity. **Significance:** Understanding the contribution of receptor binding changes in host switch events and pathogenesis in influenza is crucial for pandemic preparedness.

Development of Conjugate Vaccines from Synthetic Carbohydrates

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To develop conjugate vaccines for bacterial diseases, we synthesize oligosaccharides that mimic structure of carbohydrates on the surface of bacterial pathogens and attach them chemically to protein carriers. Conjugation is essential because carbohydrates themselves are poor immunogens. Currently, we work, among other things, on developing conjugate vaccines for cholera and anthrax. Structural requirements for a potent, medically acceptable synthetic vaccine are not known and, therefore, we want to look at the effect of fundamental variables in the synthetic antigen upon immunogenicity. Such are, for example, the size of the oligosaccharide, oligosaccharide-carrier ratio, the nature of the linker that separates the antigen from the carrier protein, the conjugation chemistry, and some other details in the architecture of the antigen. This requires preparation of a large number of conjugates with defined carbohydrate protein ratios. It will be shown that conjugation utilizing the squaric acid chemistry combined with monitoring of the conjugation reaction by SELDI TOF MS brings making neoglycoconjugates to an unprecedented, new, higher level of sophistication. It not only allows preparation of substances of this class in a reproducible way, but series of tailor-made conjugates can be made with pre-determined carbohydrate-protein ratios in one pot, and the use of large excess of the precious synthetic oligosaccharide is not required.

The Broad Spectrum Antiviral Lectin Griffithsin

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Background: The antiviral protein griffithsin (GRFT), isolated from the red alga *Griffithsia* sp., is a 12.7 kDa protein originally identified based on its picomolar activity against HIV in cell-based in vitro assays. GRFT was found to have a unique three-dimensional structure with three carbohydrate binding sites per monomer and to block viral fusion and entry. Here we describe how such a mechanism of action can lead to broad spectrum antiviral activity. **Methods:** Here we report recent progress on the development of GRFT for use as a systemic, topical and intranasal antiviral agent. Results from recent ex vivo and in vivo studies showing GRFT's activity against HIV, ebola Zaire and the SARS coronavirus will be presented. **Results:** GRFT was shown to be potently active both in vitro and in vivo against several pathogenic viruses. **Conclusion:** GRFT shows promise as a potential prophylactic or therapeutic antiviral agent. **Significance:** GRFT is the most potent anti-HIV carbohydrate binding protein yet described and its antiviral activity extends to additional enveloped viruses.

Brain sialoglycan biosynthesis and function

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Background: Sialoglycans are major molecular determinants on mammalian cell surfaces. As common termini on glycoproteins and glycolipids, sialoglycans play an enhanced role in cell surface recognition. Brain sialoglycans are diverse and serve diverse functions in neuronal development, physiology and pathology. Quantitatively, brain sialoglycans are dominated by gangliosides, sialylated glycosphingolipids. Among the functional roles of gangliosides, two major brain gangliosides, GD1a and GT1b, serve as receptors for a brain glycan binding protein, myelin-associated glycoprotein (MAG, Siglec-4). MAG is expressed exclusively on myelin, the multi-layered membrane that wraps, insulates and nurtures neuronal axons. MAG is found on the inner-most wrap of myelin, directly apposed to the axon surface. We have studied the functional roles of MAG and its ganglioside receptors in axon stability, axon protection, and the control of axon regeneration. **Methods:** Using glycan binding (ganglioside "ELISA"), mouse genetics, ganglioside analytical chemistry, neuronal cell culture, and preclinical models of nerve injury, we have probed the functional roles of MAG and sialoglycans in central and peripheral nerve function. **Results:** MAG binds selectively to the "NeuAc α 2-3 Gal α 1-3 GalNAc" terminal arm of the common brain gangliosides GD1a and GT1b. Two similar common brain gangliosides that lack this target determinant (GM1 and GD1b) fail to bind MAG. Mouse genetic studies, including those in the laboratory of Dr. Richard Proia (NIDDK), support the conclusion that this terminus on gangliosides defines a functional receptor responsible for MAG-mediated stabilization of axons over time, protection of axons from acute toxic insults, and control of axon regeneration. Animal models of nerve injury indicate that sialoglycan modulation, specifically by delivering sialidase to injured nerves, enhances recovery after nerve injury. **Conclusions:** Brain gangliosides GD1a and GT1b, among the most abundant sialoglycans in the brain, are functional receptors for the glycan binding protein MAG, enhancing axon-myelin stability and modulating axon regeneration. **Significance:** Brain sialoglycans underlie key cell-cell interactions in the brain, and their manipulation may provide opportunities for therapeutic intervention.

Cancer Cells in Transit: The Vascular Interactions of Tumor Cells

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Background: The efficiency of secondary tumor establishment is controlled by the ability of tumor cells to withstand a barrage of mechanical and immunological stresses during their passage through the circulatory system. Accumulating evidence suggests that the selectin-dependent interactions of circulating tumor cells with host cells promote their survival and extravasation from the vasculature, therefore representing a critical checkpoint for colonization of distant organs. These observations have motivated the identification and biochemical characterization of functional selectin ligands present on the surface of metastatic colon carcinoma cells. We previously identified CD44 variant isoforms (CD44v) as functional P-, but not E- or L-, selectin ligands on colon carcinoma cells. During those studies, immunoblot and blot rolling assays using CD44-knockdown cells revealed the presence of a ~170-180 kDa sialofucosylated glycoprotein(s) capable of supporting selectin-dependent adhesion. Thus, our studies aimed at identifying and characterizing the aforementioned glycoprotein(s). Methods: Using biochemical, molecular and cell biology techniques (e.g. blot rolling assays, immunoaffinity chromatography, HPLC interfaced to electrospray ionization tandem mass spectrometry (HPLC-MS/MS), immunoprecipitation studies, short-hairpin (sh)RNA technology and cell-free flow-based adhesion assays), we isolated the major functional E- and L-selectin ligands from LS174T colon carcinoma cells, and characterized their critical selectin binding determinants. Results: Using immunoaffinity chromatography and tandem mass spectrometry, we identified carcinoembryonic antigen (CEA) and podocalyxin-like protein (PCLP) as alternative selectin ligands. Blot rolling and cell-free flow-based adhesion assays disclose that CEA on LS174T colon carcinoma cells possesses E- and L-, but not P-, selectin binding activity. CEA on CD44-knockdown LS174T cells exhibits higher HECA-452 immunoreactivity than CEA on wildtype cells, suggesting that CEA functions as an alternative acceptor for selectin-binding glycans. The enhanced expression of HECA-452 reactive epitopes on CEA from CD44-knockdown cells correlates with the increased CEA avidity for E- but not L-selectin. Through the generation of stable knockdown cell lines, we demonstrate that CEA serves as an auxiliary L-selectin ligand, which stabilizes L-selectin-dependent cell rolling against fluid shear. Moreover, CEA and CD44v cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin at elevated shear stresses. Similarly, PCLP expressed on LS174T colon carcinoma cells possesses E- and L-, but not P-, selectin binding activity. The selectin binding determinants on LS174T PCLP are non-MECA-79-reactive sialofucosylated structures displayed on O-linked glycans, distinct from the MECA-79-reactive O-glycans on PCLP expressed by high endothelial venules which is an L-selectin ligand. Flow-based adhesion assays also suggest that PCLP functions as an alternative acceptor for selectin-binding glycans. Conclusion: The novel findings that CEA and PCLP are E- and L-selectin ligand may explain the enhanced metastatic potential associated with tumor cell CEA and PCLP overexpression and the supportive role of selectins in metastasis. Significance: Understanding the molecular underpinnings of tumor cell-host cell adhesive interactions may provide guidelines for developing promising antimetastatic therapies when initiated early in the course of disease progression.

Roles of Galectins in Infection: The First Barrier (and the Trojan Horse?)

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Background: Galectins are proteins characterized by a unique binding site sequence motif, affinity for β -galactosides, and wide taxonomic distribution in vertebrates, invertebrates, protista, and fungi. Galectins were initially thought to only bind endogenous (“self”) glycans and mediate developmental processes, including cell differentiation and tissue organization, and more recently, regulation of immune homeostasis. In the past few years, however, evidence has accumulated to suggest that galectins also bind “non-self” glycans on the surface of potentially pathogenic microorganisms (viruses, bacteria, protista, and fungi) and parasitic worms, and mediate recognition and effector functions in innate immunity. Methods: Molecular, genomic, biochemical, and immunological approaches were used to identify, clone, and characterize galectin repertoires and their interactions with microbial pathogens and eukaryotic parasites. Results: The experimental evidence for galectin binding to the surface of virus, bacteria, protista and helminth pathogens and parasites, suggests that galectins can potentially function as effective pattern recognition receptors (PRRs). Further, the considerable diversity of the galectin repertoire in each organism, and the substantial or subtle variations in specificity of each galectin towards the target glycans determined by either oligosaccharide repeats, branchings, or substitutions, suggest an extensive diversity and plasticity in the galectin’s capacity for non-self recognition. Moreover, the multivalent binding properties of galectins enables their effective participation both in direct recognition of pathogens and parasites, and downstream processes that lead to activation of innate and adaptive immune responses. Some pathogens and parasites, however, subvert the roles of galectins as PRRs to either attach to suitable epithelia in their insect vector or final host, or to enter the host cells to proliferate and disseminate systemically. Conclusion: Recent evidence indicates that in addition to functions mediated by binding to endogenous ligands, host galectins can function as recognition receptors that target “non-self” glycans on the surfaces of viruses, bacteria, protista, and helminth and parasites, and either prevent or facilitate infection. Whether galectin-mediated recognition of pathogen and parasites is an effective defense mechanism with a clear benefit for the host is not entirely clear except for a few examples. It is noteworthy that a particular glycan on the surface of a microbe or parasite can be recognized by multiple galectins, and the outcome of the interaction differ substantially depending on the galectin type involved, and its concentration in a particular cell surface or extracellular microenvironment, which in turn determines the degree of oligomerization and cooperative binding to ligand. Significance: The observation that galectins are directly involved in pathogen recognition has opened new avenues of research aimed at disrupting their roles in parasite-vector interactions or host invasion. Significant gaps in our knowledge about the diversity of the host galectins repertoire, their subcellular compartmentalization and secretion, and structural and biophysical aspects of their interactions with the microbial carbohydrate moieties warrant further investigation. (Supported by grant R01 GM070589-01 from the NIH, grants IOB-0618409 and IOS-0822257 from the NSF, and grant NA05NMF4571243 from NOAA to G.R.V.)

Chemoenzymatic synthesis of N-glycoproteins

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Background: Protein N-glycosylation is one of the most common posttranslational modifications in eukaryotes and is involved in many important biological processes. However, a major challenge in studies involving glycoproteins comes from the structural micro-heterogeneity of natural and recombinant glycoproteins that are extremely difficult to isolate in homogeneous glycoforms by current analytical methods. In addition, it becomes clear that different N-glycans can exhibit significantly different biological and structural impacts on glycoprotein function. An efficient synthesis of pure glycoforms is important not only for structure-activity relationship studies, but also for biomedical applications. Approaches and Results: We have explored the transglycosylation activity of endo-beta-N-acetylglucosaminidases (ENGases) for glycoprotein synthesis. ENGases are a class of hydrolyases that cleave the N-glycan from N-glycoproteins. But some family 85 ENGases possess transglycosylation activity and are able to transfer a releasing oligosaccharide to a GlcNAc-containing peptide/protein acceptor in a single step, thus providing a convergent synthetic approach. We have found that the use of synthetic oligosaccharide oxazolines, the presumed transition state mimics, as donor substrates not only expanded the substrate availability, but also resulted in substantial enhancement of transglycosylation yield. The chemoenzymatic method has permitted the synthesis of large, homogeneous N-glycopeptides that are hitherto difficult to obtain, and has been successfully extended to glycosylation engineering of glycoproteins. To address the problem of product hydrolysis involving natural N-glycans, we have recently discovered novel glycosynthase mutants that can promote transglycosylation, but lack the activity to hydrolyze the product. Conclusion: a highly efficient chemoenzymatic method for the construction of homogeneous glycoproteins is described. This chemoenzymatic approach has shown a great potential for glycosylation engineering of glycoproteins. The next step is to further enhance the transglycosylation efficiency of the enzymes by site-directed mutagenesis, and to expand the synthetic scope of the method. A wide application of the chemoenzymatic method in glycobiology and biomedical studies involving tailor-made glycoproteins is expected. (This work was supported by NIGMS grant: R01 GM080374).

Glycosphingolipid-mediated restriction of lateral mobility of HIV-1 Receptors: Implications on HIV-1 Entry" Dr. Anu Puri, Staff Scientist, Center for Cancer Biology Nanobiology Program, NCI,

Background: Cellular ceramide, sphingolipids and their metabolites have been suggested to play a role in HIV-1 infection and pathogenesis. GM3, a major ganglioside of T lymphocytes interacts HIV-1 receptors (CD4, CXCR4 and/or CCR5) and the HIV-1 fusion protein (gp120-gp41) leading to productive infection. An upregulation of GM3 and other GSLs has been noted during progression of Cancer and AIDS. Therefore, an understanding of interactions between GM3, HIV-1 envelope glycoprotein and the viral receptors at the molecular level is warranted. Methods: Our previous studies show that receptor bearing B16 mouse melanoma cells are resistant to gp120-gp41-mediated membrane fusion and the fusion activity is restored by pre-treatment of the B16 targets with PPMP, a glycosphingolipid biosynthesis inhibitor. B16 cells express exceptionally high levels of GM3, a raft-associated ganglioside that interacts with CD4. Hence, we postulated that the block in fusion was due to immobilization and/or segregation of the CD4 receptor in the plasma membrane of these cells. We investigated GM3-mediated modulation of CD4 localization and diffusion in the plasma membrane of B16 cells by utilizing a CD4 mutant (RA5) that supports HIV-1 entry despite its preferential localization into non-raft fraction. Lateral mobility was determined by monitoring fluorescence recovery after photobleaching technique (FRAP). Results: B16 cells expressing the RA5 mutant and the cognate coreceptors (CXCR4 or CCR5) readily fused with cells expressing the corresponding HIV-1 Envs. In contrast, B16 cells expressing the coreceptors and wild type CD4 (Wt-CD4) failed to support fusion under similar conditions. PPMP pre-treatment of B16 cells expressing Wt-CD4 restored fusion. Inhibition of fusion in the presence of Leu3A and C34 confirmed that fusion was mediated by CD4, CXCR4 and gp120-gp41 interactions. Conclusions: (a) wt-CD4 was associated with raft fraction, whereas RA5 mutant preferentially localized in non-raft fraction indicating the high GM3 levels did not modulate CD4 partitioning into rafts, (b) Lateral diffusion of plasma membrane lipids and CCR5 was similar in B16, GM95 and NIH3T3 cells, (c) Wt-CD4 mobility was significantly restricted in B16 cells ($1.04 \pm 0.08 \times 10^{-11} \text{cm}^2/\text{sec}$) when compared with the RA5 mutant ($3.23 \pm 2.77 \times 10^{-11} \text{cm}^2/\text{sec}$). In contrast, wt-CD4 and RA5 mutant when expressed in GSL-deficient mutant GM95 cells or NIH3T3 cells did not show any significant difference in CD4 diffusion. Significance: Our findings indicate that the lateral mobility of CD4 is an important determinant for HIV-1 Env-mediated membrane fusion and also provide a novel mechanism of interplay between membrane lipids and receptors by which host cells can escape viral infections.

Streptococcal Receptor Polysaccharides: Recognition Molecules for Oral Biofilm Development.

John O. Cisar, Ph.D., Chief, Microbial Receptors Section, Oral Infection and Immunity Branch, NIDCR. Background: Colonization of the human tooth surface involves the growth of commensal oral streptococcal species and other bacteria as a mixed-species biofilm community. Different members of this community typically interact with one another through binding of GalNAc- and/or Gal-specific surface adhesins on one cell type to cell wall polysaccharides, referred to as coaggregation receptor polysaccharides (RPS), on the other. We hypothesize that RPS-mediated interactions between different bacteria contribute to the development of biofilm communities. Methods: Laser confocal microscopy performed in conjunction with different specific antibodies was used to examine the distributions of RPS-bearing streptococci and other bacteria in early biofilms formed in vivo on retrievable enamel chips. Different types of RPS were isolated from bacteria by anion exchange column chromatography of mutanolysin-cell wall digests and structurally characterized by high resolution NMR. The corresponding gene clusters for RPS biosynthesis were identified by sequencing and characterized by methods that included the structural characterization of genetically modified polysaccharides obtained by replacing genes in one cluster with those from another cluster. Results: *Streptococcus sanguinis*, *S. gordonii* or *S. oralis* are prominent early colonizers of the human tooth surface. Six structural types of RPS have been identified from strains of these three species. These polysaccharides are composed of structurally distinct hexa- or heptasaccharide repeating units that contain conserved Galf linked α 1-6 to a host-like recognition motif, which is GalNAc α 1-3Gal (Gn) in certain types of RPS and Galb1-3GalNAc (G) in others. Adhesins present on other oral bacteria, such as those associated with the type 2 fimbriae of *Actinomyces naeslundii*, recognize both Gn and G types of RPS while those on other species are either Gn- or G-specific. The host-like features of these polysaccharides, although critical for interbacterial adhesion, contribute little to RPS serotype specificity, which instead reflects the immunogenic features of these polysaccharides. The genetic loci for biosynthesis of these polysaccharides resemble the capsular polysaccharide (CPS) loci of closely related *S. pneumoniae* and include genes for glycosyltransferases that synthesize a lipid-linked oligosaccharide repeating unit and a polymerase that links these units into a linear polysaccharide chain. The similarities noted between different types of RPS and CPS are consistent with the evolution of RPS on modern day commensal species from the CPS of ancestral *S. pneumoniae*-like pathogens during the co-evolution of these bacteria with man. Selective pressures for the evolution of host-like recognition motifs in RPS from structural features in CPS may include the advantage gained from adhesin-mediated recognition of bacteria by members of the commensal flora, which in turn, appears to be a prerequisite for the establishment of mutualism in biofilm communities. Conclusion: The structural, functional and molecular properties of streptococcal RPS support a recognition role for these cell-surface molecules in biofilm development. Significance: The specificity of RPS-mediated interbacterial adhesion may promote development of functionally distinct microbial communities. Further study to define the ecological properties of these communities could provide important insights into the pathogenesis of dental plaque-related oral diseases.

Meeting Abstracts - Posters

Chemical Biology

#1 Progress in the Chemoenzymatic Synthesis of Glc1Man9GlcNAc2-Protein for Functional Studies.

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N-Glycosylation of proteins is important posttranslational modification of proteins. A dolichyl pyrophosphate linked tetradecasaccharide (Glc3Man9GlcNAc2-PP-Dol) is transferred to Asn-X-Ser/Thr site of nascent polypeptide by oligosaccharyl transferase. Then glycoproteins are processed by several enzymes to produce diverse high mannose type glycoforms. Among those, the monoglucosylated glycan (Glc1Man9GlcNAc2) is directly involved in the protein quality control and folding. Our project aims to accomplish the total synthesis of Glc1Man9GlcNAc2-Protein and its related derivatives (such as; Gal1Glc1Man9GlcNAc-Protein), which will be highly valuable for structural and functional studies. We decide to apply chemoenzymatic methodology, which has been recently developed from our laboratory. The method utilizes the oxazoline donor as substrate for Endo-A to coupled with a GlcNAc moiety. Thus, Gal1Glc1Man9GlcNAc-oxazoline can be coupled with GlcNAc-Protein by Endo-A. Galactose residue can be trimmed enzymatically to obtain naturally occurred derivative. We began the synthesis of the oxazoline, which has retrosynthetically analyzed to three main building blocks: a core disaccharide acceptor (Man α 1 \rightarrow 3GlcN3; azido as masked NAc), two pentasaccharide donors: Man5-thio donor and Gal1Glc1Man3-thio donor. The core disaccharide (Man α 1 \rightarrow 3GlcN3) has been synthesized by glycosylation of a thioglycoside (Man-SPh) with 2-azido Glc derivative. The Man5 donor has been obtained by double glycosylation of Man α 1 \rightarrow 2Man disaccharide donor with a mannose acceptor has free OH at 3- and 6-positions. Man α 1 \rightarrow 2Man disaccharide donor was afforded from 2-position orthogonally protected monosaccharide blocks. In order to synthesize another pentasaccharide donor we chose three building blocks: an armed perbenzylated lactosyl fluoride, a mannose derivative has free OH at 3-position and a disaccharide acceptor (Man α 1 \rightarrow 2Man-SPh) has free OH at 2-position. The syntheses of these building blocks have been accomplished. The coupling of these blocks will provide desired pentasaccharide donor (Gal1Glc1Man3). Afterwards, two different pentasaccharide donors will be glycosylated with core disaccharide (Man α 1 \rightarrow 3GlcN3). The progress and results of these chemical reactions will be presented in the symposium.

#2 A glycosyltransferase is required for proper cell adhesion during Drosophila development

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Background: Mucin-type O-glycosylation is a conserved post-translational modification of secreted and membrane-bound proteins that is initiated in the Golgi apparatus by a family of enzymes known as the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (ppGalNAcTs). Mucin-type O-glycans have been implicated in a variety of biological roles including protection from proteolysis, defense against pathogenic microorganisms, lubrication and leukocyte homing. However, direct insight into the function of ppGalNAcTs has been hampered because of functional redundancy amongst the 20 family members found in mammals. To elucidate the developmental roles of O-linked glycans, we have begun to investigate mutations in members of this family in the fruit fly, *Drosophila melanogaster*, where there is less functional redundancy and more sophisticated genetic tools. Our aim is to define key biological processes influenced by O-linked glycosylation. Methods: A transposon insertion mutation in one member of this family (pgant3) was used identify phenotypic consequences of the loss of O-glycosylation. Real-time PCR was performed to compare gene expression of pgant family members between mutant and wild type flies. In vitro glycosylation reactions were performed to identify which candidate peptides PGANT3 can glycosylate. An antibody that recognizes O-linked glycans was used to compare extracts from wild-type and mutant, to identify proteins that are differentially glycosylated. RNAi to pgant3 in *Drosophila* cell culture was performed to dissect the roles of O-glycosylation in cell adhesion and morphology. Results: Mutations in pgant3 result in a blistered wing phenotype, characteristic of genes regulating cell-cell and cell-extracellular matrix (ECM) interactions. Expression of the wild-type pgant3 in mutant flies rescued the wing blistering phenotype. Glycosylation reactions demonstrated that PGANT3 glycosylates extracellular matrix proteins known to be involved in integrin binding. Western blotting using a specific O-glycan antibody showed the loss of O-glycoproteins in mutant wing discs. Western blotting and immunoprecipitation experiments identified the ECM protein, Tiggrin, as a substrate of PGANT3. RNAi to pgant3 in S2R+ cells caused cell adhesion defects and Golgi disorganization. Conclusion: PGANT3 is responsible for modifying extracellular matrix proteins within the wing disc that are responsible for proper cell adhesion through integrin binding during *Drosophila* development. Significance: This study provides the first evidence for the role of O-glycosylation in a developmentally-regulated, integrin-mediated, cell adhesion event and reveals a novel player in the regulation of wing blade formation during *Drosophila* development.

#3 A Preliminary SAR Study for the Carbohydrate Segment of the Antiproliferative Factor from Interstitial Cystitis Patients

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Background: Interstitial cystitis (IC) is a chronic bladder disorder characterized by thinning of the bladder epithelium that can lead to ulceration, voiding urge and severe pain. It affects approximately 1 million Americans. The hallmark of IC is a production of minute amounts of an antiproliferative factor (APF) by bladder epithelial cells. APF is not detectable in urine of healthy people. It inhibits normal bladder epithelial cell growth (IC₅₀ = ~1 nM) as well as proliferation of several tumor cell lines. APF was identified as a short glycopeptide containing a sialylated TF antigen, Neu5Ac α 2-3Gal β 1-3GalNAc α -O-TVPAVVVA. Methods: Fmoc-based Solid phase glycopeptide synthesis was used to prepare analogues of APF in both the peptide and carbohydrate portions of the molecule. Thymidine incorporation assays with normal bladder epithelial cells were used to evaluate the antiproliferative capabilities of the various derivatives. Results: An intriguing SAR was developed by evaluation of ~50 peptide-segment derivatives suggesting that small changes in the APF structure cause diminished activity. Analysis of changes to the carbohydrate portion allowed us to conclude that very minor changes to this structure also cause reduction or abrogation of antiproliferative activity, while other changes (removal of sialic acid, LacNAc disaccharide derivatives) maintained activity. Conclusions: Most of the APF structure is important for interaction with cellular receptors that cause reduction of cell proliferation. The fact that some carbohydrate derivatives maintained activity suggests that analogues could be designed with higher potency or with inhibitory characteristics. Significance: The APF structure has incredible potential as a scaffold to design novel therapies for IC and now for various cancers. Definition of the structural units that are important for activity and/or inhibition of APF activity may allow the design of peptidomimetics with favorable drug-like properties for preclinical development.

#4 Chemoenzymatic Synthesis of Conjugatable Oligosialic Acids

Sylvester Mosley, Pumtiwitt C. Rancy, Dwight C. Peterson, and Willie F. Vann

Background: Antibodies against the capsular polysaccharides coating encapsulated pathogens have been shown to be protective against infection of their mammalian host. This fact is the basis the development of bacterial vaccines based on capsular polysaccharides. Several encapsulated pathogens are encapsulated with polysialic acids. Escherichia coli K1, Neisseria meningitidis Group B and Group C are coated with either α 2,8 neuNAc or α 2,9 neuNAc. Capsular polysaccharides conjugated to proteins have been demonstrated to be effective vaccines for infants and adults. Results: We have devised a method for chemoenzymatic synthesis of oligosialic acids possessing a conjugatable aglycon. Lactosides were chemically synthesized with either an aliphatic alkyne or azide as the aglycon. Oligosialic acids were enzymatically formed on the lactosides using the bifunctional Campylobacter jejuni sialyltransferase, CST II and a bacterial polysialyltransferase. The resulting oligosialic acids are conjugatable under mild conditions. This was demonstrated by coupling the oligosialic acids to fluorogenic azides or alkyne substrates in the Cu⁺ catalyzed "Click" reaction. Significance: The method of preparation of these oligosialic acids should provide an avenue to the synthesis of better defined conjugate vaccines and to reagents for the analysis of antibodies and polysialic acid binding proteins.

5 Expression, glycoform characterization, and antigenicity of a HIV-1 V3 domain fused to human IgG1 Fc

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Background: 2G12 is a broadly neutralizing antibody which target the high mannose (HM) N-glycan in the envelope protein gp120 of HIV-1. Many evidences suggest the specific epitope is the 295 and 332 N-glycan which located on the root of various loop 3 (V3). The latest data from our lab also suggest V3 itself may weakly contribute to the binding with 2G12. To induce 2G12 like antibody, we designed an antigen of V3 with three N-glycosylation site (295, 301, 332) and fused it to human IgG1-Fc domain (which contain one N-glycosylation site Fc2797) to enhance its immunogenicity. Method: The fusion protein construct was arranged by putting Fc domain of Human IgG1 to the C-terminus of V3 fragment and adding leader sequence of human CD5 antigen to the N-terminus. The fusion protein was expressed in HEK293T cells. Above 2 μ g/ml of mannosidase inhibitor—kifunensine was introduced into the culture system to obtain the high-mannose (HM) glycoform. Secreted HM type V3-Fc glycoprotein was purified to homogeneity with protein A affinity chromatography. N-glycans was released from protein with PNGaseF and subjected to MALDI-TOF MS analysis. The oligomerization state of V3-Fc in native condition was accessed by size-exclusion chromatography. The affinity of V3-Fc to 2G12 and anti-V3 antibody 447-52D was analyzed by Surface plasmon resonance (SPR). As two additional HM N-glycan (301 and Fc297) were introduced into the V3-c fusion protein compared to native V3 domain, single mutants were generated for each site to evaluate their contribution to affinity to 2G12. Result: V3-Fc fusion protein was expressed and purified to homogeneity. The glycoforms of fusion protein were controlled by with/without the introduction of kifunensine (an inhibitor to the mannosidase): complex type without inhibitor, high mannose type with inhibitor. Both glycoforms were confirmed by MALDI-TOF MS analysis. Both glycoforms are dimmer in native condition. Surface plasmon resonance (SPR) measurement indicated that HM type V3-Fc has significant affinity with 2G12 (estimated kD for HM V3-Fc: 1147 nM; estimated kD for gp120: 182 nM) and its V3 domain retains normal interaction with anti-V3 antibody 447-52D. Mutagenesis studies on two additional position (301 and Fc 297) demonstrated that both of them contribute to the affinity to 2G12. Conclusion: HM type V3-glycopeptide fused to IgG-Fc demonstrated significant affinity to 2G12 which is within the comparable level to gp120. Significance: a valuable potential candidate for HIV which may induce both anti-V3 and anti-HM N-glycan antibodies.

#6 Chemo-enzymatic remodeling IgG1-Fc with homogeneous glycoforms

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Background: IgG1 antibody is a class of glycoproteins which has a single Asn glycosylation site at its Fc domain (Asn 297). The glycosylation extent as well as the composition of glycans at this particular position affects a wide range antibody dependent activity in vivo, through modulating the binding between IgG-Fc and its Fc receptors. Understanding the functional relevance of different glycoforms of antibody is critical for engineering antibody with improved efficacy. Pure, homogenous glycoform of IgG1 is needed for this research purpose. However, up to today, it remains to be a difficult task to prepare antibody of homogenous glycoform. Here we described a method which combines the yeast *P. pastoris* protein expression system and our chemo-enzymatic method to produce IgG1-Fc fragment with homogenous glycans attached. Approaches and Results: We expressed and purified recombinant IgG1-Fc from Yeast *P. pastoris* in large quantity. We then used Endoglycosidase H to remove the high mannose glycans attached to the IgG1-Fc at Asn 297 position. After that we then use chemo-enzymatic method to link synthesized, pure glycans to IgG1-Fc to form homogenous form of IgG1-Fc. The resulted IgG1-Fc was then purified and their biological activity was assessed by measuring their binding affinity to Fc Gamma IIIa receptor. We are able to synthesize IgG1-Fc of homogeneous glycoforms and the newly synthesized glycoforms of IgG1-Fc have shown distinct affinity to Fc γ IIIa receptor, indicating the distinct effects of different glycans on the activity of IgG1-Fc. Conclusion: This method demonstrates the feasibility of this combined yeast expression and enzymatic method for the remodeling of antibody with homogeneous glycans. The chemo-enzymatic glycosylation can be performed under native conditions. It can be a general method to prepare homogeneous glycoprotein for the structure-function relationship research of the glycoproteins.

Structural Biology/Technology Development

#7 Use of a Mathematical Model of Glycosylation to Characterize Disease States of Cells

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Background: Predicting the impact of possible variations associated with biosynthetic pathways of eukaryotic glycosylation can potentially lead to an improved ability to identify glycan biomarkers as well as modifications of glycoform distributions to enhance therapeutic performance of glycoprotein products. Effective representation of these pathways and manipulation of the parameters associated with them can be aided by mathematical modeling. A comprehensive model for N-linked glycosylation of CHO cells was developed by Krambeck and Betenbaugh (2005) (KB 2005). Methods: Here we describe the extension of the KB 2005 model and its application to analyze MALDI-TOF mass spectra for human N-glycans in terms of underlying enzyme activities. A new, more general, methodology and computer program were developed to generate complete glycosylation reaction networks automatically based on a simple scheme for expressing reaction specificities of the glycosylation enzymes. Software was also developed to calculate synthetic mass spectra from model-calculated glycan profiles and to adjust assumed model parameters, such as enzyme concentrations, to bring the theoretically calculated glycan mass spectrum into agreement with experimental mass spectra. Results: The generated reaction network allows prediction of the complete glycan profile for any set of assumed enzyme concentrations and reaction rate parameters. The result of this process is a complete characterization of a measured glycan mass spectrum in terms of a relatively small number of enzyme activities. In addition a complete annotation of the mass spectrum in terms of glycan structure is produced from the model which can be compared to the experimental mass spectra profile. Conclusion: The validity of the method is illustrated using a comparison of normal human monocytes versus monocytic leukemia (THP1) cells. Significance: This technology will be useful for understanding and characterizing glycosylation changes associated with disease states at the cellular level based on both mass spectra and genomic data in order to identify novel biomarkers and suggest new directions in the treatment of disease.

#8 Hydrogen Bonding in Uniformly ¹⁵N/¹³C Labeled Carbohydrates in Water

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The polysaccharide portion of the enteropathogenic *Escherichia coli* O142 lipopolysaccharide was previously characterized as being composed of 15-16 repeating pentasaccharide units including L-rhamnose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, in the ratio of 1:1:3.1 The pentasaccharide repeat unit's structure was generated by selectively degrading the polymer with anhydrous hydrogen fluoride and calculated using NOE buildup curves and restrained MD (molecular dynamics) simulations. These simulations generated glycosidic torsion angles and predicted amide donors of hydrogen bonds from the three N-acetyl-D-galactosamine (GalNAc) residues.² Experimental methods to define conformation in aqueous solution have been developed for proteins,³ and here are effectively applied to polysaccharides. We used uniform ¹⁵N and ¹³C enrichment to assign ¹H, ¹⁵N and ¹³C resonances via triple resonance HNCA and HNCOC experiments. The assignments enabled measurement of the amide protons' (¹HN) Δ dHN/DT. Temperature dependence of dHN in GalNAc residues A and B and GlcNAc residue E (Figure 1) differs from that of bulk H₂O. Δ dHN/DT alone is not sufficient to determine the presence of hydrogen bonds in solution,⁴ so we measured the temperature dependence of ¹JHN to validate the Δ dHN/DT results. The $\frac{1}{T} \frac{dJHN}{dT}$ data confirms the presence of weak or partial hydrogen bonds originating in the ¹HN of two GalNAc residues, A and B. However the GlcNAc (residue E) dHN temperature gradient is determined to result from trans-glycosidic motion. These data demonstrate the presence of hydrogen bonding in the *E. coli* O142 polysaccharide under ambient conditions with no co-solvent. This raises the possibility that carbohydrates utilize hydrogen bonding to form transient structure, a basis for protein-carbohydrate recognition and interaction.

#9 A Mass Spectrometry Based Glycomic Approach for Identification of Carbohydrate Dependant Virulence Factors Using *Caenorhabditis elegans* as a Surrogate Host

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Background: *Caenorhabditis elegans* is a soil-borne nematode bacterivore that encounters numerous bacterial species including human pathogens and their relatives in its natural environment. Wild type strains of the *C. elegans* are infected by a coryneform bacterium, *Microbacterium nematophilum* that has similarities with human coryneform pathogens. Little is known about the early stages of infection that occur during host cell invasion by coryneform bacteria. Previous work with infection resistant *C. elegans* glycosyltransferase mutants has shown that infection appears to be carbohydrate dependant. Using a mass spectrometry based approach, the identification and comparison of N- and O-glycans with those of the wild type nematode will play a major role toward the determination of carbohydrate ligands involved in these infective processes, leading to a greater understanding of host-pathogen interactions. Methods: Three mutant strains of *C. elegans* along with their parental strain were chosen for study. To isolate the glycans, the worms were disrupted using bead-beater and subjected to chloroform:methanol:water extraction followed by methanol precipitation to remove lipids and free saccharides respectively. The enzymes PNGaseF and PNGaseA were used to release N-glycans from the precipitated proteins and were purified by solvent- and solid phase extraction procedures. The O-glycans were subsequently released by beta-elimination using NaBH₄ which were purified by cation and anion exchange chromatography. Both N- and O-glycans were subsequently permethylated and analyzed on a Voyager-DE RP MALDI-TOF MS instrument. Tandem MS analysis was performed on an Applied Biosystems Qstar XL mass spectrometer. Preliminary Results: Permethylated of both N- and O-glycans has significantly improved the ionization efficiency in MALDI-TOF analysis of sample oligosaccharides. Altered distributions of both N- and O-glycans were observed in mutants in comparison to the wild type strain. Linkage analysis of some of the core-1 type O-glycans using tandem MS suggests that branched O-glycans are major isomers in the *C. elegans* mutants. Localization studies are underway to visualize the glycan tissue expression pattern and distribution. Conclusion: Preliminary results indicate that both O- and N-glycans may be involved in bacterial infection to *C. elegans*. Significance: This ongoing study will lead to a better understanding of the interplay between host glycoconjugates and pathogen receptors.

#10 Purification and Quantitative Analysis of Engineered Heparan Sulfate Binding Proteins

Lauren Wagner and Kenneth Kramer

Background: Heparan sulfate (HS) proteoglycans mediate how cells respond to extracellular signals, bind to the matrix, and interact with other cells. Proteoglycan function and specificity are primarily mediated by their HS polysaccharide chains. HS chains are unbranched disaccharide repeats that can be modified at up to five positions, leading to immense complexity in the pattern of HS modifications. Using techniques with limited sensitivity and/or specificity, several labs have demonstrated that HS exhibits temporal and spatial patterns of modification that can mediate a cell's ability to respond to cell-cell signaling molecules. These observations have led to the hypothesis that a cell's developmental potential may be regulated by the pattern of HS modifications presented at the cell surface. However, this hypothesis has been difficult to evaluate experimentally for lack of tools to block specific HS patterns. To address this question, we are developing a system to inhibit cell-surface presentation of distinct HS modifications. The most critical element of our system is a protein backbone, designated SAPERE, engineered to specifically bind HS. Our current efforts are focused on preparation of µg to mg quantities of pure SAPERE protein. Quantitative ELISA will be used to assess binding affinities of SAPERE protein for different polysaccharide modifications. Methods: The SAPERE protein is substituted at 5 moderately conserved amino acids in the putative binding pocket. In the positive control, these amino acids were replaced with arginine because it is prevalent in HS binding proteins. The negative control contains residues not known to interact with HS. We are purifying SAPERE protein with an engineered affinity tag and column chromatography. Our strategy utilizes a bacterial system and an expression construct containing an N-terminal secretion leader and a C-terminal StrepTagII purification tag. The StrepTagII is designed to bind a column with a matrix composed of streptactin tetramers, which is based on the well-characterized high-affinity, high-specificity streptavidin-biotin interaction. To evaluate the specificity and affinity of SAPERE proteins for HS, we are using ELISAs to determine K_Ds. Our protocol includes titrating amounts of HS bound to a charged plate, incubating with SAPERE protein, and detecting by immunofluorescence. The binding of fibroblast growth factor (FGF), originally identified as a heparan binding protein, serves as a positive control. Results: Initial experiments with SAPERE protein suggested the substitutions introduced instability into the positive control. By targeting each residue individually, and altering it to the respective amino acid from the negative control, we identified a critical Leu residue. Purification of secreted SAPERE by StrepTagII affinity chromatography results in clean product with the negative control. However, the positive control has recently demonstrated aggregation. Preliminary studies suggest that it will be possible to use ELISAs to rank SAPERE proteins based on their relative affinities for HS. Conclusion: SAPERE

stabilization by rational design has been effective, so SAPERE aggregation may be alleviated by modifications in the ankyrin backbone. The next phase of research will include in vitro and in vivo functional studies. Significance: These reagents hold great promise as novel tools for elucidating the molecular mechanisms of HS function.

#11 Targeted Analysis of N-Linked Glycopeptides from H5N1 Influenza Hemagglutinin by Liquid Chromatography–Tandem Mass Spectrometry Thomas A. Blake, Tracie L. Williams, James L. Pirkle, and John R. Barr

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Background: Influenza hemagglutinin (HA) is the antigenic glycoprotein that binds and fuses to the cell membrane during infection and is also the primary component of seasonal vaccines. N-linked glycosylation of HA is thought to affect the virulence of an influenza strain by interfering with the cell recognition binding site or by masking antigenic regions of the protein. Identification of differences among strains in the number/conservation of glycosylation sites as well as the size/complexity of the glycans themselves is necessary for characterizing HA from emerging strains and for determining the efficacy of alternative virus propagation platforms for vaccine production. Methods: Whole virus reagents from three strains of H5N1 influenza (rgA/Vietnam/1203/2004; A/Indonesia/05/2005; A/Bar-headed goose/Qinghai Lake/1A/05) and a reassortant virus (Ind05/PRB-RG2) grown in embryonated chicken eggs were analyzed. Solid-phase extraction (SPE) via hydrazide capture was utilized to specifically isolate tryptic glycopeptides generated from HA in order to determine glycosylation site occupation via deglycosylation and subsequent reverse-phase liquid chromatography–tandem mass spectrometry (LC-MS/MS). Hydrophilic interaction liquid chromatography (HILIC) was also utilized to improve LC-MS/MS analysis of intact glycopeptides. De novo peptide sequencing was used for peptide confirmation. ExPASy's GlycoMod Tool was utilized to aid in the interpretation of intact glycopeptide MS/MS data. Results: All six predicted N-linked glycosylation sites within the N-terminal ectodomain of HA were found to be occupied for the reagent strains examined. This approach identified the presence of glycosylation site 3 for the bar-headed goose strain, even though the exact protein sequence for the selected bar-headed goose strain was not in the database and all other strains within this subset did not predict the presence of this site. This methodology also determined occupied glycosylation sites when the predicted site was ambiguous (i.e. NNST). This approach was then used to propose compositions for multiple glycoforms at the occupied glycosylation sites on HA from the reassortant strain (Ind05/PRB-RG2). Conclusion: We have applied an approach for determining N-linked glycosylation site occupation and investigating the glycans attached to those sites for tryptic digests of whole influenza virus samples via selective sample preconcentration and LC/MS/MS analysis. We have used this data on glycosylation site occupation/conservation to assist in the examination of intact glycopeptides generated from a tryptic digest of a reassortant H5N1/H1N1 influenza virus grown in eggs. We have proposed potential sugar compositions for the multiple glycoforms associated with the occupied glycosylation sites on HA. This approach can also be utilized to determine changes in glycosylation incurred changes in the virus propagation systems used. The information gained from these experiments could then be utilized to examine the relationship between changes in HA glycosylation and changes in virulence/antigenicity. Significance: This work represents a first step towards a platform for investigating differences in HA glycosylation due to virus propagation conditions. Such information may potentially be useful for deciding which strains should be included in vaccines and how strains for the vaccines should be grown. Characterization of HA glycosylation for emerging strains may also be important for understanding which strains might be implicated in a future influenza pandemic.

#12 An LC/MS Platform for Aminated Oligosaccharide Analysis in Both Positive and Negative modes: towards more complete structural assignment Ewa Jankowska; John F Cipollo Food and Drug Administration CBER, Bethesda, MD

Novel Aspect: Facile analysis and assignment of oligosaccharide structure
Background: Structural analysis of oligosaccharides presents a formidable analytical challenge due to their highly complex and heterogeneous nature. Further, often limited quantities of these compounds are available when a thorough analysis is desired such as in vaccine research and therapeutic glycoprotein quality control. Here we present an LC/MS platform for analysis of aminated oligosaccharide derivatives in both positive and negative modes. We analyze the suitability of these amine derivatives over a broad range of oligosaccharides in both positive and negative ion modes. Methods: Oligosaccharides were reductively aminated with either 2-aminopyridine (PA), 2-aminobenzamide (2AB) or 2-aminobenzoic acid (2AA) in acidified sodium cyanoborohydride. Aminated glycans were enriched using Amide-80 solid phase extraction. The aminated derivatives were analyzed by LC/MS on a Thermo Finnigan LCQ ion trap equipped with an ESI source coupled to a Surveyor capillary LC system. The LC system consisted of a Tosho HILIC Amide-80 1 x 150 mm column and an acetonitrile/water/ammonium acetate buffered system. Data were collected in positive and negative ion modes. Tandem mass spectrometry experiments were performed and the resultant fragmentation patterns analyzed and structures assigned manually. Preliminary results: High mannose, hybrid and complex N-glycans and maltooligosaccharide aminated derivatives were examined. Under experimental conditions in positive mode the [M+H]⁺ ion was dominant. In positive ion mode all derivatives produced

predominantly reducing end glycosidic fragments while in negative mode the [M-H]⁻ ion form was dominant and fragment types were mixed. Reducing end fragments dominated although non-reducing end fragments were present. Significantly, informative cross ring fragments were readily produced in negative ion mode.

A-type fragments readily defined linkage configuration in saccharide pairs and D-ions aided in branch structure assignment in negative mode. Multiple bond cleavage in the reducing end fragment enriched spectra aided in branch structure assignment in positive ion mode. In general the positive and negative mode spectra were complementary in nature leading to more complete structural elucidation than either one on its own.

Conclusion: Under experimental conditions aminated oligosaccharide derivatives can be analyzed in both positive and negative mode whilst using the same LC system. Under experimental conditions a single ion form, [M+H]⁺ in positive and [M-H]⁻ in negative modes, is dominant, thus simplifying spectral analysis and interpretation. Spectra from positive and negative modes are complementary and together aid in more complete sample coverage and structural interpretation. Significance This analytical platform allows for more complete and facile assignment of oligosaccharide samples and their structure than single ion mode analysis.

#13 Gold Nanoparticles Bearing Tumor-Associated Carbohydrate Antigens: A Novel Cancer Vaccine Platform

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Background: Many cancer cell surface glycoproteins display aberrant glycosylation. The oligosaccharides found on some tumor glycoproteins (mucins) are truncated into smaller fragments. Some of these, like the human tumor-associated Thomsen-Friedenreich (TF) disaccharide, have been shown to be potent antigens for cancer immunotherapies. Presenting these antigens in an appropriate multivalent fashion for strong immune responses has always been a challenge. One way of addressing this issue is to use a scaffold, such as metal nanoparticles, capable of multivalent presentation and multicomponent functionalization. For example, gold nanoparticles (AuNPs) are very efficient scaffolds because of their favorable properties such as large surface area to volume ratio and ease of surface passivation with different biomolecules. Methods: We designed a novel vaccine platform where both antigen and adjuvant are presented multivalently on a AuNP scaffold. The AuNPs were synthesized by the standard sodium borohydride reduction of chloroauric acid in the presence of the thiolated ligands. Place exchange reactions were performed by incubating an excess of the displacing ligands with precursor AuNPs over 72 hours. Modification of the thiol linker with a lipoic acid (disulfide) moiety was accomplished by solid-phase synthesis on Rink resins. Immunological experiments involved vaccinating mice subcutaneously with AuNPs every two weeks over a 12-week period. Results: We have synthesized several AuNPs coated with the TF disaccharide in the 3-5 nm size range. The TF disaccharide, which was O-linked to both serine and threonine residues of a specific, thiol-functionalized 16-mer peptide repeating unit from MUC4, a mucin that is a biomarker for pancreatic adenocarcinomas. The AuNPs were further functionalized with a 28 residue segment from the complement-derived protein, C3d, as a molecular adjuvant to assist with B-cell activation. AuNPs consisting of various MUC4 glycopeptides interspersed with C3d and a synthetic linker were synthesized and used in the immunological studies. A statistically significant immune response developed against TF containing MUC-4 peptides using all the AuNP vaccine constructs. This preliminary result prompted us to optimize the design of the vaccine construct by first improving the preparation of AuNPs using place-exchange reaction, which allows for control of size and uniformity. We found that citrate-stabilized and tetraoctylammonium bromide-stabilized AuNPs were very good precursors especially for preparing AuNPs bearing carbohydrates and peptides. Also, we modified the ligand by introducing a multidentate lipoic acid moiety as the anchoring group to the surface of AuNPs for increased stability. Conclusion: Although the magnitude was moderate, the statistically significant immune response to our novel AuNP-based hybrid vaccine construct indicates that this design is quite promising. Further optimization of the design factors such as AuNP size, particle dispersity and nature of the ligands should lead to enhanced immune responses. This was addressed by using place exchange method as an alternative method for preparing the AuNPs and also by modifying the ligand structure with multidentate anchoring group. Significance: This study demonstrates that AuNPs can be a versatile and controllable platform for multivalent presentation of antigens that may lead to a new paradigm for vaccine construction.

Carbohydrate Biosynthesis, Metabolism and Function

#14 Investigation the Role of Mucin-Type O-Linked Glycosylation During Murine Submandibular Gland Development

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Background: Glycosylation is one of the most fundamental and abundant post-translational protein modifications found in nature, having roles in protein stability and processing, conserved signaling events during development and certain genetic diseases. Epithelial tubes are essential for the proper function of a diverse array of higher eukaryotic organs. Previous work from our laboratory demonstrated that initiation of mucin-type O-linked glycosylation by the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase enzyme family (pGalNAcTs in mammals or PGANTs in *Drosophila*) is essential for viability during *Drosophila* development by influencing cell polarity and diffusion barrier formation in the developing embryonic tracheal system. Here we describe the role of mucin-type O-linked glycosylation in mouse embryonic submandibular gland (SMG) development. Methods: Agilent microarray and GeneSpring analysis were used to detect pGalNAcT expression during mouse SMG development. SybrGreen quantitative PCR was used to verify gene expression. Whole-mount in situ hybridization was used to detect pGalNAcT mRNA localization in the SMG. Staged mouse SMGs were used for ex vivo organ culture, antibody immunostaining and lectin staining. Western blots probed with lectins were used to screen for potential substrates. Results: Microarray analysis of staged SMGs (E12-adult) demonstrated unique temporal expression patterns for 17 pGalNAcTs during mouse SMG development. Additionally, certain isoforms were expressed predominantly in the epithelium or mesenchyme of developing glands, indicating tissue-specific expression. To examine the biological role of the pGalNAcTs, we examined SMGs from mice deficient for pGalNAcT-1 (T1), an isoform that is expressed early during SMG development. Glands from T1 knockout animals showed reduced bud size and gland growth rate at early embryonic stages (E12). However, growth rate of glands returned to wild type levels by later embryonic stages (E13-E14), indicating a stage-specific developmental delay in T1 knockout animals. Glands from T1 knockout animals also showed reduced PNA staining during early development, suggesting that glycosylation of specific proteins may be responsible for the developmental delay observed. We are currently trying to identify the substrates of T1 in the developing SMG. Conclusion: Our work highlights the coordinated tissue and stage specific expression of members of the pGalNAcT family during SMG development. Furthermore, ablation of one member of this family results in a stage specific developmental delay in gland development. Significance: This study illustrates the importance of the pGalNAcT family and mucin-type O-linked glycosylation during murine branching morphogenesis.

#15 Altered metabolism and signaling in Mouse Embryonic Fibroblasts derived from an O-GlcNAcase (OGA) knockout

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Background: O-GlcNAc addition to serine and/or threonine residues is a dynamic post-translational modification analogous to phosphorylation. Like phosphorylation, O-GlcNAc modification impacts many cellular signaling pathways, including those that regulate stress and the growth response. Cycling of this covalent modification on proteins is mediated by the enzymes O-GlcNAc Transferase (OGT), and O-GlcNAcase (OGA). Previous studies demonstrated that the O-GlcNAc modification is sensitive to nutritional status and is a possible master regulator that coordinates cellular activities according to changing metabolic conditions. Perturbation of O-GlcNAc cycling is thought to be associated with diabetes, Alzheimer's disease and cancer. In humans, the OGA gene is a documented diabetes susceptibility locus associated with high incidence of diabetes among Mexican Americans. Methods: In this study, we generated an OGA knockout mouse model to investigate the role of the O-GlcNAc in diabetes and cellular signaling. An OGA floxed construct was designed by inserting a neomycin cassette flanked by lox P sequences between exon1 and 2, with a third lox P site in front of exon1. The homozygous floxed allele did not produce viable offspring. Therefore, heterozygous floxed mice were bred with cre-recombinase expressing mice to obtain a heterozygous knockout allele of OGA. MEFs were collected at embryonic day 12.5. Genetic and protein expression patterns of wild type, heterozygous and homozygous MEFs were compared. Gene expression profiles of MEFs were investigated by microarray using Affymetrix array chips. Body composition and metabolic data were compared from heterozygous and wild type littermates. Results: The Homozygous OGA KO embryos developed past day 18 when perinatal death was observed. Histopathological examination revealed no structural defects on Day 16 or Day 18 OGA KO embryos compared to their WT littermates. RT-PCR assay confirmed that all OGA isoforms were ablated in the OGA KO cell lines. O-GlcNAcylated protein levels were increased in OGA KO cells compared to wild type and heterozygous. Further

studies indicated that expression levels of several metabolic genes, and protein phosphorylation were altered in OGA deleted MEF cells including AMPK and GSK3 beta. Heterozygous mice tend to have a lower body weight, and fat mass. Plasma Free Fatty Acid (FFA), Triglycerides (TG) and insulin levels were also lower in heterozygous mice compared to wild type. Glucose and insulin tolerance tests demonstrated improved insulin sensitivity in OGA heterozygous mice compared to wild type littermates. Heterozygous mice also have significantly lower circulating plasma glucose. Conclusion: Results indicate that loss of mammalian OGA leads to perinatal lethality and altered cellular signaling cascades. Microarray data revealed that the OGA deletion has significant impact on cytokine signaling, innate immunity and calcium signaling pathways. The altered signaling cascades, detected in heterozygous mice, suggest a compensatory mechanism. Significance: O-GlcNAcase expression is critically important for survival at or immediately after the birth. These data also highlight the importance of O-GlcNAc cycling for signaling, particularly in the innate and adaptive immune system.

#16 Glypicans Affect Distinct Signaling Pathways in Zebrafish Development

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Background: Heparan sulfate proteoglycans (HSPGs) are essential molecules that mediate multiple cell-cell signaling pathways during embryonic development. Signal transduction can be regulated either by core proteins, including membrane-anchored glypicans, or interactions between signaling molecules and highly modifiable sugar chains. To better understand how glypicans function in zebrafish development, we specifically knocked down glypican expression using antisense morpholinos. In knockdowns of three out of the ten known zebrafish glypican genes, we observed strong dorsiventral phenotypes that were visible between gastrulation and 24hpf. Based on previous studies and known cell signaling interactions, we hypothesized that the dorsiventral phenotypes observed in glypican knockdowns may be caused by a disruption of BMP, FGF, and/or Wnt signaling. In order to distinguish which signaling pathway is affected by genetic knockdown of the glypicans, we identified transcriptional changes caused by glypican knockdowns using real-time PCR of candidate gene markers. Methods: Zebrafish embryos were injected with one of the three glypican morpholinos being studied or a randomized control morpholino that was used for a comparison. Embryos were collected at the shield stage and total RNA was isolated and digested with DNase to eliminate genomic DNA contamination. Using reverse transcriptase, RNA was converted to cDNA, which was subsequently used as the template for QPCR studies. Target gene transcriptional expression levels of glypican MO injected samples were compared to control MO injected samples using the $\Delta\Delta C_t$ method with β -actin2 as the endogenous control. Results: Genetic knockdown using morpholino injection of glypicans 2, 6A, and 6B induced dose dependent lethal phenotypes that were first visualized at 13 hpf. The phenotypes include necrosis of the head/brain, flattening of the ventral structures and elongation or bulging of the dorsal region. This phenotype indicates glypicans may be involved in dorsiventral patterning. To better understand the molecular pathways in which glypicans are involved, we used a candidate gene approach to study changes in transcription of BMP target genes, a cell-cell signaling pathway known to regulate dorsiventral patterning. Using QPCR, we identified seven genes within the BMP pathway that are significantly up regulated in at least one of the three glypican knockdowns. The gene expression patterns were different between each glypican knockdown. Conclusions: Genetic knockdown of glypicans 2, 6A, 6B cause an early developmental phenotype that appears to affect dorsiventral patterning. Several genes that are regulated by BMP signaling were significantly changed in at least one of the glypican homolog knockdowns compared to control, suggesting that glypicans play a role in early development through interacting either directly or indirectly with the BMP pathway. In addition, the transcriptional changes were unique to each glypican knockdown suggesting differential roles for each homolog. Significance: Our preliminary results suggest that three glypicans regulate zebrafish dorsiventral development by coordinately regulating distinct steps, an observation that would add a new dimension to our understanding of how proteoglycans might work together to mediate development.

#17 Specificity of protein-protein interactions of glypican and syndecan core proteins

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Background: Heparan sulfate proteoglycans (HSPGs) mediate signaling events in development, homeostasis and disease by interacting with major cell-signaling molecules. Glypicans and syndecans, the main cell-surface HSPGs, have been shown to bind several extracellular molecules in a heparan sulfate (HS)-dependent manner. It was recently reported, however, that the HS chains may not be required for some interactions between glypicans and their ligands, indicating that their core proteins may directly bind some signaling molecules. Therefore, with an aim to better understand the roles of HSPGs in regulating signaling, our goal here is to determine whether HSPG core proteins have their own binding specificity to different signaling molecules. To address this question, we have adopted two different systems to measure the protein-protein interactions, one for HS-dependent processes and the other for HS-independent processes. Methods: To measure the HS-independent protein-protein interactions, we set up a yeast two-hybrid system. We cloned the ten zebrafish glypicans and three syndecans into the two-hybrid cloning vectors

and constructed fusion proteins to the GAL4 DNA-binding domain (BD) and to the activation domain (AD). AH109 yeast strain was used for in vivo color assay. Cells co-transformed with the baits of AD- and BD-fusion constructs were streaked onto nutrient selection plates and the protein-protein interactions were detected by assaying the GAL4-activated reporter genes. To detect HS-dependent HSPG interactions, we are developing an alkaline phosphatase-tag assay in a tissue culture system. Results: We began by testing the ability of the core proteins to form homo- and heterodimers. Preliminary data indicate that there may be several glypican-glypican and glypican-syndecan core protein heterodimer interactions. Conclusion: According to our data, the core proteins of various glypicans and syndecans may be able to bind other core proteins without requiring their heparan sulfate chains. These results implicate that the core protein-protein interactions may contribute to an additional level of regulation and fine tuning in signaling events. This binding specificity is to be further investigated. We are also currently preparing the baits of signaling molecules to measure their protein interactions with the glypican and syndecan core proteins. Significance: Defining the biochemical properties of HSPG core proteins in their binding specificity to major signaling molecules as well as to their own molecules will contribute to the understanding of the molecular mechanisms underlying the regulation of major signaling pathways.

#18 Chondroitin-4-sulfation regulates axonal guidance and growth

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Background: Emerging evidence suggests that oligosaccharides harbor a “glyco code” that is waiting to be deciphered in various contexts of biological and medical importance. Particularly, glycosaminoglycans (GAGs), linear carbohydrates such as chondroitin sulfate (CS) and heparan sulfate, which have been implicated in cell division, development, and neuronal guidance, have the potential for extraordinarily sophisticated signaling mechanisms regulated by modifications to their carbohydrate chains. Previous studies with heparan sulfate demonstrate that such a signaling mechanism may exist through specific sulfation motifs, introducing the presence of a “sulfation code.” However, little has been demonstrated regarding cellular responses elicited by a sulfation code in chondroitin sulfate. A unique property of the mammalian central nervous system (CNS) is a failure to regenerate after injury. Production of chondroitin sulfate proteoglycan (CSPG) by activated glial cells at injury sites is a major negative regulator of neuronal growth. Removal of CS GAG chains with chondroitinase ABC enhances axonal growth and functional recovery after spinal cord injury, demonstrating the importance of CS GAG chains in the CNS. However, the distinctive features of CS GAG chains involved in these processes have not been fully identified. Methods: We used axonal guidance/growth and specific modifications of the sulfation of CS GAG chains as a model to decipher the nature and importance of specific sulfation and the mechanisms by which it coordinates biological events. Results: We found that chondroitin-4-sulfate, but not chondroitin-6-sulfate, exhibits a strong negative guidance cue to mouse cerebellar granule neurons and activates RhoA. Enzymatic and gene-based manipulations of 4-sulfation in the GAG side chains alter their ability to direct growing axons. Furthermore, 4-sulfated CS GAG chains are rapidly and significantly increased in regions that do not support axonal regeneration proximal to spinal cord lesions in mice. Conclusion: We present evidence that small changes in 4-sulfation of CS GAG chains have major effects on the potency of CSPGs to impart guidance cues to neurons. These results support the concept that distinct sulfation along the carbohydrate backbone carries instructions to regulate neuronal function. Significance: Using a physiologically relevant system, we found that modulation of the sulfation in astrocytic CSPGs changes the interaction between astrocytes and neurons in vitro. Combined with our observation that 4-sulfated CSPGs are robustly and rapidly deposited within CNS lesions in animals, these findings suggest that modulation of sulfation in CSPGs serves as a signal to restrict axonal regrowth and may be an important new therapeutic direction for regenerative biomedicine.

#19 Studies on the combined effects of sugar analogs and three dimensional growth substrates in modulating immune and cancer cell behavior

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Previously, the Yarema Laboratory synthesized a novel ManNAc analog, peracetylated N-thiolglycolyl-D-mannosamine (Ac5ManNTGc) that biosynthetically endows cell surface sialic acids with thiol groups. Unlike natural thiols found in cysteine, the sialic acid-carried thiols are located at the outer periphery of the glycocalyx, which allows them to be highly accessible and spontaneously form high affinity interactions with the growth substrate when cells are cultured on a gold surface. In the past, this technology has been used to activate the Wnt pathway in human embryonic cells and induce neuronal differentiation. In this study, based on the ubiquitous role of sialic acids in the immune system and cancer, our objective was to investigate the role of synthetic sugars and chemically-complementary microenvironments in immunity and on the metastatic potential of tumor cells. First, to mimic the native extracellular matrix structure leukocytes encounter during extravasation or cancer cells during metastasis, we sought to move from the 2D surfaces used previously to a 3D system and employed an electrospinning technique to fabricate

synthetic nanofiber scaffolds. These scaffolds were coated with gold to enhance the adhesion of cells displaying thiolated sialic acids. In preliminary studies Jurkat cells – a T-lymphoma line that is normally nonadhesive – were incubated with Ac5ManNTGc in suspension (in a conventional two dimensional culture dish) resulting in self-assembly of the cells into large clusters. Interestingly, when these cells were incubated in a 3D environment in the presence of gold-coated nanofibers, a large percentage of the cells (70-80%) attached to the nanofibers even in the absence of Ac5ManNTGc. In the presence of this analog, Jurkat cells, which are rich in N-linked glycoproteins, displayed cell spreading morphology that resembled basement membrane. By contrast, the HL-60 human promyelocytic leukemia cell line rich in O-linked glycoproteins, showed no morphology change when treated with thiol-derivatized GalNAc analog. In addition, real time PCR analysis of the Ac5ManNTGc treated Jurkat cell line grown under 3D culture conditions showed that β 1 integrin and MMP-9 mRNA expression was down-regulated compared to cells grown in comparable culture conditions on a 2D surface. Because β 1 integrin and MMP-9 play important roles in adhesion events in leukocyte migration and cancer metastasis, this system is anticipated to provide insight to both of these complex biological processes. Current evidence indicates that integrins are important in cancer progression leading us to speculate that integrin function can be altered through a combination of metabolic sialic acid glycoengineering and complementary 3D microenvironments in a therapeutically promising manner. For example, disruption of tumor cell interaction with the extracellular matrix by down regulation of adhesion molecules activities could reduce metastatic lesions in highly aggressive tumors by preventing a migrating cell from attaching to the ECM and growing into a second tumor. Although much work remains to reach this objective, our extant results have established a novel mechanism for the down regulation of adhesion and deadhesion molecules that modulate the immune response and the metastatic potential of tumor cells and thus form a scientific foundation for continuing investigation.

#20 O-GlcNAc cycling impacts Insulin signaling and Transcription

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Back ground: UDP-GlcNAc, the end product of Hexosamine signaling pathway, is utilized by numerous pathway in the cell. One of them, OGT (O-GlcNAc tranferase) uses this substrate to attach N-acetyl glucosamine to the serine or threonine residue of a large number of proteins in the nucleus and cytoplasm. Another sister enzyme OGA (O-GlcNAcase) removes this post-translational modification in the protein. O-GlcNAcase serves to control the amount of O-GlcNAc modified proteins. This dynamic addition and removal of O-GlcNAc process is known as O-GlcNAc cycling. Altered O-GlcNAc cycling changes the insulin-signaling pathway in *C. elegans*. This alters metabolism, life span and the Dauer pathway. Methods: Isolated adult and L4 larval stage in a synchronous manner of *C. elegans* after growing on NGM-OP50 (Agarose coated for RNA isolation) plate. Due to slow growth pattern of ogt mutant synchronous adult culture were sorted by biosorter for N2 and ogt mutant also. Released the glycan and sugar nucleotide from adult *C. elegans* chemically and analyzed using HPAE-PAD. Also isolated RNA from L4 larvae; converted to cDNA by reverse transcription; Perform expression arrays and validate by quantitative PCR Results: UDP-GlcNAc accumulates in the O-GlcNAc cycling mutants (specially in ogt KO mutant). This cytoplasmic pool of UDP-GlcNAc may be a privileged pool, since it is not reflected in changes in N-glycosylation. The composition of PNGase released glycans does not change dramatically compared to the wild type N2. Also, we observed global transcriptional deregulation especially in ogt KO mutant. The genes of hexosamine synthesis (F22B3.4 (gfat), gna-1, gna-2, ppp-1) were all substantially elevated in the ogt-1 mutant. Genes of carbohydrate and lipid metabolism, hexosamine biosynthesis, and C-type lectins are also highly deregulated in the O-GlcNAc cycling mutants. Conclusion: Hexosamine biosynthesis is linked to insulin signaling. O-GlcNAc impacts both signaling and transcription. Significance: O-GlcNAc cycling is likely to be a key epigenetic regulator of signaling and transcription. Changes in O-GlcNAc cycling could lead to epigenetic reprogramming in the intrauterine environment and contribute to human disorders such as obesity and diabetes mellitus.

#21 Antibody against *C. perfringens* Neuraminidase Recognizes Human Sialidase 3 (neu3)

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Background: We previously observed that rabbit polyclonal antibody against *C. perfringens* neuraminidase (anti-NANA) recognized cell-surface molecule(s) on murine PMN after IL-8 stimulation in vivo and reduced their transendothelial migration. We hypothesize that this antibody recognizes mammalian sialidases and inhibits their functions. Human sialidase 3 (huNeu3), the membrane protein upregulated with stimulation, is one of the potential targeted molecules. We tested if huNeu3 is recognized by anti-NANA. Methods: Rabbit were immunized with *Clostridium perfringens* neuraminidase (NANA) in CFA, followed by multiple immunizations with NANA in CIFA and saline to generate hyperimmune rabbit serum. Total IgG from serum was purified via protein G column and quantified by ELISA using NANA, rNeu1 or rNEU3 as antigens. Preimmune serum and normal rabbit IgG were used as negative

controls. cDNA encoding huNeu3 and huNeu1 were synthesized and recombinant Neu3 (rNeu3) and rNeu1 were expressed in E. coli and purified. The genes were also cloned into adenovirus vectors (adenoNeu3, adenoNeu1) which were used to infect HEK 293 cells. Cell lysates from adenoNeu3, adenoNeu1 or empty vector-infected cells as well as the rNeu3, rNeu1 and NANA were used for western blot. Cell lysates were also assayed for sialidase activity using 4-MU-NANA as substrate. Amino acid sequences of NANA and human sialidase 1 to 4 were compared in alignment analysis. Results: On Western blot, anti-NANA recognized the rNeu3 and HEK293 lysates infected with adenoNeu3 but not rNeu1 or lysates infected with adenoNeu1 or empty vector. Antibody against rNeu3 was easily detectable by ELISA, though its titer was lower than that against NANA. Sequence alignment analysis revealed the similarity in protein structure among NANA and human Neu2, Neu3 and Neu4, but not Neu1. Conclusion: Rabbit anti-NANA can recognize human Neu3 but not Neu1. Coupled to the sequence analysis, these data suggest that while there may be a superfamily of mammalian and microbial sialidase, human Neu1 appears to be quite different and may reflect a different cellular function. Significance: Understanding the structure of microbial and human sialidases may lead to the design of improved sialidase inhibitors which may be used in the treatment of inflammatory processes.

#22 Specificity of protein-protein interactions of glypican and syndecan core proteins

Laura Belland, Yun Kee, Kenneth Kramer — LDB/GDBC/NHLBI/NIH

Background: Heparan sulfate proteoglycans (HSPGs) mediate signaling events in development, homeostasis and disease by interacting with major cell-signaling molecules. Glypicans and syndecans, the main cell-surface HSPGs, have been shown to bind several extracellular molecules in a heparan sulfate (HS)-dependent manner. It was recently reported, however, that the HS chains may not be required for some interactions between glypicans and their ligands, indicating that their core proteins may directly bind some signaling molecules. Therefore, with an aim to better understand the roles of HSPGs in regulating signaling, our goal here is to determine whether HSPG core proteins have their own binding specificity to different signaling molecules. To address this question, we have adopted two different systems to measure the protein-protein interactions, one for HS-dependent processes and the other for HS-independent processes. Methods: To measure the HS-independent protein-protein interactions, we set up a yeast two-hybrid system. We cloned the ten zebrafish glypicans and three syndecans into the two-hybrid cloning vectors and constructed fusion proteins to the GAL4 DNA-binding domain (BD) and to the activation domain (AD). AH109 yeast strain was used for in vivo color assay. Cells co-transformed with the baits of AD- and BD-fusion constructs were streaked onto nutrient selection plates and the protein-protein interactions were detected by assaying the GAL4-activated reporter genes. To detect HS-dependent HSPG interactions, we are developing an alkaline phosphatase-tag assay in a tissue culture system. Results: We began by testing the ability of the core proteins to form homo- and heterodimers. Preliminary data indicate that there may be several glypican-glypican and glypican-syndecan core protein heterodimer interactions. Conclusion: According to our data, the core proteins of various glypicans and syndecans may be able to bind other core proteins without requiring their heparan sulfate chains. These results implicate that the core protein-protein interactions may contribute to an additional level of regulation and fine tuning in signaling events. This binding specificity is to be further investigated. We are also currently preparing the baits of signaling molecules to measure their protein interactions with the glypican and syndecan core proteins. Significance: Defining the biochemical properties of HSPG core proteins in their binding specificity to major signaling molecules as well as to their own molecules will contribute to the understanding of the molecular mechanisms underlying the regulation of major signaling pathways.

#23 Spatial and temporal expressions of glypicans in zebrafish embryonic development

Nisha Puntambekar, Yun Kee, Kenneth Kramer — LDB/GDBC/NHLBI/NIH

Background: Glypicans are a family of heparan sulfate proteoglycans found attached to the cell surface membrane by a glycosylphosphatidylinositol anchor and contain 14 conserved cysteine residues. Previous studies identified six vertebrate glypicans that interact with numerous cell-cell signaling proteins to mediate multiple roles in development, homeostasis, and disease. Two glypican homologs have previously been identified in zebrafish, but little is known about the cellular and molecular mechanisms by which glypicans interact to mediate zebrafish embryonic development. Here we describe our initial findings of glypican expressions in zebrafish. Methods: We cloned eight novel zebrafish glypicans, investigated their sequence relationship, assigned their nomenclature based on similarities to orthologs and homologs in other vertebrates, and examined their expression patterns during early embryonic development. Wild-type (TuAB) zebrafish embryos were collected and fixed at 24, 48, and 72 hours post fertilization (hpf), and whole mount in situ hybridization was performed using digoxigenin-labeled probes. Results: Spatial and temporal analysis of embryos revealed expression of glypicans at 24hpf in the head and diffused throughout the trunk region. At 48hpf and 72hpf, glypican expression becomes more concentrated in the head, specifically in the eyes and brain region. In order to determine the correlations between expression patterns of different glypicans, we drew upon the distinct layers of zebrafish eyes to make our observations. Because localized glypican expression occurs in the layers of the eye, the Pearson Correlation Coefficient was used to assess the degrees of association between

glypicans. We found few trends between the evolutionary relationship of glypicans and their expression patterns. However, we are finding several correlations between the various genes, and will be carrying out further expression analysis. Conclusion: After examining the spatial and temporal expression patterns of these genes in zebrafish, we can confidently state that expression primarily occurs in the brain suggesting that glypicans are essential for normal brain development. Additionally, the layers of the eye provide us with a value tool and practical assay system for investigating the correlations and contrasts between different glypicans. Finally, it is clear that syntenic glypicans do not have correlating expression patterns despite their evolutionary relationship. This evolutionary conservation over time and across species implies that glypicans play a significant role in development.

Significance: By examining the spatial and temporal expressions of glypicans, we will be able to advance current understanding of the developmental and functional roles of glypicans in zebrafish morphogenesis.

#24 Identification of subtype-specific glycoproteins from ovarian tumors

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Background: Ovarian cancer is the most lethal gynecologic malignancy with epithelial ovarian tumors comprising ninety percent of ovarian tumors in adult women. The origin of epithelial ovarian tumors are heterogeneous and can be subclassified histologically into serous, mucinous, endometrioid, clear-cell, transitional-cell types, squamous cell, mixed, and undifferentiated subtypes. Different subtypes of ovarian tumors have differences in clinical outcome and response to chemotherapy. Discovery of molecular basis of these subtypes of ovarian tumors is very important to understand mechanism of tumor genesis and predict responses to targeted biological therapeutic agents. Therefore, the direction of ovarian cancer biomarkers discovery for therapy would be toward the subtypes with early stage diagnosed cancers. Methods: The glycoproteins from seven subtypes of ovarian tumors and normal control ovarian tissues were isolated from three individuals separately using solid-phase extraction of glycopeptides isolation method. The isolated glycopeptides were analyzed by quantitative proteomic analysis method using the LC-MS and LC-MS/MS data generated by ESI-QSTAR. In addition, spectral count quantitation from LC-MS/MS data generated by multiple analyses using LTQ was also employed to increase the confidence of identification and quantification. The proteins commonly or uniquely expressed in subtypes of ovarian tumors were chosen and validated by selected reaction monitoring (SRM). Results: We were able to determine around 1000 N-linked glycosylation sites from cancer tissues and identify subtype-specific glycoproteins for ovarian tumor. Several candidate proteins including CA125, mesothelin and Niemann-Pick C1 were identified as commonly or uniquely expressed in subtypes of ovarian tumors and validated by SRM. Conclusions: Glycoproteins commonly or uniquely expressed in subtypes of ovarian cancer were identified in this study. Two known ovarian cancer related proteins, mesothelin and CA125, and a new candidate glycoprotein, Niemann-Pick C1, showed the different expression in different subtypes of ovarian tumors. And CA125 and Niemann-Pick C1 were validated by SRM, indicating that the glycoproteomic strategy using glycopeptides capture, MS/MS, and SRM is specific and sensitive to identification and validation of ovarian cancer-specific markers. Significance: This will facilitate the understanding of molecular mechanism of ovarian cancer subtypes and differentiate them in molecular level. The results also provide the candidate glycoproteins as molecular basis for detection and treatment of different ovarian tumors and further development and validation are required for clinical usage.

#25 Characterization of sialic acid transport activity of the lysosomal membrane transporter SIALIN.

G Nesterova, D Adams, M Huizing, WA Gahl

Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Background: SIALIN is a lysosomal membrane transporter for sialic acid. It is a member of the pH-dependent anion-cation symporter family. Two related autosomal-recessive human diseases are known to be caused by mutations in the SIALIN gene, SLC17A5. Salla disease is caused by a p.R39C missense variation in SLC17A5. This mutation causes a partial loss of sialic acid transport activity. The clinically more severe Infantile Sialic Acid Storage Disease (ISSD) is caused by more severe SLC17A5 mutations (predominantly frame shift or nonsense mutations), which result in little or no sialic acid transport activity. The drug cysteamine is a free thiol that has been shown to restore some arginine-like characteristics to cysteine residues that are present due to arginine to cysteine mutations. We used a cell-culture based sialic acid transport assay to test whether the impaired sialic acid transport activity of the SIALIN p.R39C mutant could be rescued with cysteamine treatment. Methods: Expression vectors (pcDNA3) with wild type and p.R39C mutated SLC17A5 inserts were obtained, each of which contained a C-terminal HA tag and a modified lysosomal targeting signal. After transfection of these constructs into HeLa cells, translated SIALIN was targeted to the plasma membrane, due to loss of the lysosomal sorting signals. Transfected HeLa cells were then incubated with radiolabeled sialic acid (with and without cysteamine) to assess cellular uptake of sialic acid, which was used to calculate SIALIN transport activity. Results: Sialic acid transport differences between wild type and p.R39C mutated SIALIN were clearly discernable by the uptake assay, indicating validity of our transport assay. However, treatment

with cysteamine (2-4 mM per well) did not appear to improve (or diminish) the defective transport activity of p.R39C mutated SIALIN. Conclusions: In vitro cysteamine treatment of p.R39C mutated SIALIN does not improve sialic acid transport. It is not, therefore, likely to be a promising therapy for humans with Salla disease.

#26 Characterization of sialic acid transport activity of the lysosomal membrane transporter SIALIN.

G Nesterova, D Adams, M Huizing, WA Gahl

Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Background: SIALIN is a lysosomal membrane transporter for sialic acid. It is a member of the pH-dependent anion-cation symporter family. Two related autosomal-recessive human diseases are known to be caused by mutations in the SIALIN gene, SLC17A5. Salla disease is caused by a p.R39C missense variation in SLC17A5. This mutation causes a partial loss of sialic acid transport activity. The clinically more severe Infantile Sialic Acid Storage Disease (ISSD) is caused by more severe SLC17A5 mutations (predominantly frame shift or nonsense mutations), which result in little or no sialic acid transport activity. The drug cysteamine is a free thiol that has been shown to restore some arginine-like characteristics to cysteine residues that are present due to arginine to cysteine mutations. We used a cell-culture based sialic acid transport assay to test whether the impaired sialic acid transport activity of the SIALIN p.R39C mutant could be rescued with cysteamine treatment. Methods: Expression vectors (pcDNA3) with wild type and p.R39C mutated SLC17A5 inserts were obtained, each of which contained a C-terminal HA tag and a modified lysosomal targeting signal. After transfection of these constructs into HeLa cells, translated SIALIN was targeted to the plasma membrane, due to loss of the lysosomal sorting signals. Transfected HeLa cells were then incubated with radiolabeled sialic acid (with and without cysteamine) to assess cellular uptake of sialic acid, which was used to calculate SIALIN transport activity. Results: Sialic acid transport differences between wild type and p.R39C mutated SIALIN were clearly discernable by the uptake assay, indicating validity of our transport assay. However, treatment with cysteamine (2-4 mM per well) did not appear to improve (or diminish) the defective transport activity of p.R39C mutated SIALIN. Conclusions: In vitro cysteamine treatment of p.R39C mutated SIALIN does not improve sialic acid transport. It is not, therefore, likely to be a promising therapy for humans with Salla disease.

#27 Transcription Initiation Requires An O-GlcNAcylation Cycle on the Human RNA Polymerase II CTD.

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Background: During the initiation of transcription, the CTD becomes phosphorylated, serving as a scaffold for the recruitment of elongation and RNA processing machinery. It has long been known that the C-terminal domain of RNA polymerase II contains a GlcNAc post-translational modification, but the function of the GlcNAc PTM has not been clear. However, we do know that the GlcNAc PTM prevents the phosphorylation of CTD peptides by the TFIIF cdk7 kinase, which is activated upon initiation. These data suggest the hypothesis that GlcNAcylation of the CTD occurs prior to initiation and that it is important for events leading up to initiation. Finally, the hypothesis also implies that the addition and removal of GlcNAc must occur during this period. Methods: In vitro transcription assays, immunoprecipitations, western blotting, preinitiation complex assembly assays. Results: We found that inhibitors of either the O-GlcNAc transferase (OGT) or the O-GlcNAc amidase (OGase) blocked transcription in crude HeLa nuclear extracts. Furthermore, this block occurred during preinitiation complex (PIC) assembly: if PIC assembly occurred before the addition of either inhibitor, transcription was not affected. We could rescue the amidase inhibition by the addition of unmodified RNA pol II, indicating that pol II was the direct target of the OGase inhibitor. We showed that OGT is recruited to the PICs and that this recruitment is negated by OGT inhibitors. The OGase inhibitor PUGNAC resulted in the accumulation of a reduced mobility pol II species on the promoter that comigrates with GlcNAc modified pol II. OGT inhibitors cause the accumulation of prematurely phosphorylated pol II on the promoters, which is an event usually seen after initiation and not before as in this instance. Finally, native RNA polymerase II that has been GlcNAcyated in vitro is refractory to phosphorylation by the elongation-specific pTEF-b kinase. Conclusions: These data indicate that both the addition and removal of O-GlcNAc from the CTD of RNA polymerase II are essential for transcription initiation. Secondly, they show that the CTD itself is required for transcription initiation. Mechanistically, the cycling of GlcNAc on and off of the pol II CTD is necessary for PIC assembly steps and the prevention of aberrant phosphorylation of the CTD, a known inhibitory event to transcription. Significance: These findings are counter to the prevailing dogma that transcription initiation is largely independent of the CTD, although there is data in the literature contradicting this point of view. Our results however, provide direct evidence of a CTD requirement, provide the first functional evidence of a GlcNAc PTM requirement on RNA polymerase II, showing that it is necessary for transcription initiation, and shed light on the mechanism through which the CTD requirement manifests itself. Secondly, this is the first evidence of any PTM requirement on RNA polymerase II or any other component of the general transcriptional machinery being required for transcription initiation.

#28 3-O-sulfation increases Fgfr2b function during submandibular gland epithelial branching morphogenesis

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Background: Fgf10-Fgfr2b signaling is critical for mouse submandibular gland (SMG) development as the glands are absent in Fgfr2b or Fgf10 null embryos. Heparan sulfate (HS) mediates high affinity binding of FGFs to their receptors. During synthesis, HS is modified by a series of sulfotransferase enzymes. Although 3-O-sulfotransferases (Hs3st) are the largest sulfotransferase family, with seven isoforms, they act late in HS biosynthesis forming the rarest sulfate modifications. Hs3st1 forms the antithrombin binding epitope whereas Hs3st3 forms the herpes simplex virus-gD1 binding epitope however their role during organ development has not been studied. Methods: Ex vivo organ culture of isolated E13 SMG epithelia was cultured in laminin-111 ECM with exogenous FGF10 and was used to assay the role of HS on branching morphogenesis. Results: FGF10-mediated proliferation occurs only at the tips of branching epithelium in culture, whereas its receptor, FGFR2b, is localized throughout the epithelium, suggesting an HSPG co-receptor is located on the tip cells. We profiled gene expression of the HS biosynthetic enzymes in the proliferating tip of the epithelium, and identified a striking increase in 3-O-ST isoforms. In situ analysis of intact SMGs confirmed that the Hs3st isoforms were localized in the peripheral epithelial end bud cells. We confirmed that 3-O-sulfated epitopes were present on the end bud HS with labeled antithrombin and HSV-gD1 binding protein. To study the function of 3-O-sulfation, kidney HS, which is not endogenously 3-O-sulfated but contains about 45 sulfates/HS chain, was treated with either Hs3st1 or Hs3st3 enzymes, adding only two 3-O-sulfates/HS chain. Over 3-O-sulfated HS increased branching and proliferation of FGF10-cultured epithelia, which was mediated via FGFR signaling. There was increased gene expression of , Etv4 and Etv5, end bud markers downstream of FGF10 signaling, and Aqp5, an acinar cell differentiation marker. In loss of function experiments, a reduction in Hs3st expression inhibited Fgf10-dependent morphogenesis, which was restored by adding exogenous over-3-O-sulfated HS. Further, over 3-O-sulfated HS increased the amount of FGF10 bound to Fgfr2b in pull-down assays, suggesting that 3-O-sulfation helps stabilize FGF10-Fgfr2b complex. Interestingly, we found Fgfr2b but not FGF10 specifically bound immobilized Hs3st3-modified kidney HS. Furthermore, Hs3st-modified kidney HS increased FGF10-FGFR2b-mediated cell proliferation in BaF3 lymphoid cells. Conclusion: Here we identify a novel role for 3-O-sulfated HS, which binds Fgfr2b and increases FGF10-dependent submandibular gland epithelial proliferation and morphogenesis. Collectively, 3-O-sulfated HSPGs localized at the peripheral end buds in SMG epithelium specifically bind Fgfr2b, increasing signaling resulting in end bud proliferation, differentiation and branching morphogenesis. Significance: 3-O sulfated HS may be useful for targeting Fgfrb-mediated regeneration of salivary gland tissue.

#29 Hyposialylation of muscle glycoproteins in a mouse model of HIBM

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Background: Hereditary Inclusion Body Myopathy (HIBM) is a recessive adult-onset neuromuscular disorder, characterized by progressive muscle weakness caused by mutations in GNE, coding for the key enzyme in sialic acid biosynthesis. The exact pathology of HIBM is unknown, but patients show decreased muscle sialylation of the glycoproteins alpha-dystroglycan, PSA-NCAM, and neprilysin. We created a Gne knock-in mouse model harboring the human Persian-Jewish M712T founder mutation. These mice died of unexpected glomerular disease, involving effacement of podocytes due to hyposialylation, before postnatal day 3. Administration of the sialic acid precursor ManNAc partially rescued the kidney phenotype and survival of mutant mice. Here we evaluate the later onset of a muscle phenotype and the effects of ManNAc treatment in surviving mutant mice. Methods: Wild type and mutant Gne M712T mice (untreated or ManNAc treated) were euthanized between the ages of 2 and 15 months and muscle tissues, serum, and other organs were harvested. Muscle tissues (gluteus, gastrocnemius, and quadriceps) were analyzed by immunohistochemistry (IH), electron microscopy (EM) and western blotting (WB), using a variety of lectins (PNA, HPA, WGA) and antibodies (alpha-dystroglycan, PSA-NCAM, neprilysin, LC3). Results: Muscles of older (over 5 months) surviving male mice showed tubular aggregates (TAs) on EM, which appeared to be a common phenomenon in all male inbred mice. However, Gne M712T mutant mice muscles also showed increased 'cellular debris' and some autophagosome-like structures on EM. ManNAc administration seemed to not only to reduce TA size and abundance, but no 'cellular debris' was found in ManNAc treated mutant mice muscles. These findings are further pursued and verified by IH and WB analysis. IH staining on mutant muscle slides showed a marked decrease for PSA-NCAM signal, which recovered after ManNAc feeding. Alpha-dystroglycan, PNA, HPA and WGA lectins did not show striking differences between wild type and mutant mice. Neprilysin and LC3 stainings are being pursued.

Conclusions: Older Gne M712T mutant mice appeared to develop a muscular phenotype of TA formation (not HIBM-specific) and areas of increased muscular 'cellular debris' (likely HIBM-specific). ManNAc administration seemed to reduce TA size and abundance, indicating that TA formation may be due to hyposialylation. IH showed reduced PSA-

NCAM staining in mutant muscle tissues, indicating hyposialylation. ManNAc administration recovered the PSA-NCAM signal, showing that ManNAc can provide sialic acid as a substrate to deprived cells. Significance: Our Gne M712T mutant mice may be a relevant model for the muscular phenotype in human HIBM, as evidenced by increased cellular debris and autophagosome-like structures in skeletal muscle on EM. ManNAc is not only a promising therapeutic patients with HIBM, but may also be beneficial for several other human muscular disorders characterized by TAs, including sporadic limb girdle weakness, familial myasthenia gravis, and unexplained exercise-induced muscle cramps. These conditions may be caused by local sialic acid deficiency. Our preliminary results suggest that the Gne M712T mutant mice are an excellent model for further evaluation of these aspects.

#30 Searching For The Substrates Of The UDP-GalNAc:Polypeptide N-Acetylgalactosaminyltransferases That Are Expressed During Drosophila Development

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Background: Mucin-type O-glycosylation is a widespread modification of eukaryotic proteins. In *Drosophila*, O-glycans play important roles during development, including roles in epithelial tube formation and cell adhesion. The transfer of N-acetylgalactosamine (GalNAc) to selected serine or threonine residues is catalyzed by UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (PGANTs in *Drosophila*). Thus far, 9 pgant genes have been identified in *Drosophila*. These pgant genes display dynamic spatial and temporal regulation during development, suggesting that the sequential and coordinated expression of these genes determines which proteins will be glycosylated at different stages during development. Defining the O-glycosylated substrate proteins and their functions will provide insights into the biological role of O-linked glycosylation. Methods: To identify substrates of the PGANTs, we employed fly lines expressing double-stranded RNA (dsRNA) to knock down expression of specific pgant genes via RNA interference (RNAi). Glycoproteins from lines expressing dsRNA in all tissues were compared to wild type at various stages of development. Proteins were separated under reducing conditions in 4-12% SDS-PAGE gradient gels. Gels were western blotted and incubated with horseradish peroxidase-conjugated lectins that specifically recognize various O-linked sugars. We used lectins from *Helix pomatia* (HPA), which recognizes GalNAc, and from *Arachis hypogaea* (PNA), which recognizes Gal β 1-3GalNAc. We will then use lectin affinity columns combined with mass spectroscopy to purify and identify the target proteins. Candidate proteins will be validated in vivo and in vitro. Results: Initial HPA lectin blots of proteins extracted from a line expressing dsRNA to CG30463 displayed alterations in high molecular weight bands when compared to wild type. Additionally, PNA lectin blots of extracts prepared from a line expressing dsRNA to CG9520 identified a protein band corresponding to 55 KDa that was stronger than in the wild-type sample. Conclusion: Initial lectin blots identified a number of specific bands that are reduced or stronger in intensity in the dsRNA-expressing lines relative to wild type. Significance: The proteins corresponding to these bands might be the potential substrates of the PGANTs. Knowing the functions of these substrate proteins will provide insights into the mechanisms by which O-glycosylation affects various developmental processes.

#31 Studies Investigating the Role of Protein O-Glycosylation During Drosophila Development Using RNAi in vivo.

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Background: Protein O-glycosylation represents a major form of post-translational modification that is conserved across most eukaryotic species. One type of O-glycosylation, known as mucin-type O-glycosylation, is initiated by a family of enzymes (pGalNAcTs in mammals and PGANTs in *Drosophila*) that catalyzes the transfer of GalNAc to the hydroxyl group of either serine or threonine in protein substrates. There are twelve pgant genes in *Drosophila melanogaster*. Previous work from our group demonstrated that these genes are expressed in distinct spatial and temporal patterns throughout *Drosophila* development, suggesting unique functions for the enzymes they encode. Loss-of-function mutations in one family member, pgant35A, resulted in death early in development and irregularities in epithelial tube formation, indicating that this protein is essential for viability. Additionally, the activity of another family member, pgant3, is required for proper cell adhesion during development. Together these data suggest that the PGANT family of transferases plays crucial and unique roles during *Drosophila* development through their modification of distinct substrates. However, it remains unclear what roles the other family members may play during development. Methods: In an effort to define the developmental roles of the remaining ten family members, we have employed RNA interference (RNAi)-mediated knockdown of individual genes in the fly. Transgenic fly lines containing inverted repeats specific to each gene under the control of the Gal4-inducible UAS promoter were crossed to lines that express the Gal4 transcriptional activator constitutively (to induce RNAi throughout development). Progeny from these crosses were then scored for viability. Results: Using this technology we have obtained specific decreases in gene expression from each pgant family member. We have identified three additional members of the PGANT gene family that are required for viability. These results further demonstrate the importance of this enzyme family in *Drosophila*. To further define the role of each pgant, tissue and stage-specific Gal4 expressing lines are being used to induce RNAi to each gene. Conclusions: Our results suggest that multiple members of the PGANT gene family

serve essential functions during *Drosophila* development. These differing functions indicate that these transferases serve non-overlapping roles within the cell presumably through the modification of distinct substrates. Significance: These results further demonstrate the importance of this family of enzymes in *Drosophila*. Our studies aim to elucidate the role of mucin-type O-glycosylation throughout *Drosophila* development as well as provide insight into the role of this highly conserved protein modification during mammalian development.

#32 Exploring stem cell biology and tissue engineering with sugar analogs

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Regeneration is the process of restoring cells, tissues and structures that are lost during disease, injury or aging. Natural regenerative processes are facilitated by stem or progenitor cells and the progressive loss of these capabilities throughout adulthood contributes to impaired healing and degenerative disease in the elderly. Hence, small molecules that promote stem cell proliferation and/or differentiation are highly sought after as molecular tools to study stem cell biology and, ultimately, as new therapeutic agents to enhance tissue repair and regeneration. In this study, we used sugar analogs as small-molecule tools to create a 'glycoengineered stem cell niche' using metabolic glycoengineering methods. In this approach, synthetic monosaccharides bearing unnatural functionalities (such as thio-, keto-, and azido-groups) but otherwise similar in structure to natural metabolites found in a biosynthesis pathway for cell-surface glycans are incubated with living cells. These analogs are incorporated into the glycocalyx of living cells and – because quantitative and structural variations of the glycocalyx can profoundly influence specific cell-cell and cell-matrix interactions – this method provides an opportunity to modulate a diverse range of biological activities and endow the cell surface with unique adhesive properties. We have shown that ManNAc analogs can be exploited to express thiols and ketones in glycocalyx, consequently endowing the cell surface sialosides with novel chemical properties that can be exploited for new carbohydrate-based modes of cell adhesion. Particularly intriguing results were obtained when the ManNAc analog 'Ac5ManNTGc' was used to install the thiol-bearing sialic acid 'Neu5TGc' on the surfaces of human embryonic cells and the cells were consequently grown on a high affinity gold surface. In these experiments, the thiol-bearing analogs altered morphology and displayed biochemical markers (e.g., nestin and β -catenin) indicative of neural differentiation. Subsequently, we have expanding testing to additional growth substrates (e.g., polysialic acid and maleimide-derivatized polymers), additional cell lines (e.g., immune cells and additional human embryonic lines), and 3D topology (e.g., electrospun nanofibers). In conclusion, the modification of cell surface properties provides new opportunities to exploit the complex interplay between adhesion, signal pathways, and stem cell fate through the 'glycoengineered stem cell niche.' This finding is significant because, although stem cells hold tremendous promise for tissue engineering and regenerative medicine, reliable methods for maintaining these cells and inducing their self-renewal, as well as controlling their differentiation to specific and homogeneous lineages, remain problematic. Up to now, efforts to control stem cell biology have focused on the use of large biological molecules (proteins such as growth factors) or genetic alteration (mutants, knockouts, loss- or gain-of-function studies). Here, we show that non-natural sugar analogs as small molecules act on receptors involved in various signaling pathways and induce lineage-specific differentiation of stem cells and thereby provide a class of small molecules capable of influencing stem cell fates through glycosylation pathways.

#33 The O-GlcNAc Modification Modulates Nutrient Stress and Differentiates Insulin Signaling Phenotypes in *C. elegans*

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Background: O-linked-N-acetyl glucosamine (O-GlcNAc) is a post-translational modification implicated in transcription, translation, proteasome activity, and the stress response. Over 500 nuclear and cytoplasmic proteins are O-GlcNAc modified, including insulin signaling components. OGA-1 is a human type II diabetes susceptibility locus. Over expression of the O-GlcNAc transferase OGT-1 or inhibition of the O-GlcNAcase OGA-1 causes insulin resistance in mammalian cells. Glucose levels influence O-GlcNAc modification levels, suggesting that this pathway is a nutrient sensor. Although these enzymes are essential in mammals and highly conserved in *C. elegans*, knockouts of *oga-1* and *ogt-1* are viable in the worm, making it a good model system for genetic studies of the O-GlcNAc modification. Methods: Simple genetic assays (fertility, lifespan, and dauer) are used to elucidate the role of O-GlcNAc. The *C. elegans* dauer assay is an indirect measure of insulin signaling: mutations in the insulin-like receptor DAF-2 result in an alternative dauer larval stage that can be identified visually. Previous work in our laboratory demonstrated that loss of OGT-1 in a *daf-2* mutant partially suppresses dauer formation, indicating insulin hypersensitivity. Results: Loss of OGA-1 also results in a decrease in dauer formation in *daf-2* mutants. Dauer formation in *daf-2 ogt-1* and *daf-2;oga-1* double mutants is dependent on the downstream transcription factor DAF-16/FOXO. *ogt-1* and *oga-1* mutations have different effects on different DAF-16-dependent *daf-2* phenotypes: although both mutations

suppress dauer formation, neither has an effect on *daf-2* fertility and they have opposing effects on *daf-2* lifespan (in collaboration with CA Wolkow). Since O-GlcNAc levels respond to nutrient levels, we tested the role of O-GlcNAc in the genetic response to excess nutrition. *ogt-1* mutants, but not *oga-1* or *daf-2* mutants, show decreased fertility on high glucose (3-5X fewer progeny compared to wild type). High glucose suppresses dauer formation in *daf-2* single mutants and *daf-2 ogt-1* and *daf-2;oga-1* double mutants, but not in the TGF- β mutants *daf-1* and *daf-7*.
 Conclusions: Our data suggest that dynamic cycling of the O-GlcNAc modification is important for *daf-2* dauer formation. Different phenotypes in the *daf-2* dauer, fertility, and lifespan assays suggest that the regulation of insulin signaling may occur downstream of DAF-16, which has hundreds of transcriptional targets. Because mammalian FOXO is O-GlcNAc modified, we are testing whether DAF-16 is also O-GlcNAc modified and whether differential DAF-16 modification leads to these different phenotypes. The glucose effect on fertility suggests that O-GlcNAc, but not insulin signaling, is necessary for the response to nutrient stress. In contrast, the glucose effect on dauer formation is specific to insulin signaling and independent of O-GlcNAc. Significance: Mammalian cell culture experiments have provided important information about the function of O-GlcNAc in insulin signaling, but are limited by the essential nature of the genes and a lack of integrated signals between genetic networks and between tissues. Studying this pathway in *C. elegans* allows us to observe the effects of *ogt-1* and *oga-1* in the context of the whole organism and provides new mechanistic insights.

Carbohydrate Binding Proteins

#34 Carbohydrate specificity of the eastern oyster (*Crassostrea virginica*) galectin CvGal1: Recombinant expression of selected carbohydrate-recognition domains

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Background: It is widely accepted that recognition of exposed glycans on the cell surface of potential pathogens by host humoral or cell-associated lectins is a key component of the innate immune response of vertebrates and invertebrates. However, the protozoan parasite *Perkinsus marinus* causes “Dermo” disease in the eastern oyster *Crassostrea virginica*, and is responsible for catastrophic damage to shellfisheries in North America. Until recently, the parasite’s mechanism(s) for entry into the hemocyte had remained obscure. We identified and characterized in oyster hemocytes a galectin [*C. virginica* galectin, CvGal (hereafter CvGal1)] of unique carbohydrate-recognition domain (CRD) organization that, unlike most mammalian galectins, recognizes exogenous carbohydrate ligands. CvGal1 binds to a variety of potential microbial pathogens, phytoplankton components, and *Perkinus* trophozoites, suggesting that it functions as a hemocyte surface receptor for this parasite, and facilitates its entry into the host cells. Unlike all galectins known so far, CvGal1 displays four CRDs. These contain seven of the nine amino acid residues that bind ligand in the bovine galectin-1. Because the CvGal1 CRDs are similar but not identical to each other, their carbohydrate specificities may be also different. Methods: To enable the rigorous analysis of the binding specificity and affinity of the CvGal1 CRDs, we initiated their recombinant expression, individually and as combinations of 2 and 3 CRDs. Expression constructs were developed in the pET expression vector for all four individual CRDs, as well as CRDs 2-3 and CRDs1-2-3 were developed and the selected plasmids were transferred into expression hosts *E. coli* BL21(DE3) (Novagen). The recombinant CvGal1 CRDs were isolated by affinity chromatography on lactosyl-Sepharose. Results: SDS-PAGE analysis of the IPTG-induced *E. coli* cultures of each of four individual CRDs revealed a protein band corresponding to their calculated molecular weight, demonstrating their expression in *E. coli*. Most recombinant CvGal1 CRD 2 was localized in the insoluble fraction, but the recombinant CvGal1 CRD 3 and 4 were both in the soluble and insoluble fractions. In general, IPTG induction at lower temperature (room temperature) favored higher proportions of soluble recombinant protein. Optimization of recombinant expression, purification, and characterization of each recombinant CRD are underway. Conclusion: We have demonstrated recombinant expression of CvGal1 individual CRDs, which will be used for analysis of their binding specificity and affinity. Significance: The biological roles of galectins have only been partly elucidated, encompassing embryogenesis and indirect roles in innate and adaptive immunity mediated by the binding to endogenous ligands. In contrast, CvGal1 recognized both exogenous and endogenous ligands. The present study will contribute to understand the diversity in non-self recognition of CvGal1, and its biological role(s) in microbial infection. (Supported by NIH Grant RO1 GM070589-01, NOAA Grant NA05NMF4571243, and National Science Foundation and NSF Grant IOB 0618409 to G.R.V.)
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#35 Comparative Structural, Functional and Molecular Characterization of Closely Related *Streptococcus oralis* Coaggregation Receptor Polysaccharides and *Streptococcus pneumoniae* Capsular Polysaccharides

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Background. The coaggregation receptor polysaccharides (RPS) of *Streptococcus oralis* C104 and SK144 function as receptors for GalNAc-binding lectin-like adhesins present on other members of the oral biofilm community. The structures of these polysaccharides resemble those reported for the capsular polysaccharides of serogroup 10 *S. pneumoniae* and indeed, the RPS and CPS gene clusters of these bacteria are closely related. However, certain previously determined features of these gene clusters were not compatible with the available structures of *S. oralis* RPS and *S. pneumoniae* CPS10F. These discrepancies have now been fully resolved based on the revised structure of CPS10F determined by NMR in the present study. Methods. The CPS10F gene cluster was previously sequenced from genomic DNA of *S. pneumoniae* 34355. Consequently, we isolated CPS10F from the culture supernatant of this strain and determined the structure of this polysaccharide from high resolution heteronuclear NMR data. Novel polysaccharides whose structures were also determined by NMR data were obtained by genetic engineering of the RPS gene cluster of *S. oralis* C104 with homologous genes from *S. pneumoniae* CPS serotypes 10A and 10F. Results. The specificities of the glycosyl transferases and polymerases encoded by genes in the RPS gene cluster of *S. oralis* and CPS gene cluster of *S. pneumoniae* were fully compatible with the resulting polysaccharide structures identified by NMR. In *S. oralis* C104, these genes direct the synthesis of a linear polysaccharide that contains the trisaccharide motif, Gal β 1-6GalNAc β 1-3Gal β , which is the proposed coaggregation receptor of RPS. In contrast, similar genes in the CPS cluster of *S. pneumoniae* 10F direct synthesis of a polysaccharide that contains a β 1-6 linked Galf branch to GalNAc β 1-3Gal β . Formation of the Galf branch was attributed to the action of the *S. pneumoniae* polymerase (Wzy), which linked the reducing end of each CPS hexasaccharide repeating unit to the adjacent unit through a β 1-4 linkage to subterminal GalNAc β of Gal β 1-6GalNAc β 1-3Gal β rather than through a β 1-6 linkage to terminal Galf, as in *S. oralis* RPS. *S. pneumoniae* CPS10F is not recognized as a receptor for interbacterial adhesion. The critical contribution of the internal β 1-6 linked Galf to receptor recognition was further established by engineering a linear surface polysaccharide in *S. oralis* that was identical to RPS except for the linkage between Galf and GalNAc β 1-3Gal β . This involved deletion of *wefE* for the transferase that forms the β 1-6 linkage between Galf and GalNAc and expression of a related gene (*wcrD*) for a β 1-3 transferase from serotype 10A *S. pneumoniae*. The engineered polysaccharide, which was linear and contained repeating Gal β 1-3GalNAc β 1-3Gal β motifs, did not promote interbacterial adhesion. Conclusion. Adhesin-mediated recognition of internal GalNAc β 1-3Gal β in wild type *S. oralis* RPS depends on the adjacent β 1-6 linked Galf unit. Significance. Our findings, which illustrate a molecular approach akin to site-directed mutagenesis for relating bacterial polysaccharide structure to function, provide insight into the possible evolution of *S. oralis* RPS from *S. pneumoniae* CPS. They also indicate that it may be possible to engineer the production of pneumococcal capsular polysaccharide vaccines in closely related non-pathogenic streptococci.

#36 Identification and characterization of a second galectin (CvGal2) from the eastern oyster (*Crassostrea virginica*)

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Background: *Perkinsus marinus* is a protozoan parasite causes "Dermo" disease in the eastern oyster *Crassostrea virginica*, and is responsible for catastrophic damage to shellfisheries and the estuarine environment in North America coasts. We have previously shown that oyster hemocytes recognize *Perkinsus marinus* via a novel galectin (C. virginica galectin (CvGal, hereafter CvGal1)). Upon attachment and spreading of hemocytes on foreign surfaces, CvGal1 localizes to the cell periphery and is secreted to the environment. This process is further enhanced by exposure of hemocytes to *Perkinsus* spp. trophozoites. CvGal1 recognized preferentially, *Perkinsus* spp. trophozoites, and their phagocytosis could be partially inhibited by pretreatment of the hemocytes with anti-CvGal1 antibodies. Because most metazoans exhibit galectin repertoires of variable complexity we searched for additional galectins on the oyster, and recently identified and partially characterized a second galectin (CvGal2). Methods: Molecular, genomic, biochemical, and immunological approaches were used to identify, clone, and characterize CvGal2, both structurally and functionally. Results: We have partially characterized the gene organization of CvGal2, which is substantially similar to CvGal1; however, both galectins differ in their intron sizes. In addition, we have identified two putative allelic variants for CvGal2. Further, cDNA analysis indicate the presence of alternative spliced messages. The primary structure of CvGal2 also reveals four CRDs in which from the nine seven amino acid residues that in the canonical mammalian (bovine) galectin-1 bind the carbohydrate ligand, seven are conserved. The full-length cDNA was isolated and cloned into pET30 for recombinant production of rCvGal2, that will be used to compare its carbohydrate specificity profile with CvGal1, generate specific antibodies, and study its potential binding to microorganisms and phytoplankton components and to *Perkinsus* spp. trophozoites. Conclusion: We identified in the eastern oyster a

second galectin homologue of CvGal1, of unique carbohydrate-recognition domain organization that, unlike most mammalian galectins, recognizes both endogenous and exogenous carbohydrate ligands. Based on their gene organization CvGal1 and CvGal2 appear to be a paralogues. CvGal2 is alternatively expressed. Significance: In the oyster hemocytes, CvGal1 appears to play a role on the passive entry of the protozoan parasite *P. marinus* suggesting that the parasite may have evolved to adapt the trophozoite's glycocalyx to be selectively recognized by the oyster hemocyte CvGal1, thereby subverting the oyster's innate immune/feeding recognition mechanism to gain entry into the host cells. The identification of CvGal2 revealed the presence of multiple galectins in the oyster, and it is possible that it might also contribute to parasite entry. Understanding the complexity of the oyster galectin repertoire and its role in parasite/host interactions may lead to novel intervention strategies since, so far, no effective preventive or treatment methodologies for "Dermo" disease have been developed. (Supported by NIH Grant RO1 GM070589-01, NOAA Grant NA05NMF4571243, and National Science Foundation and NSF Grant IOB 0618409 to G.R.V.)¹ Baltimore City Community College, Biotechnology

#37 Structural Specificity of gp120 Carbohydrates for Binding to HIV-fusion Blocking Cyanobacterial Proteins Determined by NMR and other Biophysical Techniques

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Number of cyanobacterial lectins has been identified in recent years as the potential antiviral candidates on account of their binding ability to envelope carbohydrates of virus. We have described here the structural specificities of gp120 carbohydrates required for binding to two cyanobacterial lectins. These lectins by binding to gp120 inhibit the fusion of HIV-1 to the host cell. We have also described the enzymatic ability of one these two lectins which cleaves the straight chain chitooligosaccharides into monosaccharides. We used Saturation Transfer Difference (STD), chemical shift mapping and intermolecular NOE techniques of NMR, Isothermal Titration Calorimetry (ITC) and site-directed mutagenesis in these studies. *Microcystis aeruginosa* lectin MVN shares 33 % identity with known potent anti-HIV protein cyanovirin-N. We have studied the binding specificity of this lectin using fragment-based approach of high-mannose carbohydrates and determined the solution structure partly by using three-dimensional NMR experiments. *Microcystis viridis* lectin (MVL) is known to recognize Man2A, a tetrasaccharide as the smallest structure of the high-mannose carbohydrates of gp120. Our results suggested GlcNAc2, a disaccharide, as the smallest carbohydrate unit recognized by this protein. MVL was also found having enzymatic ability to cleave glycosidic linkage of GlcNAc2 and straight chain chitooligosaccharides into N-acetyl glucosamine. Analysis based upon site directed mutagenesis revealed only one of the two carbohydrate binding sites of this protein was involved in the enzymatic activity and aspartic acid-75 and glutamic acid-76 were identified as the key residues involved in the catalytic hydrolysis. We also determined the structure of MVL-GlcNAc3 complex using intermolecular NOE after assigning the NMR chemical shifts of protein and carbohydrate. These two lectins with different structural properties and different binding specificities, therefore, represent important leads for the anti-HIV protein.

#38 Genetic deletion of galectin-3 accelerates disease onset and demise in a G93A SOD1 mouse model of ALS.

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Background: Inflammation associated with amyotrophic lateral sclerosis (ALS) is known to influence disease onset and progression. We recently observed elevated galectin-3 (gal-3) mRNA and protein in the spinal cord taken from a mouse model of ALS (SOD1G93A), at early presymptomatic time points (56 and 70 days) and at the end stage of disease (126 days). Galectin-3 was selectively upregulated in the spinal cord, rather than in other CNS regions, or skeletal muscle. Galectin-3 expression was also increased in the spinal cord of patients with ALS. The primary source of gal-3 in the mouse and human was activated microglia, rather than astrocytes or neurons. Methods: Based on this information, and the lack of good, selective, CNS-permeable galectin-3 inhibitors, we crossed SOD1G93A mice with gal-3 knock-out mice of the same background and followed the onset and progression of motor neuron disease in the progeny using a variety of neurobehavioral tests: splay, rotarod, grip strength, activity as well as overall survival (defined as the time until loss of righting reflex). Results: SOD1G93A/Gal-3 (-) mice exhibited symptoms of earlier disease onset (splay test) and expired, on average, 30 days earlier than did SOD1G93A/Gal-3 (+) cohorts. Rotarod, grip strength, and activity tests also indicated a more severely impaired neurological phenotype in SOD1G93A / Gal-3 (-) mice during the progression of disease, relative to SOD1G93A/(Gal-3 +) mice. Conclusions: Based on these observations, we suggest that secretion of galectin-3 by activated microglia may provide neuroprotective support during the progression of motor neuron disease. We hypothesize that gal-3 may bind to N-glycans on cell membrane glycoproteins and create lattices that stabilize, and consequently increase, receptor activation for certain growth factors and cytokines that are enriched with N-glycan motifs. This, in turn, may enhance trophic and neuroprotective support, which may delay further neurodegeneration, and prolong overall survival.

Significance: An emerging body of evidence suggests that neuronal-glia cross-talk is an important determinant of the progression of ALS; the present data represent a new and striking example in support of this concept. Moreover, they suggest that by understanding the mechanism of action of gal-3 we may form a basis for development of novel therapeutic approaches that slow or halt this devastating disease.

#39 Development of an SPR-based assay to facilitate the development of immunoglobulin products for prophylaxis and treatment of avian influenza

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Background: Due to a threat of a possible avian influenza pandemic there will be a need for the development of a flu specific immunoglobulin product (FLUIGIV) for prophylaxis and treatment. Selection of plasma with high neutralizing activity is essential if high potency FLUIGIV is to be produced. In the past, subpotent specific immune globulins have resulted from using plasma screened by ELISA methods, and neutralization assays are time-consuming and expensive. A rapid, high-throughput, specific inhibition assay to identify H5N1-specific plasma with high neutralizing activity would accelerate development and manufacturing of a FLUIGIV product. Methods: Since influenza hemagglutinin (HA) binding to glycans is essential for viral infectivity, our goal was to develop a Surface Plasmon Resonance (SPR) – based assay that measures antibody-mediated inhibition of HA binding to corresponding glycan receptors on cells. Briefly, biotinylated glycans were immobilized on a Biacore streptavidin chip at varying densities. Following immobilization, HAs with different specificities toward the glycans were then passed over the chip and binding was assessed by an increase in resonance units (RU). Results: The results of the assay indicate that glycan/HA binding is specific and can be observed and characterized using Biacore technology. H5 HA (Vietnam/1203/04) showed high affinity binding to known glycan partners, including the saccharide structures Neu5Ac α 2,3Gal β 1,4[Fuc α 1,3]GlcNAc β Osp-LCLC-Biotin and Neu5Ac α 2-3Gal β 1-4GlcNAc β -SpNH-LC-LC-Biotin. These glycans showed no binding to H3 HA (Wyoming/3/03). The saccharide structure Neu5Ac α 2-3[GalNAc β 1-4]Gal β 1-4GlcNAc β -Sp-LC-LC-Biotin which is known to have no binding to HA showed no binding to either H5 HA or H3 HA. Conclusion: The results of the binding experiments indicate that the glycan/HA binding is specific and that the Biacore instrument is an appropriate technology for the development of our alternate screening assay. Significance: The optimization of the glycan/HA binding is the essential first step towards the ultimate development of a rapid, high-throughput, specific inhibition assay to identify H5N1-specific plasma with high neutralizing activity. “The findings and conclusions in this poster have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.”

#40 Expression of galectins in cell lines derived from teleost fish

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Background: Galectins, β -galactoside-binding proteins, are present in the cytosol, nucleus, and the extracellular space, where they bind to cell surface and extracellular matrix glycans. Galectins are classified into three basic structural types: Proto-type galectins contain one carbohydrate recognition domain (CRD) and form homodimers. Chimera-type galectins have a single CRD, and may oligomerize. Tandem-repeat-type galectins are comprised of two CRD joined by a linker peptide. In homeotherm vertebrates, fragmentary evidence suggests that galectin-virus interactions can affect viral infectivity and/or pathogenicity. Galectin-1 blocks infection by paramyxovirus, whereas it facilitates infection by HIV. We hypothesize that in teleost fish galectin interactions with viral proteins modulate viral infectivity. We have shown that zebrafish galectins interact with glycoproteins of the rhabdovirus IHNV propagated in the EPC cell line. EPC also expresses a galectin-1-like protein that potentially interacts with IHNV. In this study we examined additional fish cell lines for expression of galectin-like proteins that may interact with fish virus and modulate their infectivity. Methods: (a) Western blot was used to identify galectin-like protein expression in minnow (EPC), trout (RTG-2), bluegill (BF-2), salmon (CHSE-214, ASK), zebrafish (ZFL), and catfish (COO, 42TA, G14D, 28S.3, 1G8, and 3B11) cell line lysates. Antibodies (Ab) that recognize the proto, chimera, and tandem-repeat type galectins of zebrafish (Drgal) were employed. We further (b) identified galectin-like transcripts by RT-PCR using primers designed to amplify zebrafish galectins. Results: (a) Identification of galectin-like proteins in fish cell lines. All tested cell lines expressed a protein of \sim 15kDa that is recognized by the anti-Drgal1-L2 Ab. Similarly, all tested cell lines expressed a protein recognized by the anti-Drgal3-L1 Ab. While in 42TA, ASK, COO, G14D, 1G8, ZFL, RTG-2, and BF-2 cells these proteins were \sim 25kDa, in EPC, RTG-2, and ASK the Ab recognized higher molecular weight galectin-3-like proteins. Results with anti-Drgal9-L2 Ab have been inconclusive so far. (b) Identification of galectin-like mRNA in fish cell lines: All tested cell lines exhibited Drgal1-L2-like mRNA. In addition, of the 12 tested cell lines, 10 expressed Drgal1-L3-like mRNA, 7 expressed Drgal1-L1-like mRNA, and 6 expressed Drgal1-L4-like mRNA. With the exception of 3B11, all tested cell lines

expressed Drgal3-L1-like mRNA. In addition, 8 of the 12 expressed Drgal3-L2-like mRNA. Two of the 12 cell lines expressed Drgal9-like mRNA. Conclusions: A variety of fish cell lines express both proto and chimera type galectin-like mRNA and proteins. Future studies will expand our analyses of galectin interaction with viruses, and will address the potential for such galectins to modulate viral infectivity. Significance: Evidence suggests that galectins interact with viral pathogens. These studies will help elucidate the mechanisms by which galectin-virus interactions modulate viral infectivity and host responses to viral challenge. (Supported by an NIH Ruth L. Kirschstein National Research Service Award to S.S.J., a pre-doctoral fellowship from the Ministry of Education and Science of Spain (MEC) to A.I.H.S., and Grant RO1 GM070589-01 to G.R.V.)

Glycans in Genetic Disorders/Disease

#41 Mass Spectrometric Analysis of Glycans in Liver Disease

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Background: Increasing incidence of hepatocellular carcinoma (HCC) in the world and in the US is associated with hepatitis C viral (HCV) infection. Our mass spectrometric study evaluated changes in glycans during the progression of HCV infection to cancer. Glycosylation of proteins in serum reflects the function of the liver except for immunoglobulins. We describe methods for MS analysis of permethylated glycans from immunoglobulins and other serum proteins. Application of the methods to the samples of patients with liver disease is expected to provide new information about this growing health problem. Methods: Glycans enzymatically released from serum proteins were analyzed by MALDI-TOF/TOF following solid phase permethylation. Analysis of less than 0.02 ml of serum allowed relative quantification of 70 glycan structures.

Results: We observed interesting trends in changes of some glycans. Specific glycans were associated with immunoglobulins. Some glycan types changed with gender and age of the participants. Substantial changes were associated with chronic HCV infection. The progressive glycan changes in liver disease differ by structural class; complex glycans decrease in abundance with several other N-glycan classes increasing with disease progression. A set of 3 glycans was sufficient to classify HCC with 90 % prediction accuracy. Conclusion: In conclusion, our results show that mass spectrometric analysis of permethylated N-glycans in serum is useful in studies of the natural progression of HCV infection to HCC. Significance: This study demonstrates simultaneous analysis of 70 N-glycans at multiple stages of liver disease progression. Glycosylation of immunoglobulins is distinct from the glycosylation of other serum proteins. Evaluation of N-glycan abundance suggests the potential to use glycans for the detection of liver disease.

#42 Galectin-1 is a novel functional receptor for tissue plasminogen activator in pancreatic cancer.

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Background: Tissue plasminogen activator (tPA) exerts many different functions in addition to its role in fibrinolysis. In pancreatic ductal adenocarcinoma (PDA), tPA is overexpressed and plays an important role in proliferation, invasion and angiogenesis. tPA interaction with cell membrane receptors has been related to increased proteolytic activity and to signal transduction through non-enzymatic mechanisms. The aim was to analyze the role of galectin-1 (Gal-1), an endogenous lectin that is also overexpressed in PDA, as a new functional receptor for tPA. Methods: Gal-1/tPA interaction was analyzed using surface plasmon resonance and pull-down assays. Pancreatic cells and tumors were used to study Gal-1 expression and localization by WB and immunostaining. Down-regulation of Gal-1 by siRNA was used to analyze the involvement of Gal-1/tPA interaction in ERK1/2 activation, cell proliferation and invasion in pancreatic and fibroblastic cells.

Results: Gal-1/tPA interaction is direct, specific and of high affinity. Gal-1 moderately increases the catalytic activity of tPA. High Gal-1 levels were detected in PDA cells in culture, where it concentrates at the migration front, and in tissues, where it is expressed in epithelial cells and in the stroma. Down-regulation of Gal-1 abolished the effects of

tPA on ERK1/2 activation, cell proliferation and invasion, both in pancreatic and in tumor-derived fibroblasts. Conclusions: These findings support a new molecular mechanism by which Gal-1 interaction with tPA contributes to PDA progression involving both transformed epithelial cells and tumor fibroblasts. Significance: Glycosylation has been reported to be very relevant in tumor progression in different tumor types. In this article, we report a new molecular mechanism by which a Galectin mediates pathological effects from a glycoprotein in pancreatic cancer cells.

#43 Structural Characterization of N-linked Glycans on Prostate Specific Antigen and Prostatic Acid Phosphatase Derived from Proximal Fluids of the Prostate

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Background: Prostate epithelial cells secrete a large repertoire of glycoproteins, including prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP). Both glycoproteins have a long clinical history of use as biomarkers for prostate cancer. Relative to serum concentrations, these two proteins are present at >10,000-fold higher concentrations in proximal fluids of the prostate like seminal plasma or expressed prostatic secretion fluids.

Methods: Using proximal fluid samples representative of normal control, benign prostatic disease and prostate cancers, PAP and PSA were enriched by thiophilic absorption chromatography. Released N-linked glycan constituents from both proteins were analyzed by a combination of normal phase HPLC and MALDI-TOF spectrometry. Individual glycopeptides were further analyzed using a hybrid linear ion-trap triple quadrupole mass spectrometer. Results: For PSA, which has only one glycosylation site, 40 putative glycoforms were determined. The predominant structures were complex bi-, tri- and tetra-antennary sialylated glycans. For PAP, which has three N-linked glycan sites, 21 glycoforms were determined. Following glycopeptide analysis, specific glycoforms of PAP were further assigned to each of the three possible N-linked sites. Conclusions: Proximal fluids of the prostate are an excellent clinical fluid source for purifying enough PSA and PAP for characterization of prostate disease associated changes in glycan structures. Significance: The glycan differences identified in these studies will allow for more defined targeting of prostate disease-specific changes for PAP, PSA and other secreted prostatic glycoproteins. These glycan differences represent new biomarker candidates for detection and differentiation of benign prostatic disease and prostate cancers.

#44 Mutation in Fukutin related protein helps to elucidate the disease mechanism of dystroglycanopathies

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Background: Alpha dystroglycan is an important component of the dystrophin-glycoprotein complex (DGC). Muscular dystrophies with reduced glycosylation of α -dystroglycan (α -DG) are a clinically and genetically heterogeneous group of autosomal recessive muscular disorders. With variable neurological and ophthalmic involvement they are referred to as the dystroglycanopathies. Reported phenotypes range from severe congenital onset of muscular dystrophy with CNS and eye involvement, to a later onset form of limb girdle muscular dystrophy (LGMD2I), without any CNS or eye involvement. To date, six genes have been identified which alter the glycosylation pattern of α -dystroglycan, all of which are known or putative glycosyltransferases. One of these putative glycosyltransferases is fukutin-related protein (FKRP) that participates in the glycosylation of α -dystroglycan in the muscle fiber. Methods: To further define the mutation frequency, phenotypes and understand the disease mechanisms associated with mutations in the FKRP gene, we studied a large cohort of patients (1088) with unknown muscular dystrophy. All subjects had progressive muscle weakness and myopathic findings on muscle biopsy. Initial screening was done for the common mutation c.826C>A (p.L276I) reported in the FKRP gene by using Taqman probes, followed by sequencing. Immunostaining on muscle fibers for α -dystroglycan was performed in the mutation positive patients. Muscle biopsies from mutation positive patients were subjected to mRNA profiling using Affymetrix Human U133 plus 2 arrays and data was analyzed using PLIER algorithm. Results: Mutations were identified in 26 patients. Of these 26, ten were homozygous and sixteen were heterozygous for the common mutation. In 6 of the 16 patients with a heterozygous mutation, there was another identifiable mutation found in the gene, but in the other nine cases, the second mutation was not identified by sequencing the whole coding region. Pattern of α -dystroglycan staining in the muscle fiber of these patients with mutations ranged from partial to complete absent. Muscle mRNA expression profiles were generated from 10 patients with mutations in FKRP and compared to a disease control, 10 (LGMD2B) dysferlin mutation positive patients, 14 (BMD) dystrophin mutation positive patients, and normal muscle samples. Conclusion: There is a large range of phenotypic variability associated with mutations in FKRP gene in our patients. An apparent clinical delineation was found between patients homozygous and compound heterozygous for the c.826C>A mutation. Homozygous patients had later onset, milder clinical progression, and less muscle weakness compared with

compound heterozygous patients. This was due to greater number of complete loss of function alleles in the compound heterozygotes. mRNA profiling showed an unexpected pattern in FKRP group, compared to muscle from patients with other muscular dystrophies and normal controls. We observed decreased expression of genes involved in the TGF- β and IGF-II signaling networks in muscle from patients with glycosylation defects. Based on the result we hypothesize that abnormal glycosylation in muscle alters the extracellular matrix leading to alteration of intracellular signaling. Significance: We present an extensive series of genotype, phenotype and molecular signatures of partial loss of function of O-linked glycosylation pathways. These pathways may help to explain disease etiology in the dystroglycanopathies.

#45 Identification of Cancer Specific Glycosylation Patterns of PSA for Prostate Cancer Diagnosis

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Background: Prostate specific antigen (PSA) is the best-known serum marker for prostate cancer diagnosis and monitoring. However, one of the challenges it faces is its high false positive rate resulting in many unnecessary biopsies for cancer patients in the diagnostic grey zone. One possible method to improve the detection specificity of prostate cancer using PSA is to use the glycosylation patterns of PSA. If the PSA glycosylation pattern is associated with prostate cancer development, the specific glycosylation forms can be potentially used to improve the specificity of cancer detection.

Method: Two immunoassay-based technologies: a high-density lectin microarray and an electrochemiluminescent (ECL)-based lectin-antibody immunoassay were used to analyze glycosylation patterns of PSA in this study. Both methods are able to provide a sensitive, reproducible, and high through-put analysis. Result: We first extracted PSA proteins from tissue and serum samples from cancer and non-cancer patients. The same amount of PSA protein was used to probe high-density lectin arrays containing 94 lectins to globally profile PSA carbohydrates. Two lectins that have different binding ratios to PSA were then selected as targeted marker candidates, and the lectin-antibody immunoassays have been developed using this ECL-based platform. The limit of detection of these two assays were down to ng/mL level and the CV < 5%. The validation test was preformed using tissue samples from cancer and non-cancer patients with the same amount of PSA protein to measure targeted glycan level. Conclusion: In this study, we first established a two-step analytical platform for glycosylation pattern analysis. The high-density lectin array was used to global profile glycosylation patterns and ECL-based lectin-antibody immunoassay was applied to quantitatively analyze the targeted glycan-lectin expression for validation. Certain lectins have showed differential lectin-PSA binding ratios between prostate cancer and normal control, indicating that these lectins can potentially be used to detect cancer-specific glycosylation patterns in prostate cancer. Significance: The sensitive and high throughput platform is expected to be useful to profile glycosylation patterns of other glycoprotein markers.

#46 Cellular expression and localization of the key enzymes in sialic acid biosynthesis.

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Background: UDP-GlcNAc 2-epimerase/ManNAc Kinase (GNE/MNK) is a rate limiting, bifunctional enzyme in sialic acid (SA) synthesis, which is feedback inhibited by CMP-SA in its allosteric site. Mutations in GNE/MNK cause two human disorders, Hereditary Inclusion Body Myopathy (HIBM) and sialuria. Sialuria, clinically characterized by variable symptoms, including hepatomegaly and mental retardation, is caused by dominant mutations in the allosteric site of GNE/MNK, leading to a loss of feedback-inhibition and increased excretion of SA. HIBM is characterized by adult onset progressive muscle wasting and results from recessive, mostly missense mutations, leading to decreased GNE/MNK enzyme activities, and decreased SA production. Recent immunofluorescence studies showed that GNE/MNK not only resides in the cytoplasm and Golgi, as expected, but also in the nucleus. This nuclear localization is remarkable, since GNE/MNK performs its enzymatic role within the cytoplasm. However, GNE/MNK contains a putative nuclear export signal and the nucleus contains CMP-SA synthase, which converts all cellular SA to CMP-SA. Methods: Skin Fibroblast cultures of normal, sialuria and HIBM individuals were subjected to several cellular techniques including subcellular fractionation, immunofluorescence confocal microscopy, western blot analysis, epimerase enzymatic assays, and high pH anion-exchange chromatography. Results: Preliminary results showed that not only GNE/MNK but also GlcNAc 2-epimerase (RENBP), another key enzyme in SA biosynthesis, is localized to the nucleus. Sialuria cells appear to down regulate expression of RENBP. Subcellular fractionation followed by Western blotting showed nuclear bands of GNE/MNK and RENBP proteins of abnormal size. We are now analyzing nuclear posttranslational modification, the role of CMP-SA, and the function of the GNE/MNK nuclear export signal to obtain insights into the regulation of cellular SA synthesis. Enzyme activity measurements of cellular fractions showed epimerase activities of GNE/MNK isolated for the nucleus to be lower than the epimerase activity of GNE/MNK isolated from the cytosol. Furthermore

cytosolic GNE/MNK from HIBM fibroblasts displayed reduced activities when compared to cytosolic GNE/MNK for control cell lines. Reduced nuclear enzyme activities appeared similar between HIBM and control cell lines. Conclusion: We propose GNE/MNK is 'stored' in the nucleus, in its inactive, CMP-SA bound, feedback-inhibited form. If the cell needs SA, more CMP-SA is released from the nucleus, so there is more 'free' GNE/MNK to translocate to the cytoplasm through its nuclear export signal to produce more SA. GNE/MNK modifications in sialuria and HIBM CMP-SA may prevent the cell from compartmentalizing GNE/MNK which affects sialic acid production. RENBP expression may function as a rescue mechanism for rescue of critically increased or decreased cellular SA levels. Significance: Subcellular localization of GNE/MNK and RENBP may provide intriguing insights into the regulation of cellular SA synthesis, and may reveal novel therapeutic opportunities for patients with disorders of sialic acid metabolism.

#47 Podocyte glycocalyx alterations in the GneM712T/M712T HIBM mouse model

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Background: Hereditary inclusion body myopathy (HIBM) is an autosomal recessive inherited, adult-onset, progressive myopathy linked to the GNE gene, which encodes UPD-GlcNAc 2-epimerase/ManNAc kinase, the bifunctional, rate-limiting enzyme in sialic acid (SA) biosynthesis. Gne knockout mice are embryonic lethal, so our lab created a knock-in mouse model of HIBM mimicking the M712T founder mutation. Unexpectedly, over 95% of mutant mice died in the first three days of life (P3); necropsy revealed grossly hemorrhagic kidneys, while histology showed erythrocyte accumulation in glomeruli and tubules with podocyte foot process fusion and glomerular basement membrane splitting on EM. Maternal dietary supplementation with ManNAc raised mutant survival to ~50%, while partially restoring the histology defects. We are investigating the renal glycocalyx alterations in this mouse model, particularly focusing on the podocyte due to its importance in glomerular filtration. Methods: Lectin staining was performed on paraffin-embedded renal sections from all genotypes for untreated (P2) and ManNAc treated mice (P6) using HPA-FITC, PNA-FITC, WGA-FITC, LFA-FITC, and LPA-TRITC. Imaging was performed using a Zeiss LSM 510 Meta. Results: Lectin staining revealed several differences between control mice (+/+ or +/-) and homozygous mutant mice (-/-). HPA staining for GalNAc labeled only renal tubules in control mice, while an abnormal pattern of both podocytes and tubules labeling is found in mutant mice. PNA staining for β -galactose also labeled only tubules in control mice, with podocyte labeling in mutant mice. WGA staining for GlcNAc or SA labeled podocytes in all genotypes. Both LFA and LPA staining for SA (including free SA and CMP-SA) revealed a predominantly nuclear label in control mice, while mutant mice showed very little staining. Lectin staining of ManNAc treated kidney tissues showed no differences in control mice, while HPA, PNA, and WGA still abnormally labeled podocytes in mutant mice. However, LFA and LPA staining showed a restoration of the predominantly nuclear staining pattern after ManNAc feeding.

Conclusion: This lectin study revealed several alterations in sialylation patterns of the podocyte glycocalyx in GneM712T/M712T mutant mice as compared to control mice. As GalNAc is the most proximal carbohydrate on O-linked glycans, the HPA staining of podocytes in mutants presents several possibilities: 1) hyposialylation destabilizes O-linked glycans to the core structure, 2) hyposialylation upregulates the expression or activity of O-GalNAc transferases, or 3) the O-GalNAc-SA structure is more prevalent on the podocyte than previously believed. Increased PNA staining in mutants was expected, as β -galactose is proximal to SA on glycans. Decreased LFA and LPA staining in mutants was also expected due to hyposialylation, but the staining in control mice and ManNAc-treated mutants showed a mostly nuclear pattern that implies an abundance of CMP-SA at this stage of renal development.

Significance: While HIBM is not associated with any renal defects in human patients, our findings indicate that hyposialylation and other glycocalyx defects in the mouse model contribute to the observed renal failure. Glycosylation defects might be a feature of human renal diseases of unknown etiology with features similar to our mouse model, including FSGS, minimal change disease, and IgA nephropathy.

#48 Down regulation of galectin-3 expression in prostate adenocarcinoma is caused by its promoter hypermethylation: Development and validation of a methylated marker for early diagnosis of prostate cancer

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Background: Prostate cancer (PCa) is the second most common cancer in men, and the second leading cause of cancer death. However, if PCa is diagnosed in its early stages such as stages I and II when the tumor is still confined to the prostate gland, it can be effectively treated and cured. But, unfortunately, the widely used PSA test is flawed by highly prevalent false positive and negative results. Therefore, a reliable marker for early detection of prostate cancer is urgently needed. Genes, whose expression changes dramatically in the early stages of PCa, should serve as ideal markers in this regard. Galectin-3 (gal3), a member of the galactose-binding protein family, has been found to be

silenced in the early stages of PCa. Methods: The expression of gal3 from the prostate cancer cell lines (LNCaP, PC-3, and DU-145) was determined by RT-PCR. To analyze methylation pattern of the gal3 gene promoter, cytosine deamination by bisulfite treatment of single-stranded DNA (nonmethylated C changes to U) was achieved by EZ Gold Methylation kit (Zymo Research) and subsequent PCR amplification of the bisulfite-treated DNA was performed using Multiplex PCR kit (Qiagen). The PCR product was cloned into a pGEM-T (Promega) vector and sequenced. Gal3 MS-PCR using the methylated primers was performed to detect early stages of PCa. Results: In PC-3 and DU-145 cells, gal3 is present at normal levels, whereas in LNCaP its expression is silenced. In LNCaP, the gal3 promoter was heavily methylated, whereas PC-3 or DU-145 cells showed negligible or no methylation in the gal3 promoter indicating a negative correlation between gal3 promoter methylation and its expression. On immunohistochemical analysis of normal and tumor prostate tissues, gal3 was found expressed both in nucleus and cytoplasm of normal, benign prostatic hyperplasia (BPH), high-grade prostatic intraepithelial neoplasia (HGPIN) and stage I. The expression of the gal3 was found drastically down-regulated in advanced stages, and interestingly, mostly in the cytoplasm. On methylation analysis, the gal3 promoter in stage II PCa was found heavily methylated, whereas in stage III and IV, it was only lightly methylated. However, in stage I PCa, both heavy and light methylation was observed in the gal3 promoter. In normal and BPH tissues, the gal3 promoter was almost unmethylated. Based on the cytosine methylation in the gal3 promoter in stages I-IV of PCa, we developed sensitive and specific methylation-specific PCR assay that clearly identified the early stages of prostate cancer in tissues as well as in biological fluids such as serum and urine (100% sensitive, nearly 100% specific). Conclusion: Expression of gal3 is negatively correlated with its promoter hypermethylation. Differential cytosine methylation has led to develop a sensitive, specific, and non-invasive assay platform for the detection of early and advanced PCa. Significance: Early detection of PCa is critical for effective treatment and cure. This non-invasive diagnostic tool is not only for early detection, but can also be applied for therapeutic guidance and recurrence monitoring of PCa in urine samples. (Supported by UMBI Presidential Proof of Concept Award to H.A.)

#49 Glycoproteomics for Prostate Cancer Detection: Changes in Serum PSA Glycosylation Patterns

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Background: Prostate cancer (PCA) is the most common cancer of men in the United States. Early detection using prostate-specific antigen (PSA) has significantly decreased mortality and improved the treatment outcome. However, it also led to an increased number of unnecessary biopsies in patients with benign diseases due to PSA's low cancer specificity. Because aberrant glycosylation is a fundamental characteristic of tumor genesis and progression, the objective of this study was to investigate whether changes in PSA glycosylation may allow distinction of PSA from tumor and non-tumor origins. Methods: PSA is a glycoprotein with a single N-oligosaccharide chain. In this study, the glycosylation patterns of PSA in serum were analyzed using five lectin immunosorbant assays (total PSA with SNA, total PSA with MAL I, total PSA with MAL II, free PSA with MAL I, and free PSA with MAL II). In these assays, either total or free anti-PSA antibodies are used to capture PSA molecules from serum. PSA glycosylation is then detected using biotinylated lectin SNA, MAL I, or MAL II. Finally, streptavidin labeled with ruthenium compound is used for electrochemiluminescence (ECL) detection in the Meso Scale Discovery platform. Results: Analytical performance of these assays were evaluated by limit of detection (LOD) and quantification (LOQ), and within-run reproducibility. Their clinical performance were initially evaluated using 3 pools of PCA sera vs. 3 pools of non-cancer (non-CA) sera with matched total PSA and free PSA levels. While glycosylation patterns determined by these assays showed less variation among the 3 pools of non-CA sera, the glycosylation among 3 pools of PCA sera were significantly different, which may indicate more heterogeneous glycosylation pattern of PSA from cancer than non-cancer origins. Further, these assays were applied to 52 individual patient's serum (26 biopsy confirmed PCA and 26 biopsy confirmed non-CA). ROC analysis showed that although clinical performance of %free PSA (AUC 0.85) was superior than all the five lectin immunosorbant assays when applied to all 52 individuals, SNA assay for total PSA performed better when %free PSA was in the range of 10-20% (AUC 0.71 vs. 0.54). This indicate the potential application of SNA assay for total PSA in the diagnostic gray zone of %free PSA. Conclusion: Five lectin immunosorbant assays were developed to detect sialylation and glucosylation of total and free PSA in serum. Application of these assays to PCA and non-CA sera indicates that glycosylation patterns of PSA from the former are more heterogeneous than from the latter. SNA assay for total PSA showed better clinical performance than %free PSA in its diagnostic gray zone (%free PSA in 10-20%) in a study of 21 patients. A larger sample size will be used to validate this finding. Significance: These lectin immunosorbant assays may be very useful in understanding perturbed glycosylation in tumor genesis and progression and could be used clinically to distinguish cancer from non-cancer.

Glycans role in immune function/carbohydrate based vaccines

#50 MINIATURIZED ASSAY FOR ANTIBODY-MEDIATED INHIBITION OF SIALIC ACID CLEAVAGE FROM A LARGE GLYCAN SUBSTRATE BY INFLUENZA NEURAMINIDASE

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Background: An assay for influenza virus NA inhibition (NI), based on colorimetric measurement of sialic acid cleaved from fetuin, has long been available for serological studies. Despite the capacity of NA-specific antibodies to reduce viral replication and disease severity, NI is not routinely examined in influenza vaccine trials, partly due to the cumbersome nature of the assay. To prevent HA antibody-mediated interference with NA activity, the optimal virus target for an NI assay should possess an HA subtype to which the host is naïve. Methods: We have reformatted the established colorimetric NA assay such that virus-fetuin incubation, chemical reactions, and chromophore extraction are performed in 96-well PCR plates, and analysis is performed with a microplate absorbance reader. Adapting an established strategy, we used 8-plasmid transfection to rescue reassortant viruses bearing NA genes of contemporary influenza A strains, an H6 subtype HA gene, and the complementary genes of A/Puerto Rico/8/34. Antigenic drift of NA in vaccine strains of the past 1-2 decades was characterized using CDC reference antisera. Human sera pre- and post-immunization with trivalent seasonal influenza vaccine were analyzed for changes in NI titer. Results: Titrated concentrations of free sialic acid are measured with high linearity. NAs of different H1N1 and H3N2 strains vary in their dependence on divalent cations. Ferret antiserum incubated with a standardized quantity of homologous virus inhibits NA signal in a subtype-specific manner; endpoint titers are comparable or higher than those obtained in a traditional assay format. Substantial changes in antigenicity of NA are sometimes observed across sequential vaccine strains. Analysis of human sera detects small rises in NI titer after trivalent influenza vaccination. Conclusion: Our data provide quantitative evidence for the contribution of NA to the antigenic character of recently predominating influenza A strains and suggest the importance of NA to the selection of a well-matched vaccine strain. Significance: This streamlined methodology can support more efficient and comprehensive serological monitoring in experimental or clinical vaccine studies.

#51 Immunomodulatory effect of Neisseria meningitidis type C Polysaccharide (MCPS) on the BAFF system.

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Background and hypothesis: *Neisseria meningitidis* is a leading cause of bacterial meningitis worldwide. The polysaccharide (PS) capsule is a major virulent factor and also the major target of the host immune system. This large PS capsule interacts directly with B cells inducing serogroup specific antibodies. However PS based vaccines are known to be poorly immunogenic especially in infants where the incidence of the disease is highest. TNF family cytokines BAFF and APRIL and their receptors on B cells, namely BAFF-receptor, BCMA and TACI form the so-called BAFF system which is shown to be essential in the development, survival, proliferation and homeostasis of B cells with TACI playing a pivotal role in the development of antibody response against bacterial PS. In this study, we sought to determine the effect of capsular PS of *N. meningitidis*-C (MCPS) on the BAFF system. Methods: Splenocytes or B cells from BALB/c mice were stimulated in vitro with varying concentrations of MCPS. Cell surface levels of TACI, BAFF receptor, CD19, CD40, MHC-I, MHC-II, CD83 and CD80 were determined by flow cytometry. B cells were either co stimulated and/or re-stimulated with BAFF and APRIL ligands and assayed for Ig secretion by ELISA after seven days of culture and for plasma cell generation after three days of culture. Apoptosis was assayed by levels of annexin/PI binding by flow cytometry. TACI levels in cells stimulated with heat inactivated whole bacterial preparations were also compared in capsulated and non-capsulated bacteria by flow cytometry. Results: B cells stimulated with MCPS secreted significantly reduced amount of Igs in response to BAFF and APRIL. Correlating with this, a significant decrease in the B cell surface expression of TACI was observed although among other assessed B cell surface markers CD19, CD40 and MHC-I remained unaffected, while MHC-II, CD83 and CD80 were upregulated. Since TACI is crucial for development of antibody response against bacterial polysaccharides, we investigated if MCPS affected plasma cell development or cell survival. Indeed, cells treated with MCPS showed increased apoptosis as determined by Annexin-V binding assay and decreased number of Sydecan-1 positive cells in response to BAFF and APRIL. The down regulation of TACI increased with increasing concentrations of MCPS and was specific for MCPS since NP-ficoll, also a T-independent-II Ag did not inhibit TACI expression. Furthermore, mouse splenocytes stimulated with encapsulated *N. meningitidis* strain FAM 18 C+ showed decreased levels of TACI as compared to its unencapsulated isogenic counterpart, FAM 18 C- Conclusion: Taken together our data demonstrates that meningococcal capsular polysaccharide inhibits B cell response to BAFF and APRIL, and suggests that MCPS mediated suppression of TACI expression may be the underlying mechanism for this outcome. Significance: The down regulation of TACI on B-cell

surface could be a possible mechanism by which meningococci evade host immune responses and decreased expression of TAC1 may explain the poor immunogenicity of PS based vaccines.

#53 Maturation of myeloid precursor cells into mature dendritic cells is associated with changes in expression of polysialic acid

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Background: Polysialic acid (polySia) is a linear homopolymer of α 2-8-linked sialic acid that has been identified as a posttranslational modification on only six mammalian proteins. Studied extensively on neural cell adhesion molecule (NCAM) during development of the vertebrate nervous system, polySia modulates cell-cell interactions. We recently demonstrated that neuropilin-2 (NRP-2), a receptor for semaphorins and vascular endothelial growth factor, is expressed on the surface of human dendritic cells and is polysialylated. This suggested polySia is expressed more broadly in cells of the immune system than previously appreciated and that this unique glycan may play a vital role in hematopoiesis, differentiation and cell function. **Methods:** Primary human monocytes and murine bone marrow cells were maintained in culture in medium containing GM-CSF and IL-4 to generate dendritic cells. Expression of polySia was determined by flow cytometry and immunoblot using mAb 735 and GFP-endosialidaseNF. PolySia was digested from the cell surface using endosialidaseNF, that cleaves specifically chains of at least five α 2-8-linked sialic acids. Proliferation of lymphocytes after exposure to dendritic cells was measured by incorporation of $[^3H]$ -thymidine or expression of IFN- γ . **Results:** Expression of polysialylated NRP-2 is up-regulated during dendritic cell maturation, coincident with increased expression of ST8Sia IV, one of the key enzymes of polySia biosynthesis. Removal of polySia from NRP-2 on the surface of dendritic cells promoted dendritic cell-induced activation and proliferation of T lymphocytes. Monocyte-derived macrophages also express ST8Sia IV and NRP-2, yet they express a single polysialylated cell surface protein other than NRP-2. Sequence analysis of RNAs encoding NRP-2 in dendritic cells and macrophages demonstrates the presence of the NRP-2 2a(17) isoform only in dendritic cells, suggesting that this isoform might have special features that promote polysialylation. Murine bone marrow-derived dendritic cells also express polysialylated NRP-2. Furthermore, polySia is present on the surface of myeloid cells in the bone marrow and a subset of hematopoietic stem cells. NCAM (CD56) is the lone polysialylated protein in these cells, as demonstrated by the loss of polySia on murine bone marrow cells from NCAM $-/-$ mice. **Conclusion:** Polysialic acid is expressed on myeloid cells during differentiation from bone marrow precursor cells into mature dendritic cells. CD56 is the carrier of polySia on myeloid precursor cells in murine bone marrow, whereas NRP-2 is the predominant polysialylated protein in dendritic cells. Polysialic acid and NRP-2 influence dendritic cell-T lymphocyte interactions. A protein other than NRP-2 is polysialylated in human macrophages, suggesting that a precise mechanism controls the selection of polySia carriers in myeloid cells during their maturation. **Significance:** In light of what is known about NRP-2 and polySia in the nervous system, it is likely that both molecules will have important roles in cell-cell and ligand-cell interactions of myeloid cells, as well as in migration to sites of inflammation and to lymph nodes. The presence of polySia on myeloid cells in the murine bone marrow also raises the possibility that this glycan influences the lineage commitment of hematopoietic stem cells.