PROGRAM AND ABSTRACTS FOR THE 2007 MEETING OF THE SOCIETY FOR GLYCOBIOLOGY

November 11–14, 2007
Boston, MA

PRELIMINARY PROGRAM OVERVIEW

Sunday, November 11	9:00 am – 1:00 pm	**SATELLITE SYMPOSIUM: GLYCOBIOLOGY AND GLYCOCHEMISTRY CHALLENGES IN BIOTECHNOLOGY** *($40 extra)* Organized by Shekar Ganesa, *Genzyme* and Joseph Siemiatkoski, *Biogen Idec*
	8:00 am – 5:00 pm	**SATELLITE MEETING: GLYCANS IN CELL COMMUNICATION** Annual meeting of the Consortium for Functional Glycomics Participating Investigators Attendance is open to all interested scientists and is *free of charge*. **Please notify Anna Crie (annacrie@scripps.edu) before October 12**
	6:45 – 7:00 pm	**CONFERENCE OPENING** Opening Remarks: **Jacques U. Baenziger,** *President, Society for Glycobiology*
	7:00 – 8:55 pm	**SESSION I: GLYCOCONJUGATE BIOSYNTHESIS**
	9:00 – 10:00 pm	**WELCOME RECEPTION**
Monday, November 12	8:30 – 10:05 am	**SESSION II: GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT** Chair, Carlos B. Hirschberg, *Boston University, MA*
	10:45– 11:50 am	**SESSION III: GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT II** Chair, Carlos B. Hirschberg, *Boston University, MA*
	2:00 – 4:00 pm	**POSTERS and EXHIBITS**
	4:00 – 5:55 pm	**SESSION IV: PROTEIN FOLDING AND QUALITY CONTROL** Chair, Armando Parodi, *Instituto Leloir, Argentina*
Tuesday, November 13	8:30 – 10:10 am	**SESSION V: BIOLOGY AND BIOCHEMISTRY OF SULFATASES** Chair, Steven Rosen, *UCSF, CA*
	10:45 am – 12:20 pm	**SESSION VI: QUANTATATIVE GLYCOBIOLOGY** Chair, Jefrey Esko, *UCSD, CA*
	2:00 – 4:00 pm	**POSTERS and EXHIBITS**
	4:00 – 4:30 pm	**BUSINESS MEETING**
	4:45 – 5:45 pm	**KARL MEYER LECTURE**
	7:00 – 9:30 pm	**BANQUET.** Nominal fee. Extra tickets for guests may be ordered.
Wednesday, November 14	8:30 – 10:05 am	**SESSION VII: RECOGNITION IN GLYCAN BIOSYNTHESIS AND FUNCTION** Chair, Karen J. Colley, *Univ. of Illinois-Chicago*
	10:45 am – 12:15 pm	**SESSION VIII: STRUCTURAL INSIGHTS OF PROTEIN-CARBOHYDRATE INTERACTIONS** Chair, Nancy Dahms, *Medical College of Wisconsin*
	2:00 – 4:00 pm	**SESSION IX: GLYCOSYLATION IN THE CYTOSOL** Chair, John Hanover, *National Institutes of Health, MD*
	4:00 – 6:00 pm	**POSTERS and EXHIBITS**

SUNDAY, NOVEMBER 11
9:00 AM – 1:00 PM
SATELLITE SYMPOSIUM: GLYCOBIOLOGY AND GLYCOCHEMISTRY CHALLENGES IN BIOTECHNOLOGY, *Berkeley/Clarendon rooms*
($40 extra) Organized by Shekar Ganesa, *Genzyme* and Joseph Siemiatkoski, *Biogen Idec*

9:00 **Increasing Antibody ADCC through Carbohydrate Modification with Glycosylation Inhibitors**, Qun Zhou, *Genzyme Corporation, Framingham, MA*

9:25 **Tackling N-Glycan Heterogeneity: Glyco-Optimization of Therapeutic Glycoproteins in Lemna**, Jason Sterling, *Biolex Therapeutics, Pittsboro, NC*

9:50 **Challenges in Structural Charcterization of Heparin and GAG containing Polysaccharides**, Parastoo Azadi, *Complex Carbohydrate Research Center, Athens, GA*

10:15 **2D-LC MS Analysis of Bioreactor Samples**, Zoran Sosic, *Biogen-Idec, Cambridge, MA*

10:40 – 11:10 **BREAK**

11:10 **Uncovering Unique Structural Isomers in Cancer via MSn Disassembly**, Justin M Prien, *University of New Hampshire, Durham, NH*

11:35 **The Importance of Monitoring, Maintaining and Analyzing Sialic Acids on Therapeutic Proteins**, Tom Warner, *Prozyme, San Leandro, CA*

12:00 **Assessing the Impact of Glycosylation Profiles on the Binding of mAbs to the Fc gamma RIII Receptor**, Xianzhi Zhou, *Pfizer Global R&D, St. Louis, MO*

12:25 **Characterization of Glycan Structures of a Lysosomal Enzyme and Correlation with Biological Activity**, Manas Ray, *Shire HGT, Cambridge, MA*

SUNDAY, NOVEMBER 11
8:00 AM – 5:00 PM
SATELLITE MEETING: GLYCANS IN CELL COMMUNICATION, *Imperial Ballroom*
Organized by James C. Paulson, *The Scripps Research Institute*
Annual meeting of the Consortium for Functional Glycomics Participating Investigators Attendance is open to all interested scientists and is *free of charge*. Please notify Anna Crie (annacrie@scripps.edu) if you are planning to attend on or before October 12, 2007

Confirmed Speakers

Parainfluenza Virus Receptors, Gillian Air, *The University of Oklahoma Health Sciences Center*

The Changing Glycome of the Developing Myocardium, Eric S. Bennett, *University of South Florida*

Glycan Binding Properties of Galectins, Richard D. Cummings, *Emory University*

Immune Cell C-Type Lectins that Recognize Viruses, Kurt Drickamer, *Imperial College*

Glycomic Analysis of Human and Murine Leukocytes, Stuart Haslam, *Imperial College*

Role of Siglec-G in B Cell Signaling, Lars Nitschke, *Universitty Erlangen-Nornberg*

Galectin-1 in the Regulation of Immune Cell Homeostasis: Lessons from Experimental Models *in vivo*, Gabriel Rabinovich**,** *University of Buenos Aires*

Strategies for Building Interfaces to Glycomics Databases, Rahul Raman, *Massachusetts Institute of Technology*

Myelin-Associated Glycoprotein (Siglec-4) in Axon Regeneration, Ronald L. Schnaar**,** *The Johns Hopkins School of Medicine*

***Streptococcus mitis* Platelet Aggregation Factor: A Cholesterol-dependent Cytolysin with a Novel Fucolectin Domain**, Rodney Tweten, *The University of Oklahoma Health Sciences Center*

C-type Lectins in Innate and Adaptive Immunity, Yvette van Kooyk, *Vrije University Medical Center*

World Efforts for Building Glycan Structure Databases, Claus-W. von der Lieth, *German Cancer Research Center*

Mincle is a Novel Macrophage Receptor for the Yeast *Candida Albicans*, Christine Wells, *Griffith University*

SUNDAY, NOVEMBER 11
6:45 – 8:55 PM
SESSION I
GLYCOCONJUGATE BIOSYTHESIS, *Imperial Ballroom*
Chair, Anant Menon, *Cornell University, NY*

Time **Abstract Number**

6:45 **OPENING REMARKS**, Jacques U. Baenziger, *President, Society for Glycobiology*

7:00 ***In vivo* Roles for Mammalian Oligosaccharyltransferase Isoforms**; Catalina Ruiz Canada; Daniel Kelleher; Reid Gilmore; *University of Massachusetts Medical School, Worcester, MA* 1

7:25 **Lipid Flip-Flop during Glycoprotein Biosynthesis**; Anant K. Menon; *Weill* Cornell *Medical College, New York, NY* 2

7:50 **Coupling Polymerization, Chain Termination and Export in the Biosynthesis of Bacterial Lipopolysaccharide O Antigens**; Chris Whitfield; Leslie Cuthbertson; Bradley R. Clarke; Matthew S. Kimber; *University of Guelph, Guelph, ON, Canada* 3

8:15 **Mycobacterial Cell Wall Glycomics: Recent Revelations**; Delphi Chatterjee; Jian Zhang; Devinder Kaur; Patrick J. Brennan; Kay-Hooi Khoo; *Colorado State University, Fort Collins, CO* 4

8:40 **Synthetic Acceptor Analogs for the Characterization of Glucosyltransferases Involved in the Biosynthesis of O56 and O152 Antigens of *Escherichia coli***; Inka Brockhausen[1]; Bin Liu[2]; Bo Hu[2]; Kenneth Lau[1]; Walter A. Szarek[1]; Lei Wang[2]; *[1]Queen's University, Kingston, Ontario, Canada; [2]Nankai University, Tianjin, China* 5

8:45 **Glycoengineering using Bacterial Oligosaccharyltransferases**; Amirreza Faridmoayer; Messele A. Fentabil; Dominic C. Mills; John S. Klassen; Mario F. Feldman; *University of Alberta, Edmonton, Canada* 6

8:50 **Mycobacterial Cell-wall Biosynthesis: Exploitation of Chemistry and Biology**; Jian Zhang[1]; Kay-Hooi Khoo[2]; Delphi Chatterjee[1]; *[1]Colorado State University, Fort Collins, CO; [2]Institute of Biological Chemistry, Academia Sinica, Nankang, Taipei 115, TW* 7

9:00 – 10:00 PM
WELCOME RECEPTION

MONDAY, NOVEMBER 12
8:30 – 10:05 AM
SESSION II
GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT I, *Imperial Ballroom*
Chair, Carlos B. Hirschberg, *Boston University, MA*

Time **Abstract Number**

8:30 **The Nucleotide Sugar Transport/Antiport Cycle in Eukaryotic Development and Disease**; Carolina E Caffaro; Carlos B Hirschberg; *Boston University, Boston, MA* 8

8:55 **Extracellular Matrix-Glycan Interactions in the Peripheral Nervous System**; David J Carey; *Weis Center for Research, Danville, PA* 9

9:20 **Regulation of Skeletal Morphogenesis by Sulphated Proteoglycans in Zebrafish**; Aurélie Clément[1]; Malgorzata Wiweger[1]; Sophia von der Hardt[2]; Scott Selleck[3]; Melissa Rusch[3]; Chi-Bin Chien[4]; Henry Roehl[1]; *[1]University of Sheffield, Sheffield, UK; [2]MPI für Entwicklungsbiologie, Tubingen, DE; [3]University of Minnesota, Minneapolis, MN; [4]University of Utah, Salt Lake City, UT* 10

9:45 **Exploring the Pathogenesis of Mucolipidosis II in Zebrafish.**; Heather R Flanagan-Steet; Richard A Steet; *University of Georgia, Athens, GA* 11

9:50 **An O-Glycosyltransferase is Required for Proper Cell Adhesion in Drosophila**; Liping Zhang; Kelly G. Ten Hagen; *NIDCR, National Institutes of Health, bethesda, MD* 12

9:55 **The Importance of alg10 and Regulated N-Glycosylation during Drosophila Development**; Erica M. Selva; *University of Delaware, Newark, DE* 13

10:00 **Genetic Background Influences Glycosylation and Developmental Phenotypes Associated with the *Sugar-Free Frosting* Mutation in *Drosophila***; Sarah Baas; Mary Sharrow; Megan Middleton; Samuel Lee; Kazuhiro Aoki; Michael Tiemeyer; *Complex Carbohydrate Research Center, UGA, Athens, GA* 14

10:05 – 10:45 AM
COFFEE BREAK

MONDAY, NOVEMBER 12
10:45 – 11:50 AM
SESSION III
GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT II, *Imperial Ballroom*
Chair, Carlos B. Hirschberg, *Boston University, MA*

Time **Abstract Number**

10:45 **Novel Chondroitin Proteoglycans Play an Essential Role in Early *C. elegans* Development**; Sara K. Olson[1]; Jeffrey D. Esko[2]; Karen Oegema[1]; *[1]Ludwig Institute for Cancer Research, La Jolla, CA; [2]University of California, San Diego, La Jolla, CA* 15

11:10 **HSPG Signaling at the Drosophila NMJ**; Cheryl Thompson[1]; April Duckworth[1]; Graeme Davis[2]; Julie Gates[3]; Joseph Loureiro[4]; Mark Peifer[3]; Frank Gertler[4]; David L. Van Vactor[1]; *[1]Harvard Medical School, Boston, MA; [2]University of Californai, San Francisco, San Francisco, CA; [3]University of North Carolina, Chapel Hill, NC; [4]Massachusetts Institute of Technology, Boston, MA* 16

11:35 **Mutation in the Key Enzyme of Sialic Acid Biosynthesis Causes Severe Glomerular Proteinuria and is Rescued by *N*-Acetylmannosamine**; Enriko D Klootwijk[1]; Irini Manoli[1]; Belinda Galeano[1]; Dominic Hickey[1]; Wesley Bond[1]; Carla Ciccone[1]; Daniel Darvish[2]; Donna Krasnewich[1]; William A. Gahl[1]; Marjan Huizing[1]; *[1]NIH, NHGRI, Bethesda, MD; [2]HIBM Research Group, Encino, CA* 17

11:40 **Roles for the Bisecting GlcNAc in the Development and Metastasis of Mammary Tumors**; Yinghui Song; Pamela Stanley; *Albert Einstein College of Medicine, New York, NY* 18

11:45 **Identification of a Protein *O*-Glucosyltransferase Required for Notch Signaling in *Drosophila***; Hideyuki Takeuchi[1]; Melih Acar[2]; Hamed Jafar-Nejad[3]; Akhila Rajan[2]; Dafina Ibrani[2]; Nadia A. Rana[1]; Honglin Pan[2]; Hugo J. Bellen[2]; Robert S. Haltiwanger[1]; *[1]Stony Brook University, Stony Brook, NY; [2]Baylor College of Medicine, Houston, TX; [3]Univ. of Texas Health Science Center at Houston, Houston, TX* 19

2:00 – 4:00 PM
POSTER SESSION I, *Georgian, Arlington, Berkeley & Clarendon*

MONDAY, NOVEMBER 12
4:00 – 5:55 PM
SESSION IV
PROTEIN FOLDING AND QUALITY CONTROL, *Imperial Ballroom*
Chair, Armando Parodi, *Instituto Leloir, Argentina*

Time **Abstract Number**

4:00 **ER Resident Lectins and Glycosylhydrolases Determine the Fate of Newly Synthesized Glycoproteins**, Maurizio Molinari, *Institute for Research in Biomedicine, Bellinzona, Switzerland* 20

4:25 **Carbohydrate-Mediated Protein Maturation and Quality Control in the Endoplasmic Reticulum**; Daniel N. Hebert; James Cormier; Brad Pearse; Taku Tamura; Ning Wang; *University of Massachusets, Amherst, MA* 21

4:50 **Extensive Mannose Trimming and Mannosidase Compartmentalization in Glycoprotein ER-Associated Degradation**; Edward Avezov; Gerardo Z. Lederkremer; *Dept. of Cell Research, Tel Aviv University, Tel Aviv, Israel* 22

5:15 **The Biophysics of Quality Control**; Julio J Caramelo; *Fundación Instituto Leloir, Buenos Aires, Argentina* 23

5:40 **ER α1,2-Mannosidase I is not the Only Family Member that Trims Mannose to Create the ERAD Targeting Signal.**; Annette Herscovics; *McGill University, Montreal, Canada* 24

5:45 **Differential Protein Glycosylation Activity by the Two Vertebrate Oligosaccharyltransferases A- and B-OST.**; Catalina Ruiz-Canada; Reid Gilmore; *BMP, UMass Medical School, Worcester, MA* 25

5:50 **N-Glycosylation in *Trypanosoma brucei***; Luis Izquierdo; Deuan C. Jones; Dan C. Turnock; Angela Mehlert; Michael A. Ferguson; *University of Dundee, Dundee, Scotland, UK* 26

TUESDAY, NOVEMBER 13
8:30 – 10:10 AM
SESSION V
BIOLOGY AND BIOCHEMISTRY OF SULFATASES, *Imperial Ballroom*
Chair, Steven Rosen, *UCSF, CA*

Time **Abstract Number**

8:30 **Introductory Comments: Structure and Function of Sulfatases**

8:40 **Sulfatase Modifying Factor-1 Trafficking through Cells: Implications for Sulfatase Activities**, Maria Pia Cosma, Telethon Institute of Genetics and Medicine, *Napoli, Italy* ..... N/A

9:05 **Sulf Regulation of Heparan Sulfate-Mediated Signaling during Development and Tissue Regeneration**; Xingbin Ai; Charles P. Emerson, Jr.; *Boston Biomedical Research Institute, Watertown, MA* 28

9:30 **The Sulfs – Dynamic Regulators of Cell Signaling and Development via Heparan Sulfate Editing**; Ina Kalus[1]; William C Lamanna[1]; Benedikt Salmen[2]; Marc-André Frese[1]; Rudi D'Hooge[3]; Thomas Dierks[1]; *[1]Biochemistry I, Bielefeld University, Bielefeld, Germany; [2]Neuroscience Research Center, Charité Berlin, Berlin, Germany; [3]Biological Psychology, University of Leuven, Leuven, Belgium* 29

9:55 **Regulation of wnt Signaling, Cell Growth, and Tumorigenicity in Human Lung Cancer Cells by SULF2, an Extracellular Endosulfatase**; Hassan Lemjabbar Alaoui[1]; Durwin Tsay[1]; Roman Nawroth[2]; Steven D Rosen[1]; *[1]University of California at San Francisco, San Francisco, CA; [2]Klinikum Rechts der Isar, Munich, Germany* 30

10:00 **TGF-β1 Induces Heparan Sulfate 6-O-Endosulfatase 1 Expression *in vitro* and *in vivo***; Xinping Yue; Xian Li; Hong T Nguyen; Dawn R Levy; Joseph A Lasky; *Tulane University, New Orleans, LA* 31

10:05 **The Expression of Sulf-1, a Heparan Sulfate Glucosamine 6-O-Endosulfatase, in Wounded Mouse Cornea.**; Inna Maltseva; Steven D. Rosen; *University of California of San Francisco, San Francisco, CA* 32

10:10 – 10:45 AM
COFFEE BREAK

TUESDAY, NOVEMBER 13
10:45 AM – 12:20 PM
SESSION VI
QUANTATATIVE GLYCOBIOLOGY, *Imperial Ballroom*
Chair, Jefrey Esko, *UCSD, CA*

Time **Abstract Number**

10:45 **Systems Biology and Computational Modeling: Why Glycobiologists should Rejoice**; Arthur D. Lander; *University of California, Irvine, Irvine, CA* 33

11:10 **A *Virtual* Glycan Array (VGA) for High Throughput Receptor Screening**; Robert J. Woods; *Complex Carbohydrate Research Center, U of Georgia, Athens, GA* 34

11:35 **Binding Studies of GalNAc Specific Lectins to the Tn-Antigenic Form of Porcine Submaxillary Mucin and Its Fragments**; Tarun K. Dam[1]; Thomas A. Gerken[2]; Benildo S. Cavada[3]; Kyria S. Nascimento[3]; Moura R. Tales[3]; C. Fred Brewer[1]; *[1]Albert Einstein College of Medicine, Bronx, NY; [2]Case Western Reserve University School of Medicine, Cleveland, OH; [3]Universidade Federal do Ceara, Fortaleza, Brazil* 35

11:40 **Unprecedented *in vivo* Efficacy of Pre-ordered Polymeric Heterobifunctional Ligands**; David R. Bundle[1]; Pavel I. Kitov[1]; Tomasz Lipinski[1]; Eugenia Paszkiewicz[1]; Joanna M. Sadowska[1]; Dmitry Solomon[1]; George L. Mulvey[2]; Glenn D. Armstrong[2]; *[1]University of Alberta, Edmonton, Canada; [2]University of Calgary, Calgary, Canada*..... 36

11:45 **Chemical Glycobiology and Vaccine Development**; Geert-Jan Boons; *Complex Carbohydrate Research Center, Athens, GA* 37

12:10 **IDAWG: A Novel Quantitative Method for Glycomics**; Lance Wells; Meng Fang; Jae-Min Lim; Stephen Dalton; Kelley Moremen; Michael Pierce; Michael Tiemeyer; William York; James Atwood; Ron Orlando; *Complex Carbohydrate Research Center, UGA, Athens, GA* 38

12:15 **Glycan Reductive Isotope Labels (Gril) for Precision Glycomics**; Baoyun Xia[1]; Goverdhan P. Sachdev[2]; Christa L. Feasley[2]; David F. Smith[1]; Richard D. Cummings[1]; *[1]Biochem. Dept, Emory University School of Medicine, Atlanta, GA; [2]University of Oklahoma Health Sciences Center, Oklahoma City, OK*..... 39

2:00 – 4:00 PM
POSTER SESSION II, *Georgian, Arlington, Berkeley & Clarendon*

4:00 – 4:30 PM
BUSINESS MEETING, *Imperial Ballroom*

4:30 – 5:30 PM
KARL MEYER, *Imperial Ballroom*

7:00 – 9:30 PM
BANQUET, *Ticket Required*

WEDNESDAY, NOVEMBER 14
8:30 – 10:05 AM
SESSION VII
RECOGNITION IN GLYCAN BIOSYNTHESIS AND FUNCTION, *Imperial Ballroom*
Chair, Karen J. Colley, *Univ. of Illinois-Chicago*

Time **Abstract Number**

8:30 **Sequence Requirements for NCAM Polysialylation**; Karen J. Colley; Deirdre A. Foley; Kristin G. Swartzentruber; Matthew G. Thompson; Shalu Shiv Mendiratta; *University of Illinois at Chicago, Chicago, IL*..... 40

8:55 **The Diverse Location and Composition of the N-Glycomes (Glycoproteins with N-Glycans) of Parasitic Protists**; John Samuelson; Daniel Ratner; Sulagna Banerjee; Anirban Chatterjee; Phillips Robbins; *Boston University, Boston, MA*..... 41

9:20 **LNFPIII neo-Glycoconjugates Activate Antigen Presenting Cells *in vivo* and *in vitro* and have Immunomodulatory Activity *in vivo***; Donald Harn[1]; Olga Atochina[1]; Akram Da'dara[1]; Dita Mayerova[2]; Christophe Benoist[2]; Dianne Mathis[2]; Paul Thomas[4]; *[1]Harvard School of Public Health, Boston, MA; [2]Joslin Diabetes Reseacrh Institute, Boston, MA; [3]Children's Hospital, Boston, MA; [4]St. Jude's Children's Hospital, Memphis, TN*..... 42

9:45 **Mice Lacking Ganglioside GM3 Synthase Exhibit Complete Hearing Loss Due to Selective Degeneration of the Organ of Corti**; Jin-ichi Inokuchi[1]; Misato Yoshikawa[1]; Kotaro Takasaki[2]; Yasuhiro Kakazu[3]; Mitsuru Ohashi[3]; Shinji Go[1]; Masakazu Nagafuku[1]; Kazuya Kabayama[1]; Takashi Kimitsuki[3]; Nozomu Matsumoto[3]; Shizuo Komune[3]; Kazutaka Takaiwa[3]; Masaki Saito[4]; Michihiro Fujiwara[2]; Katsunori Iwasaki[2]; *[1]Tohoku Pharmaceutical University, Sendai, Japan; [2]Fukuoka University, Fukuoka , Japan; [3]Kyushu University, Fukuoka , Japan; [4]Meiji Pharmaceutical University, Akitsu, Japan*..... 43

9:50 **Plasma Concentrations of Glycoproteins Terminating with Siaα2,6Gal are Regulated by the Asialoglycoprotein Receptor**; Lindsay M. Steirer[1]; Eric I. Park[2]; R. Reid Townsend[1]; Jacques U. Baenziger[1]; *[1]Washington University in St. Louis, St. Louis, MO; [2]University of North Carolina at Chapel Hill, Chapel Hill, NC*..... 44

9:55 **Carcinoembryonic Antigen is an E- and L-, but not P-, Selectin Ligand on Colon Carcinoma Cells**; Susan L. Napier; Ronald L. Schnaar; Konstantinos Konstantopoulos; *The Johns Hopkins University, Baltimore, MD*..... 45

10:00 **Role of the Sulfotransferase, GlcNAc6ST-2, in a Mouse Model of Rheumatoid Arthritis**; Hanayo Arata-Kawai; Kenji Uchimura; Steven D Rosen; *University of California, San Francisco, California*..... 46

10:05 – 10:45 AM
COFFEE BREAK

WEDNESDAY, NOVEMBER 14
10:45 – 12:15 PM
SESSION VIII
STRUCTURAL INSIGHTS OF PROTEIN-CARBOHYDRATE INTERACTIONS, *Imperial Ballroom*
Chair, Nancy Dahms, *Medical College of Wisconsin*

Time **Abstract Number**

10:45 **Carbohydrate-Based Pharamcological Chaperones for Treatment of Lysosomal Storage Diseases**, Gregory A. Petsko, *Brandeis University, MA*.....N/A

11:10 **Heparin and Heparan Sulfate on Proteins of the Hedgehog Signaling Pathway**; Robert J Linhardt[1]; Fuming Zhang[1]; Alondra M Ayala[1]; Jason S McLellan[2]; Daniel J Leahy[2]; *[1]Rensselaer Polytechnic Institute, Troy, NY; [2]Johns Hopkins University School of Medicine, Baltimore, MD*..... 48

11:35 **A Model for Processive Glycan Chain Synthesis**, Suzanne Walker, *Harvard University, MA*.....N/A

12:00 ***N*-Glycolyl GM1 Ganglioside as a Host Cell Receptor for Simian Virus 40 (SV40)**; Maria A Campanero-Rhodes[1]; Alicia Smith[2]; Wengang Chai[1]; Sandro Sonino[3]; Laura Mauri[3]; Robert A Childs[1]; Yibing Zhang[1]; Helge Ewers[2]; Ari Helenius[2]; Anne Imberty[4]; Ten Feizi[1]; *[1]Imperial College London, London, UK; [2]Institute of Biochemistry, ETH, Zurich, Switzerland; [3]Center of Excellence on Neurodegenerative Diseases, Milan, Italy; [4]CERMAV-CNRS, Grenoble, France*..... 50

12:05 **In Silico Prediction of Carbohydrate Binding Sites: Prospects and Current Limitations**; Frank Martin ; von der Lieth Claus-W; *German Cancer Research Center, Heidelberg, Germany*..... 51

12:10 **Structural Characterisation of Glycosaminoglycans: First Steps of a Glycomics Strategy**; Berangere Tissot[1]; Alessio Ceroni[1]; Andrew K. Powell[2]; Nijole Gasiunas[3]; Yassir Ahmed[2]; Zheng-liang Zhi[2]; Stuart M. Haslam[1]; Howard R. Morris[4]; Jeremy E. Turnbull[2]; John T. Gallagher[3]; Anne Dell[1]; *[1]Imperial College, London, United Kingdom; [2]University of Liverpool, Liverpool, United Kingdom; [3]University of Manchester, Manchester, United Kingdom; [4]M-SCAN Ltd, Wokingham, United Kingdom*..... 52

WEDNESDAY, NOVEMBER 14
2:00 – 4:00 PM
SESSION IX
GLYCOSYLATION IN THE CYTOSOL, *Imperial Ballroom*
Chair, John Hanover, *National Institutes of Health, MD*

Time **Abstract Number**

2:00 **SECRET AGENT and SPY Inform about the Role of Protein O-GlcNAc Modification in Plant Processes**; Lynn M. Hartweck; Young-Cheon Kim; Harriëtte Oldenhof; Qiuxia Wu; Manjula Gopalraj; Neil E. Olszewski; *University of Minnesota, St. Paul, MN*..... 53

2:25 **Breaking the O-GlcNAc code: O-GlcNAc and Cellular Signaling**; Dona C Love; Salil Gosh; Michele E Forsythe; Brooke D Lazarus; Eun Ju Kim; Chithra Keembiyehetty; Michael W Krause; John A Hanover; *National Institutes of Health, Bethesda, MD*..... 54

2:50 **Significance of Cytoplasmic Glycosylation for O2-signaling in Dictyostelium and other Protists**; Christopher M. West; Hanke van der Wel; Zhuo A. Wang; Ira J. Blader; *Oklahoma Center for Medical Glycobiology, OUHSC, Oklahoma City, OK*..... 55

3:15 **A novel and Sensitive Method for Absolute Quantification of Protein GlcNAcylation**; Wagner B. Dias; Win D. Cheung; Gerald W. Hart; *Johns Hopkins University, Baltimore, MD*..... 56

3:20 **A Case for Functional O-GlcNAc Modification of IRS Proteins**; Chin Fen Teo; Lance Wells; *Complex Carbohydrate Research Center, UGA, Athens, GA*..... 57

3:25 **The Role of O-GlcNAcylation in Metastasis**; Liz Cohen; Isam Khalaila; *Ben-Gurion University of the Negev, Beer-Sheva, Israel*..... 58

3:30 **CLOSING COMMENTS**

4:00 – 6:00 PM
POSTER SESSION III, *Georgian, Arlington, Berkeley & Clarendon*

MONDAY, NOVEMBER 12
2:00 – 4:00 PM
POSTER SESSION I, *Georgian, Arlington, Berkeley & Clarendon*
Topics:
GLYCOBIOLOGY AND GLYCOCHEMISTRY CHALLENGES IN BIOTECHNOLOGY
GLYCANS IN CELL COMMUNICATION
GLYCOCONJUGATE BIOSYNTHESIS
GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT I
These posters should be set up by 8:00 am and removed by 7:00 pm

Poster Topic
GLYCOBIOLOGY AND GLYCOCHEMISTRY CHALLENGES IN BIOTECHNOLOGY

Poster Number **Abstract Number**

1 **Glycoprotein Oligosaccharide Structural Characterization using Solid-Phase Permethylation Approach and Arabinosazone as MALDI TOF MS Matrix**; Mei Lin; Caesar Almaraz; Steve Gozo; Michael Grace ; Yunping Huang; *Bristol Myers Squibb, Pennington, NJ*..... 59

2 **Anti- Human Immunodeficiency Virus Type 1 (HIV- 1 IIIB) Activity of Lectin from Sea Mussel Crenomytilus Grayanus**; Wei Li[1]; Chuannan Xiong[2]; Jianhua wang[3]; Yongtang Zheng[3]; Qiukuan Wang[1]; *[1]Dalian Fisheries University, Dalian, China; [2]Dalian Institute of Chemical Physics, CAS, Dalian, China; [3]Kunming Institute of Zoology, CAS, Kunming, China*..... 60

3 **The Effect of the Length of Arginine Chain on the Internalization into the SMC Cell**; Kürşrt Oğuz Yaykaşli; Toshitaka Oohashi; Yoshifumi Ninomiya; *Okayama University-Graduate School of Medicine, Okayama, Japan*..... 61

Poster Topic

GLYCANS IN CELL COMMUNICATION

Poster Number **Abstract Number**

Poster Topic

GLYCOCONJUGATE BIOSYNTHESIS

Poster Number **Abstract Number**

42 **Synthetic Acceptor Analogs for the Characterization of Glucosyltransferases Involved in the Biosynthesis of O56 and O152 Antigens of *Escherichia coli***; Inka Brockhausen[1]; Bin Liu[2]; Bo Hu[2]; Kenneth Lau[1]; Walter A. Szarek[1]; Lei Wang[2]; [1]*Queen's University, Kingston, Ontario, Canada;* [2]*Nankai University, Tianjin, China* 5

43 **Glycoengineering Using Bacterial Oligosaccharyltransferases**; Amirreza Faridmoayer; Messele A. Fentabil; Dominic C. Mills; John S. Klassen; Mario F. Feldman; *University of Alberta, Edmonton, Canada* 6

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BIOLOGY AND BIOCHEMISTRY OF SULFATASES
QUANTATATIVE GLYCOBIOLOGY
RECOGNITION IN GLYCAN BIOSYNTHESIS AND FUNCTION I
These posters should be set up by 8:00 am and removed by 7:00 pm

Poster Topic
GLYCANS ARE REQUIRED FOR EUKARYOTIC DEVELOPMENT II

Poster Number **Abstract Number**

Poster Topic
PROTEIN FOLDING AND QUALITY CONTROL

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QUANTATATIVE GLYCOBIOLOGY

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RECOGNITION IN GLYCAN BIOSYNTHESIS AND FUNCTION I

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GLYCOSYLATION IN THE CYTOSOL
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(1) *In vivo* Roles for Mammalian Oligosaccharyltransferase Isoforms

Catalina Ruiz-Canada; Daniel Kelleher; Reid Gilmore
University of Massachusetts Medical School, Worcester, MA

The genomes of metazoan organisms contain two genes (STT3A and STT3B) that encode catalytic subunits of the oligosaccharyl-transferase. STT3A or STT3B are assembled together with a shared set of non-catalytic subunits (ribophorin I, ribophorin II, OST48, DAD1, OST4 and IAP or TUSC3) to form distinct isoforms of the OST complex. *In vitro* assays have shown that the STT3A catalytic subunit confers enhanced selectivity for the fully assembled dolichol-linked oligosaccharide, while the STT3B catalytic subunit confers a higher turnover rate and a relaxed specificity for the donor substrate. mRNAs encoding both catalytic subunits (STT3A and STT3B) are widely expressed in human tissues, and both STT3A and STT3B have been detected at the protein level in all mammalian cell lines analyzed to date. Here, we have used a siRNA knockdown approach in Hela cells to determine whether STT3A and STT3B have identical, partially overlapping or non-overlapping roles in asparagine-linked glycosylation in the endoplasmic reticulum. Ten-fold reductions in STT3A or STT3B content do not cause growth rate defects, in part due to a compensatory increase in expression of the other catalytic subunit. Depletion of STT3A, but not STT3B, causes a modest induction of the unfolded protein response (UPR) pathway. Analysis of pulse-labeled glycoproteins reveals that specific glycosylation sequons (N-X-T/S) are not modified upon selective depletion of STT3A or STT3B. Evidence will be presented showing that mammalian OST isoforms have partially non-overlapping roles in oligosaccharide transfer and cooperate to mediate full glycosylation of specific substrates.

(2) Lipid Flip-Flop during Glycoprotein Biosynthesis

Anant K. Menon
Weill Cornell Medical College, New York, NY

N-glycosylation of secretory proteins in the ER involves transfer of a 14-sugar glycan from the glycolipid Glc3Man9GlcNAc2-diphosphate-dolichol (G3M9-LLO) to select asparagine residues in the protein. G3M9-LLO assembly requires the flipping of three glycolipids, Man5GlcNAc2-PP-dolichol (M5-LLO), Man-P-dolichol and Glc-P-dolichol, from the cytoplasmic leaflet to the lumenal leaflet of the ER membrane. Since the translocation of these lipids across the ER, like that of 'membrane-building' glycerophospholipids such as phosphatidylcholine, does not occur spontaneously at an appreciable rate because of the considerable energy barrier to flipping, it is hypothesized that specific translocators (flippases) are required. None of these translocators has been identified although Rft1, an ER-localized membrane protein, has been proposed to function as a flippase for M5-LLO. Here we reconstitute M5-LLO flipping in proteoliposomes generated from a detergent extract of S. cerevisiae microsomes. We assay flipping by exploiting the ability of the lectin Concanavalin A to bind and capture M5-LLOs that are present in, or gain access to, the outer leaflet of the reconstituted vesicles. Our data indicate (i) that M5-LLO flipping in the reconstituted system is ATP-independent and requires specific membrane proteins that sediment at ~4S in velocity gradients and (ii) that Rft1 is not required. The M5-LLO flippase implicated by these results is distinct from the ER glycerophospholipid flippase activity that is necessary for bilayer propagation: M5-LLO flippase is ~3-fold less abundant and sediments more rapidly than the glycerophospholipid flippase. These results indicate the ER has at least two distinct flippase proteins and set the stage for identifying these transporters.

(3) Coupling Polymerization, Chain Termination and Export in the Biosynthesis of Bacterial Lipopolysaccharide O Antigens

Chris Whitfield; Leslie Cuthbertson; Bradley R. Clarke; Matthew S. Kimber
University of Guelph, Guelph, ON, Canada

Lipopolysaccharides are complex glycolipids that play critical roles in the viability and pathogenesis of gram-negative bacteria. These molecules often contain a long-chain polysaccharide called the O antigenic polysaccharide (O-PS) whose role is to protect the cell from host defenses. The structure of the O-PS, its chain length, and the extent to which it covers the cell surface are all critical elements in its function in virulence. There are two distinct assembly mechanisms for O-PS and these employ different methods to regulate O-PS length. The work presented here describes an ATP-binding cassette (ABC) transporter-dependent assembly system, established in prototypes from *Escherichia coli*. The O-PS glycan chain is synthesized in the cytoplasm. Chain termination is achieved by capping the growing non-reducing terminus with a residue that is not found in the repeating unit structure. In addition to determining chain-length, this terminal addition serves as an essential signal for the ABC transporter which moves the nascent glycan across the inner membrane. Once exported, the O-PS it is ligated to the rest of the lipopolysaccharide molecule and translocated to the cell surface. The ABC transporter recognizes the terminated glycan via a discrete domain linked to the nucleotide-binding domain (NBD) of the ABC transporter. The recognition domain is a modified immunoglobulin-binding fold and exists as a dimer stabilized by inter-monomer strand exchange. This assembly mechanism effectively couples glycan chain polymerization and termination to its export. Bioinformatic studies suggest this strategy may be conserved in the assembly of other bacterial glycoconjugates.

(4) Mycobacterial Cell Wall Glycomics: Recent Revelations

Delphi Chatterjee; Jian Zhang; Devinder Kaur; Patrick J. Brennan; Kay-Hooi Khoo
Colorado State University, Fort Collins, CO

One of the most prominent components of the mycobacterial cell wall is arabinan, a constituent present in two distinct settings, arabinogalactan (AG) and lipoarabinomannan (LAM). The latter is implicated as an important virulence factor for the bacterium. Structurally, the D-arabinofurans are unique. They consist of biologically rare sugar, D-arabinofuranose (Araf) and are almost unknown outside the member of the *Actinomycetales*. Functionally, the arabinan in AG tether the unusual α branched mycolic acids to peptidoglycan, which then contributes to the permeability barrier.

An exciting key development over the last two years is a gradual recognition of the structural differences encoded within the arabinan architecture, which may determine its immunomodulatory functions. The branching pattern and overall size of the arabinan thus synthesized is distinctively regulated in different species with an unexpected twist that those of major human pathogens, e.g. *M. tb* and *M. leprae*, differ significantly from the non-pathogenic genetic model, *M. smegmatis*.

AftA, AftB and the Emb proteins have been identified as the arabinosyltransferases to date. The genes have been shown to be essential indicating physiological significance of arabinosyltransferases in *M. tb* and the potential of being a drug target.

The fundamentals of how these arabinans are assembled-if on a lipid carrier, growing from the reducing or non-reducing end, built *en bloc*, assembled on an enzyme complex as in the polyketides, adding one residue at a time- is not known. Recent findings contributing to the intricate pathway of the cell wall formation will be the highlight of this work.

(5) **Synthetic Acceptor Analogs for the Characterization of Glucosyltransferases Involved in the Biosynthesis of O56 and O152 Antigens of Escherichia Coli**

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O antigens of the LPS on Gram-negative bacteria contain oligosaccharide repeats of various structures and antigenicities. The repeat units are assembled by glycosyltransferases that utilize nucleotide sugar donors and undecaprenol-phosphate as the natural acceptor substrate. The O56 and O152 antigens of Escherichia coli both contain the Glc-beta1-3GlcNAc- linkage within the repeat unit. We have cloned two genes within the O antigen gene cluster encoding putative Glc-transferases synthesizing this linkage (wfaP of O56 and orf9 of O152). The open reading frame orf9 shares 59% identity to wfaP, and 47% identity at the amino acid level. A synthetic substrate analog of the natural undecaprenol-phosphate lipid (GlcNAc-PP-PhU) was previously successfully used in characterizing a Gal-transferase of E.coli O7, unrelated in sequence to the Glc-transferases. Here, we show that both orf9 and wfaP encode highly active Glc-transferases, using GlcNAc-PP-PhU as acceptor substrate. Both Glc-transferases require divalent cation, are deactivated by detergents, and require the pyrophosphate-sugar linkage in the substrate. Substrates lacking phosphate groups are inactive. Although the natural linkage of GlcNAc in O56 and O152 repeat units is beta, the enzymes readily recognize the alpha-linkage in the acceptor substrate. The length of the lipid moieties of substrates appears to be less important for activity. This shows that synthetic substrates are of general application to O-antigen synthesizing glycosyltransferases, and that these enzymes share properties and substrate recognition, even in the absence of sequence identity.

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(6) **Glycoengineering using Bacterial Oligosaccharyltransferases**

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Protein glycosylation is an important posttranslational modification that occurs in all domains of life. In bacteria, N-glycosylation has only been demonstrated in *Campylobacter jejuni* whereas O-glycosylation has a broader distribution. Pilins, the structural components of type IV pili, are glycosylated in *Neisseria meningitidis*, *N. gonorrhea*, and some strains of *Pseudomonas aeruginosa*. In this work, we functionally reconstitute the *P. aeruginosa* 1244 and *N. meningitidis* MC58 O-glycosylation systems in *Escherichia coli*. In both cases sugars are transferred *en bloc* by an oligosaccharyltransferase (OTase), named PglL in *N. meningitidis* and PilO in *P. aeruginosa*. Both OTases possess relaxed sugar specificity and are sufficient for protein glycosylation. Both enzymes require translocation of the undecaprenol-pyrophosphate–linked oligosaccharides substrates into the periplasm for activity. Furthermore, we show that PglL is able to transfer diverse oligo- and polysaccharides, whereas PilO activity is restricted to short oligosaccharides. Based on this functional characterization we propose that, despite their low sequence similarity, PilO and PglL belong to a new family of "O-OTases" that transfer oligosaccharides from a lipid carrier to hydroxylated amino acids in proteins. To date, such activity has not been identified in eukaryotes. To our knowledge, this is the first report describing recombinant O-glycoproteins synthesized in *E. coli*.

(7) **Mycobacterial Cell-Wall Biosynthesis: Exploitation of Chemistry and Biology**

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The D-arabinans in Mycobacterium are essential, extraordinarily complex entity comprised of D-arabinofuranose residues, which are rarely found in nature. Despite the well recognized importance of the mycobacterial arabinan, delineation of the arabinosylation process has been severely hampered due to lack of positively identified arabinosyltransferases. Identification of genes involved in arabinan biosynthesis entailed the use of ethambutol (EMB), a first line antituberculosis agent that is known to inhibit cell wall arabinan synthesis. The three genes (embA, embB and embC) encode novel membrane proteins, implicated as the only known mycobacterial arabinosyltransferases to this date. We have now adapted a multi-faceted approach involving development of convenient arabinosyltransferase assay using novel synthetic acceptors to identify arabinosyltransferase/s that will be distinct from the Emb proteins. In our present work, Mycobacterium smegmatis mc2155 was used as a model to study the biosynthesis of cell wall arabinan. In an *in vitro* assay, we demonstrate that, transfer of only α-Araf had occurred from decaprenylphosphoryl-D-arabinose (DPA) on a newly synthesized branched acceptor [α-D-Araf]2-3,5-α-D-Araf-(1→5)-α-D-Araf-(1→5)-α-D-Araf with an octyl aglycon. Higher molecular weight (upto Ara10) oligomers were also detected in a parallel reaction using cold Phosphoribosylpyrophosphate (pRpp). On the other hands, a linear pentasaccharide incorporated an early branch, which indicate that the arabinan formation is tightly regulated. These results have given us a new study within arabinan biosynthesis and leaded new hypothesis on the formation of the arabinan in mycobacterium.

(8) **The Nucleotide Sugar Transport/Antiport Cycle in Eukaryotic Development and Disease**

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Transport of nucleotide sugars into the lumen of the Golgi apparatus is mediated by specific transporters, which are antiporters, and have been found to occur in every eukaryote studied to date. Mutants in these transporters have been characterized in mammals, yeast, plants, leishmania, Entamoeba, Drosophila, nematodes and Giardia. In many of these organisms mutations of transporter proteins result in striking developmental phenotypes including diseases such as Leukocyte Adhesion Deficiency Syndrome II and Complex Vertebral Malformation. Recent studies with C. elegans suggest functional redundancy among these transporters. Supported by NIH grant GM 30365.

(9) **Extracellular Matrix-Glycan Interactions in the Peripheral Nervous System**

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Interactions between Schwann cells and proteins in the peripheral nerve extracellular matrix (ECM) are essential for Schwann cell terminal differentiation and function, including the establishment of myelin sheaths. Interactions of cells with ECM are mediated by cell surface receptors that bind ECM proteins and regulate intracellular signaling pathways that affect critical cellular functions such as cell adhesion, cell migration, cell morphology and cytoskeletal organization, and terminal differentiation. Our laboratory identified a novel mechanism for Schwann cell-ECM adhesion that involves glypican-1 on the Schwann cell membrane and type V collagen in the nerve ECM. Glypican-1 is a glycosylphosphatidylinositol (GPI)-anchored cell surface heparan sulfate proteoglycan (HSPG). In peripheral nerves glypican-1 is

localized to the outer Schwann cell membrane that contacts the basal lamina matrix. A major HS-binding ECM protein in developing peripheral nerve tissue is type V collagen, which binds to HS molecules via a high affinity binding site in the non-collagenous N-terminal domain (NTD) of the □4(V) collagen chain. In peripheral nerves Type V collagen is synthesized by Schwann cells and incorporated into fibrillar and basal lamina ECM. NTD binding to glypican-1 mediates Schwann cell adhesion and cytoskeletal organization by a HS-dependent mechanism that requires the high affinity HS-bind site in the NTD. In primary co-cultures of Schwann cells and dorsal root ganglion neurons suppression of glypican-1 or Type V collagen expression inhibits Schwann cell myelination. Type V collagen-glypican-1 appears to affect Rho pathway signaling and works in concert with other ECM receptors receptors to regulate Schwann cell cytoskeletal organization.

(10) **Regulation of Skeletal Morphogenesis by Sulphated Proteoglycans in Zebrafish**

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One of the earliest signs of skeletal development is the formation of pre-cartilage condensations. Although condensations form the basis for morphogenesis of much of the skeleton, little is known about the mechanism by which cells within the condensation become organised. Here we show that in two zebrafish mutants, dackel (dak) and pinscher (pic), skeletal morphogenesis is disrupted due to a loss of polarised flattening and intercalation of chondrocytes. We have previously shown that dak corresponds to a human tumour-suppressor gene that is required for heparan biosynthesis (Exostosin-2). Positional cloning of pic reveals that it encodes a sulphate transporter required for sulphation of proteoglycans (Papst1). Whereas early differentiation of chondrocytes is unaffected in both mutants, chondrocyte hypertrophy and bone formation are reduced or absent. Transplantation analysis indicates that dak-/- cell morphology is usually rescued by neighbouring wild-type chondrocytes. In contrast, pic-/- cells always act autonomously and can disrupt the morphology of neighbouring wild-type cells. Our data provides the first clues for understanding the molecular mechanism underlying condensation morphogenesis and furthers our understanding of the aetiology of hereditary osteochondromas.

(11) **Exploring the Pathogenesis of Mucolipidosis II in Zebrafish**

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The severe lysosomal storage disorder, mucolipidosis II (ML-II or I-cell disease), is caused by defects in GlcNAc-1-phosphotransferase, the initial enzyme responsible for the biosynthesis of mannose 6-phosphate (M6P) residues. Patients with ML-II exhibit multiple clinical features shortly after birth including skeletal, craniofacial, neurological and cardiac defects. In an effort to explore the mechanisms by which loss of M6P residues on proteins results in the developmental abnormalities of the disease, we have previously generated a vertebrate model for ML-II in zebrafish (Danio rerio) using a morpholino-based knockdown strategy. These morphant zebrafish exhibit several striking developmental anomalies consistent with the human disease, including cartilage morphogenesis defects. Analysis of Alcian blue-stained craniofacial cartilage reveals misshapen and missing jaw structures and changes in extracellular matrix composition. Using transgenic zebrafish in which the neural crest-derived chondrocyte progenitors are labeled with EGFP, we have begun to analyze the cellular and molecular basis for impaired craniofacial development in GlcNAc-1-phosphotransferase deficient embryos. Our preliminary studies indicate that the migration and differentiation of chondrocyte precursors is abnormal in morphant zebrafish. Interestingly, these precursors appear to precociously differentiate after entering the cranial facial field, as evidenced by differences in the timing and distribution of type II collagen expression. Together, these data suggest that alterations in the composition and deposition of the extracellular matrix are central to the craniofacial phenotype associated with ML-II. This model offers a valuable new tool that can be used to investigate ML-II pathogenesis.

(12) **An O-Glycosyltransferase is Required for Proper Cell Adhesion in Drosophila**

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Cell-cell adhesion and the factors that govern it are crucial in many aspects of eukaryotic development. Here we demonstrate that an enzyme responsible for the initiation of protein O-linked glycosylation is involved in epithelial cell adhesion in the Drosophila wing blade. Mutations in a member of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family result in a blistered wing phenotype, characteristic of genes regulating cell-cell and cell-ECM interactions. The frequency of this phenotype is exacerbated in certain genetic backgrounds. Expression of the wild-type O-glycosyl-transferase in mutant flies rescues the wing blistering phenotype. RNAi to this transferase in Drosophila cell culture causes Golgi apparatus misorganization and cell adhesion defects. We postulate that this transferase is involved in proper Golgi organization as well as processing/secretion of components involved in maintaining cell-cell adhesion. Lectins that recognize the O-linked glycans are being used as affinity reagents on wild type wing disc extracts to identify proteins that are normally glycosylated by this transferase and are therefore candidates for components mediating proper cell adhesion. This study demonstrates the role for a new class of genes in the regulation of cell-cell interactions in this system.

(13) **The Importance of alg10 and Regulated N-Glycosylation during Drosophila Development**

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Proteins involved in extracellular signaling events must first traverse the secretory pathway where they can undergo a variety of different posttranslational alterations before the fully mature protein is able to execute its function outside the cell. In this study, Drosophila harboring mutations in alg10 are characterized. alg10 encodes a glycosyltransferase that catalyzes the addition of terminal glucose residues to the growing dolichol-linked oligosaccharide just prior to its en masse transfer to nascent polypeptides. Notably, this terminal glucose is removed from the oligosaccharide following transfer and alg10 is not required in tissues that express oligosaccharyltransferase isoforms that transfer under glycosylated substrates. Our study of P-element excision alleles of alg10 demonstrates that this gene product is of surprisingly critical importance during Drosophila development. Removal of both zygotic- and maternal-derived alg10 alleles results in severe and pleiotropic deficits in embryos, demonstrating the importance of regulated N-glycosylation during the initial phases of Drosophila development. During larval development, removal of alg10 from the eye imaginal disc leads to a disordered eye of reduction size. These effects might be mediated through the Sevenless activated MAP kinase cascade, as we find alg10 mutant eye imaginal discs display pathway gain-of-function phenotypes. These data suggest that regulated N-glycosylation of a component

of the Sevenless receptor tyrosine kinase pathway, perhaps the receptor itself, is one important target of Alg10 function. Together, our data suggest that tissue specific addition of terminal glucose to the dolichol-linked oligosaccharide is an essential regulatory event in executing the Drosophila developmental program.

(14) **Genetic Background Influences Glycosylation and Developmental Phenotypes Associated with the *Sugar-Free Frosting* Mutation in *Drosophila***

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The *Drosophila* embryo expresses a family of related N-linked glycan structures, known as HRP-epitopes, that are enriched in neural tissue and carry Fuc linked α3 to the reducing terminal GlcNAc of the chitobiose core. In a screen for mutations that affect tissue specific glycosylation, we generated a new mutation, named *sugar-free frosting* (*sff*), that specifically abolishes almost all HRP-epitope expression. Our single mutant allele of *sff* (sff^{B22}) is semi-viable. Homozygous adults display a deficit in geotaxis, which is rescued by acute administration of drugs that affect biogenic amine metabolism. We have noticed that specific genetic backgrounds, particularly those that include mutations in the *white* gene (*w*), affect viability and the efficacy of drug treatment in sff^{B22}. Therefore, characterization of the N-linked glycan profile of sff^{B22} embryos was performed in two different genetic backgrounds, either wild-type or mutant for white (w^{+}, and w^{1118}, respectively). In either background, sff^{B22} reduces both α3-linked Fuc and the prevalence of some minor complex glycans, while other changes reflect the genetic background. In parallel, we compared the glycan profiles of wild-type (OreR) and w^{1118} embryos to provide a background for assessing gene-specific effects. The predominant glycans of both genotypes, primarily pauci- and high-mannose structures, exhibit similar prevalences. However, significant differences were apparent among less prevalent, complex glycans. Therefore, differences in the genetic backgrounds of nominally wild-type strains can influence the flux and nature of glycan processing and should be taken into account when interpreting mutant phenotypes. Supported by funding from NIH/NIGMS.

(15) **Novel Chondroitin Proteoglycans Play an Essential Role in Early C. elegans Development**

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Chondroitin sulfate proteoglycans have been well-documented in vertebrate systems where they play roles in biological processes ranging from structural support in cartilage, to integrity of the dermis, to nervous system axon guidance and inhibition of mature neurite outgrowth. Some of these functions are specific to organisms with more complex body plans, but previous work has shown chondroitin also plays a fundamental biological role in simpler organisms such as Caenorhabditis elegans. Studies of the squashed vulva (sqv) mutants demonstrated that chondroitin is required for proper cell division of the single-celled embryo, as well as larval vulval morphogenesis. Interestingly, in silico analysis showed that C. elegans does not express obvious homologs of any mammalian CSPG core proteins that would harbor these chondroitin chains. We previously utilized a biochemical purification and mass spectrometry approach to identify nine novel chondroitin proteoglycan (CPG) core proteins in the worm, none of which are present in the mammalian genome. We selected two of these proteoglycans, CPG-1 and CPG-2, for further functional studies. While RNAi depletion of either gene alone shows no phenotype, simultaneous depletion of both genes results in multinucleated embryos that die at the single cell stage. The embryonic lethal phenotype resembles that seen in sqv mutants, suggesting CPG-1 and CPG-2 are two novel, functionally redundant chondroitin proteoglycans. CPG-1 and CPG-2 both contain functional chitin binding domains, suggesting potential interaction with the chitinous embryonic eggshell. Our current work aims to understand the role of these proteoglycans in eggshell structure and function.

(16) **HSPG Signaling at the Drosophila NMJ**

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Formation of the chemical synapse. the essential unit of neural function, relies upon coordinated assembly of intricate pre and postsynaptic protein complexes. On the presynaptic membrane, neurtransmitter release is orchestrated by the active zone. While many components of the active are known, the information and signaling pathways that shape and modulate its form and function remain an active area of discovery. Recent genetic manipulations in Drosophila identified heparan sulfate proteoglycans (HSPGs) in the Syndecan and Glypican families as important and specific regulators of synaptic morphogenesis and active zone structure. While these initial studies showed that both Syndecan (Sdc) and the Glypican Dallylike (Dlp) act through a receptor protein tyrosine phosphatase (the Leukocyte Antigen Related protein ortholog, LAR), little is known about the intracellular signaling machinery downstream of LAR at the synapse. Our current study is focused on the constituents and organization of this intracellular pathway. We find that the tyrosine kinase Abl and its substrate Enabled, form part of this pathway, presumably linking LAR to cytoskeletal dynamics in order to coordinate both morphological growth of presynaptic terminals and appropriate assembly of the active zone.

(17) **Mutation in the Key Enzyme of Sialic Acid Biosynthesis Causes Severe Glomerular Proteinuria and Is Rescued by *N*-Acetylmannosamine**

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Mutations in the key enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE/MNK), result in hereditary inclusion body myopathy (HIBM), an adult-onset, progressive neuromuscular disorder. We created knock-in mice harboring the M712T Gne/Mnk mutation. Homozygous mutant ($Gne^{M712T/M712T}$) mice did not survive beyond postnatal day 3 (P3). At P2, significantly decreased Gne-epimerase activity in $Gne^{M712T/M712T}$ muscle, but no myopathic features were apparent. Rather, homozygous mutant mice had glomerular hematuria, proteinuria, and podocytopathy. Renal findings included segmental splitting of the glomerular basement membrane, effacement of podocyte foot processes and reduced sialylation of the major podocyte sialoprotein, podocalyxin. Administration of the sialic acid precursor *N*-acetylmannosamine (ManNAc) yielded survival beyond P3 in 43% of the $Gne^{M712T/M712T}$ pups. Survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne/Mnk protein expression and Gne-epimerase activities. These findings establish this $Gne^{M712T/M712T}$ knock-in mouse as the first genetic model of podocyte injury and segmental glomerular basement membrane splitting due to hyposialylation. The results also support evaluation of ManNAc as a treatment not only for HIBM, but also for renal disorders involving proteinuria

and hematuria due to podocytopathy and/or segmental splitting of the glomerular basement membrane.

(18) **Roles for the Bisecting GlcNAc in the Development and Metastasis of Mammary Tumors**

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The *Mgat3* gene codes for *N*-acetylglucosaminyltransferase III (GlcNAcT-III) that transfers the bisecting GlcNAc to complex and hybrid N-glycans. Overexpression experiments in which an *Mgat3* cDNA is introduced into tumor cells suggest that it may suppress transformed properties, and the *Mgat3* gene maps to a region of chromosome 22 that exhibits loss-of-heterozygosity (LOH) in human cancers. To investigate whether GlcNAcT-III may act as a tumor suppressor *in vivo* we used the MMTV-PyMT mouse which develops mammary tumors that metastasize to lung. The *Mgat3* gene was not expressed in normal mammary glands, but was upregulated with the onset of lactation. Mammary tumors from *Mgat3*$^{+/-}$PyMT mammary tumors expressed the *Mgat3* gene and tumor glycoproteins bound E-PHA. There was no apparent difference in the stages of tumor development between mutant and control females. In addition, the metastatic index based on real-time PCR of PyMT expression in lungs at 17 weeks, correlated with tumor burden rather than *Mgat3* genotype. However, *Mgat3*$^{-/-}$ PyMT females developed tumors more quickly and had a significantly increased tumor burden. This contrasts with mice lacking GlcNAcT-V in which mammary tumor development is severley retarded. GlcNAcT-V initiates the β1,6 branch on the Man(α1,6) arm of complex N-glycans. The mechanism by which the presence of a bisecting GlcNAc on cell surface N-glycans retards the growth of mammary tumor cells that express GlcNAcT-V is under investigation. The data are consistent with a role for the *Mgat3* gene in tumor suppression.

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(19) **Identification of a Protein *O*-Glucosyltransferase Required for Notch Signaling in *Drosophila***

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Notch is modified with two unusal types of *O*-linked glycosylation in its extracellular EGF repeats: *O*-fucose and *O*-glucose. The *O*-fucose modifications are known to be essential for Notch signaling, but little is known about the significance of *O*-glucose. In an unbiased genetic screen designed to isolate new components of the pathway in *Drosophila*, we identified a novel gene, *rumi*, with a temperature-sensitive Notch phenotype. rumi encodes a soluble, ER-retained protein which contains a CAP10 domain, found in sugar-modifying enzymes in fungi. RNAi-mediated reduction in *rumi* caused a decrease in *O*-glucose levels on a Notch extracellular fragment expressed in S2 cells, suggesting Rumi may mediate addition of *O*-glucose to EGF repeats. To examine whether Rumi has protein *O*-glucosyltransferase activity, a FLAG-tagged version of Rumi was overexpressed in S2 cells and utilized in *in vitro O*-glucosyltransferase assays. Rumi showed significant *in vitro O*-glucosyltransferase activity, and the activity was dependent on the amount of the Rumi, concentration of factor VII EGF repeat, and concentration of UDP-glucose. Product analysis demonstrated that Rumi transfers a single glucose in *O*-linkage to an EGF repeat. Mouse Rumi protein expressed in 293T cells also showed *in vitro* protein *O*-glucosyltransferase activity. Our data suggest that Rumi is a protein *O*-glucosyltransferase and an essential component of Notch signaling. We are currently examining the mechanistic basis for the effects of *O*-glucose modification of Notch in both *Drosophila* and mouse systems. This work was supported by NIH grant GM61126 (RSH) and HHMI (HJB).

(20) **ER-resident Lectins and Glycosylhydrolases Determine the Fate of Newly Synthesized Glycoproteins**

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The ER is the site of synthesis and maturation for proteins destined for the membrane and lumen of the ER, Golgi, endosomes, lysosomes, for the plasma membrane and for the extracellular space. Most of the proteins synthesized in the ER are covalently modified by co-translational addition of pre-assembled glucose$_3$-mannose$_9$-*N*-acetylglucosamine$_2$- (Glc_3-Man_9-$NAcGlc_2$) core oligosaccharides. Protein-bound oligosaccharides are exposed to several ER-glycanases that sequentially remove terminal glucose or mannose residues. Rapid generation of a mono-glucosylated (Glc_1-Man_9-$GlcNAc_2$) trimming intermediate is required to enter the calnexin chaperone system in which protein folding progresses with highest efficiency. Removal and re-addition of the innermost glucose residue activate cycles of dissociation/re-association with calnexin that may facilitate, and in some cases is required for acquisition of the polypeptide's native structure. Slower removal of terminal □1,2-bonded mannose residues from *N*-linked glycans occurs upon persistent polypeptide retention in the ER, which is symptom of defective folding. Substrate de-mannosylation eventually interrupts *futile* folding attempts, results in substrate exclusion from the calnexin chaperone system and promotes retro-translocation into the cytosol for degradation operated by the 26S proteasome. De-glucosylation and de-mannosylation activities must be tightly regulated because the *N*-glycan composition will determine if the associated protein will be subjected to folding-attempts in the ER lumen or if it will be retro-translocated into the cytosol and degraded.

(21) **Carbohydrate-Mediated Protein Maturation and Quality Control in the Endoplasmic Reticulum**

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N-linked glycans act as maturation and quality control tags in the early secretory pathway. Their role starts cotranslationally as proteins emerge into the ER by directing the timing and location of lectin chaperone (calnexin and calreticulin) binding. These interactions persist post-translationally due to the action of the UDP-Glucose: glycoprotein glucosyltransferase (GT), which recognizes late folding intermediates, unassembled multimeric proteins or terminally misfolded proteins. Continued reglucosylation by GT eventually leads to the degradation of the aberrant glycoprotein by the ER-associated degradation process. Finally, the role of EDEM1 in sorting defective proteins for degradation will also be discussed.

(22) **Extensive Mannose Trimming and Mannosidase Compartmentalization in Glycoprotein ER-Associated Degradation**

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When ER chaperones fail to correct folding or processing defects on newly-synthesized glycoproteins within a certain time interval, these are targeted for ER-associated degradation (ERAD). The timing of this interval is linked to trimming of mannose residues from the precursor $Glc_3Man_9GlcNAc_2$, after the glucose residues

have been excised. Although initially thought to involve the trimming of only one specific mannose residue (the terminal middle branch mannose), recent evidence from our lab and others implies further trimming in mammalian cells of α1,2-linked mannose residues to form $Man_{5-6}GlcNAc_2$. A possible candidate for this activity is ER mannosidase I (ERManI). Although *in vitro*, at low concentrations, ERManI removes only one mannose residue, at very high concentrations it can excise up to four. Our recent evidence indicates that ERManI is required for trimming to $Man_{5-6}GlcNAc_2$ and for ERAD in cells *in vivo*. Trimming by ERManI to the smaller oligosaccharides would remove the glycoprotein from reglucosylation and calnexin binding cycles. ERManI appears strikingly concentrated together with the ERAD substrate in the pericentriolar ER-derived quality control compartment (ERQC) that we had described previously. This compartment recruits ERAD machinery components and the chaperones calnexin and calreticulin but not other chaperones or ER resident proteins like BiP, PDI, UGGT and ERp57. The ERQC provides a high local concentration of ERManI, and cycling through this compartment would allow timing of ERAD by trimming of a critical number of mannose residues, triggering a signal for degradation.

(23) **The Biophysics of Quality Control**

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Folding intermediates and irreparable folding species are retained in the ER by the lectins calreticulin (CRT) and calnexin. Central to this process is the enzyme UDP-Glc:glycoprotein glucosyltransferase (GT), which regenerates the monoglucosylated glycan recognized by both lectins. *In vitro* experiments show that this enzyme recognizes exposed hydrophobic aminoacids, preferentially when they are forming patches in advanced folding intermediates. This property allows the participation of GT not only in the quality control of protein tertiary structure, but also in the supervision of oligomers assembling. Interestingly, the different components of the folding quality control system seem to work in coordination. For instance, recent evidence suggests that the long term activity of GT is strongly dependent on the presence of CRT, as this lectin prevents GT inhibition by monoglucosylated glycans. Finally, besides working as a lectin-chaperone, CRT is one of the main calcium buffers of the endoplasmic reticulum. Although it was originally described that the lectin activity of CRT depends on calcium, we found that both activities are mutually independent *in vitro*. Accordingly, ER calcium depletion in Trypanosoma cruzi did not affect the association of cruzipain with CRT.

(24) **ER α1,2-Mannosidase I Is not the only Family Member that Trims Mannose to Create the ERAD Targeting Signal**

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Mannosidase activity is important for ERAD of misfolded glycoproteins in mammalian cells since degradation is prevented by GH family 47 α1,2-mannosidase inhibitors (1). It was originally proposed that ERAD targeting is initiated by recognition of the Man8GlcNAc2 isomer B product of ER α1,2-mannosidase I by EDEM, a GH family 47 member suggested to be a lectin (2). However, this model needs to be modified. First, we showed that ER α1,2-mannosidase I cleaves additional mannose residues from Man8GlcNAc2 both *in vitro* (3) and *in vivo* while its overexpression stimulates ERAD of misfolded α1-antitrypsin NHK (4). We then demonstrated that overexpression of EDEM3, but not a catalytic residue mutant, enhances mannose trimming *in vivo* while stimulating ERAD of NHK (5). We recently showed that overexpression of Golgi α1,2-mannosidases IA, IB or IC stimulates NHK degradation as well as trimming of its N-glycans to Man5-7GlcNAc2 and that some NHK can be transported to the Golgi (6). Therefore, ER α1,2-mannosidase I is not the only GH family 47 member implicated in mannose trimming of terminally misfolded glycoproteins to generate the recognition signal for ERAD of misfolded glycoproteins.

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(25) **Differential Protein Glycosylation Activity by the Two Vertebrate Oligosaccharyltransferases A- and B-OST**

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N-Glycosylation of proteins is a common modification of nascent polypeptides in the endoplasmic reticulum (ER). It is involved in key functions such as the exit of glycoproteins from the ER, and quality control of protein folding. The eight-subunit complex (RbI, RbII, DAD1, Ost48, Ost4, N33/IAP, STT3, Ost5) formed by the Oligosaccharyltransferase (OST) catalyzes the transfer of blocks of 14 sugars to the NXS/T motif of polypeptides. Previous studies show that the catalytic subunit of OST is STT3 and that vertebrates contain two isoforms STT3A and B. These assemble in different complexes, here called A-OST and B-OST. By using RNAi we have induced knockdowns of either STT3A, STT3B, both, or the essential subunit RbI in HeLa cells. Knockdown of both STT3s, or RbI result in defective glycosylation measured by *in vitro* OST activity, as well as glycosylation of endogenous glycoproteins. On the other hand, single knockdowns of STT3A or B do not induce major changes in steady state levels of glycoproteins. However, we could distinguish different functions for A and B-OSTs by short radio labeling of the protein Factor VII, known to contain two glycosylation sites modified one co- and the other posttranslationally. We suggests that A-OST functions as the major co-translational glycosylation OST in cells, whereas B-OST is the sole responsible for posttranslational glycosylation. In addition, in the absence of A-OST, B-OSTs are capable of co-translational glycosylation.

(26) **N-Glycosylation in Trypanosoma Brucei**

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Trypanosoma brucei, the parasite that causes Sleeping Sickness in humans, shows an aberrant N-glycosylation pathway. In T. brucei, while the insect-dwelling procyclic form makes and transfers Man9GlcNAc2-PP-Dol to the procyclins, the main surface glycoprotein in this life stage, the bloodstream form of the parasite transfers both Man9GlcNAc2-PP-Dol and Man5GlcNAc2-PP-Dol to the Variant Surface Glycoprotein (VSG). The generation of a null mutant of the gene encoding for the UDP-Glc:glycoprotein glucosyltransferase (UGGT), suggest that this protein appear to prefer the Man5GlcNAc2 precursor as its substrate. As in other organisms, UGGT was not essential for the growth of T. brucei *in vitro* or *in vitro*. As it has been reported before for Schizosaccharomyces pombe, UGGT is essential for T. brucei viability under ER stress conditions. However we did not detect the upregulation of Grp78/BiP or any other components of the unfolded protein response to allow the T. brucei ER processing machinery to cope with the lack of UGGT.

The analysis of another bloodstream form null mutant in T. brucei allowed us to determine that the lack of the ALG12 enzyme did not

compromise the ability of the parasite to grow in culture or in the mammalian host. However, by using the abundant VSG as a reporter to assess the status of the N-glycosylation sites we observed that the pattern of glycosylation of the protein was dramatically altered. Essentially, alg12 null mutant VSG presented a higher complexity in the N-glycans, perhaps to compensate for the lack of Man9GlcNAc2 N-glycans on its surface.

(28) **Sulf Regulation of Heparan Sulfate-Mediated Signaling during Development and Tissue Regeneration**

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Sulf1 and Sulf2 are novel heparan sulfatases that mediate the selective 6-0 desulfation of cell surface heparan sulftate proteoglycans to control the formation of heparan sulftate-mediated receptor-ligand complexes. Sulf1 and Sulf2 are differentially expressed by a diversity of embryonic and adult stem cell progenitor lineages and have dual regulatory functions as enhancers of HS-dependent Wnt and GDNF signaling and as repressors of HS-dependent FGF and EGF signaling. Mice with single Sulf1 and Sulf2 are fully viable and fertile, consistent with biochemical evidence of their redundant enzymatic functions. However, double-mutant mice have molecular, developmental, and regenerative defects. These include: 1) elevated levels 6-0 sulfation of heparan sulfate, consistent with the central role of Sulfs as regulators of 6-0 sulfation states of heparan sulfate; 2) developmental defects in esophageal muscle innervation, attributed to reduced GDNF signaling between innervating neurons and their target muscles; and 3) delayed skeletal muscle regeneration, attributed to hyperactive FGF signaling in activated satellite cells that promotes their protracted proliferation and delays their differentiation. These findings establish a key regulatory role for Sulf enzymes in heparan sulfate-mediated signaling in development and tissue regeneration.

(29) **The Sulfs – Dynamic Regulators of Cell Signaling and Development via Heparan Sulfate Editing**

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Heparan sulfate (HS) is a cell surface carbohydrate polymer modified with sulfate moieties whose highly ordered composition is central to directing specific cell signaling pathways underlying development and homeostasis. The discovery of two HS endosulfatases, Sulf1 and Sulf2, with the unique ability to edit these sulfation patterns, has opened up a new understanding of HS-dependent signal transduction regulation in space and time. To elucidate their *in vivo* function we generated Sulf1 and Sulf2 knock-out mice. Sulf2-/- but not Sulf1-/- mice showed increased embryonic lethality and non-penetrant malformations in particular of the brain. Analysis of these mice demonstrates that both Sulf1 and Sulf2 fulfill non-redundant functions in the development and maintenance of the murine nervous system. More specifically, the Sulfs are differentially involved in neuronal and behavioral plasticity. Sulf1−/−/2−/− double knock-out mice have a dramatic phenotype. They show a high degree of embryonic lethality and typically have a very short life span as well as an obvious reduction in body weight. Structural analysis of HS from knock-out fibroblasts had shown a striking increase in 6-O-sulfation in Sulf1−/− and, even more pronounced, in Sulf1−/−/2−/− double knock-out, but not in Sulf2−/− HS. These data imply that Sulf1 and Sulf2 are functionally co-operative. The mechanism underlying this co-operativity, which likely involves also the HS biosynthetic enzymes, and its tissue specificity are unknown. Likewise the biochemical and structural basis of endosulfatase activity, and of Sulf functioning at the cell surface and possibly also in the Golgi and/or the extracellular matrix requires thorough investigation.

(30) **Regulation of wnt Signaling, Cell Growth, and Tumorigenicity in Human Lung Cancer Cells by SULF2, an Extracellular Endosulfatase**

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Lung cancer is the leading cause of cancer death worldwide, and greater than 90% of lung cancers are cigarette smoke-related. An increased understanding of the molecular pathogenesis of lung cancer is required to develop more effective therapies.

Activation of Wnt signaling appears to play an important role in carcinogenesis in several cancers including lung cancer. Recently, we found that Wnt signaling is a critical mediator in cigarette smoke-induced malignant transformation of human bronchial epithelial cells. Heparan sulfate proteoglycans (HSPGs) are known as modulators of Wnt signaling, which regulate the availability of Wnt ligands to their signaling receptors. Two recently identified elements of Wnt signaling are the extracellular sulfatases, SULF1 and SULF2. These enzymes act as endosulfatases for HSPGs and mobilize Wnt ligands from HSPG sequestration and thereby can switch on Wnt signaling. In this report, we show that either SULF1 or SULF2 is expressed in 9 of the 17 human non small cell lung carcinomas (NSCLC) cell lines tested including smoke-transformed bronchial epithelial cells. We further show that exposure of cells expressing SULF2 to a catalytically inactive form of SULF2 or siRNA-mediated silencing of endogenous SULF2 inhibited both Wnt signaling and cell growth. Also, siRNA-mediated SULF2 silencing produced a marked increase in apoptosis and a decrease of anchorage- independent growth. Finally, SULF2 silencing in smoke-transformed bronchial epithelial cells and 2 lung cancer cell lines resulted in markedly reduced growth of tumors in nude mice.

(31) **TGF-β1 Induces Heparan Sulfate 6-O-Endosulfatase 1 Expression *in vitro* and *in vivo***

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Transforming growth factor (TGF)-β1 plays an important role in the development of pulmonary fibrosis. In this study we examined the relationship between TGF-β1 stimulation and the expression of heparan sulfate (HS) 6-O-endosulfatase 1 (Sulf1) in cultured normal human lung fibroblasts (NHLFs) and in murine lungs *in vivo*. By removing 6-O-sulfates from specific HS intra-chain sites on the cell surface, Sulf1 has been shown to modulate the activities of many HS binding growth factors and morphogens including fibroblast growth factor (FGF)-2. Real time RT-PCR analysis revealed that TGF-β1 increased Sulf1 expression in NHLFs in a dose- and time-dependant manner, which was accompanied by a decrease in 6-O-sulfated disaccharides as revealed by HPLC analysis. Decreased ERK activation following FGF-2 stimulation was observed in TGF-β1 treated NHLFs compared to control cells, which could be restored by exogenous heparin or siRNA against Sulf1, further confirming the 6-O-desulfation carried out by Sulf1. To study the function of Sulf1, negative control or Sulf1 specific siRNA transfected NHLFs were stimulated with TGF-β1. Interestingly, enhanced Smad2/3 phosphorylation and elevated total Smad2 protein level were observed in Sulf1 siRNA transfected cells, which were accompanied by enhanced expression of alpha-smooth muscle actin and fibronectin. In addition, Sulf1 siRNA transfection enhanced the anti-proliferative effect of TGF-β1. Finally Sulf1 expression was upregulated in the lungs of mice treated with adenovirus encoding active TGF-β1. Taken together,

our data indicate that Sulf1 is a TGF-β1 responsive gene both *in vitro* and *in vivo*, and may function as a negative regulator of TGF-β1-induced fibrogenesis.

(32) The Expression of Sulf-1, a Heparan Sulfate Glucosamine 6-O-Endosulfatase, in Wounded Mouse Cornea

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The behavior of cells during wound healing is influenced by their responses to growth factors. Heparan sulfate proteoglycans (HSPGs) act as modulators of growth factor signaling. Glucosamine 6-O-endosulfatases (Sulf-1 and Sulf-2) remove 6-O sulfate groups from trisulfated disaccharides present on heparan sulfate chains. We hypothesized that the structural modifications of HSPG chains provided by these enzymes might regulate epithelial re-growth during wound healing. We examined the expression and functional activity of Sulfs in a wound using a mouse corneal scratch model, and investigated the role of Sulfs in repair using an *in vitro* scratch-wound-closure assay. Linear scratches were applied to one cornea of male BALB/C mice, while the contralateral cornea served as control. By immunohistochemistry, Sulf-1 expression was restricted to the limbal epithelium in the unperturbed cornea. In contrast, Sulf-1 expression was detected in the corneal basal epithelium between 8 and 72 hrs post-scratch. Sulf-1 expression inversely correlated with the presence of the trisulfated disaccharide epitope. Monolayers of mock or Sulf-2 silenced human corneal epithelial cell (HCEC) line were wounded by a linear scratch and observed immediately and at 8, 16 and 24 hrs post-scratch. Gap closure was delayed in Sulf-2 deficient cells at all time points measured as compared to the controls. Our findings provide evidence that Sulf-1 may have critical functions during corneal repair.

(33) Systems Biology and Computational Modeling: Why Glycobiologists Should Rejoice

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There are nearly as many definitions for Systems Biology as proponents of this (seemingly) new approach to biological and biomedical research. Often identified with the gathering and mining of the ever-more-massive datasets bequeathed to us by molecular biology (genomes, proteomes, interactomes, phenomes, etc.), what really distinguishes Systems Biology is not the datasets, but the changes they provoke in how we think about biology. To the systems biologist, for example, the importance of system components (e.g. genes, proteins) is judged by their ability to contribute to performance objectives (tasks that a system has been selected, by evolution, to carry out), and not the severity of the phenotypes that result when they are deleted. This is one of several breaks with classical molecular biology that allow systems biologists to emphasize strategies for control—e.g. robustness, adaptability, noise-suppression—over mere mechanisms of action. A particularly exciting development is the use of mathematical and computational modeling in non-traditional ways to identify control relationships. I will illustrate this point through investigations of models of morphogen-mediated developmental patterning that suggest that heparan sulfate proteoglycan co-receptors are utilized because of their ability to overcome certain robustness tradeoffs. This conclusion offers a deeper understanding of what co-receptors do than the standard view that they are simply "modulators". Given how much of the glycome also seems "merely modulatory" when probed using the traditional tools of molecular biology, glycobiologists should rejoice at the opportunities offered by systems biology to uncover control strategies in which glycoconjugates take center stage.

(34) A *Virtual* Glycan Array (VGA) for High Throughput Receptor Screening

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Small molecule virtual libraries are frequently employed in computational pharmacophore screening and lead generation. Here we present the first development of a Virtual Glycan Array (VGA) and screening protocol for 3D carbohydrate structures that are equivalent to those in the glycan array provided by the Consortium for Functional Glycomics.

Although experimentally-observed specificities may correlate with distinct glycan sequences, array technology alone does not provide insight into the 3D character of the interaction or of the ligand. In contrast to an experimental array, in principle the VGA enables: the identification not only of ligands that bind, but also putative structures of the complexes; precise control of the 3D glycan presentation relative to the surface to which it is anchored; and the ability to include any glycans of interest or their derivatives. The VGA may include glycans that are not readily amenable to current experimental array techniques, or those that are difficult to produce or isolate, such as glycolipids, complex bacterial surface glycans, and GAGs.

While many anecdotal success stories have been reported for predicting the structure of carbohydrate-protein complexes, these studies have generally been limited to a single glycan-protein interaction and have rarely included either a large range of ligands or negative controls. By working with the publicly available screening data from the CFG glycan array, we will be able to validate and advance the docking strategies for carbohydrates and proteins.

Preliminary results are presented for several representative receptors including antibody fragments, hemagglutinins, and galectins.

(35) Binding Studies of GalNAc Specific Lectins to the Tn-Antigenic Form of Porcine Submaxillary Mucin and its Fragments

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Isothermal titration microcalorimetry (ITC) and hemagglutination inhibition measurements demonstrate that a chemically and enzymatically prepared form of porcine submaxillary mucin that possesses a molecular mass of ~106 daltons and ~2300 α-GalNAc residues (Tn-PSM) binds to the soybean agglutinin (SBA) with a Kd of 0.2 nM which is ~106-fold enhanced affinity relative to GalNAcα1-O-Ser (Tn), the pancarcinoma carbohydrate antigen. The enzymatically derived 81 amino acid tandem repeat domain of Tn-PSM containing ~23 α-GalNAc residues binds with ~103-fold enhanced affinity, while the enzymatically derived 38/40 amino acid cleavage product(s) of Tn-PSM containing ~11-12 α-GalNAc residues shows ~102-fold enhanced affinity. A natural carbohydrate decorated form of PSM (Fd-PSM) shows ~104 enhanced affinity for SBA. Vatairea macrocarpa lectin (VML), which is also a GalNAc binding lectin, displays a similar pattern of binding to the four forms of PSM, although there are quantitative differences in its affinities as compared to SBA. The higher affinities of SBA and VML for Tn-PSM relative to Fd-PSM indicates the importance of carbohydrate composition and epitope density of mucins on their affinities for lectins. The higher affinities of SBA and VML for Tn-PSM relative to its two shorter

chain analogs demonstrate that the length of a mucin polypeptide and hence total carbohydrate valence determines the affinities of the three Tn-PSM analogs. The results suggest a binding model in which lectin molecules "bind and jump" from α-GalNAc residue to α-GalNAc residue along the polypeptide chain of Tn-PSM before dissociating. The results have important implications for the biological activities of mucins.

(36) **Unprecedented *in vivo* Efficacy of Pre-Ordered Polymeric Heterobifunctional Ligands**

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A novel approach for inhibition of multivalent receptors utilizes the supramolecular effect. A low molecular weight, hetero-bifunctional compound mediates high avidity, supramolecular assembly between the target receptor and an endogenous multivalent protein "TRAP" [1]. Incorporation of pre-ordered ligands into a polymeric format significantly augments avidity gains while addressing the issue of circulation half life and clearance.

Our approach is exemplified by the design and activity of Shiga toxin (Stx) inhibitors that mediate specific aggregation of Stx with serum amyloid P component (SAP). Structural information for both proteins as complexes with their ligands was used to design compact hetero-bifunctional ligands, containing the P^k trisaccharide fragment for recognition by Stx and a cyclic pyruvate for binding to SAP. The inhibitory activity of the ligands was strongly dependent on SAP concentration and rivals that of the decameric STARFISH inhibitor (10^6 times P^k trisaccharide activity).

In the polymeric format biological activity was ~10^9 fold higher than P^k trisaccharide. Furthermore, *in vivo* protection of mice against a lethal dose of Stx1 was achieved with less than 200 μg/mouse. The concept embodied in this approach is general and may be applied to soluble multivalent receptors or cell surface receptors [2].

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(37) **Chemical Glycobiology and Vaccine Development**

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The over-expression of saccharides such as Globo-H, LewisY and Tn antigen is a common feature of oncogenic transformed cells. Endeavors to exploit this aberrant glycosylation for cancer vaccine development has been complicated by difficulties of eliciting high titers of IgG antibodies against classical conjugates of tumor-associated carbohydrates to carrier proteins. We have designed, chemical synthesized and immunologically evaluated a number of fully synthetic vaccine candidates to establish strategies to overcome the poor immunogenicity of tumor-associated carbohydrates and glycopeptides. We have found that a three-component vaccine composed of a Toll Like receptor 2 (TLR2) agonist, a promiscuous peptide T-helper epitope and a tumor-associated glycopeptide, can elicit in mice exceptionally high titers of IgG antibodies that can recognize and kill cancer cells expressing the tumor-associated carbohydrate. The superior properties of the vaccine candidate are attributed to the local production of cytokines, upregulation of co-stimulatory proteins, enhanced uptake by macrophages and dendritic cells and avoidance of epitope suppression. The methodology has been extended to the generation of monoclonal antibodies against other types of self-antigens such as a beta-GlcNAc modified glycopeptide derived from CKII.

(38) **IDAWG: A Novel Quantitative Method for Glycomics**

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One of the major challenges in the –omics field is the development of technologies that allow for quantitative analysis between samples. In proteomics, stable isotope approaches, such as SILAC, have been developed to address this need. Here we report a methodology that takes advantage of stable isotope labeling of glycans in cell culture for performing relative quantitative glycomics. This methodology termed IDAWG, isotopic detection of aminosugars with glutamine, relies on the hexosamine biosynthetic pathway that uses the side-chain of glutamine as its sole donor source of nitrogen for aminosugars in the production of sugar nucleotides. Thus, introduction of heavy glutamine (^{15}N) into Gln-free media allows for all aminosugars to become labeled and shifted in mass by +1 dalton. Here we demonstrate that this methodology allows for rapid and nearly complete incorporation of ^{15}N into GlcNAc, GalNAc, and sialic acids of N-linked and O-linked glycans in various mammalian cell culture systems. Besides aiding in the assignment of structures via LC-MS^n approaches, this method allows us to determine whether the glycans isolated from a sample result from cellular processes or serum glycoproteins. Importantly, this method also allows us to compare in a quantitative manner the glycans between two cell populations. Furthermore, half-life studies can be performed on glycan structures by switching a cell population from heavy to light labeling conditions and harvesting and analyzing the glycans by LC-MS^n approaches at multiple time points afterwards. Thus, the IDAWG approach is an easily applied and powerful new tool in the glycomics toolbox.

(39) **Glycan Reductive Isotope Labels (GRIL) for Precision Glycomics**

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Changes in glycan structures are associated with many human diseases. Modern approaches to analyzing qualitative and quantitative changes in glycans rely largely on comparative analytical techniques including HPLC- and mass spectrometry (MS)-based methods. Here we describe a novel method in which glycan samples are labeled by reductive amination with either [12C6]aniline or [13C6]aniline and then mixed together for MS analysis. We refer to these labels as Glycan Reductive Isotope Labels or GRIL. Identical glycans in the mixture appear as doublets differing by 6 mass units; the absence of such doublets indicates the absence of a corresponding structure. The hydrophobic and absorptive properties of aniline permit isolation of derivatives on reversed phase resins and a method of quantifying the labeled glycans. We analyzed N-glycans released from human and mouse serum glycoproteins. The results show that N-glycans released from total glycoproteins in individual human serum samples do not differ significantly among the healthy donors we examined. Unexpectedly, there was little similarity between human and mouse serum-derived N-glycans. We also used GRIL to compare mucin O-glycans from normal and Cystic Fibrosis patients, and readily observed specific and quantitative differences between the samples. The use of GRIL provides qualitative and

quantitative measurements of glycan mixtures from different samples, and the ability to assess quantitative and qualitative changes in glycan structures accompanying cellular differentiation, animal development, and disease-associated differences in glycan expression. [This work was supported in part by NIH Grant HL065509 to GPS and resources from the Glycomics Center at Emory University.]

(40) Sequence Requirements for NCAM Polysialylation

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Polysialic acid (PSA) is a developmentally regulated, anti-adhesive glycan that is found predominantly on the neural cell adhesion molecule, NCAM. The PSA negatively modulates cell adhesion and is critical for a variety of processes including brain development, learning and memory, and cancer cell invasiveness. Because NCAM is one of only a few mammalian polysialylated proteins, we hypothesize that polysialylation is a protein-specific modification that requires an initial protein-protein interaction between enzyme and substrate. We have demonstrated that the first fibronectin type III repeat (FN1) of NCAM is required for the polysialylation of the N-glycans on the adjacent Ig5. The crystal structure of NCAM FN1 revealed that it possesses a unique acidic surface patch and a novel α helix that links strands 4 and 5 of its β sandwich structure. Replacement of the acidic patch reduces or eliminates polysialylation, while replacement of the α helix shifts the addition of PSA from Ig5 N-glycans to FN1 O-glycans. This suggests that the acidic patch plays a role in polysialyltransferase recognition, while the α helix is critical for positioning the Ig5 N-glycans for polysialylation. We can restore polysialylation to an unpolysialylated NCAM-OCAM chimera by inserting specific NCAM FN1 amino acids into the OCAM FN1 sequence. Different combinations of β 1- β 2 strand sequences (acid patch, α helix, and $P_{501}YS/Q_{506}VQ$) lead to varying levels of polysialylation and ratios of N- and O-linked polysialic acid, suggesting the presence of multiple, overlapping enzyme recognition sites. We are currently refining the requirements for polysialyltransferase-NCAM recognition and interaction.

(41) The Diverse Location and Composition of the N-Glycomes (Glycoproteins with N-Glycans) of Parasitic Protists

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These experiments follow on the observation that protists that cause dysentery (Entamoeba) and vaginitis (Trichomonas) build their N-glycans from a truncated 7-sugar precursor (Man5GlcNAc2), while protists that cause diarrhea (Cryptosporidium and Giardia) build their N-glycans from GlcMan5GlcNAc2 and GlcNAc2, respectively. We show here that for the most part, these protist N-glycans are not further modified, so that Man5GlcNAc2 of Entamoeba, Trichomonas, and Cryptosporidium is recognized by the anti-retroviral lectin cyanovirin-N, and glycoproteins containing Man5GlcNAc2 (the so-called N-glycome) are rapidly purified using the lectin Concanavalin A. While cyanovirin-N binds extensively to the vacuolar membranes of Entamoeba and caps its surface, cyanovirin-N binding is restricted to a discrete set of vesicles in both Trichomonas and Cryp-tosporidium. In Cryptosporidium, there is a non-overlapping set of vesicles, which contain O-linked glycans that bind to the GalNAc-binding lectin MPA. The N-glycome of Entamoeba is dominated by ER proteins (e.g. calreticulin and PDI), lysosomal enzymes (e.g. cysteine proteinases), and plasma membrane proteins (e.g. GalNAc lectins that are involved in pathogensis and are vaccine candidates). In contrast, the N-glycome of Trichomonas is dominated by unique proteins, which have previously been designated as "hypothetical proteins." The Giardia N-glycome, which is localized with wheat germ agglutinin to perinuclear and peripheral vesicles, is composed of ER proteins, lysosomal enzymes, and unique plasma membrane proteins that contain Cys-, Leu, or Gly-rich repeats. These experiments suggest that the compartmentalization of the N-glycome and the glycoproteins that compose the N-glycome vary dramatically among the protists studied.

(42) LNFPIII Neo-Glycoconjugates Activate Antigen Presenting Cells *in vivo* and *in vitro* and Have Immunomodulatory Activity *in vivo*

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Helminth parasites drive Th2-type and anti-inflammatory responses. Monoclonal antibodies to Schistosoma mansoni egg antigens detected LNFPIII/Lewis X expression by this parasite. LNFPIII neo-glycoconjugates were administered to mice and shown to be immunomodulatory *in vivo*. *In vivo* injection of LNFPIII conjugates rapidly expands a population of immunomodulatory macrophages. Additionally, stimulation of immature dendritic cells with LNFPIII conjugates drives their maturation towards a DC2 phenotype. Adoptive transfer of antigen pulsed LNFPIII activated DCs or macrophages to naïve mice confers a Th2-type and anti-inflammatory CD4+ Th response in recipients characterized by IL-10 and IL-13. LNFPIII may activate APCs via one or more C-type lectin, specifically MMR, MGL-1 and MSIGN. Using mice deficient in single C-type lectins we have shown that mice deficient in MGL-1 respond to LNFPIII identical to wild-type mice. We are attempting to define the C-type lectin receptors and the activation mechanisms by which LNFPIII activates antigen presenting cells *in vivo* and *in vitro*, inducing an anti-inflammatory phenotype. The ability of LNFPIII conjugates to function as an immunodulatory agent *in vivo* for treatment of pro-inflammatory autoimmune diseases was tested in murine disease models. To date we have shown that treatment with LNFPIII conjugates prevents development of psoriasis, diabetes and colitis in the majority of animals treated. LNFPIII conjugates also function to reduce clinical disease in the EAE mouse model. Thus LNFPIII conjugates may be useful for treatment of autoimmune diseases. We believe that LNFPIII/Lewis X expression on cells is part of an endogenous immunoregulatory system.

(43) Mice Lacking Ganglioside GM3 Synthase Exhibit Complete Hearing Loss Due to Selective Degeneration of the Organ of Corti

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During observations of the general behavior of mice lacking the ganglioside GM3 synthase gene (SAT-I -/- mice), we found that the mice exhibited no acoustic reflex, indicating a loss of hearing. Electrophysiological studies, including auditory brain stem response and electrocochleography, revealed that SAT-I -/- mice, but not their SAT-I +/- and SAT-I +/+ littermates, exhibit severe defects in the cochlear function of the inner ear. Histological examination indicated the selective absence and degeneration of the organ of Corti in SAT-I -/- mice. The major gangliosides of the mouse inner ear are GM3, GM1, GD1a, GD1b, and GT1b. In SAT-

I -/- mice, these a- and b-series gangliosides were completely absent, and the 0-series gangliosides GM1b and GD1alpha were generated due to the lack of GM3 synthesis. Thus, our finding strongly suggests that GM3 synthase plays a pivotal role in the auditory system.

(44) **Plasma Concentrations of Glycoproteins Terminating with Siaα2,6Gal Are Regulated by the Asialoglycoprotein Receptor**

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The asialoglycoprotein receptor (ASGP-R) is an abundant hepatic receptor with few known endogenous ligands and an unclear function. The ASGP-R rapidly clears glycoproteins bearing terminal Gal or GalNAc from the circulation; however, endogenous glycoproteins with terminal Gal/GalNAc have not been identified in wild-type or ASGP-R-/- mice. We reported that glycoproteins bearing N-linked structures that terminate with Siaα2,6GalNAc are recognized by the ASGP-R and rapidly cleared from circulation. The small number of glycoprotein hormones selectively modified with this structure may represent the first examples of endogenous ligands for the ASGP-R. The ASGP-R also binds glycoproteins bearing terminal Siaα2,6Gal, suggesting they may represent a major group of endogenous ligands. Using two-dimensional difference gel electrophoresis to compare the levels of plasma glycoproteins in wild-type and ASGP-R-/- mice we have identified a number of glycoproteins that are elevated in ASGP-R-/- mice. The elevated glycoproteins are reactive with SNA-I, a lectin specific for Siaα2,6Gal/GalNAc, supporting a role for the ASGP-R in their clearance. We have confirmed that two of these glycoproteins, haptoglobin and SAP, are 2-5 fold elevated in ASGP-R-/- mice by Western blot analysis using specific antibodies. Haptoglobin from wild-type and ASGP-R-/- mice is quantitatively bound by SNA-I, indicating that the accumulation of haptoglobin and other glycoproteins in ASGP-R-/- is due to a reduced rate of clearance and not to a change in their carbohydrate structure. We propose that the ASGP-R plays an important role in regulating the concentration of plasma glycoproteins that bear terminal Siaα2,6Gal.

(45) **Carcinoembryonic Antigen is an E- and L-, but not P-, Selectin Ligand on Colon Carcinoma Cells**

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Selectin-mediated adhesion of tumor cells to platelets, leukocytes, and vascular endothelium may regulate their hematogenous spread in the microvasculature, enhancing tumor metastasis. Variant glycoforms of CD44 expressed on tumor cells (CD44v) support P-, E- and L-selectin-mediated tumor cell adhesion. siRNA knockdown revealed that CD44v was the major functional ligand for P-selectin on LS174 colon carcinoma cells. However, when CD44v was absent, alternate ligands mediated E- and L-selectin binding. To identify these alternate ligands, sialofucosylated glycoproteins in CD44-knockdown cell lysates were purified by HECA-452 immunoaffinity chromatography and identified by mass spectrometry. We found that GPI-linked carcinoembryonic antigen (CEA) on LS174T cells mediates high affinity E- and L-, but not P-selectin binding. Selectin-binding determinants on CEA from LS174T cells are sialofucosylated O-linked glycans, akin to those on PSGL-1 and CD44v. CEA expressed by CD44-knockdown cells is more densely decorated with sialofucosylated (HECA-452-reactive) epitopes than CEA expressed by control cells. We characterized shear-dependent binding of E- and L-selectin to CEA derived from control and CD44-knockdown LS174T cells to reveal how the extent of sialofucosylation regulates selectin-ligand affinity. Moreover, PIPLC-treated CD44-knockdown LS174T cells rolled faster over E-, but not L- or P-selectin compared to untreated cells, implicating GPI-linked molecules in E-selectin adhesion by carcinoma cells. The novel finding that CEA is a selectin ligand may explain the enhanced metastatic potential associated with tumor cell CEA overexpression and supports a critical role of selectins in metastasis. Supported by National Institutes of Health Grant RO1 CA 101135 and the National Science Foundation Graduate Research Fellowship.

(46) **Role of the Sulfotransferase, GlcNAc6ST-2, in a Mouse Model of Rheumatoid Arthritis**

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Two high endothelial venues (HEV)-expressed sulfotransferases, GlcNAc6ST-1 (ST-1) and GlcNAc6ST-2 (ST-2), are L-selectin ligand-forming enzymes involved in normal lymphocyte homing. They contribute to the L-selectin ligand activity and the expression of the epitope for MECA-79, a monoclonal antibody that stains HEV. The pathophysiological significance of L-selectin ligands expressed in rheumatoid arthritis (RA) joints has not been defined. To elucidate a role of the two enzymes in lymphocyte recruitment to the RA synovial membrane, we employed a collagen-induced arthritis (CIA) model in DBA/1J mice. Chicken type II collagen (CII) was injected intradermally. At several time points, ankles were processed for immunohistochemistry or digested and analyzed for leukocyte populations by FACS. Quantitative PCR was also conducted with RNA obtained from the joints. In addition, we compared wild-type (WT) and ST-2 null mice (ST-2 -/-) on DBA/1J background for their clinical score and CII-specific responses of B-cell and T-cell. We found a modest number of MECA-79+ vessels and somewhat more ST+ vessels. FACS analysis revealed that the main leukocyte populations were CD3+ T cells and F4/80+ macrophages. ST-2 mRNA was induced prominently in the joints. Compared with WT mice, ST-2 -/- mice showed a significant decrease in clinical score. The joints of ST-2 -/- mice lacked MECA-79+ vessels and had fewer T cells and macrophages. There were no defects in CII specific B cell and T cell responses. These results suggest that ST-2 may contribute to the development of inflammation and disease in this CIA model.

(48) **Heparin and Heparan Sulfate on Proteins of the Hedgehog Signaling Pathway**

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Heparan sulfate (HS) proteoglycans (PGs) interact with a number of extracellular signaling proteins thereby playing an essential role in the regulation of many physiological processes. These interactions are important for both normal signal transduction as well as regulation of the tissue distribution of signaling molecules. In the present study, we use surface plasmon resonance (SPR) to study interactions of HS and structurally related heparin with proteins in Hedgehog signaling pathway. SPR analysis shows that heparin binds with different affinity to active fragments of the proteins Hedgehog (Hh), Interference Hedgehog (Ihog), Cam-related/Down-regulated by Oncogenes (CDO) and Sonic Hedgehog (Shh). Solution competition studies show that the minimum size of a heparin oligosaccharide capable of interacting with Ihog is larger than a tetrasaccharide, and for interacting with Shh is larger than an octasaccharide. In comparison with heparin, Ihog and Shh showed lower affinity to HS than to heparin, and CDO and Hh show negligible binding to HS. The current study clearly demonstrates Shh and Ihog are heparin/HS binding proteins and that both molecules preferentially bind heparin or HS having a high level of sulfation.

(50) *N*-Glycolyl GM1 Ganglioside as a Host Cell Receptor for Simian Virus 40 (SV40)

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Simian virus 40 (SV40) is an extensively studied oncogenic virus. Its major protein VP1 assembles into virus-like particles (VLPs) that retain many properties of the infectious virus including endocytosis by host cells. We have used microarrays with 190 sequence-defined oligosaccharides[1] in the form of neoglycolipids and natural glycolipids representative of diverse mammalian glycans to examine interactions of SV40-VLPs with potential carbohydrate receptors. While corroborating the specificity of SV40 for the ganglioside GM1[2], our results revealed the *N*-glycolyl-GM1, characteristic of simians and other non-human mammals, to be a better ligand than the *N*-acetyl-GM1 found in mammals including humans. When added to glycolipid-deficient GM95 cells, both the *N*-glycolyl-GM1-ganglioside and the neoglycolipid constructed from its pentasaccharide moiety, supported binding and infection better than the respective *N*-acetyl-analogs, indicating that superior receptor activity is conferred by the NeuGc moiety. By molecular modeling we identified a conformation of *N*-glycolyl-GM1 ganglioside in complex with the virus VP1-pentamer compatible with its presentation as membrane receptor. Our results have relevance to tropisms of SV40 in simians and other mammals including humans. The high infection rate in simians may reflect the prevalence[3] of *N*-glycolyl-GM1. Paucity of *N*-glycolyl-GM1 in humans may be an example of evolutionary genetics whereby humans evolved and became relatively resistant to infection. (Supported by MRC and U.K.Research Councils' Basic Technology Grant 'Glycoarrays').

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(51) *In silico* Prediction of Carbohydrate Binding Sites: Prospects and Current Limitations

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Docking of carbohydrates to proteins using computational tools is a powerful approach for predicting and understanding the molecular mechanism of carbohydrate recognition and specificity. Prediction of protein-carbohydrate complex structures depends on two aspects: efficient search strategies to explore the conformational space in order to find structures that represent (global) energy minima, and an accurate empirical free energy or scoring function to determine the relative energies of the docked solutions. We present different strategies to explore conformational space that are particularly useful for detection of potential carbohydrate binding sites.

The search problem in docking of carbohydrates can be separated into different categories that all have their special demands with respect to the docking strategy. For example, if the binding site is unknown the complete protein surface has to be searched for potential binding sites ('blind docking'). If one is interested in multivalent interactions of carbohydrates and the location of the binding site of a fragment (e.g. a monosaccharide) is known from a crystal structure, a different strategy needs to be employed ('knowledge based docking'). Especially challenging is to include protein flexibility into the docking protocol. This might be necessary if the coordinates of the receptor structure represent an apo protein structure or have been derived from homology modelling [1]. The application of the different docking strategies will be presented and their limitations will be discussed.

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(52) Structural Characterisation of Glycosaminoglycans: First Steps of a Glycomics Strategy

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Protein-glycosaminoglycans (GAGs) interactions are amongst the most complicated protein-carbohydrate interactions to elucidate partly because of the structural complexity of the glycan partner and the difficulty to analyze small amounts of highly sulfated sugars.

We are developing a mass spectrometry glycomics strategy to profile heparin oligosaccharides. We recently published the first data obtained on di-, tetra-, hexa- and decasaccharides carrying from two to thirteen sulfate groups (Tissot et al., Glycobiology, 2007). MALDI-TOF/TOF MS/MS experiments using norharmane as matrix allowed the characterization of the glycosidic backbone and gave information about the presence and the position of N-acetylated residues in the sequence. Using the ionic liquid 1-methylimidazolium alpha-cyano-4-hydoxycinnamate we obtained information on the maximum number of sulfate groups present on the backbone. We are now testing and validating this strategy on a larger amount of heparin and heparan sulfate fractions ranging from dp4 to dp20.

A major part of this new approach relies on the use of bioinformatics tools to assist the complicated data interpretation. Our group is developing software which now enables the link between data obtained in the norharmane (length of skeleton, number of NAc groups) and in the ionic liquid matrix (number of sulfate groups) and ranks putative GAG chains structures according to their probability of matching these two sets of data.

(53) SECRET AGENT and SPY Inform about the Role of Protein O-GlcNAc Modification in Plant Processes

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Genetic studies with *Arabidopsis thaliana* indicate that posttranslation modification of nuclear and cytoplasmic proteins with O-linked N-acetylglucosamine (O-GlcNAc) is involved in gibberellin, cytokinin, light and circadian regulation. This modification is required for embryo development and thus is likely to be involved in additional response pathways. While the two O-GlcNAc transferases, SECRET AGENT and SPINDLY, responsible for this modification have been identified, lack of information about the modified proteins is a major constraint to understanding the role of the modification. We are using two approaches to identify the O-GlcNAc-modified proteins of arabidopsis. An *E. coli*-based co-expression system was used to identify SECRET AGENT substrates and map the modifications. Transcription factors known to participate in gibberellin and light signaling were among the substrates identified. We have also initiated the use of mass spectrometry-based approaches to identify and map modifications on arabidopsis proteins. Experiments assessing the role of this modification by characterizing the

functionality of non-modifiable mutant proteins in transgenic plants are in progress. Since SPINDLY and SECRET AGENT have both overlapping and unique functions, chimeric proteins have been created and are being used to map the regions responsible for the unique functions of these proteins.

(54) **Breaking the O-GlcNAc Code: O-GlcNAc and Cellular Signaling**

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The Hexosamine Signaling Pathway leads to the reversible addition of O-GlcNAc on target proteins and serves as a key cellular response to nutrient excess. The large number of O-GlcNAc modified proteins includes transcription factors, nuclear pores, proteasomal subunits and signaling kinases. Disregulation of this pathway has been linked to type II diabetes and neurodegeneration. To better understand the Hexosamine Signaling Pathway, we have focused on the enzymes of O-GlcNAc cycling in a genetically amenable organism Caenorhabditis elegans. We studied null alleles of OGT and the O-GlcNAcase (OGA) in C. elegans. These knockouts exhibited altered O-GlcNAc cycling, metabolism and dauer formation. The impact of the knockouts on transcription was examined by expression microarrays. We detected dramatic changes in genes involved in chromatin remodeling, organelle trafficking and nutrient sensing. ChIP-on-Chip tiling arrays were used to create a genome-wide map of the O-GlcNAc sites on chromatin. O-GlcNAc was restricted to the promoter regions, suggesting a direct link to transcriptional control by O-GlcNAc signaling. Thus, O-GlcNAc cycling may ''fine-tune'' insulin-like signaling and other nutrient responsive pathways in response to nutrient flux. The mutant C. elegans strains provide a unique genetic model for examining the role of O-GlcNAc in cellular signaling, insulin resistance, obesity and neurodegeneration.

(55) **Significance of Cytoplasmic Glycosylation for O2-Signaling in Dictyostelium and other Protists**

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Cells continuously sense changes in O2 and modify their metabolism and behavior accordingly. An important O2-signaling pathway in animals involves prolyl 4-hydroxylases, the transcriptional factor subunit HIFalpha (target of prolyl hydroxylation), and E3(VHL)Ub-ligase (which recognizes hydroxyproline). The model organism Dictyostelium also has a cytoplasmic/nuclear prolyl 4-hydroxylase (P4H1) required for the O-glycosylation of Skp1, a subunit of E3(SCF)Ub-ligases related to the VHL class. Sporulation is selectively dependent on O2 in Dictyostelium. P4H1 appears to regulate culmination, because genetic deletion raises the O2-requirement from >10% to ~21%, whereas overexpression of P4H1 decreases the O2-requirement to <5%. Skp1, the only detected hydroxylation target, is encoded by 2 genes and genetic modification affects the O2 threshold, suggesting that Skp1 is the functional target of P4H1. Skp1 hydroxyproline is sequentially modified by 3 glycosyltransferases yielding a novel pentasaccharide. Although their glycosyltransferase domains were founding members of new glycosyltransferase families in the CAZy database, they are evolutionarily related to known Golgi glycosyltransferases except for the absence of secretory pathway targeting signals. The function of the first sugar, added by an alphaGlcNAc-transferase homologous to mucin-type alphaGalNAc-transferases, is not known but appears to be essential. The final four sugars are dispensible for O2-regulation, suggesting a function under O2-replete conditions. Their addition is catalyzed by novel diglycosyltransferases, one with two domains and the second with one domain linked to a beta-propeller-like domain involved in conditional alpha-galactoslylation of Skp1. Bioinformatic and biochemical evidence suggests that homologous cytoplasmic hydroxylation/glycosylation is conserved in the agent for human toxoplasmosis, Toxoplasma gondii.

(56) **A Novel and Sensitive Method for Absolute Quantification of Protein GlcNAcylation**

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O-GlcNAc is an emerging and important posttranslational modification that modulates signaling, protein expression, activity, trafficking and degradation. Recently, many tools to facilitate studying O-GlcNAc have become available, including an O-GlcNAc-specific antibody, potent O-GlcNAcase inhibitors, and mass spectrometry-based techniques for site mapping. However the lack of a fast and precise method for quantifying GlcNAcylation levels is still a major problem in this field. As a result, we have developed an ultra-sensitive method for quantifying GlcNAcylation in purified proteins, cell culture extracts or tissues. GlcNAc residues released from glycoproteins after b-elimination or O-GlcNAcase treatment were oxidized with N-acylhexosamine oxidase at the C1 position, and the resultant hydrogen peroxide was determined by reaction with horseradish peroxidase and a fluorogenic substrate. Importantly, this technique can be used to precisely determine stoichiometric measurements of GlcNAc on purified proteins. In addition, this approach may also be useful for quantifying glycans containing reducing end GlcNAc or GalNAc in modified surface molecules. This method can detect free GlcNAc in low picomole quantities, and is also useful for measuring O-GlcNAcase activity toward glycopeptides or glycoproteins. Furthermore, because b-elimination also releases O-phosphate from proteins, we are able to compare the levels of phosphorylation and GlcNAcylation in the same sample. This work is supported by NIH grants CA42486 and HD13563. G.W. H. receives a share of royalty received by the university on sales of the CTD 110.6 antibody. Terms of this arrangement are managed by JHU.

(57) **A Case for Functional O-GlcNAc Modification of IRS Proteins**

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The O-GlcNAc modification, a ubiquitous and dynamic intracellular glycosylation on the serine and threonine residues of polypeptides, serves as a negative regulator of the insulin-mediated PI3K/Akt signaling cascade with a concomitant defect at or upstream of Akt activation in mammalian cell culture models. This defect results in insulin resistance, which precedes and is the hallmark of type II diabetes, in adipocytes. While activation of the PI3K/Akt pathway is a universal master switch of various receptor tyrosine kinase (RTK)-mediated signaling pathways, the impact of elevated O-GlcNAc modification on RTK pathways other than the insulin pathway has yet to be elucidated. In this study, we examined the activation status of the PI3K/Akt pathway via two other predominant RTK pathways mediated by epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) in 3T3-L1 adipocytes in response to global elevation of O-GlcNAc levels. Our results showed that the inhibition of Akt activation via elevated O-GlcNAc levels is exclusively an insulin signaling-specific phenomenon. Given that increasing O-GlcNAc levels does not affect the autophosphorylation capability of the insulin receptor, our results strongly suggest that the IRS proteins, which are O-GlcNAc modified, are the target for functional O-GlcNAc modification and fine-tuning of the insulin signal transduction pathway. Current studies are aimed at confirming these results using genetic means to alter O-GlcNAc levels in relevant cell lines

for the aforementioned and other RTK pathways. As well, mapping and mutagenesis of O-GlcNAc sites on IRS proteins for functional studies is underway.

(58) The Role of O-GlcNAcylation in Metastasis

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Proliferation invasion and metastasis are the most effective and fatal phenotypic characteristics of cancerous cells. Many works have shown that disruption of cell signaling and transcription factors regulating the mitotic process of cell cycle are involved in cancer development and metastasis. Phosphorylation, the addition of phosphate group to proteins, is one of the most investigated protein modification. Similar to protein phosphorylation is protein modification by N-acetyl-glucosamine (GlcNAc). O-GlcNAc is attached to the hydroxyl group of serine or threonine residues with the O-beta-linkage. Dysregulation of proteins O-GlcNAcylation contributes to the etiology of important human diseases and aberrant patterns of O-GlcNAcylation on transcription factors are documented in several diseases, such as diabetes, cancer, neurodegenerative diseases etc. Therefore we hypothesize that metastasis could be reflected by dysregulated O-GlcNAcylation of nuclear and cytoplasmic proteins. Our model system includes metastatic and non-metastatic clone variants of two different cancer cell lines: human colon adenocarcinoma and murine Lewis lung carcinoma. Using specific anti-O-GlcNAc antibody, western blot analysis of total cell extracts and enriched nuclear and cytoplasmic compartments shows differences in the level of O-GlcNAcylation of several proteins reflecting the metastatic state. These observations support our assumption that O-GlcNAcylation could indicate the metastatic state of tumors and thus may play a substantial role in the transformation of tumor cells toward metastasis. Identification of specific factors which are modified differently by O-GlcNAcylation in response of the metastatic state will enable the elucidation of mechanisms that initiate cancer metastasis.

(59) Glycoprotein Oligosaccharide Structural Characterization using Solid-Phase Permethylation Approach and Arabinosazone as MALDI TOF MS Matrix

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A novel permethylation method using a solid-phase approach and arabinosazone (ARA) as matrix for glycan profiling using MALDI/TOF MS was developed.

N-linked glycans with or without sialic acids were enzymatically cleaved from several glycoproteins using PNGase F. The released glycans were purified using Microcon filters and Glycoclean cartridges. The purified N-glycans were permethylated via iodomethane in DMSO using a solid-phase derivatization approach involving a microspin column packed with sodium hydroxide beads. We have optimized the permethylation reaction conditions and evaluated the potential utility of this approach. The native and permethylated glycans were analyzed using Bruka Autoflex MALDI/TOF and ABI Qstar MALDI TOF MS/MS spectrometers. ARA was synthesized in house. The resulting MS spectra using ARA as matrix were compared to that obtained using 2,5-dihydroxybenzoic acid (DHB) as matrix. The results show that the spin-column permethylation technique was far superior in reducing the side reactions and reaction time (from 3 hours to 5 minutes). It allowed multiple reactions to be automated, which increased throughput significantly. In addition, the newly synthesized arabinosazone matrix enhanced MALDI-TOF analysis by generating less fragmentation, reducing background noise, and allowing ionization with lower laser energy which improved the quality of MALDI-TOF spectrum significantly. Further, ESI-MSn analysis of the permethylated glycans were performed on LCQ Deca Xp ion trap mass spectrometer using a static nanospray source for more detailed structural elucidation.

In conclusion, these approaches can be used for glycoprotein structure characterization, product quality and process control monitoring or for product lot to lot comparability studies.

(60) Anti- Human Immunodeficiency Virus Type 1 (HIV- 1 IIIB) Activity of Lectin from Sea Mussel Crenomytilus Grayanus

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CGL, a Crenomytilus grayanus lectin was purified from sea mussel C. grayanus, and the anti- HIV activity was assessed. CGL was purified with affinity chromatograph on galactose- Sepharose 6B and following gel filtration on Sephacryl S- 200. The inhibition of syncytial cell formation induce by human immunodeficiency virus was determined under microscope, and the reduction of HIV p24 antigen expression level was measured with ELISA. The purified CGL showed GalNAc/Gal specificity. CGL inhibited cytopathic effect induced by HIV- 1 and the production of viral p24 antigen. The EC50 values were 27.88 and 47.5 mg•L-1, respectively. CGL could block the cell- to- cell fusion process of HIV infected and uninfected cells with EC50 values of 35.12 mg•L-1. CGL is a GalNAc/Gal specific lectin with anti- HIV activity.

(61) The Effect of the Length of Arginine Chain on the Internalization into the SMC Cell

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During the past 15 years, a variety of peptides called Protein Transduction Domains (PTDs) or cell penetrating peptides (CPPs), have been characterized for their ability to translocate into live cells. Polyarginine peptides, one of the most important PTDs, exhibits even greater efficiency in terms of delivery of several peptides and proteins. Using transduction technology, a wide variety of cargo molecules (Protein/Peptide, Antisense, PNA, siRNA, Iron beads, Liposomes, Plasmid) covalently linked to PTDs could be internalized into the cell. Recently, the uptake mechanism of the PTDs widely investigated, and several mechnanism were proposed. To assess the use of PTD peptide as a new therapeutic approach for the prevention of postangioplasty restenosis, the effect of arginine length for uptake mechanism was examined by using Rat Aorta smooth muscle cells (AoSMC). COS-7 cell was used a kind of positive control.

Effect of the length of arginine chain on the internalization has been investigated by using mouse macrophage RAW 264.7 cells before (Futaki Shiroh, J. Biol. Chem.2001;276:5836-5840). In this research R6 and R8 exhibited the maximum internalization.

Our experimental data indicated that R12 exhibited the maximum internalization. In addition to that R16 peptides showed the most efficient uptake activity for 10 μM peptide concentration, but as we observed from pictures R16 peptide might mainly reside on the membrane surface at 10 μM concentration. In conclusion, we have confirmed that R12 showed the most efficient uptake activity. So, PTD length and PTD concentration effect the uptake quantity.

(62) Nutritional Benefits of Lectins from Marine Food Invertebrates

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Marine invertebrates contain proteins that are usually referred to as lectins on the basis of their carbohydrate-binding properties. Lectins are major component of the diet of food-producing marine invertebrates and are increasingly important in the human diet in Asia. Although lectins are heat-labile, more people are eating heat untreatment marine invertebrates. Additionally lectins in marine food bivalves showed a significantly mitogenic response towards BALB/c M cell. Two lectins, Gal-specific lectin (SBL) was isolated from marine bivalve Scapharca broughtonii and GalNAc/Gal-specific lectin (CGL) was obtained from marine bivalve Crenomytilys grayanus as reported. The peristalsis of rabbit ileum was increased by CGL and SBL. The results indicate that lectins are capable of provoking diarrhoeal effect. This study might be helpful in the choice of safe and nutritious marine food bivalves.

(63) Studies on the Carbohydrate Binding Sites of the Mucin-Binding Lectin CAL Isolated from the Sponge Craniella Australiensis

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The binding of carbohydrates to the mucin-binding lectin CAL isolated from the sponge Craniella australiensis. From our previous results, CAL was suggested to have more than one carbohydrate-binding site. We determined the number of carbohydrate-binding sites of the protein by ELISA using mucin-HRP as ligands. The Scatchard plots suggest that CAL has two carbohydrate-binding sites per molecule. The assosiation constants for the binding of mucin-HRP were estimated to be 550000 ml/ng and 2000ml/ng. The carbohydrate side chains of porcine stomach mucin (type III) are O-glycosidically linked through GalNAc to Ser or Thr of the protein core. There are 12 carbohydrate side chains found to be present, which are composed of one to five sugar residues with Galβ1-3 GalNAc α-O-Ser/Thr as the carbohydrate core region. These findings suggest that the binding site of CAL has a structure favorable for fitting of mucin. In addition to carbohydrate-binding properties, CAL demonstrated collagen-binding ability. As soon by ELISA, the binding of CAL with both native and denatured collagen was dose-dependent and inhibited by GRGDS-peptide and pepsin-soluble low molecular weight collagen.

(64) Isolation and Characterization of Gal-Specific Lectin from the Sea Mussel Scapharca Broughtonii

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Lectins are multivalent carbohydrate-binding proteins found in plants, bacteria and animals including marine invertebrates. We isolated Gal-specific lectin (SBL) from the sea mussel Scapharca broughtonii by DEAE-Sephacel and Sephadex G-100. Gel filtration chromatography and SDS-polyacrylamide gel electrophoresis of purified lectin indicate that its molecular mass to be 15 kDa. SBL was found to agglutinate native and trypsinized rabbit erythrocytes, and don't agglutinate native and trypsinized human erythrocytes. The hemagglutination was inhibited by galactose, lactulose, lactose and porcine stomach mucin (type III). The hemagglutinating activity of SBL was dependent of the divalent cation Ca2+. The peristalsis of rabbit ileum was increased by SBL. The results reported here will be useful for understanding of biological function of SBL. On the other hand it can be used as valuable tool in biomedical research.

(65) Study on Anti-HIV Activity of Glycoprotein from Marine Alga Hyalosiphonia Caespitosa

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The ocean provides a huge resource bank to the discovery of novel compounds. Many compounds, which are diversity, novel and bioactive, have been isolated from marine algae. These compounds have the potential to become new therapeutic agent for variety of diseases. Here we report isolation of glycoprotein from the marine alga Hyalosiphonia caespitosa by ion-exchange on DEAE-Sephacel and further was purified by gel filtration on Sephadex G-100. The glycoprotein had a molecular mass of about 20 kDa by gel filtration chromatography. The amino acid composition was rich in Ile, Leu, Asp and Gly. The total carbohydrate content was found to be 58.2 %. The glycoprotein was found to agglutinate native and trypsinized human A, B erythrocytes together with mouse, rabbit and chicken. It showed anti-HIV-1 activity *in vitro*. It inhibited cytopathic effect induced by HIV-1 with EC50 at 14.53 μg/mL. The results reported here will be useful for understanding of bioactivity information about marine natural products. The elucidations of the detailed carbohydrate-binding specificity and primary structure of the extract are under progress in our laboratory.

(66) Bead-Based Glycoblotting, BlotGlyco™, for Rapid and Large-Scale Glycomics

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The significance of protein glycosylation is now widely realized by the fact that the posttranslational modification is associated with a variety of disease states, therefore much attention has been dedicated to a need for a large-scale structural and functional analysis of protein glycosylation.

Since highly efficient sample preparation is a major concern for the quantitative glycan analysis, we have recently demonstrated that utilization of BlotGlyco, a bead-based platform of *glycoblotting*, is an efficient system that integrates oligosaccharide-capturing, on-bead methyl esterification, and fluorescent-labeling in a single workflow on a multi-well filter plate.

To extend its validity, we here describe a novel strategy that allows flexible and sequential incorporation of various functional tags into oligosaccharides in order to fulfill the requirements in the following analyses. When combined with a chemoselective glycoblotting technique, our approach employing another version of BlotGlyco bead permitted a quantitative detection of more than 120 glycans on human carcinoembryonic antigens. When sera from rheumatoid arthritis patients were analyzed, we precisely confirmed well-known structural variations in N-linked oligosaccharides. Besides, we could detect novel discriminative N-glycans as potential candidates for diagnostic marker.

Moreover, upon successful tag exchange at the reducing end from one to another, we have streamlined sequential processes of oligosaccharide purification, chromatographic fractionation, and

immobilization onto solid support for the analysis of interaction involving carbohydrates.

We believe that the results demonstrated herein provide a simplified sample preparation/processing that will facilitate functional and/or clinical glycomics study employing "real world" biological materials.

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(67) Oligosaccharide Profiling of O-linked Oligosaccharides Labeled with 2 Aminobenzoic Acid (2-AA)

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The analysis of O-linked oligosaccharides has been complicated by the absence of an enzyme that removes all O-linked oligosaccharides from the glycoprotein. O-linked oligosaccharides must be removed from the glycoprotein using chemical methods and the best chemical method (Carlson β-elimination) destroys the reducing end of the glycans, preventing labeling of the glycans by reductive amination. Our goal was to find a method for chemically releasing O-glycans without destroying the reducing end so we could develop an oligosaccharide profiling method with fluorescently-labeled O-linked oligosaccharides. Using the Carlson β-elimination method we had been limited to using high pH anion exchange chromatography (HPAEC) separation methods with pulsed amperometric detection (PAD) of the oligosaccharides.

The ammonia-based β-elimination method published by Huang et al. (Anal. Chem. (2001) 73:6063) releases both O-linked and N-linked oligosaccharides from the glycoprotein. We have demonstrated that the N-links are not released quantitatively by this method. We are however, able to efficiently release O-linked oligosaccharides from the glycoprotein, label them with 2-AA and show a good separation using an amino column. The residual N-linked oligosaccharides pose a problem and the optimization of this method for maximal recovery of O-linked oligosaccharides and minimal interference from the residual N-linked oligosaccharides will be discussed.

(68) Method Development of Sialylated Glycopeptide Isolation from Serum Using Mixed Mode LC Fractionation and Affinity Solid Phase Extraction

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Many diseases are associated with an increase in sialic acid content in malignant cell membranes compare to normal cells. Therefore, the isolation of sialic acid containing glycopeptides from enzymatically digested serum can potentially lead to biomarker discovery and identification for cancer diagnosis. We have developed a novel sialylated glycopeptide enrichment method using two stage separations; a mix mode HPLC column (strong cation exchange and reverse-phase/SCX-RP) was used first to separate and fractionate the peptides and metal oxide affinity chromatography (MOAC) in micro scale solid phase extraction (SPE) was applied to further enrich the sialylated glycopeptides by removing acidic glycopeptides that do not contain sialic acids in each fraction. The peptides eluted from MOAC SPE were sequenced using nanoLC/MS/MS analysis; the glycoproteins were identified based on the peptide sequence information and glycosylation sites are identified by looking for enzymatic conversion of asparagine to aspartic acid. A total of 61 nonredundant glycoproteins were identified from serum, some of the identified proteins in serum are present in low abundance. Majority of the identified sites matches with the published results in SwissProt database since some of the glycosylation sites are not listed in the database. The results show that the enrichment method of sialylated glycopeptides using the mixed mode LC fractionation and MOAC SPE can be a useful tool for cancer biomarker discovery.

(69) The Unnatural Sialic Acid Precursor N-Propanoylmannosamine Interferes with O-GlcNAc Modification of the Tyrosine 3-Monooxygenase and Stimulates Dopamine Secretion

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The most consistent neurochemical abnormality in Parkinson's disease is degeneration of dopaminergic neurons in the substantia nigra, leading to a reduction of striatal dopamine levels. The rate-limiting step in the biosynthesis of dopamine, noradrenalin and adrenalin is catalyzed by tyrosine 3-monooxygenase (= tyrosine hydroxylase), which catalyses the formation of L-DOPA. In earlier studies we demonstrated that the novel, synthetic sialic acid precursor, N-propanoylmannosamine, is a potent stimulator of axonal growth and promotes the re-establishment of the perforant pathway from layer II of cortical neurons to the outer molecular layer of the dentate gyrus.

Our data show that application of N-propanoylmannosamine leads to increased biosynthesis and secretion of dopamine. This increased biosynthesis of dopamine is due to decreased expression of O-linked N-acetylglucosamine on tyrosine 3-monooxygenase. Intracellular attachment of O-linked N-acetylglucosamine to serine and threonine residues hinders phosphorylation, thereby regulating the activity of the protein concerned. We therefore propose a model in which the application of ManNProp leads to increased phosphorylation and activation of tyrosine 3-monooxygenase, which in turn leads to an increased synthesis of dopamine.

(70) Linking Biomolecules to Monoclonal Antibodies via Glycan Chains using Mutant Glycosyltransferases

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We recently described a highly sensitive chemoenzymatic method to tag free GlcNAc residues on N-linked glycan chains of glycoproteins using the bovine β4Gal-T1-Y289L mutant and a UDP-galactose analog with ketone functionality at the C-2 position of the galactose ring. The transfer of the galactose residue, with a chemically reactive ketone group, to GlcNAc has been followed by coupling to the aminooxy-biotinylated derivative which is detected by chemiluminescence using streptavidin conjugated to horseradish peroxidase (HRP). We showed the transfer to be strictly dependent on the presence of both the mutant enzyme and the UDP-derivatives of galactose analogs. Here we show the transfer of 2-keto-galactose or 2-azido-galactose to a panel of monoclonal antibodies and linking of IgG to biotinylated derivatives via glycan chains. The monoclonal antibodies showing various glycosylated patterns were fully de-galactosylated as monitored by MS analysis of the glycan chains released after PNGase F treatment. The subsequent coupling of the 2- keto-galactose with aminooxy-biotinylated derivative, or of 2-azido-galactose with biotinylated alkyne derivative, was also monitored by MS analysis of the glycan chains released after PNGase F treatment. The re-galactosylation and linking of the IgG's to biotinylated derivatives occurs only on the Fc fragment and not on the Fab domain of IgG via sugars at the Asn297 of the heavy chain of IgG. Our results demonstrate that the linking of cargo molecules to monoclonal IgG's via glycans could prove to be an invaluable tool for potential drug targeting by immunotherapeutic methods.

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(71) **Therapeutic Glycoproteins Produced on PER.C6® Cells**

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The number of therapeutic glycoproteins in production is expected to rise rapidly in the next years. Aspects of importance for expression platform are the speed of transition from bench to manufacturing, yield and quality of the therapeutic product. We have evaluated the human PER.C6® cell line for its ability to produce therapeutic glycoproteins.

The generation of recombinant protein-producing cell lines is fast, as rounds of amplification of inserted genes are not required for high production yields. The gene copy number of inserted genes is around 1 to 5 copies per genome. Serum-free cell lines can be generated in three months. Screening for most optimal cell lines during the cell line generation program can be taken beyond yield to quality by screening for the most optimal glycoforms with lectins.

Production yields in generic fed batch for IgG is up to 4 g/L, extreme yields for IgG (>10g/L) have been obtained with the XD process (as developed in alliance with DSM biologics). Consistent high quality of the end product is obtained for mAbs, but also for more complex glycoproteins such as erythropoietin. Because of their human nature PER.C6® cell lines don't add potentially immunogenic glycans to recombinant proteins. The glycan profile of PER.C6® produced IgG is similar to human plasma IgG. There is restricted heterogeneity in glycosylation for PER.C6® produced erythropoietin and clearance rates in rats and activity are comparable to EPREX.

(72) **The Reptilian Cation-Independent Mannose 6-Phosphate Receptor Binds Human Insulin-Like Growth Factor-II with High Affinity**

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The mammalian cation independent Mannose 6-phosphate/insulin like growth factor-II receptor (Mr 300 kDa) is a multifunctional protein that binds a number of ligands such as the mannose 6-phosphate (m6p), human IGF-II, retinoic acid. We identified homologous proteins with m6p binding abilities from fish to mammals as well as in the invertebrates (echinodermates and molluscs). The mammalian protein contains an isoleucine residue in domain 11 at its IGF-II binding site. In chicken however, leucine is present and this receptor shows low affinity to IGF-II binding. In a recent study we showed that the chicken cell MPR 300 protein is capable of binding IGF-II. There is also a report that the fish MPR 300 binds IGF-II. To understand whether the reptilian receptor which we purified can also bind IGF-II, we carried out the present study. Experimental results using the purified reptilian receptor and IGF-II, in pull down assays, and cross-linking studies, suggests specific interaction between these. This data is supported by other binding studies where the dissociation constant (Kd) was found to be 12.02. Furthermore, the cDNA sequencing of 11th domain of CI-MPR reveals the presence of an isoleucine residue in the reptilian species, similar to the mammalian protein. Taken together, these findings provide a strong evidence for high affinity binding between reptilian receptor and IGF-II and support that IGF-II binding ability of the MPR 300 protein is also evolutionarily conserved.

(73) **Production of Humanized Therapeutic Proteins in the Yeast Pichia Pastoris**

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Protein-based therapeutics are emerging as the largest class of new entities being developed by the drug industry. About 70% of all approved therapeutic proteins on the market are glycoproteins.

The production of recombinant proteins that are N-glycosylated has in most cases required mammalian expression hosts that have the ability to synthesize human-like N-glycans. However, purified proteins from a mammalian cell culture process are essentially a mixture of individual drugs, some of which are more active than others and some of which have no activity at all. Each of these glycoforms has its own pharmocokinetic, pharmocodynamic and efficacy profile.

The methylotrophic yeast Pichia pastoris is an excellent alternative for the production of therapeutic glycoproteins: the system is cheap, the yields are high and there is no risk for viral or prion contamination. However, the N-glycosylation is non-human, heterogeneous and may cause immunogenic reactions.

We are currently tackling these handicaps by engineering the P. pastoris N-glycosylation pathway to allow the synthesis of human-like oligosaccharides. The general strategy consists in knocking out endogenous glycosyltransferases (responsible for the synthesis of yeast specific N-glycans) and introducing heterologous glycosidases and glycosyltransferases (responsible for the build-up of mammalian-type N-glycans). In this way we have created a library of yeast strains that have been engineered to modify all proteins with one specific N-glycan.

Recently, we have started producing several therapeutically interesting glycoproteins in these strains and the characteristics of the individual glycoforms will be tested both *in vitro* and *in vivo*.

(74) **Glycosylation Engineering of Glycoproteins through Endoglycosidase-Catalyzed Transglycosylation**

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Glycosylation is one of the most ubiquitous posttranslational modifications of proteins, and the oligosaccharides attached can profoundly affect proteins' structure and function. As natural and recombinant glycoproteins are typically produced as a mixture of glycoforms, homogeneous glycoproteins carrying structurally defined oligosaccharides are urgently needed both for detailed structure-function relationship studies and for developing glycoprotein-based therapeutics. We describe here a highly convergent chemoenzymatic method for glycoprotein synthesis and glycosylation engineering. The approach takes advantage of the transglycosylation activity of endo-beta-N-acetyl- glucosaminidase (ENGase) that allows the attachment of an intact oligosaccharide moiety to a suitable GlcNAc-containing polypeptide or protein in a single step. We have recently found that synthetic sugar oxazolines, the mimic of the presumed transition state, could be efficiently used as donor substrate for the enzymatic transglycosylation. When combined with a high-yield yeast expression system, the method allows a quick access to homogeneous glycoproteins carrying defined natural or unnatural oligosaccharides in a two-step approach: enzymatic de-glycosylation to remove the heterogeneous N-glycan and then ENGase-catalyzed transglycosylation to attach a desired oligosaccharide to the GlcNAc-protein. The application of the method to glycoengineering of human IgG-Fc will be discussed.

(75) **Heterogeneity of Cell Wall Carbohydrates and Lipids from Clinical Isolates of Mycobacterium Tuberculosis: Impact on Interactions with the Human Host**

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A growing body of evidence suggests that genetic variation among strains of a bacterial species contributes to biomedically relevant differences in host-pathogen interactions and phenotypes such as virulence. Studies of the global population structure of Mycobacterium tuberculosis (M.tb) have revealed that a relatively limited number of distinct phylogenetic lineages is responsible for most cases of disease. However, few pathogenesis studies have been conducted in the context of the M.tb phylogenetic framework. We tested the hypothesis that phylogenetic distinct clinical isolates of M.tb differ in their interaction with human macrophages. Unexpectedly, we discovered that a group of genetically related strains highly represented in large tuberculosis case clusters has reduced association with human macrophages. These organisms do not expose mannose-capped lipoarabinomannan (ManLAM) on their cell surface, have less higher-order phosphatidyl-myo-inositol mannosides (PIMs) in their cell envelope, and do not associate with the macrophage mannose receptor. We found that these strains have a truncated ManLAM with less branching mannan and arabinan domains, and contain phenolic glycolipids (PGLs) and triglycerides. Taken together, our results show that some tuberculosis strains differ fundamentally in host-pathogen molecular interaction, and provide evidence that the clinical spectrum of tuberculosis is dictated not only by the host, but also by strain genotype. We propose a new model for how M.tb strains within different phylogenetic lineages interact with human macrophages.

(76) The Macrophage Inducible c-Type Lectin, Mincle, Is an Essential Component of the Innate-Immune Response to Candida Albicans

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The recognition of carbohydrate moieties by cells of the innate immune system is emerging as an essential element in anti-fungal immunity, but few carbohydrate receptors have been characterised, despite the number and diversity of lectins expressed by innate immune cells. We demonstrate a novel role for the c-type lectin Mincle in macrophage responses to C. albicans. Mincle was observed to localise in the phagocytic cup of macrophages in response to a range of stimuli. Loss of Mincle reduced macrophage TNF production both *in vivo* and *in vitro*, but did not alter the number of Candida yeast phagocytised. Mice lacking Mincle showed a significantly increased susceptibility to systemic candidiasis. Thus Mincle contributes a novel and non-redundant role in the induction of inflammatory signalling in response to C. albicans infection.

(77) Novel Kv3 Glyocforms Expressed throughout the Adult Mammalian Central Nervous System Contain Sialylated N-Glycans

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The N-glycan pool of mammalian brain contains remarkably high levels of sialylated N-glycans with atypical structures. Here, it was shown that voltage-gated K+ channels, Kv3.1, 3.3, and 3.4, possess distinct sialylated N-glycan structures in the different regions of adult rat brain, as well as spinal cord. Electrophoretic migration patterns of Kv3.1, 3.3, and 3.4 glycoproteins from brain and spinal cord membranes digested with and without glycosidases were utilized to identify the various glycoforms. The Kv3.1 glycoform in cerebellum was expressed at much higher levels compared to those expressed in other regions of the central nervous system. The expression levels of the Kv3.3 protein were quite similar with the exception of the glycoform in spinal cord. The Kv3.4 glycoform level was also higher in cerebellum compared to the other regions, however not as dramatic. Additionally, brain membranes digested with specific glycosidases indicated that the N-glycan structures were terminated with sialyl residues. We suggest that the novel sialylated N-glycan structures of the Kv3 glycoproteins differentially expressed throughout the central nervous system may be an important component of these channels in: cell recognition events, targeting to specific subdomains of neurons, and in modulating channel activity.

(78) Role of Siglec-G in B Cell Signaling

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B1 cells are an important cell population for production of natural antibodies and for anti-bacterial immunoglobulin responses. Here we characterize the mouse protein Siglec-G as a novel B1 cell inhibitory receptor. Siglec-G is expressed in a B-cell restricted fashion with high levels in B1 cells. When over-expressed, it can inhibit BCR-mediated Ca2+ signaling. Siglec-G-deficient mice show a massive expansion of B1a cells, which starts early in development and is B-cell intrinsic. Siglec-G-deficient mice have increased levels of natural IgM antibodies, but not a higher penetrance of IgG autoantibodies. Siglec-G-deficient B1 cells show selectively a strongly enhanced Ca2+ signaling. These results demonstrate a novel negative regulatory pathway in B1 cells which may explain the naturally muted signaling response of B1 cells.

(79) Immune cell C-Type Lectins that Recognize Viruses

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Although glycans attached to enveloped viruses are assembled by the host glycosylation machinery, they can sometimes be recognised as foreign. Many enveloped viruses interact selectively with glycan-binding receptors on cells of the immune system. These receptors probably evolved to capture viruses and present fragments to the adaptive immune system, but HIV and other viruses have been able to subvert them to gain access to lymphocytes. Receptors that bind viral glycoproteins include DC-SIGN and the related endothelial cell receptors DC-SIGNR and LSECtin. Examination of the properties of these receptors reveals that they bind distinct but sometimes overlapping sets of glycans and viruses. For example, high mannose oligosaccharides on human immunodeficiency virus and hepatitis C virus are a common target for DC-SIGN and DC-SIGNR but not for LSECtin. In contrast, Ebola virus and the SARS coronavirus interact with all three receptors. Studies with the glycan array reveal that LSECtin binds with high selectivity to glycoproteins terminating in GlcNAcβ1-2Man. This disaccharide is one of the best low molecular weight ligands known for any C-type lectin. As a result of the selective binding of this disaccharide unit, the receptor recognizes glycoproteins with truncated complex and hybrid N-linked glycans on glycoproteins. Analysis of glycans on viral envelop glycoproteins indicates that DC-SIGN, DC-SIGNR and LSECtin interact with different types of relatively poorly processed glycans present on these viruses. Such glycans, which differ in character from the typical glycans on cell surfaces, appear to be a hallmark of virus surfaces.

(80) **Galectin-1 in the Regulation of Immune Cell Homeostasis: Lessons from Experimental Models *in vivo***

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T-cell mediated processes, including activation, differentiation and homing, are accompanied by a programmed remodeling of cell surface glycans, which themselves are the products of a repertoire of glycosyltransferases acting sequentially and dictating the glycosylation "signature" of each effector cell type. The responsibility of decoding the biological information encrypted by this glycosylation "signature" is assigned, in part, to endogenous glycan-binding proteins or lectins, whose expression and function are regulated at inflammatory sites. Galectin-1, a member of a family of highly conserved glycan-binding proteins, has emerged as a regulator of T cell homeostasis and a key effector of the immunosuppressive activity of T regulatory cells. Galectin-1 plays a pivotal role in conferring immune privilege to tumor tissues by skewing the cytokine balance toward a Th2 profile. We recently found that TH1 and TH-17-differentiated cells expressed the repertoire of cell surface glycans essential for galectin-1 binding, while TH2 cells were protected from galectin-1 through differential sialylation of N- and O-glycans on cell surface glycoproteins. Consistently, galectin-1-deficient mice showed hyper-TH1 and TH-17 responses following antigenic challenge *in vivo* and show greatly enhanced susceptibility to autoimmune neuroinflammation. In addition, we observed that galectin-1 contributes to the homeostasis of other immune cell types through non-apoptotic immunoregulatory mechanisms. Our findings provide a novel molecular link between differential glycosylation of immune cells and termination of the inflammatory response with potential implications in cancer, autoimmunity, transplantation and fetomaternal tolerance.

(81) **Myelin-Associated Glycoprotein (Siglec-4) in Axon Regeneration**

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Myelin-associated glycoprotein (MAG), a member of the Siglec (sialic acid-binding immunoglobulin-like lectin) family, is expressed on myelin that surrounds axons throughout the nervous system. MAG on the inner-most myelin wrap (apposed to the axon) enhances axon stability. However, after injury (e.g. spinal cord injury) MAG on residual myelin inhibits axon regeneration. Blocking MAG-mediated inhibition may allow axons to regenerate, enhancing functional recovery. MAG inhibition of axon regeneration is mediated, in part, by its binding to axonal sialoglycans. Two spinal cord injury models were used to test whether disruption of MAG-sialoglycan binding enhances recovery. In brachial plexus injury, the nerves to the arm are often violently yanked from the spinal cord. Nerve grafts are used to bridge the gap between the spinal cord and the remaining nerve stump, but poor axon outgrowth restricts functional recovery. We cut the brachial plexus of rats and inserted a nerve graft into the spinal cord at the injury site. Delivery of bacterial sialidase to disrupt MAG-sialoglycan binding enhanced spinal axon outgrowth into the graft 2.5-fold. In a second model, rats were subjected to blunt contusion spinal cord injury. Delivery of sialidase to the injury site enhanced motor behavior and a spinally-mediated (baroreceptor) reflex that controls blood pressure, two measures of functional recovery. These data indicate that sialic acid-directed therapies might contribute to anatomical and functional recovery of the injured spinal cord. Supported by NIH grants NS046669 & NS037096 (RLS), HL16315 (LPS) and the Johns Hopkins Medicine Alliance for Science and Technology Development.

(82) **Identification of Novel Carbohydrate Binding Endothelial Receptors by a Carbohydrate-Mimicking Peptide**

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Previously, we screened a peptide-displaying phage library using monoclonal anti-carbohydrate antibodies, and identified IELLQAR (I-peptide) that binds to anti-Lewis A antibody. I-peptide mimics selectin ligand, and inhibited sialyl Lewis X binding to the selectins. When I-peptide was injected intravenously to a mouse, I-peptide inhibited a carbohydrate-dependent lung colonization of melanoma cells in mice. However, I-peptide inhibited the cancer cell lung colonization in mutant mice deficient of both E- and P-selectins, suggesting the existence of novel carbohydrate binding receptors in the lung vasculature distinct from selectins (Zhang, J. et al., Cancer Research 62: 4194-4198, 2002). We purified the putative endothelial receptors by I-peptide affinity column chromatography, and identified them by proteomics. These experiments identified two proteins: mRNA splicing factor (Sfrs) and annexin1 (Anxa1). Glycoarray analysis showed that Sfrs and Anxa1 bind to fucosylated oligosaccharides and sulfated oligosaccharides, respectively. Because Anxa1 has been identified as endothelial marker of tumor vasculature, we conjugated I-peptide with geldanamycin (GMB) and targeted the tumor vasculature in the mouse. Our results show that intravenously injected I-peptide-GMB suppress tumor growth in the mouse. These results suggest significant potential of I-peptide in therapies against cancer.

This study has been supported by grants NIH P01CA71932, and Susan Komen Breast Cancer Research Foundation grant BCTR0504175. The glycoarray resources were provided by the Consortium for Functional Glycomics GM62116.

(83) **Fluorescent Lectins Identify Carbohydrate Ligands of Pathogen Adhesins on Glycoprotein Micelles**

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Carbohydrate structures resembling surface-exposed receptors on host cells can be used to capture pathogens expressing certain lectins. We demonstrate the production of oil-filled micelles whose surface is a biocapture film. Protein-bound oligosaccharides protrude into the aqueous environment, stabilizing these micelles and forming a relatively large capture surface. In order to develop a selective biocapture film, we choose glycoproteins whose oligosaccharides contain receptors of specific pathogen adhesins. However, terminal carbohydrate structures of nascent glycoproteins typically contain some natural variation, while others may be modified by indigenous glycosidases. Further, oligosaccharides are sometimes altered during protein purification. To evaluate the relative prevalence of carbohydrate structures on biocapture micelles, we developed a novel assay using fluorescent lectins. The measurable binding of these lectins to spherical micelles provides a sensitive and inexpensive means of identifying functional sugar sequences in the oligosaccharides of native and commercially isolated glycoproteins. We performed tests involving the plant lectins, Galanthus nivalis agglutinin, Griffonia simplicifolia II and Concanavalin A, which verified the presence of typical hen egg white glycans on micelles derived from ovomucoid (trypsin inhibitor from Sigma).

(84) **Direct Effects of GnT-Vb-Mediated Glycosylation on Signaling in the Developing Nervous System**

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The functional effects of glycosylation are controlled by the coordinated expression of glycosyltransferases and the regulated expression of particular glycoprotein acceptors. O-mannosyl-linked glycosylation is abundant within the central nervous system, yet very few glycoproteins with this glycan modification have been identified, and the functional roles of this glycan modification have not been defined. Congenital diseases with significant neurological defects arise from inactivating mutations found within the glycosyltransferases that act early in this pathway. We are studying the glycosyltransferase known as GnT-Vb that is responsible for elongating O-mannosyl-linked glycans allowing for the addition of further glycan elaborations such as the HNK-1 epitope. We have identified a key substrate of GnT-Vb and provide evidence that GnT-Vb-mediated glycosylation of this receptor alters neuronal signal transduction that influences cell-cell interactions and migration in the developing nervous system.

(85) **Streptococcus Mitis Platelet Aggregation Factor: A cholesterol-Dependent Cytolysin with a Novel Fucolectin Domain**

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The characterization of purified recombinant platelet aggregation factor from Streptococcus mitis (Sm-hPAF) is described. The gene for Sm-hPAF is present in isolates of the S. mitis group of streptococci including S. mitis, S. pseudopneumoniae and S. pneumoniae. Sm-hPAF was determined to be a functional member of the cholesterol-dependent cytolysins (CDC), a family of pore forming toxins. In contrast to previous reports, SM-hPAF does not aggregate platelets, but does lyse platelets. Unlike other CDCs SM-hPAF contains an extra amino terminal domain of about 162 amino acids that encodes a putative fucolectin. The isolated fucolectin domain exhibits novel glycan specificity for Lewis Y (LeY) and b (Leb) antigens. The fucolectin glycan-binding site is not exposed in the soluble monomer of Sm-hPAF. The glycan-binding site is predicted to be exposed only upon formation of the oligomeric membrane pore complex of Sm-hPAF, thus forming a high avidity glycan-binding complex on the cell surface. Sm-hPAF defines a novel member of the CDC family of pore-forming toxins.

(86) **Role of Galectin-3 in Leukocyte Recruitment in a Murine Model of Lung Infection by Streptococcus Pneumoniae**

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Pneumonia can be induced by a variety of pathogens, among which Streptococcus pneumoniae causes one of the most common forms of community-acquired pneumonia. Depending on the invading pathogen, the elements of the immune response triggered will vary. For most pathogens, such as E. coli, neutrophil recruitment involves a well described family of adhesion molecules, β2-integrins and selectins. In streptococcal pneumonia, however, neutrophil recruitment occurs mainly through a β2-integrin-independent pathway. Despite decades of research on this issue, the adhesion molecules involve in neutrophil recruitment during lung infection by S. pneumoniae have not been identified. We have previously shown that galectin-3, a soluble mammalian lectin, can be found in lungs infected by S. pneumoniae, but not by E. coli, and can mediate the tight adhesion of neutrophils on the endothelial cell layer *in vitro*, implying its role in the recruitment of neutrophils to lungs infected with S. pneumoniae. In this study, using galectin-3 deficient mice, we report further evidence of the involvement of this soluble lectin in the recruitment of neutrophils to S. pneumonia infected lungs. Indeed, in the absence of galectin-3, lower numbers of leukocytes, mainly neutrophils, were recruited to the infected lungs during infection by S. pneumoniae. In the case of β2-integrin-dependent recruitment induced by lung infection with E. coli, the number of recruited neutrophils was not reduced. Thus, taken together, our data suggest that galectin-3 plays a role as a soluble adhesion molecule in the recruitment of neutrophils to lungs infected by S. pneumoniae, which induces β2-integrin-independent migration.

(87) **Parainfluenza Virus Receptors**

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Human parainfluenza viruses (hPIV) bind receptors containing sialic acids in α2-3 linkage. We investigated the binding of hPIV types 1 and 3 to the Glycan Array of the Consortium for Functional Glycomics under conditions where neuraminidase is inactive or active. Both hPIV1 and hPIV3 bind to modifications of Neu5Acα2-3Galβ1-4GlcNAc, including the sialyl-Lewisx motif and structures containing 6-sulfogalactose. hPIV-1 and hPIV-3 thus bind structures found on N-linked glycans in contrast to avian influenza H5 hemagglutinin (J. Stevens, O. Blixt, T. M. Tumpey, J. K. Taubenberger, J. C. Paulson, and I. A. Wilson, Science 312:404-410, 2006) which binds mucin-like motifs. The different α2-3 sialic acid receptors for hPIV and H5 influenza may contribute to their different pathologies.

(88) **Mici Lectin of T.GONDII Induces IL-12 Production by Antigen Presenting Cells**

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Toxoplasma gondii is an obligate intracellular protozoan capable of invading and replicating within nucleated cells of warm-blooded animals. MIC1 and MIC4, endowed of lectin activity, are micronemes proteins that constitute a lactose-binding fraction (Lac+) purified from soluble tachyzoites antigen (STAg) of T. gondii RH strain. Crude STAg is a potent inducer of IL-12 production by dendritic cells (DC), an activity presumably due to antigen interaction with TLR(s) on DC surface. Considering that the active STAG component(s) was not identified and lectins are potential ligands for TLRs, we evaluate if MIC1 can account for the IL-12 induction activity of STAg. Lac+ fraction, containing MIC1/4 complex, was isolated from STAg of RH strain, by affinity to immobilized lactose. MIC1/4 preparation (1ug/ml) was used for *in vitro* stimulation of murine spleen cells, which produced high levels of IL-12 (718 +/- 15 pg/ml). In the presence of 0,05M lactose the IL-12 production was inhibited in 30%. These results suggest that IL-12 is at least partially due to MIC1, which has its lectin property implicated in the activity. In order to unambiguously attribute the activity to MIC1, and not to MIC4, we prepared the recombinant protein. Therefore the cloned MIC1 gene was expressed in BL21 (DE3) E. coli strain. The purified recombinant MIC1 was assayed for the IL-12 inducing activity on purified DCs which produced significant levels of these cytokine (1350+-/30). We postulate that MIC1 from T. gondii induces APCs

to release IL-12, a property that is possibly due to the MIC-1 lectin nature.

(89) **Neuropilin-2 Is a Newly-Recognized Polysialylated Protein Expressed in Human Dendritic Cells that Modulates Dendritic Cell-T Lymphocyte Interactions**

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Polysialic acid (PSA) has been identified as a posttranslational modification on only five mammalian proteins. Polysialylation of NCAM, the most extensively studied polysialylated protein, plays a significant role in migration, axonal guidance, synapse formation and functional plasticity of neurons by preventing formation of stable cell contacts mediated by NCAM and other cell surface molecules. An isoform of NCAM (CD56) on natural killer (NK) cells is the only protein known to be polysialylated in cells of the immune system, yet the function of PSA in NK cells remains unclear. Given the similarities between neurons and dendritic cells, we determined whether PSA plays a role in the function of human dendritic cells. We show here that neuropilin-2, a receptor for semaphorin and vascular endothelial growth factor families in neurons and endothelial cells, respectively, is expressed on the surface of human dendritic cells and is polysialylated. Expression of neuropilin-2 is upregulated during dendritic cell maturation, coincident with increased expression of ST8Sia IV, one of the key enzymes of PSA biosynthesis, and with appearance of PSA on the cell surface. Removal of PSA from neuropilin-2 or binding of neuropilin-2 with specific IgG promoted dendritic cell-induced activation and proliferation of T lymphocytes. Thus, this newly-recognized polysialylated protein on the surface of dendritic cells influences dendritic cell-T lymphocyte interactions through one or more of its distinct extracellular domains. Current studies are exploring the molecular mechanism(s) by which PSA on neuropilin-2 affects the interaction of dendritic cells with T lymphocytes and other cells of the immune system.

(90) **Sialidase Activity and Metastatic Characteristics of Tumor Cell Clones *in vivo* of Rat Rhabdomyosarcoma RA-23**

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Introduction. It has been found that lysosomal sialidase (LS) can significantly modulate invasive and metastatic characteristics of tumor cells. We have earlier found that the low metastatic potential is of characteristic of rat rhabdomyosarcoma RA-23 tumor cells with high level of genome instability. It was reasonable to elucidate LS activity in tumor cell clones which are differing in their ability to metastasis. Methods. The clones of tumor cells with high (n=10) and low (n=10) ability to metastasis were assayed for LS activity as follows: clones were homogenized in buffer and suspension was centrifuged at 3,000 r.p.m. One ml of supernatant (fraction I) was again centrifuged at 16,000 r.p.m. The supernatant was discarded and pallet was obtained as fraction II. 4-methylumbelliferyl-N-acetylneuraminic acid was used as a substrate to assay LS activity in fractions I and II. Results. There was no difference in LS activity in fraction I from clones with low and high ability to metastasis. The enzyme assay elucidated that more than 60% LS activity was concentrated in fraction II for clones with high ability to metastasis as well as for clones with low ability to metastasis. The former was characterized lower LS activity (1,46±0,10 nm/mg/h) whereas the later showed more high LS activity (2,71±0,31 nm/mg/h) ($P<0,01$, Students's t-test). Conclusion. The high ability to metastasis *in vivo* of rat rhabdomyosarcoma RA-23 clones is closely related to decreased LS expression in tumor cells.

(91) **Glycomic Analysis of Human and Murine Leukocytes**

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The objective of the Analytical Glycotechnology Core C of the Consortium for Functional Glycomics (CFG) is to is to acquire and disseminate glycan structural data from the carbohydrates of glycoconjugates that are involved in cell-cell communications. This is being achieved by focused, in depth structural analyses of the glycoprotein and glycolipid glycomes of purified cell populations of the human and murine immune systems supplied by CFG Participating Investigators (PI's). This has been facilitated by enhanced access to new technologies at Core C including state-of-the-art MALDI-TOF-TOF mass spectrometric technology, as well as new integrated methodologies for concerted glycoprotein and glycolipid analysis. In addition, were ever possible, cells provided by PI'S to Core C for glycomic profiling are also provided to Core E for Glyco-Gene chip microarray analysis to facilitate integrated data analysis of both glycan profiles and glyco gene expression. Data generated from such integrated glycomic analysis of PI's human and murine leukocytes will be presented. This work was funded by The Consortium for Functional Glycomics (grant number GM62116), the The Wellcome Trust and the Biotechnology and Biological Sciences Research Council (BBSRC).

(92) **World Efforts for Building Glycan Structure Databases**

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At the NIH Workshop on "Frontiers in Glycomics and Glycobiology" (September 2006) there was a general agreement that there is an urgent need for a unified, thoroughly curated and sustainable database for carbohydrate structures. The lack of appropriate databases is regarded as one of the biggest deficiencies in glycomics. "We need to be able to search databases for what is out there. Imagine genomics and proteomics without GenBank" (Ajit Varki).

To pave the way for a central carbohydrate structure database, the existing larger initiatives agreed to immediately start with the necessary preparatory steps for the conversion of CarbBank data into the GLYDE-II format. It was agreed that the conversion will be a multi-institutional, international effort, which is coordinated by the EUROCarbDB initiative.

The lecture will summarize the efforts to develop and agree on a controlled vocabulary for the description of monosaccharide building blocks (MonosaccharideDB, GlycoCT), as well as a comprehensive encoding scheme to specify the topology of glycan structures including all types of uncertainties in their structural description (Glyde-II and GlycoCT).

With the GlycomeDB (www.glycome-db.org) a carbohydrate structure meta-database has been created which provides a structure-oriented access to all major worldwide glycan databases (CFG, KEGG, GLYCOSCIENCES.de, BCSDB and CarbBank). The currently implemented search options will be demonstrated. The retrieved structures refer back to the original databases, where users can access additional resources (like pathways, MS- and NMR data) provided by the respective initiative. The next steps towards establishing a unified Carbohydrate Structure Database will be discussed.

(93) **C-Type Lectins in Innate and Adaptive Immune Responses**

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Dendritic cells (DC) are specialized in the recognition of pathogens and play a pivotal role in the control of immunity. Yet DC are also important for homeostatic control recognizing self antigens and tolerizing its environment, indicating that the nature of the antigen it recognizes may steer a DC towards immunity or tolerance. We have shown that the C-type lectins DC-SIGN and MGL are specific antigen uptake receptors that process antigen for presentation to T cells. Modification of glycosylated antigen can strongly affect the antigen uptake and presentation capacity of DC. We have indicated that these C-type lectin receptors have a unique signalling capacity by which they can modify TLR signalling processes.

Both MGL and DC-SIGN can also mediate cellular interactions. We have demonstrated that MLG binds to a specific population of effector T cells through CD45 and affects TCR signalling by inducing apoptosis. Similarly DC-SIGN has been shown to mediate neutrophil binding which affects DC maturation. It is clearly that the regulated expression levels of glycans dictates which subsets of cells interact with DC. Understanding the diversity of C-type lectins being expressed on DC as well as their carbohydrate specific recognition profile will be instrumental to understand DC pathogen recognition in many pathogenic disorders, as well as the regulation of cellular interactions of DC that are essential in the control of immunity.

(94) **Identification of One Gene in Tobacco Leaves Induced by Oligochitosan**

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Oligochitosan has a variety of biological activities. The presence of oligochitosan triggers a wide range of cellular responses including changes in gene expression and synthesis of PR proteins. To investigate the ability of oligochitosan to affect gene transcription, the messenger RNA (mRNA) differential display technique was applied to the identification and isolation of genes whose transcription was altered in cultured Nicotiana tabacum (var. Samsun NN) plants that were treated with oligochitosan. One gene whose mRNA levels significantly changed in response to oligochitosan was isolated and identified. Reverse Northern blot and RT-PCR analysis indicated that the expression of the gene was up-regulated by oligochitosan. Sequence and homology analysis show that the fragment share 62% identity with polyubiquitin of maize. Because the ubiquitin protein is relevant to plant defense response and hypersensitive response, so these results suggested that oligochitosan can induce plant resistance and its mechanism may be relevant to ubiquitination. These findings also demonstrate the usefulness of mRNA differential display technique for the detection of plant metabolic pathways affected by oligochitosan.

(95) **Myelin-Associated Glycoprotein (Siglec-4) Protects Hippocampal Neurons and Inhibits their Axon Regeneration using Different Receptors**

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Myelin associated glycoprotein (MAG, Siglec-4) is a member of the Siglec (sialic acid-binding immunoglobulin-like lectin) family. MAG enhances axon-myelin interactions, contributing to axon stability. However, MAG also inhibits axon regeneration at sites of injury, limiting functional recovery. MAG binds to different receptors on axons, including glycosylphosphatidylinositol-anchored Nogo-receptors (NgRs) and the major brain gangliosides GD1a and GT1b. Identifying the receptors involved in each of MAG's effects provides potential targets for therapeutic intervention. The aim of the present study was to identify the receptors and signaling pathways used by MAG to mediate its dual effects: inhibition of axon regeneration and neuroprotection. Rat postnatal hippocampal neurons were plated on control surfaces or surfaces adsorbed with native MAG derived from rat myelin. Neurite outgrowth inhibition and cell survival in a model of excitotoxicity induced by exposure to kainic acid were tested. Enzymatic and pharmacological agents were used to modulate potential MAG receptors and signaling pathways. We report that MAG dependent inhibition of axon regeneration was primarily mediated by gangliosides. In contrast, neuronal protection was mediated primarily by NgRs. In both cases, Rho kinase inhibitor reversed MAG effects, implicating RhoA as a downstream signaling molecule. TAT-Pep5, an inhibitor of the signal transduction molecule P75NTR, partially reversed inhibition of neurite outgrowth but had no effect on MAG-mediated neuroprotection. From these results we conclude that MAG inhibits axon regeneration and protects neurons using different receptors in the same nerve cells. Supp. by NIH NS037096.

(96) **Mechanism of Resistance to Dexamethasone Action on Neutrophil Migration Induced by Lectin MNCF**

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Maria Cristina Roque Barreira
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The Macrophage-derived Neutrophil Chemotactic Factor (MNCF) has been characterized as a lectin able to induce neutrophil migration, which is resistant to the dexamethasone action. We studied different activation parameters on pretreated neutrophils or not with dexamethasone and following stimulation with MNCF or others inflammatory mediators. In agreement with others inflammatory mediators (fMLP or CXCL8), MNCF was able to induce L-selectin shedding and polarization of F-actin filamentous. But, polimerization of F-actin filamentous was not induced by MNCF on neutrophils not pretreated with dexamethasone. On the other hand, even after dexamethasone pretreatment, the interaction of MNCF on neutrophils surface induced *in vitro* migration, polimerization of F-actin filamentous, genic transcription and secretion of proinflammatory cytokines and chemokines. In paralell, these activities were inhibited by dexamethasone in fMLP- or CXCL8-stimulated neutrophils. So, we propose that, MNCF induces neutrophil migration, resistant to dexamethasone actions, in part because, these cells answer to MNCF stimuly suporting genic transcription and secretion of inflammatory cytokines and chemokines.

(97) **Characterisation of Siglec Ligands Expressed on Activated Murine T-Lymphocytes**

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Sialic acid-binding immunoglobulin-like lectins (Siglecs) are expressed primarily in the immune and haematopoietic systems where they bind sialylated glycoconjugates with a high degree of specificity. Activated T-lymphocytes undergo marked alterations in surface glycosylation and sialylation and this could be important in influencing their interactions with siglec-expressing cells of the immune system such as macrophages and dendritic cells. Changes in sialylation have been probed via flow cytometry using conjugated Maackia Amurensis II (MAA) (α2,3 Sia-specific) and Sambucus Nigra (SNA) (α2,6 Sia-specific) lectins following anti-CD3 and anti-CD28-mediated activation of isolated murine splenocytes cultured for 24 – 96 hours in the presence of r-IL-2. Furthermore, the binding profiles of siglec-Fc chimeras, including Siglec-E, CD22 and Siglec-F pre-complexed with FITC-goat anti-human Fc IgG have been assessed. We have shown significantly increased binding of Siglec-E to murine T-lymphocytes following 24 hours of activation. This binding is sialic acid-dependent and is

sensitive to proteinase K treatment, suggesting that the putative ligands are presented by a sialylated glycoprotein(s). Various approaches have been employed in order to isolate and characterise the putative glycoprotein counter-receptors. These have included glycoprotein enrichment with Wheat Germ Agglutinin (WGA), the use of MAA and SNA-conjugated agarose beads as well as an immunoprecipitation-based strategy in which pre-complexed recombinant siglec-E-Fc has been coupled to protein G sepharose beads using dimethylpimelimidate.

This work is supported by the Interdisciplinary Research Collaboration in Proteomic Technologies, BBSRC grant number: BB/C511613/1.

(98) **The Changing Glycome of the Developing Myocardium**

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Carbohydrates attached in large and varied numbers to proteins and lipids are vital for normal cell communication. Glycosylation-associated genes comprise ~1% of the total human genome. We set out to determine whether cardiomyocyte glycosylation-associated gene expression and glycan structures are regulated and remodeled throughout the developing heart. GeneChip microarray analyses indicated significant regulation of glycosylation-associated gene expression among myocytes from neonatal and adult atria and ventricles that was confirmed using quantitative RT-PCR. Nearly one-third of glycosylation-associated genes were significantly differentially expressed in atria versus ventricles of the newborn, while expression of nearly half of the genes was regulated throughout ventricular development. N-glycan structures were determined and compared among myocyte types. The data indicate that high mannose structures are dominant throughout the heart, but the relative abundance and types of complex N-glycan structures vary among myocyte types. This remodeled glycome apparently impacts cardiac function, with differences in voltage-gated sodium channel (Nav) activity and action potential (AP) waveforms observed in neonatal atrial myocytes isolated from polysialyltransferase (STX) knockout versus control mice. Neither AP waveform nor Nav gating were different in knockout versus control ventricular myocytes, consistent with the fact that STX is essentially expressed in the newborn atria but not in the ventricle. Together, these data indicate that remodeled glycosylation-associated gene expression throughout myocardial development results in altered N-glycan structures, particularly in the abundance and types of more complex structures. This remodeled glycome has an apparent direct effect on Nav function and overall cardiac excitability.

(99) **Administration of KM^+ Lectin Protects Mice against *Paracoccidioides brasiliensis* Infection via Interleukin-12 Production in a TLR2 Dependent Mechanism**

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KM^+ is a mannose-binding lectin from *Artocarpus integrifolia* that induces IL-12 production by macrophages and protective T helper 1 immune response against *Leishmania major* infection. In this study, we carried out experiments to evaluate the activity of jackfruit KM^+ ($jfKM^+$) and its recombinant (rKM^+) in the prophylaxis and therapy of experimental paracoccidioidomycosis. To this end, $jfKM^+$ or rKM^+ was administered to BALB/c mice either 10 or 3 days before or 10 days after the challenge with *Paracoccidioides brasiliensis*. Fourteen and 30 days postinfection, lungs from the KM^+-treated mice presented 86% less colony-forming units and absent or very few organized granulomas when compared to the controls. In addition, lung homogenates from the KM^+-treated mice presented higher levels of nitric oxide, IL-12, interferon-γ and tumor necrosis factor-α, whereas in the control group higher levels of IL-4 and IL-10 were detected. By using mice deficient in IL-12, Toll-like receptor (TLR) adaptor molecule MyD88, TLR2, and TLR4, we undeniably demonstrated that KM^+ leads to protection against *P.brasiliensis* infection through IL-12 production, which is dependent on TLR2. These results demonstrate a beneficial effect of KM^+ on the *P.brasiliensis* infection severity and expand its potential use as a novel immunotherapeutic molecule.

(100) **Design of Novel Xylosides that Prime Multiple Glycosaminoglycan Chains**

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University of Utah, Salt Lake City, UT

Proteoglycans are the most complex glycoconjugates found in nature, and often carry multiple glycosaminoglycans chains on a single core protein. It is known that specific protein sequences are required for a given serine residue to append a GAG chain. However, additional factors that are required to produce proteoglycans with multiple chains attached to a specific core protein are largely unknown. To unravel these intricate factors, we have designed and synthesized a number of cluster xylosides. We have determined the specific structural features that are required to prime several glycosaminoglycans chains within the given molecular scaffold. This work will lead to further our understanding of biosynthetic mechanisms and further our ability to modulate signaling pathways dictated by proteoglycans that are essential for human health and development.

(101) **Detailed Structural Analysis of the Lipid-Linked Oligosaccharide Pathway via MS^n Disassembly**

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Since the introduction of electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) into biomolecular mass spectrometry (MS), MS-based analysis with superior sensitivity, low sample consumption, and aptitude for High-Throughput automation (HTP) is the predominant method for glycan structural analysis. Preparing glycan samples as methyl derivatives analyzed in an ion-trap mass spectrometer provide an effective method for uncovering structural detail and isomers in biological samples. Here we extend ion-trap applications to the lipid linked oligosaccharide (LLO) pathway. The LLO pathway is a glycosyltransferase driven process conserved in all eukaryotes. This enzymatic process begins on the cytosolic surface of the endoplasmic reticulum (ER) and continues in the lumen creating the mature precursor dolichol-P-P-$Glc_3Man_9GlcNAc_2$, which is then transferred *en bloc* to a nascent polypeptide. In humans, genetic defects in this pathway lead to severe multi-systemic disorders, referred to as congenital disorders of glycosylation type I (CDG-I). Although rare, CDG-I demonstrate the essential nature of the LLO pathway in terms of human development. Using the model organism *C. elegans*, we have characterized the LLO pathway components in fine structural detail, uncovering the expected structures ($Man_5GlcNAc_2$ through $Glc_3Man_9GlcNAc_2$) as well as several unexpected structural isomers. Aberrant protein *N*-

glycosylation is associated with many disease states and our method will probe the LLO pathway to observe any structural changes in the pentasaccharide core.

(102) **Identification and Characterization of Two Novel Human Collagen Galactosyltransferases**

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Collagen is the most abundant protein found in the human body and is an essential component of the extracellular matrix. Collagen is characterized by a right handed triple helix formed out of three left handed alpha-chains with glycine-X-Y repeats, where X and Y are often represented by (hydroxy)proline and (hydroxy)lysine. Selected hydroxylysines are further modified by the addition of either galactose or the disaccharide glucosylgalactose. The glycosylation of collagen takes place in the ER before triple helix formation and is mediated by beta-1-O galactosyl- and alpha-1-2 glucosyl-transferases. The molecular nature of these glycosyltransferase activities has long remained unknown. We have now identified novel collagen galactosyltransferase enzymes (ColGalTs) using affinity chromatography and mass spectrometry. Homology searches revealed that the ColGalTs belong to a family of structurally related genes. We cloned three of these genes, expressed them in Sf9 insect cells, and confirmed the ColGalT-activity for two of these transferases (ColGalT1 and ColGalT2). The ColGalT genes are differentially expressed in human tissues, suggesting that they may show preference for different types of collagens or contribute to the varying extent of collagen glycosylation throughout tissues. This was supported by showing a selective preference for collagen type-I and collagen type-IV acceptors. ColGalT1 shows a higher enzymatic activity on collagen type-IV than ColGalT2, whereas the latter preferentially glycosylates collagen type-I. ColGalT1 and ColGalT2 have a much higher galactosyltransferase activity than the lysylhydroxylase PLOD3, previously reported to possess a low glucosyl- and galactosyltransferase activity.

(103) **Polymerization of Sialic Acid by Bacterial Polysialyltransferases**

Justine Vionnet; Dwight C. Peterson; Willie F. Vann
Food and Drug Administration, Bethesda, MD

The polymerization reaction of *Escherichia coli* and *Neisseria meningitidis* polysialyltransferases has been examined. Pathogenic gram negative bacteria produce three types of polysialic acid. The α(2,8)neuNAc is produced by *E. coli* K1 and *N. meningitidis* Group B and is the polysialic acid structure commonly found in eukaryotic cells. The α(2,9) neuNAc is less common and is produced by *N. meningitidis* Group C. *E. coli* K92 produces a polysialic acid containing both α(2,8)neuNAc and α(2,9)neuNAc. The polysialyltransferases of these bacteria are firmly associated with the cytoplasmic membrane. We have partially purified the *N. meningitidis* Group C polysialyltransferase as a soluble protein. We have compared the polymerization reaction of polysialyltransferases of *E. coli* K1 and K92 and of the *N. meningitidis* Group B and C using a fluorescence based HPLC assay. All of these polysialyltransferases will extend a fluorescent oligosaccharide containing α(2,8) linked disialic acid. The polysialyltransferases extend these substrates to form long polymers in a non-processive fashion. While the *E. coli* K92 polysialyltransferase appears to have a size preference for the rate of polymerization, the *Neisseria* polysialyltransferases do not. In spite of differences in enzyme structure and the structure of the polysialic acid products our data suggest significant similarity in the overall mode of polymerization.

(104) **Characterization of ST6 beta-Galactosamide alpha-2, 6-Sialytranferase I N-Glycans by Exoglycosidase Digestions and Mass Spectrometry**

Parastoo Azadi; Seongha Park; Lu Meng; Kelly Moremen
Complex Carbohydrate Research Center, Athens, GA

ST6 beta-galactosamide alpha-2, 6-sialyltranferase I (ST6 Gal I) enzyme is responsible for the transfer of sialic acid to the 6-hydroxyl group of galactose. The carbohydrate parts in ST6 play an important role for the function of the enzyme. Therefore analysis of the carbohydrate structure is necessary for characterization of ST6 Gal I. Sialylation by ST6 Gal I occur mainly at the galactose residue of the terminal Gal-GlcNAc of either N-linked or O-linked carbohydrates.

The N-glycans were released by treatment with PNGase F and subsequently permethylated prior to MS analysis. Complex mixture of N-glycans was detected and the main component was found to be the HexNAc5.Hex4.NeuAc2.Fuc1 structure. The above structure corresponded to an unusual fucosylated biantennary structure with two sialic acids and a HexNAc-HexNAc structure on one of the biantennary arms. The glycoprotein was sequentially digested with α 2-3 Neuraminidase, β N-acetylhexosaminidase, α 1-2,3 mannosidase, and α 1-6 mannosidase followed by permethylation. The structure of derivatized glycan residues were elucidated by MALDI-TOF-MS and ESI-MS/MS. The presence of GalNAc-GlcNAc structure on the biantennary arm instead of the usual Gal-GlcNAc was confirmed by specific exoglycosidase digestions. The linkage of the GalNAc-GlcNAc-residues to the α-mannose (either 1-3 or 1-6 linkage) is being confirmed. Preliminary data indicates that the GalNAc-GlcNAc structure is attached to the 3-arm..

(105) **CHO Cells Differentially Resistant to Modeccin Isolectins**

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We have isolated a new CHO cell mutant resistant to a specific isoform of modeccin. Modeccin is a disulfide-linked dimer consisting of an A-chain N-glycosidase that inactivates ribosomes and a B-chain lectin domain. Five isoforms of modeccin were isolated from the plant Adenia digitata by ammonium sulfate precipitation and affinity chromatography. The new mutant line was highly resistant to modeccin isolectins purified by asialofetuin-affinity chromatography, but it was not resistant to other modeccin isolectins affinity-purified on Sepharose 6B and 4B. Changes in sensitivities to a battery of other toxic lectins revealed small increases in resistance to the plant lectins L-PHA (P. vulgaris leukoagglutinin) and WGA (wheat germ agglutinin). However, no cross-resistance to the modeccin-related, galactoside-binding lectins ricin or abrin was observed. Thus, the modeccin resistance of the new mutant cannot be due to resistance to A chain activity, which would also induce resistance to ricin and abrin. Previously characterized modeccin-resistant CHO glycosylation mutants including Lec21 were resistant to all five modeccin isolectins. Therefore the novel mutant may display a distinct complement of cell surface glycans. Unlike receptors for ricin, modeccin receptors are of low abundance. Attempts to identify a modeccin receptor will include structural characterization of cell surface glycans of the new mutant and the modeccin-hypersensitive CHO mutant Lec3.

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(106) **Comparative N-Glycan Profiles of CHO Cells Expressing GlcNAcT-III (LEC10) or Lacking GlcNAcT-V (Lec4)**

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Chinese hamster ovary (CHO) glycosylation mutants offer a system for examining the effects of a change in a single glycosyltransferase on the overall N-glycan profile of a cell. To initiate this analysis, the N-glycans released by peptide N-glycosidase F from total cellular glycoproteins of CHO cells and the CHO mutants Lec4 and LEC10 were permethylated and examined by MALDI-TOF/(TOF) mass spectroscopy. Lec4 cells have a mutation in the Mgat5 gene and lack GlcNAcT-V activity. Consequently they cannot make complex N-glycans with a β1,6GlcNAc branch. LEC10 CHO cells express the Mgat3 gene that encodes GlcNAcT-III, the enzyme that transfers the bisecting GlcNAc to N-glycans. To compare the glycosylation profiles of CHO cells and the two mutants, the relative intensities of isotope clusters were determined for signals with masses corresponding to compositions of known N-glycans. The most abundant complex N-glycans from LEC10 cells grown in monolayer are bisected whereas these glycans are only present in trace levels in CHO and Lec4 cells. The abundance of polylactosamine-containing N-glycans appears to be similar in LEC10 and CHO cells. The spectrum of N-glycans from Lec4 cells growing in monolayer shows structures carrying up to at least 11 LacNAc units despite the absence of GlcNAcT-V. MALDI-TOF/TOF MS/MS analysis indicates that the abundance of polylactosamine components is greater in Lec4 cells than CHO cells.

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(107) **Glyco-DNA: DC-SIGN Receptor-Targeted DNA Vaccines**

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It is well known that high-mannose N-glycans bind lectins on dendritic cells in the course of a normal immune response. The DC-SIGN receptor recognizes branched high mannose structures and reducing di-mannoses, while the mannose receptor binds di-mannose clusters and reducing single mannose structures. However, these receptors also serve as a means of entry for sustained infection and on occasion, transmission of pathogen. These receptors bind an assortment of viruses, bacteria, and parasites including HIV, Ebola virus, Mycobacteria tuberculosis, and Leishmania pifanoi. Our aim is to target a DNA vaccine to the DC-SIGN receptor on DCs using the high mannose N-glycan Man9GlcNAc2. The plasmid DNA would code for antigenic protein, thereby stimulating an adaptive immune response.

This approach requires the purification of soybean agglutinin, which is both a galactose specific lectin and a glycoprotein containing Man9GlcNAc2. A galactose affinity column was constructed and used to isolate SBA on a gram scale in a single chromatographic step from soy flour extract. The affinity purified SBA was homogenous by SDS-PAGE. Purified SBA was digested with Pronase to release the high mannose N-glycan. We have installed an intercalating molecule on the amino-terminus of Asparagine on the reducing end of the glycan for non-covalent coupling to DNA. These N-glycans bind to plasmid DNA and are under investigation as novel DNA vaccines.

(108) **Glycomics of Proteoglycan Biosynthesis in Murine Embryonic Stem Cell Differentiation**

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Glycosaminoglycans (GAGs) play a critical role in binding and activation of growth factors and chemokines involved in cell signaling critical for developmental biology. The biosynthetic pathways for GAGs have been elucidated over the past decade and it is now possible to determine GAG composition in as few as 10 million cells. A glycomics approach was used to examine GAG content, composition and the expression of transcripts encoding for GAG biosynthetic enzymes as murine embryonic stem cells (mESCs) differentiate to embryoid bodies (EBs) and to extraembryonic endodermal cells (ExE) to better understand the role of GAGs in stem cell differentiation. Hyaluronan synthesis was enhanced by 13- to 24-fold, most likely due to increased expression of hyaluronan synthase-2. Chondroitin sulfate (CS)/dermatan sulfate (DS) synthesis was enhanced by 4- to 6-fold and heparan sulfate (HS) synthesis was enhanced by 5- to 8-fold following the transition from mESC to EB and ExE. Transcripts associated with the synthesis of the early precursors were largely unaltered, suggesting other factors account for enhanced GAG synthesis. Interestingly, CS type E and highly sulfated HS both increase as mESCs differentiate to EBs and ExE, as well as, enhanced 2-sulfation in both CS/DS and HS families. Transcript levels for core proteins generally showed increases or remained constant upon mESC differentiation in both CS/DS and HS proteoglycan families. Transcripts encoding selected enzymes, including GlcNAc-4,6-O-sulfotransferase, C5-epimerases, and 3-O-sulfotransferases involved in late GAG biosynthesis were also enriched. [Supported by NIH grants HL62244, HL52622, GM38060 (to RJL) and RR018502 (to KWM and JMP)].

(109) **A General pH Indicator Assay for High-Throughput Screening of Glycosyltransferase Libraries**

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Protein engineering using directed evolution or saturation mutagenesis at hot spots is often used to improve enzyme properties such as substrate selectivity or stability. This requires access to robust high-throughput assays for analysis of enzyme libraries. Relatively few studies on directed evolution or saturation mutagenesis of glycosyltransferases have been reported in part due to a lack of suitable screening methods. We report a general screening assay for glycosyltransferases that has been developed using the blood group galactosyltransferase (GTB) as a model. GTB utilizes UDP-Gal as a donor and alpha-Fuc(1,2)beta-Gal (H-antigen) as an acceptor to synthesize the blood group B antigen alpha-Gal(1,3)[alpha-Fuc(1,2)]beta-Gal. A closely related (1,3)-N-acetylgalactosaminyltransferase (GTA) uses UDP-GalNAc as the donor with the same H acceptor, yielding the A antigen alpha-GalNAc(1,3)[alpha-Fuc(1,2)]beta-Gal. GTA and GTB are highly homologous enzymes differing by only 4 of 354 amino acids. The screening assay we developed is based on the colour change of the pH indicator bromothymol blue when a proton is released during the transfer of Gal/GalNAc from UDP-Gal or UDP-GalNAc. Saturation mutagenesis of the GTB enzyme at M214, a hot spot adjacent to the 211/DVD/213 metal binding motif was performed and 350 of the clones were screened for elevations in UDP-Gal and UDP-GalNAc transfer activity. M214G, M214S and M214Q were identified by pH indicator screening to have wild-type Gal transfer activity. The M214G and S mutants exhibited two-fold higher activity than wild-type GTB with UDP-GalNAc confirming the importance of residue M214 for donor enzyme specificity.

(110) **Chemoenzymatic Synthesis of ABO(H) Blood Group and Lewis Antigens as Terminal Structures on Natural bi-antennary N-linked Glycopeptides**

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We have undertaken to do chemoenzymatic synthesis of ABO(H) blood group and Lewis antigens (Lea, Lex, Leb and Ley) as terminal structures on natural bi-antennary N-glycopeptides to provide well defined compounds for the glycan array of the Consortium for Functional Glycomics. The glycopeptides were isolated from bovine fibrinogen and egg yolk. After pronase and neuraminidase digestions they were purified. Some of them were further degalactosylated. Both the asialo- and agalacto-glycopeptides were used as substrates for the enzymatic modifications. We explored the acceptor specificities of several glycosyltransferases, such as b1-3galactosyltransferase 5 (b1-3GalT5), b1-4 galactosyltransferase, a1-2fucosyltransferase 2 (a1-2FucT2), a1-3/4fucosyltransferase 5 (a1-3/4FucT5), glycosyl-transferase A (GTA) and glycosyltransferase B (GTB), to transfer different sugar residues from the nucleotide sugar donors to both branches of bi-antennary N-linked glycopeptides. Some of the modifications required two and three consecutive incubations with specific glycosyltransferases. Glycopeptides with terminal ABO(H) blood groups (type 1 and type 2) and Lewis antigens were purified and analyzed by mass spectrometry. We fount out that a1-2FucT2 transferred well to both terminal type 1 and type 2 chains yielding blood group O(H), type 1 and type 2. Both blood group O(H) type 1 and type 2 were also good acceptors for GTA and GTB. Blood group A and B, type 1 and type 2 were synthesized, respectively. a1-3/4FucT5 successfully fucosylated both blood group O, type 1 and type 2, as well and the resulting terminal structures were Leb and Ley, respectively. (Supported by NIH grant GM62116).

(111) **Mutagenesis and Kinetic Characterization of Mouse Lunatic Fringe**

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Disrupted Notch signaling causes lethality in an embryo and is implicated in many disease states postnatally. Interaction between Notch and ligands of the DSL family can be modulated by O-fucose on extracellular Notch. The fucose can be further elongated to a tetrasaccharide beginning with N-acetylglucosamine (GlcNAc), added by one of three fringe β1,3-GlcNAc transferases (Lunatic (Lfng), Manic, or Radical) in mammals. Lfng is involved in vertebrate segmentation, and disruption of its function results in the human genetic disorder, Spondylocostal Dysostosis. A recent crystal structure for Mfng allowed us to produce a homology model for Lfng. Using the program HEX4.5, we docked an O-fucosylated EGF repeat onto the Lfng model. We manually culled inappropriate solutions and found the O-fucose of the docked EGF clustered in two groups between the putative catalytic Asp289 and GlcNAc of the donor. Based on this model we chose residues in Lfng to mutate and analyzed their activity with an *in vitro* assay. We categorized the mutants as Vmax or KM defects and attempted to determine their role in enzyme function. Based on these results, we propose that one of the O-fucose clusters is the most likely orientation. We also have evidence that a small loop not observed in the crystal structure may become ordered upon substrate binding, closing one side of the catalytic pocket. Finally we have characterized the inhibition of the enzyme by UDP and UMP.

This work was supported by GM61126.

(112) **Manipulation of Glycosaminoglycan Biosynthesis by Suppression of Glycosyltransferases**

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To bring glycoprotein pharmaceuticals into production utilizing mammalian cells, it is important to control the glycan biosynthesis and to keep the glycan structure as uniform as possible. This issue is especially challenging in case of glycosaminoglycans (GAGs) on recombinant proteoglycans, since biosynthesis of GAGs is not fully understood. Indeed, we previously expressed an artificial proteoglycan (PG-FGF1) in which GAG modification sites were partially modified with heparan sulfate (HS) and partially with chondroitin sulfate (CS). Here we aimed at manipulating GAG biosynthesis by silencing responsible glycosyltransferases in CHO cells. To modulate CS biosynthesis, chondroitin N-acetylgalactosaminyltransferase (CSGnT)-1 and -2 genes were suppressed in the cells by stable expression of the respective siRNAs. The CHO cell clones in which mRNA expression levels of the target genes were suppressed were used to express decorin and syndecan-4 ectodomain as a model CSPG and a HSPG, respectively. Analysis of GAG modifications on the secreted proteins revealed that knocking down CSGnT-2 gene, but not CSGnT-1, greatly suppressed CS modification on decorin. Neither affected HS modification on syndecan-4 ectodomain. Similar attempt was then made to modulate heparan sulfate (HS) biosynthesis. CHO transfectants in which EXT-1 and/or EXT-2 genes were knocked down were isolated, and their products were analyzed. We found that the syndecan-4 expressed by the EXT-1 knocked down cells were modified with very short HS chain, whereas CS modification on decorin was not affected at all. These results indicate the feasibility of manipulating GAG biosynthesis through suppression of responsible glycosyltransferases.

(113) **Structural and Enzymatic Bases of N-Glycans of alpha-Fetoprotein L2 and L3 from Hepatoma Cell Lines and Hepatocellular Carcinoma Patients**

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Alpha-fetoprotein L3 (AFP-L3) is a tumor marker for hepatocellular carcinoma (HCC) and patients with higher AFP-L3 levels often receive a poor diagnosis. Alpha 1,6 fucosyltransferase (fucosyltransferase VIII; FUT8) catalyzes the transfer of fucose to the proximal GlcNAc of N-glycan in an alpha 1,6 linkage—a process known as "core-fucosylation." The present study examined the relationships among glycosyltransferase expression, glycan structures, and the pattern of Lens culinaris agglutinin (LCA)-affinity electrophoresis of AFP. A hepatoma cell line, Huh7 was transfected with either N-acetylglucosaminyltransferase III (GnT-III) or N-acetylglucosaminyltransferase V (GnT-V). GnT-III catalyzes the formation of bisecting GlcNAc of N-glycan, and GnT-V transfers alpha 1,6-linked GlcNAc residues on Man alpha 1,6-arm branched tri-antennary (2,2,6-Tri) glycans. AFPs were purified from the cells and LCA-reactive AFPs (AFP-L3 and -L2) were isolated using LCA-sepharose chromatography. Determination of N-glycan structures, using HPLC and MALDI-TOF, showed that the major constituent of AFP-L2 was a core-fucosylated N-glycan with bisecting GlcNAc. 2,2,6-Tri glycan with alpha 1,6 core-fucose was recovered in AFP-L3. Suppression of Fut8 by siRNA led to a reduction in AFP-L3 along with an increase in AFP-L1. In addition, AFP from HCC patients also was carefully analyzed. The amount of core-fucosylated N-glycans with and without bisecting GlcNAc were highly correlated with the amount of AFP-L2 and -L3, respectively. These results clearly

demonstrate that the ratio of AFP-L1 to -L3 is regulated by glycosyltransferase activities and by the affinities of AFP N-glycans for LCA.

(114) α1,4GlcNAc-Capped Mucin-Type *O*-Glycan Inhibits Cholesterol α-Glucosyltransferase from *Helicobacter pylori* and Suppresses *H. pylori* Growth

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Helicobacter pylori infects over half the world's population and is thought to be a leading cause of gastric ulcer, gastric carcinoma, and gastric malignant lymphoma of mucosa-associated lymphoid tissue type. Since only a fraction of those infected develop these diseases, it has been suggested that there is a natural defense mechanism that inhibits *H. pylori* growth. Previously, we reported that gland mucin (MUC6) present in lower portion of the gastric mucosa containing α1,4GlcNAc-capped core 2-branched *O*-glycans suppressed *H. pylori* growth by inhibiting the synthesis of α-glucosyl cholesterol, which is a major constituent of *H. pylori* cell wall (Science, 305, 1003-1006, 2004). Because of this importance, we cloned genomic DNA encoding cholesterol α-glucosyltransferase (CHLαGcT), and its soluble form was expressed in *Escherichia coli*. Using this soluble CHLαGcT, we show herein that CHLαGcT sequentially acts on UDP-Glc and cholesterol in an ordered Bi-Bi manner. We found that both newly synthesized α-glucosyl cholesterol and α1,4GlcNAc-capped core 2-branched *O*-glycans inhibit CHLαGcT in a mixed, non-competitive manner. In contrast, UDP competitively inhibits CHLαGcT. We also found that α1,4GlcNAc-capped core 2-branched *O*-glycans most efficiently inhibited *H. pylori* growth. Since those IC_{50} toward CHLαGcT gave similar effect, these findings together indicate that α1,4GlcNAc-capped *O*-glycans suppress *H. pylori* growth by inhibiting CHLαGcT, and α1,4GlcNAc-capped *O*-glycans may be useful to treat patients infected with *H. pylori*. This work was supported by NIH grants CA71932 (to M.F. and P.H.S.) and CA33000 (to M.F.).

(115) Functional Glycosylation of α-Dystroglycan by Mouse Large

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Alpha-dystroglycan (α-DG) is an important component of the dystrophin glycoprotein complex (DGC) that links extracullar matrix proteins to intracellular cytoskeleton. Mutations in genes encoding glycosyltransferases (POMT1, POMT2, and POMGnT1) and putative glycosyltransferases (LARGE, Fukutin and FKRP) lead to hypoglycosylation of α-DG which inhibits binding to laminin and results in congenital muscular dystrophies termed dystroglycanopathies. LARGE has two glycosyltransferase domains respectively related to unfolded glycoprotein glucosyltransferases (UGGT) and β(1,6)GlcNAc-T6. Interestingly, overexpression of LARGE in muscle cells from patients with several dystroglycanopathies bypasses their defects in the functional glycosylation of α-DG. Similar results were reported from our laboratory when mouse Large was overexpressed in Lec15 Chinese hamster ovary (CHO) mutant cells defective in the transfer of O-Mannose glycans. The glycans modified by Large in CHO cells were partially sensitive to N-glycanase and glycosylation was enhanced when core 2 GlcNAcT was introduced indicating that Large modifies both N- and mucin O-glycans. To further investigate this question, we have developed a new cell line termed Lec15.Lec1. This mutant cannot synthesize O-Mannose glycans or complex N-glycans. We have previously shown that the oligomannosyl N-glycans of Lec1 cells are not a substrate for Large. When Large was introduced into Lec15.Lec1 cells, functionally glycosylated α-DG was made, providing additional evidence that Large modifies mucin O-glycans. To further investigate, co-expression of Large and a secreted form of α-DG (DGFc5) is being examined in ldlD and ldlD.Lec1 cells under culture conditions in which mucin O-glycans are either present or absent.

(116) Xylosyltransferase 2 Deficiency Results in Polycystic Disease and Nephron Dysfunction

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The basic biochemical mechanism(s) underlying many heritable human polycystic diseases is unknown despite evidence that most cases are caused by mutations in members of several protein families, the most prominent being the polycystin gene family, whose products are found on the primary cilia. Inherited polycystic kidney disease, the most prevalent polycystic disease, currently affects approximately 500,000 people in the United States and the autosomal dominant form (ADPKD) ranks as the third most common cause of end stage renal disease worldwide. Decreases in proteoglycans (PGs) have been found in tissues and cultured cells from patients that suffer from ADPKD and this decrease has been hypothesized to be responsible for cystogenesis. This is possible since alterations in PG concentrations would be predicted to disrupt many homeostatic mechanisms of growth, development, and metabolism. To test this hypothesis we have generated mice lacking xylosyltransferase II (XylT2) an enzyme involved in PGs biosynthesis. We have found XylT2 deficiency results in substantial reduction in PGs and polycystic disease phenotypically similar to many aspects of polycystic liver and kidney disease including biliary epithelial cysts, renal tubule dilation, organ fibrosis, and basement membrane abnormalities. In addition, we find that nephron function is compromised as demonstrated by proteinuria likely resulting from glomerular dysfunction. This is significant since ADPKD patients with proteinuria have a less favorable prognosis. These novel findings demonstrate that reduced PGs can induce cyst development, and furthermore, that XylT products are important in glomerular function and structure.

(117) Enzymes Responsible for Synthesis of Corneal Keratan Sulfate Glycosaminoglycans

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Keratan sulfate glycosaminoglycans are among the most abundant carbohydrate components of the cornea and are suggested to play an important role in maintaining corneal extracellular matrix structure. Keratan sulfate carbohydrate chains consist of repeating *N*-acetyllactosamine disaccharides with sulfation on 6-*O* positions of *N*-acetylglucosamine and galactose. Despite its importance for corneal function, the biosynthetic pathway of the carbohydrate chain and particularly the elongation steps are poorly understood.

Here we analyzed enzymatic activity of two glycosyltransferase, β1,3-*N*-acetylglucosaminyltransferase-7(β3GnT7) and β1,4-galactosyltransferase-4(β4GalT4), in production of keratan sulfate carbohydrate *in vitro*. These glycosyltransferases produced only

short, elongated carbohydrates when they were reacted with substrate in absence of a carbohydrate sulfotransferase; however, they produced extended GlucNAc-sulfated poly-*N*-acetyllactosamine unit in presence of corneal *N*-acetylglucoamine 6-*O* sulfotransferase(CGn6ST). Moreover, we detected production of highly sulfated keratan sulfate by two-step reaction *in vitro* with a mixture of β3GnT7/ β4GalT4/CGn6ST followed by keratan sulfate galactose 6-*O* sulfotransferase treatment. We also observed that production of highly sulfated keratan sulfate in cultured human corneal epithelial cells was drastically suppressed when expression of β3GnT7 or β4GalT4 was suppressed by siRNAs, indicating that these glycosyltransferases are responsible for elongation of keratan sulfate carbohydrate backbone.

(118) **Characterization of POMGnT1 Acceptor Specificity using Synthetic O-Mannosylated Peptides Designed from the Mucin-like Domain of alpha-Dystroglycan**

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Alpha-Dystroglycan (αDG) possesses a mucin-like domain with multiple serine (S) and threonine (T) residues, that express O-linked mannosylated (O-Man) moieties that are then elongated by O-mannosyl-β1,2-N-acetylglucosaminyltransferase (POMGnT1) and other glycosyltransferases. Mutations in POMGnT1 result in truncated structures that cause muscle-eye-brain disease. In order to understand the acceptor specificity of POMGnT1, we have synthesized eight peptide sequences derived from the mucin-like domain of αDG (which spans from residues 316 to 489), each with one or multiple O-Man sites. These peptides were designated as M1 (residues 416-420 with O-Man at T418), M2 (residues 429-433 with O-Man sites at S430 and T431), M3 (residues 326-331 with O-man sites at T328 and T329), M4 (residues 411-416 with O-Man at T414), M5 (residues 461-466 with O-Man sites at T463 and T464), M6 (residues 480-487 with O-Man sites at T482, T483, T484, and S485), M7 (residues 419-427 with O-Man sites at T421 and T424), and M8 (residues 419-427 with O-Man sites at T421, T422, T423, and T424). These peptides were used as *in vitro* acceptors for recombinant POMGnT1 secreted by HEK-293 cells, and kinetic parameters (Km, Kcat, and Km/Kcat) were determined for each substrate. Results show a higher specificity of POMGnT1 for substrates M2, M3 and M4. Mass spectrometric analysis of the reaction products of the M2 and M3 substrates (each with two O-Man sites) show that only one mannosylation site is utilized by the enzyme. These results suggest that POMGnT1 has increased affinity for specific amino acid sequences in the mucin-like domain of αDG.

(119) ***N*-Glycan Structural Analysis of a CDG-II/COG1 Condition in the *C. elegans cogc-1(k179)* Strain**

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The Golgi apparatus is vital in the glycosylation and trafficking of glycoproteins, glycolipids and proteoglycans. The Conserved Oligomeric Golgi (COG) complex is an eight subunit protein that is essential for normal Golgi morphology and structure. It is believed that COG complexes are involved in membrane trafficking and/or compartment function. Proper sequential modifications of glycoproteins by glycosyltransferases depend on the distribution of resident enzymes within the Golgi. Defects in COG function can cause abnormalities in intracellular protein sorting, protein secretion, cell growth and glycoconjugate synthesis. Mutations of the COG complex subunit 7 and 8 appear to cause a congenital disorder of glycosylation type IIe and IIh in humans. Additionally, mutations in subunit 1 also cause a CDG type II/COG1 illness. The COG complex has been studied considerably at the cellular level, but its role in animal development remains unknown. Despite growing understanding of the COG complex, the overall implications involved in glycosylation are uncertain. Structural analysis of glycoconjugates of COG deficient cells have yet to be explored. This study will examine the structural consequences of COG deficient cells using the capability of mass spectrometry and the known glycome of *C. elegans*. Preliminary data reveals an increase in high-mannose and fucosylated structures as well as a reduction of endogenous O-methylated structures in *cogc-1(k179)* worms.

(120) **Analysis of Glycosylinositol Phosphorylceramide Expression in Cryptococcus Neoformans Xylose Pathway Knockout Strains**

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The pathogenic basidiomycete Cryptococcus neoformans causes serious disease in immunocompromised patients. A characterizing feature of C. neoformans is its polysaccharide capsule, which is required for virulence. Xylose is a key component of both of the major polysaccharides comprising the capsule, glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), and is essential for proper capsule formation and virulence. Xylose is also present in C. neoformans glycosylinositol phosphorylceramides (GIPCs) [1], characteristic glycosphingolipids of fungi whose biosynthesis is essential for normal growth and life cycle. Interestingly, structural features are shared between GIPCs and capsular polysaccharides of C. neoformans, especially GalXM. Thus, GIPCs of C. neoformans possess a core structure incorporating a branching Xylβ1,2 residue, with overall sequence Manα3(Xylβ2)Manα4GalβManα2InsPCer [1]. We speculate that one or more Xylβ1,2-transferases involved in capsule synthesis might also add xylose to GIPCs. We are comparing strains disrupted in genes responsible for xylose metabolism to test our hypothesis with respect to GIPC biosynthesis as well. Strains examined include wild type JEC21 and a strain lacking a Xylβ1,2-transferase (Cxt1p [2]; see presentation by J. S. Klutts, T. L. Doering et al). Significantly, the characteristic Xylβ1,2 residue was missing from GIPCs of the Cxt1p knockout. Furthermore, a Manα6 residue, which elongated the Manα3(Xylβ2)Manα4GalβManα2InsPCer core in JEC21, was also absent in the mutant, suggesting that the presence of the Xylβ2 residue could be required for further elongation of the core by a Manα1,6-transferase. [1] Heise et al (2002) Glycobiology 12:409-420; [2] Klutts et al (2007) J Biol Chem 282:17890-17899.

(121) **Structural Analysis of *N*-Linked Oligosaccharides Reveals an Essential Role of *YlMPO1* for Mannosylphosphate Transfer in the Dimorphic Yeast *Yarrowia lipolytica***

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Structural analyses of *N*-linked oligosaccharides using high performance liquid chromatography (HPLC) indicated that the *N*-linked glycans of *Yarrowia lipolytica* appeared to be consisted of major neutral and minor acidic oligosaccharides. The presence of phosphorylated mannoses in the *Y. lipolytica* *N*-glycans was confirmed by mass spectrometric analysis of glycans derived from the recombinant EGI using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). The major peaks detected in the negative mode of MALDI-TOF had a higher molecular weight than those of neutral sugars, which nearly

correspond to the molecular mass of monomannosylphosphorylated $Man_{7-9}GlcNAc_2$ species. A putative open reading frame, designated as *Y. lipolytica MPO1* (*YlMPO1*), was predicted to encode a protein (YlMpo1p) with 40% identity to the *Saccharomyces cerevisiae MNN4* protein. Disruption of *YlMPO1* in the wild type background (*ylmpo1Δ*) and in the *yloch1Δ* mutant (*yloch1Δ /ylmpo1Δ*) strains led to the disappearance of the minor acidic sugar peaks on HPLC. Furthermore, alcian blue staining showed a decreased color intensity of the *ylmpo1Δ* mutant strain compared to that of the parental strain. Taken together, the results strongly supported that YlMpo1p plays an essential role in mannosylphosphorylation of the core glycan moiety on glycoproteins in *Y. lipolytica*.

(122) **The Cytosolic Region of GM3 Synthase Defines its Stability and Subcellular Localization**

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GM3 synthase (SAT-I) is the first enzyme of ganglioside biosynthetic cascade to produce GM3. Recently, it has been reported that GM3 is involved in the pathological condition of type 2 diabetes. Mouse SAT-I (mSAT-I) possesses two transcriptional variants (mSAT-Ia and mSAT-Ib). mSAT-Ia and mSAT-Ib have the alternative initiator codons in their mRNAs, resulting in the generation of two polypeptides, M1-SAT-I and M2-SAT-I, respectively. Interestingly, M1-SAT-I has a long N-terminal cytosolic region as compared to M2-SAT-I. In this study, we examined whether the difference of cytosolic region between M1-SAT-I and M2-SAT-I affects their activity and stability. The activity of M1-SAT-I was quite similar to that of M2-SAT-I. However, it was found that M1-SAT-I has a long half-life relative to M2-SAT-I. Based on this result, we assumed the different subcellular localization of M1-SAT-I and M2-SAT-I. Accordingly, we expressed fusion proteins of each N-terminus of M1-SAT-I and M2-SAT-I containing transmembrane region and enhanced green fluorescent protein (EGFP)(M1-/M2-SAT-I(N)-EGFP) in CHO cells. M1-SAT-I(N)-EGFP localized in endoplasmic reticulum (ER), while M2-SAT-I(N)-EGFP localized in Golgi apparatus. Moreover, mutational analyses revealed that multiple arginines (R) in the cytosolic region of M1-SAT-I were responsible for ER targeting. The minimal requirements of this targeting motif are RR, RXXXXR, and RXXXXXR. It has been reported that RR motif functions as a retrograde transport signal in type II membrane proteins. To our knowledge, RXXXXR and RXXXXXR are the new consensus sequences for retrograde transport. This is the first report demonstrating that the cytosolic region of glycosyltransferase determines the subcellular localization and stability.

(123) **Incorporation of D-Arabinopyranose into Leishmania Major Glycoconjugates Requires Golgi Nucleotide-Sugar Transport Activity**

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Polysaccharides composed of arabinose residues are important constituents of glycoconjugates from many bacteria, protozoa and plants. Unlike L-arabinofuranose, D-arabinopyranose is not widespread among living organisms. Rare examples are protozoan parasites of the genus Leishmania (causative agents of the human disease leishmaniasis), which in part of their life cycle use D-arabinopyranose as a critical component of glycoconjugates implicated in virulence, including phosphoglycans (PGs) and small glycosylinositolphospholipids (GIPLs). Despite its importance, the D-arabinopyranose metabolic pathway remains largely unexplored. In previous studies, it was shown that the donor for the incorporation of D-arabinopyranose into Leishmania glycoconjugates is GDP-D-arabinopyranose. Furthermore, null mutants of the L. major GDP-sugar transporter LPG2 were severely compromised in macrophage survival and induction of acute pathology in susceptible mice, yet were able to persist indefinitely. In this current work of D-arabinose metabolism, 3H-arabinose incorporation of the PGs and GIPLs was abolished in the L. major lpg2-/- cells and similar results were also observed in mutants lacking the LPG5A and LPG5B genes which encode for two Golgi UDP-Gal transporters. One explanation is that lack of the LPG2 transporter precludes GDP-Ara translocation or, alternatively, it may also be possible that arabinosylation requires the presence of mannose or galactose residues as acceptor sites in glycoconjugates. As the synthesis of PGs is LPG2-dependent whereas GIPLs are LPG2-independent, our results indicate that LPG2 alone, and not another of the large family of Leishmania nucleotide-sugar transporters, is responsible for GDP-D-Ara transport into the secretory pathway *in vivo*.

(124) **The Complete Elucidation and Pro-Inflammatory Activity of LPSs from the Burkholderia Cepacia Complex**

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Members of genus Burkholderia comprise opportunistic pathogens responsible for important infections in the immunocompromised and in cystic fibrosis (CF) patients. The B. cepacia complex (Bcc) is a group of microorganisms composed of at least nine closely related genomovars. All genomovars have been shown to cause infections although B. cenocepacia and B. multivorans (genomovars III and II, respectively) are the most frequently isolated genomovars from CF patients. Notably B. cenocepacia is the most feared Burkholderia cepacia complex pathogen isolated from CF patients as it is widely recognized to cause epidemics associated with excessive mortality. One major factor of virulence of Gram negative bacteria infection is the Lipopolysaccharide (LPS). The knowledge of the LPS structure is an essential pre-requisite to the understanding of the molecular mechanisms involved in the inflammatory process. The primary structure of the LPS from B. cenocepacia ET-12 has been determined and its pro-inflammatory activity analyzed, in particular, the ability of purified LPS at inducing TNF-alpha production in human myelomonocytic U937 cells. Moreover, we have investigated and compared the structures of lipopolysaccharides from clonally identical Burkholderia multivorans strains isolated pre- and post- lung transplantation through compositional analysis, mass spectrometry and 2D NMR spectroscopy. The pro-inflammatory activity of extracted LPS was tested as a stimulant of U937 cells cytokine induction. Such data are essential to elucidate the molecular modifications involved in the inflammatory process that occur pre- and post- surgery. Understanding how bacteria modify and adapt their LPS in new physiological conditions may allow greater understanding of LPS associated signalling.

(125) **Synthesis of Heparan Sulfate O-Sulfotransferase Inhibitors to Modulate Heparanome**

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Heparan Sulfate Proteoglycans regulate various pathological, physiological and developmental processes. Gene knockout experiments in model organisms have demonstrated the importance of sulfate codes in numerous developmental processes. However, it is difficult to unravel the role of individual sulfate groups in signaling pathways that are critical for many biological actions in a spatiotemporal manner. To overcome this barrier, we propose a chemical approach to modulate actions of biosynthetic enzymes using a library of novel sulfotransferase inhibitors. The molecular scaffolds are synthesized and examined how they modulate heparanome. We envision that these sulfotransferase inhibitors will enhance our ability to understand the role of HS sulfation patterns that are critical for a range of biological events.

(126) **Lec36: A New Ricin-Resistant Human Cell Line for Engineering Glycoproteins with Hybrid-Type Glycosylation**

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Glycosylation can have a significant impact on the pharmacological properties of therapeutic glycoproteins. Here, we isolate a human embryonic kidney 293T cell line which exhibits hybrid-type glycosylation. The cell line displays sensitivity to ricin that lies between the parental 293T cells, whose secreted and cell-surface tethered proteins are dominated by complex-type glycosylation, and 293S Lec1 cells, which only produce oligomannose-type N-linked glycans. The stem cell marker, 19A, was transiently expressed in the 293T Lec36 cells, and in parental 293T cells with and without the potent Golgi alpha-mannosidase II inhibitor, swainsonine. Negative-ion nano-electrospray ionization mass spectra of the 19A N-linked glycans from 293T Lec36 and swainsonine-treated 293T cells were qualitatively indistinguishable and, as shown by collision-induced dissociation spectra, dominated by hybrid-type glycosylation. Nucleotide sequencing revealed a mutation in Man2a1, encoding Golgi alpha-mannosidase II that maps to the active site. 293T Lec36 cells provide a model for human type II congenital dyserythropoietic anemia, wherein Golgi alpha-mannosidase II activity is disrupted, and a vehicle for the expression of therapeutic glycoproteins with hybrid-type glycosylation.

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(127) **Identification of a Novel Putative Glycosyltransferase in Trypanosoma Brucei**

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Trypanosoma brucei is the causative agent of human African sleeping sickness, a fatal disease if untreated. The parasites are able to evade the host's immune system and establish chronic infections aided by their unique surface coat. It undergoes antigenic variation and is composed of 10 million Variant Surface Glycoprotein (VSG) molecules. Besides VSG, Trypanosomes contain many other less abundant glycosylated proteins with various functions.

Major components of glycan chains in T. brucei are poly-N-acetyllactosamine (poly-LacNAc) structures made of Gal and GlcNAc. It has been demonstrated that Gal and GlcNAc metabolism are essential in T. brucei. UDP-Gal and UDP-GlcNAc-dependent glycosyltransferases (GTs) are therefore potential drug targets.

To identify novel Gal/GlcNAc GTs in T. brucei, we performed bioinformatic analysis of the T.brucei genome. One of the identified putative GTs was named TbGT15. It is present as a single copy gene, which is expressed in both life cycle stages of the parasite. To determine the gene function we have created null mutants in bloodstream-form (bsf) parasites by allelic replacement through homologous recombination. TbGT15 is a non-essential gene. Mutant cells were able to infect mice and were viable in culture. However, they exhibited slightly slower growth kinetics.

Here we report that total glycoprotein (GP) extracts from TbGT15 mutant cells had different lectin binding properties. The altered glycan composition of ricin-binding GPs was determined by methylation linkage analysis. According to the data TbGT15 is a GT active in bsf cells that appears to be involved in the synthesis of poly-LacNAc containing GPs.

(128) **Structural Studies of *Yersinia* Lipopolysaccharides by High-Resolution Electrospray Ionization Mass Spectrometry**

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A zoonotic pathogen *Yersinia pseudotuberculosis* causes chronic intestinal infections in human and is the recent ancestor of *Yersinia pestis*, the cause of plague. Lipopolysaccharide (LPS) is an important virulence determinant of Gram-negative bacteria. *Y. pseudotuberculosis* produces the full S-type LPS together with R- and SR-type LPS having lipid A and an oligosaccharide core with no O-antigen or one O-antigen repeat, respectively. *Y. pestis* possesses only an R-type LPS. Even small alterations in the LPS structure may significantly influence its bioactivity. Owing to the complexity, intrinsic heterogeneity, lability, and amphiphilic character of the LPS, elucidation of its structure by routine techniques is labor-consuming and requires partial degradation(s) often resulting in loss of important structural information. To solve the problem, we applied high-resolution ESI MS with the whole *Yersinia* LPS. Fourier transform ion-cyclotron resonance MS allowed precise molecular mass determination and identification of molecular species. Capillary skimmer dissociation (CSD) cleaved the linkage between ketodeoxyoctonic acid in the core and distal glucosamine of lipid A, thus separating lipid A (Y-fragments) from the core (B-fragments); the latter could easily be distinguished by accompanying fragment ions caused by decarboxylation of ketodeoxyoctonic acid (mass difference -44 amu). Using this approach, we established the LPS structure in Y. pestis wild-type strains and mutants with defects in LPS biosynthesis genes and demonstrated the identity of the R-type LPS structure in *Y. pestis* and *Y. pseudotuberculosis*, including temperature-induced core modifications. Analysis of the SR-type LPS enabled determination of the O-antigen repeat composition in *Y. pseudotuberculosis* strains from various serotypes.

(129) **Control of Sialyl Lewis X Antigen Expression in the Colon by Fucosyltransferase VI and β4GalNAcT-II.**

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Sialyl Lewis X (sLex) is a well known carbohydrate antigen whose overexpression in cancer correlates with metastasis. In colon cancer, the molecular bases of sLex overexpression remain elusive.

We have investigated sLex expression in normal and cancer colonic tissues and in cell lines as a function of the expression of fucosyltransferases and β4GalNAcT-II. This enzyme, which is downregulated in colon cancers, is responsible for the biosynthesis of the Sda antigen [Siaα2,3(GalNAcβ1,4)Galβ1,4GlcNAc], whose biosynthesis competes with that of sLex. In colon cancers and cell lines, sLex expression correlates with a fucosyltransferase activity able to fucosylate 3'sialyllactosamine at low (0.5 mM) concentration. Transfection experiments with FucT-III, IV, V, VI and VII cDNAs in COS-7 cells indicate that only FucT-VI displays this property. In gastrointestinal cell lines, high levels of this fucosyltransferase activity are shown by cell lines expressing high levels of the FucT-VI transcript. In normal colon, sLex antigen is detectable upon de-acetylation but its expression does not correlate with any fucosyltransferase activity or transcript. Rather, we observed a significant correlation between sLex and the ratio between fucosyltransferase activity with 0.5 mM 3'sialyllactosamine and β4GalNAcT-II activity. These data suggest that in both normal and cancer colonic tissues the biosynthesis of sLex is mainly due to FucT-VI or to a fucosyltransferase with similar kinetic properties, unknown at present. In normal colon, but not in colon cancers, the fucosyltransferase activity above described is counteracted by competing glycosyltransferases, like the high β4GalNAcT-II activity that synthesizes the alternative Sda antigen.

(130) **Concentrations in Rats Induced by Dietary Vitamin B6-Deficiency and its Relation to Complex N-GLYCAN Structures of Liver Membrane-Proteins**

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Rats aged 3 weeks or 2 months were fed a vitamin B6-free diet for periods varying from 3 days to 7 weeks. Uracil and cytidine nucleotides were quantified by enzymatic-photometry and liquid chromatography. In 3 week-old rats, vitamin B6-deficiency resulted in an up to 6.3-fold increase in the concentrations of UTP, UDP, UMP and UDP-sugars and less of CTP in liver, while no changes were observed in older rats. The increase started after 1 week diet, with a maximum after 2 weeks. After 5 weeks, the concentrations returned to normal values. In contrast to liver, the heart muscle uracil nucleotide concentrations were decreased by 40% after 2 weeks diet. In kidney, the sum of UTP, UDP and UMP showed a decrease of 40%, whereas UDP-sugars were increased 1.4-fold. In the lungs, nucleotide concentrations were unaffected by vitamin B6-deficiency, despite a 70% increase of UDP-GA. In brain, UDP-Glc, UDP-Gal and the sum of CTP and CDP showed an increase of 30-50%. Surprisingly, the increased UDP-sugar concentrations did not influence the structure of liver plasma membrane-N-glycans. Despite the 4 to 6-fold increase of UTP and UDP-sugars, no changes in complexity or sialylation of these N-glycans could be detected. This study demonstrates that, especially in liver, pyridoxal phosphate is closely related to the regulation of the concentration of uracil nucleotides during a defined period of development. In contrast to in vitro experiments investigating secretory proteins, *in vivo* N-glycan biosynthesis of liver plasma membrane glycans is regulated independently from the substrate concentrations.

(131) **Structural Studies of the O-Chain Polysaccharide of *Aeromonas Caviae* ATCC 15468 Lipopolysaccharide**

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Aeromonas caviae belongs to the family *Vibrionaceae* and is associated with gastrointestinal disease in adults and acute, severe gastroenteritis in children. Although *A. caviae* has been isolated from humans with diarrheal illnesses, its role as a definitive agent of diarrhea remains to be established. The role of lipopolysaccharide (LPS) in the pathogenesis of *A. caviae*-induced gastroenteritis is not well understood but it has been implicated in the adherence to human epithelial cells and biofilm formation. In this study, we report the structural characterization of the O-chain polysaccharide of *A. caviae* ATCC 15468. Mild hydrolysis of LPS afforded an O-specific polysaccharide composed of L-rhamnose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose and phosphoglycerol. Subsequent methylation and CE-ESIMS analyses and 1D/2D NMR (^{1}H, ^{13}C, and ^{31}P) spectroscopy showed that the O-chain polysaccharide is a polymer of tetrasaccharide repeating units containing a phosphoglycerol substituent and having the structure -3)-β-D-GalpNAc-(1-4)-[Gro-1(PO4 $^{-}$-3)]-β-D-GalpNAc-(1-4)-β-D-GlcpNAc-(1-4)-α-L-Rhap-(1-, where Gro is glycerol.

(132) **Probing the Role of Calcium Ion in Catalysis of Class 1 Mannosidase by Metal Reconstitution Experiments**

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Mammalian Class I α1,2-mannosidases (GH 47) play critical roles in the maturation of Asn-linked glycoproteins in the endoplasmic reticulum (ER) and Golgi complex as well as influencing the timing and recognition for disposal of terminally misfolded proteins during ER-associated degradation. Several aspects of the inverting catalytic mechanism for these enzymes are novel. First, a water residue was found to bridge between the catalytic general acid and the glycosidic oxygen during catalysis. Second, an enzyme-bound Ca^{2+} ion is directly involved in both catalysis and binding of the glycone. Third, the catalytic water nucleophile in the inverting hydrolytic mechanism is also directly coordinated to the enzyme-bound Ca^{2+} ion. Forth, previously mutagenesis and structural studies demonstrated that changing the Ca^{2+} coordination from 8-fold to 7-fold coordination greatly reduced the enzyme efficiency. We have further tested the mechanistic role of the enzyme-associated divalent cation by metal-exchange experiments. We performed a detailed study of steady state and transient enzyme kinetics, binding and structural analyses of human ER mannosidase I in the presence of various divalent and trivalent cations compared to control reactions with a bound Ca^{2+} ion. The results suggest that the nucleophilic water is deprotonated by general base and its hydroxide ion is stabilized via Lewis-acid-base interaction with the calcium ion. (Supported by NIH grants GM47533 and RR05351)

(133) **Defining the Molecular Basis for Associations among Glycosphingolipid Glycosyltransferases**

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Glycosphingolipids, molecules comprised of ceramide and various oligosaccharides, are synthesized in the ER and Golgi by numerous membrane-bound glycosyltransferases. These glycosyltransferases catalyze the addition of monosaccharides in a sequential fashion, resulting in a diverse assortment of glycosphingolipids. Glycosphingolipid biosynthetic enzymes have been shown to associate with one another, forming biosynthetic clusters. Previous work demonstrated that associations among these enzymes are controlled by their N-terminal regions, which include their single-pass transmembrane (TM) domains. To characterize interactions among the TM domains of five glycosphingolipid

glycosyltransferases, we employed an assay that measures TM domain association in SDS micelles. We discovered that three of the TM domains homo-oligomerize. We have used the same assay to identify the polar and charged amino acids that control oligomerization. In addition, we used fluorescence microscopy and Western blot analysis to investigate enzyme homo-oligomerization occurring in transiently transfected mammalian cells. In ongoing work, we are investigating how the observed enzyme oligomerization affects glycosphingolipid production.

(134) Mouse Embryo Fibroblasts from N-Acetylglucosaminyltransferase Va Null Mice Exhibit Altered Glycosyltransferase and Galectin Transcript Expression

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Many studies show that alterations in N-linked oligosaccharides of tumor cells are associated with tumorigenesis, progression and metastasis. Profound effects on cell-cell and cell-matrix adhesion are observed, for example, after changes in N-linked beta1,6 branched glycan expression synthesized by N-acetylglucosaminyltransferase Va (GnT-Va, Mgat5), that result in altered cell motility and invasiveness. Knockout of GnT-Va resulted in reduced progression of mouse mammary carcinomas driven by the polyoma middle T oncoprotein. To investigate molecular mechanisms that caused inhibition of tumor progression in GnT-Va null mice, we isolated mouse embryo fibroblasts (MEFs) from GnT-V knockout mice and studied the effects of the elimnation of GnTVa expression on N-glycans and transcript profiles of groups of glycosyltransferases and galectins related to tumor progression. GnT-V null MEFs showed decreased cell growth and survival, accompanied by reduced activation of PKB and ERK stimulated by EGF or serum. Knock-out of GnT-V caused aberrant expression of N-glycan structures detected by lectin binding, including diminished N-linked beta(1,6) branching, reduced poly-N-lactosamine, but enhanced bisecting N-GlcNAc and alpha2,6 sialic acid-terminating glycans. Using quantitative real-time PCR, altered gene expression profiling of several groups of glycosyltransferases and galectins, which showed both up- or down-regulation, were observed in GnT-V null MEFs, supporting the changes in N-glycan structures observed after deletion of GnT-Va.

Reference: Granovsky et al., Nature Med., 6, 306.

(135) Glycogene Expression Analysis of the Conjunctival Epithelium in Patients with Dry Eye

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The stratified, non-keratinized epithelia of the ocular surface mucosa are covered by hydrophilic cell surface glycans. This wet-surfaced phenotype is lost in patients with dry eye, a common ocular surface disease characterized by epithelial damage, loss of goblet cells and, in late stages, keratinization. The purpose of this study was to identify differences in the expression of genes involved in cell surface glycosylation at the conjunctival epithelium of dry eye patients, using a GLYCOv3 oligonucleotide microarray. In normal conjunctiva, 488 glycogenes (48% of the total) were expressed. In dry eye, 53 of them were significantly reduced ($p<0.05$). Among the glycosyltransferases, three genes involved in the modification of heparan sulfate, known to be present on epithelial cell surfaces, were downregulated. Those included HS3ST6 (ratio:0.7;p=0.043), EXTL2 (0.7;p=0.004), and HS2ST1 (0.6;p=0.002). Interestingly, more than one third of the genes involved in Notch signaling pathway were also downregulated: Notch receptors 1 (ratio:0.6;p=0.003), 2 (0.7;p=0.032), 3 (0.7;p=0.023), and their ligands Jagged 1 (0.7;p=0.024) and Delta 1 (0.7;p=0.023). The only gene upregulated in dry eye was interferon-induced transmembrane protein-1 (ratio:2.5;p=0.026). These data suggest that glycosaminoglycans may contribute to the hydrophilic character of the ocular surface epithelial glycocalyx. Alterations in Notch signaling could affect the normal differentiation of this epithelium and therefore play a role in the pathogenesis of dry eye. These findings open new therapeutic possibilities for dry eye. Support: NIH EY014847 and GM62116.

(136) Xylosyltransferases of the Pathogenic Fungus *Cryptococcus Neoformans*

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Cryptococcus neoformans is a pathogenic fungus responsible for serious disease in immunocompromised individuals, including both pulmonary infection and a fatal meningoencephalitis. A polysaccharide capsule made primarily of two xylose-containing polysaccharides surrounds the cryptococcal cell wall. This capsule, composed of mannose, xylose, galactose, and glucuronic acid, is essential for fungal virulence. As part of our studies of the capsule biosynthetic pathway, we have investigated xylosyltransferases of this opportunistic pathogen. We initially purified one β-1,2-xylosyltransferase with specificity appropriate for capsule synthesis, and found that it represents a new family of large fungal glycosyltransferases (Klutts *et al*, JBC (2007) 282:17890). We have now identified a second xylosyltransferase with the same activity. We have made single and double deletions of the genes encoding these enzymes, and examined the phenotypes of the resulting mutants for changes in biochemical features, morphology, and virulence. We find that these enzymes are needed for normal capsule polysaccharide synthesis, glycosphingolipid synthesis (see presentation by Owuor *et al*), capsule ultrastructure, and for fungal virulence in animal models of infection.

(137) Xylosides Modulate Zebrafish Development

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Heparan sulfate proteoglycans (HSPGs) play a major role in the development of various organisms including zebrafish. The biosynthesis of this complex glycoconjugate requires many enzymes, which are very tightly regulated. Gene knock-outs of the biosynthetic enzymes are often lethal and exemplify the importance of HSPGs in development. We hypothesize that modulation of HS biosynthesis using chemical approaches will reveal the fine structural requirements of HSPGs in developmental biology. We have examined a number of xylosides, which are known to prime heparan sulfate and chondroitin sulfate chains independent of core protein, and their effects in FGF mediated signaling in zebrafish development. Here we present the effect of these xylosides on zebrafish development and the plausible mechanism of action of these molecules in signaling pathways that are essential for normal zebrafish development.

(138) Analysis of the Role of Galectin-3 in Corneal Neovascularization

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Purpose: Corneal neovascularization (NV) is associated with a number of corneal conditions that result in blindness for example trachoma and hepatic keratitis. Furthermore, vascularization of the corneal bed in corneal transplant recipients significantly increases the risk of graft rejection and failure. The purpose of this study is to determine the role of a carbohydrate-binding protein, galectin-3, and its inhibitors in the process of NV.

Methods: Capillary tubule formation assays and the mouse corneal micropocket assays were performed using HUVEC to determine whether galectin-3 promotes angiogenesis *in vitro* and *in vivo*, respectively. Experiments were also conducted to determine whether galectin-3 induced as well as VEGF- and/or bFGF-induced angiogenesis can be inhibited by the saccharide inhibitors of galectins.

Results: Galectin-3 promoted capillary tubule formation of HUVEC cells *in vitro*, as well as, the formation of vessels *in vivo* in the corneal micropocket assay in a dose-dependent manner. Galectin-3-mediated capillary tubule formation was inhibited by competing saccharides, 0.1M β-lactose and 0.1% modified citrus pectin (MCP), but not by noncompeting saccharides, sucrose or citrus pectin (CP). Also, galectin-3 induced angiogenesis was markedly reduced in corneal pocket assays in the presence of the saccharide inhibitors of galectin-3. Of particular significance is our findings that VEGF- and bFGF- mediated migration and capillary tubule formation were also inhibited by specific inhibitors of galectin-3.

Conclusions: Galectin-3 promotes angiogenesis *in vitro* and *in vivo* via a carbohydrate-based recognition system. The carbohydrate-based recognition, most likely, also plays a role in VEGF- and bFGF-mediated angiogenesis.

(139) **An Approach for the Identification of O-Mannose Modified Proteins from Mouse Brain**

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Modification of mammalian proteins by O-Mannose-initiated glycans has received increased attention recently due to the implicated role of these structures in congenital muscular dystrophy. Multiple forms of these diseases result from defects in glycosyltransferases involved in the addition and extension of O-Mannose, including Muscle-Eye-Brain disease (MEB) that is caused by mutations in the gene encoding protein O-mannose β-1,2-glucosaminyltransferase (POMGnT1). We have characterized via LC-MS^n the permethylated O-linked glycans released by reductive elimination from brain proteins of wildtype and POMGnT1 knockout mice. As expected, ~30% of the observed O-linked glycans released from wildtype brain proteins are O-Man initiated and elongated O-Man glycans were not detected in the POMGnT1 knockout brains. To date, the only well characterized O-mannose modified mammalian protein is alpha-dystroglycan. Since the O-Man-initiated structures are so important in disease processes, we have developed a strategy for identifying the full complement of O-Man modified proteins. As proof of principal, a synthetic alpha-dystroglycan derived O-Man modified peptide has been extended with azido-modified GlcNAc using recombinant, purified POMGnT1 and UDP-GlcNAz. Staudinger ligation was subsequently used to attach a FLAG tag to this peptide for affinity purification. An alternative approach was developed in which GlcNAc was added with POMGnT1 to the O-Man peptide followed by addition of a keto-modified Gal using a mutant GalT. This keto-sugar can then be reacted with aminoxybiotin for affinity purification. A comprehensive identification of the full diversity of O-Man modified proteins will result from applying these tagging strategies to glycopeptides generated from POMGnT1 knockout brains.

(140) **Development of Novel Tissue Engineering Technologies: Metabolic Engineering of Thiol Groups into the Surface Sialic Acids of Human Stem Cells**

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In a recent pilot study, we used the 'metabolic oligosaccharide engineering (MOE)' strategy to install thiol groups into surface sialic acids of human embryoid body derived (hEBD-LVEC) stem cells using the N-acetyl-D-mannosamine (ManNAc) analog Ac_5ManNTGc (**1**) (*Nat. Chem. Biol.* **2**, 149-152 (2006)). Interestingly, cells treated with **1** underwent neuron-like differentiation via Wnt signaling. In this report, we describe the further development of this newfound method for controlling stem cell fate into a useful tool for the tissue engineering community. First, to optimize analog design, cell surface thiol (CST) expression was compared in several cell lines incubated with sulfur-containing ManNAc analogs with varying *N*-acyl chain length. Cell surface thiol (CST) expression was inversely related to the *N*-acyl side chain length when studied with 3-acetylthiopropanoyl and 4-acetylthiobutanoyl derivatives. Far-western analysis of cell lysates after CST-biotinylation showed a substantial increase in thiolated proteins, indicating analog incorporation into protein-linked glycans. CST expression was efficient in Jurkat, hEBD-LVEC, and HEK293 cells, while the blasotcyst-derived BG01 (human stem cells) exhibited only a moderate increase, possibly due to interference in analog uptake by cell-secreted ECM components. Finally, to test whether pathways beyond Wnt signaling were involved in differentiation, gene expression was evaluated in LVEC cells using focused microarrays on signaling pathways associated with stem cell maintenance and differentiation. The array results established that the chemical composition of both the growth substrate (collagen *vs* gold) and interacting cell surface glycans affect important genes – such as WNT4, WNT7B, WNT9A and DVL1 – involved in stem cell differentiation.

(141) **Hexosamine Signaling Impacts in C. elegans Carbohydrate Metabolism**

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Perhaps one of the most important post-translational modifications of nuclear and cytoplasmic proteins is the attachment of O-linked N-Acetyl- giucosamine (OGlcNAc) to the serine and threonine residues. This dynamic cycle of O-GlcNAc addition and removal is mediated via a pair of enzymes such as O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). We have previously reported that, among the several knock-out mutants of C. elegans examined, the OGT mutant, lacking the transferase, produced a significant rise in both trehalose and glycogen whereas the OGA mutant remained inert to these changes. Follow-up studies, including examination by micro array and quantitative PCR, confirmed the significant rise in specific hexosamine signaling pathway (HSP) regulatory genes for OGT and the corresponding lack of response for OGA. More recently, the inclusion of an alternate OGT knock-out allele (TM1040) has provided new insight into the manner in which the HSP is affected. These studies have now been extended to an examination of the carbohydrate pattern present during the several larval stages of this organism. In each case, the carbohydrate moiety was found to be characteristically altered during maturation up to, and including, the adult form.

(142) **Protein *O*-Mannosyltransferases 1 and 2 Are Required for Dystroglycan Function in *Drosophila***

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In vertebrates, mutations in Protein *O*-mannosyltransferase1 (POMT1) or POMT2 are associated with muscular dystrophy due to a requirement for *O*-linked mannose glycans on the Dystroglycan (Dg) protein. In this study we examine larval body wall muscles of *Drosophila* mutant for *Dg*, or RNAi knockdown for *Dg*, and find defects in muscle attachment, altered muscle contraction and a change in muscle membrane resistance. To determine if POMTs are required for Dg function in *Drosophila* we examine larvae mutant for genes encoding POMT1 or POMT2. Larvae mutant for either POMT, or doubly mutant for both, show muscle attachment and muscle contraction phenotypes identical to those associated with reduced Dg function. Further, genetic interactions between POMTs and *Dg* mutant alleles indicate these genes function in the same genetic pathway. Together these data establish a requirement for POMTs for Dg function in *Drosophila* and opens the possibility of using *Drosophila* to study the role of glycosylation in muscular dystrophy.

(143) **Galectin-3 Promotes Formation of Lamellipodia in Corneal Epithelial Cells by Interacting with α3β1 Integrins**

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Purpose: We have previously shown that: (i) re-epithelialization of corneal wounds is significantly slower in galectin-3-deficient mice compared to the wild type mice, and (ii) the exogenous addition of galectin-3 stimulates re-epithelialization of corneal wounds in a mouse animal model (J. Biol. Chem. 277:42299-42305, 2002). In an effort to define the molecular mechanism by which galectin-3 stimulates the cell migration, the goals of the present study are to: (i) characterize the lectin-induced migratory phenotypes in human corneal epithelial cells *in vitro*, and (ii) determine whether carbohydrate-based interactions between galectin-3 and integrins play a role in the lectin-induced lamellipodia formation in corneal epithelium.

Methods: The human corneal epithelial cells in culture were exposed to recombinant human galectin-3, stained with phalloidin and examined by fluorescence microscopy. The galectin-3-binding proteins were isolated by affinity chromatography of corneal epithelial cell lysates on a galectin-3-bound Sepharose column, and were identified by MALDI-TOF MS and Western analyses.

Results: As early as 30 minutes after treatment with galectin-3, a significant number of cells showed changes in cell shape with filopodial and lamellipodial extensions. In contrast, galectins-1 and -8 had no effect. Also, the galectin-3-induced lamellipodia were inhibited by a competing sugar, β-lactose. α3β1 integrins and laminin-5 were identified as major galectin-3 binding proteins in corneal epithelial cells. Preincubation of cells with anti-α3 integrin function-blocking antibody inhibited the galectin-3-induced lamellipodia significantly.

Conclusions: Galectin-3 influences re-epithelialization of corneal wounds by initiating the formation of lamellipodia and filopodia in corneal epithelial cells through its interaction with α3β1 integrins.

(144) **Regulation of HNK-1 and Polysialic Acid Expression on Neural Cell Adhesion Molecule for Distinct Roles of Neural Cells**

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Polysialic acid, a homopolymer of α2,8 linked sialic acid, and HNK-1, a sulfated and glucuronylated glycan, play important roles in brain development. Polysialic acid is mainly attached on neural cell adhesion molecule (NCAM) in embryo and neonatal brain, while HNK-1 is widely found on glycolipids, proteoglycans, and glycoproteins including NCAM throughout life. To understand roles of each glycan and whether the two molecules function cooperatively or not in neural development, we first analyzed their expression patterns. Polysialic acid was highly expressed in embryonic hippocampal neurons and gradually decreased during culture *in vitro*, in contrast to HNK-1 expression, which increases during culture. We determined that human NCAM bears HNK-1 glycan on 1st, 3rd, 5th, and 6th N-glycosylation sites, while polysialic acid on 5th and 6th sites. When glucuronyltransferase (GlcAT-P) was transiently expressed in polysialic acid-positive RD cells, the cells acquired HNK-1 instead of polysialic acid. Sialyltransferases and GlcAT-P compete in initiating the synthesis of PSA and HNK-1 glycan since both enzymes utilize the same acceptor glycan (Galβ1→4GlcNAc). Polysialic acid reduced NCAM-mediated cell aggregation, but HNK-1 did not. However, the same cell lines expressing NCAM with either polysialic acid or HNK-1 promoted neurite outgrowth at the same level, demonstrating different mechanisms by distinct carbohydrates to function in neural development. Our results suggest that a balance of two synthetic pathways can be a key factor to control of the functions displayed by these glycans in neural cells. (Supported by CA33895)

(145) **The Role of UDP-GalNAc:polypeptide N-Acetylgalactosaminyltransferases during Drosophila Development**

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Mucin-type O-linked protein glycosylation is initiated by the action of an evolutionarily conserved family of enzymes known as the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (ppGaNTases in mammals or pgants in Drosophila) (EC 2.4.1.41). The acquisition of carbohydrate side chains has a profound structural impact on a polypeptide backbone and thus highlights the unique physicochemical properties of O-glycosylated proteins such as mucin glycoproteins, which function as lubricants, protectants and mediators of signal transduction. The evolutionary conservation of members of the large ppGaNTase gene family in Drosophila and mammals suggests that certain genes serve unique and important functions in conserved aspects of development. In an effort to identify the role played by these enzymes during development, we have constructed transgenic fly lines containing Gal4-inducible RNAi vectors for the pgant genes shown to have unique developmental expression patterns during embryogenesis. Induction of RNAi to pgants at specific times and in specific organs systems resulted many unique phenotypes as well as lethality. Specifically, the knockdown of one transferase resulted in wing blisters, which are known to be caused by defects in cell adhesion between the epithelial layers that comprise the wing. Additionally, other phenotypes observed were reminiscent of those associated with disruption of signal transduction pathways. Our results suggest diverse roles for O-glycans in conserved developmental processes, such as cell adhesion and signal transduction. Current work is focused on defining the repertoire of O-glycoproteins that mediate these developmental effects.

(146) **Defective Influenza-Specific B Cell Responses in Mice Lacking Expression of ST6Gal 1**

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Post-translational modification of proteins, such as glycosylation can affect the localization of cell surface glycoproteins on the cell membrane, impacting cell signaling and function. ST6Gal 1 is a glycosyltransferase expressed by T and B cells that catalyzes the addition of alpha2,6 sialic acid to galactose, a modification typically found on N-linked glycoproteins such as CD45. In this study, we show that in contrast to activated T cells, ST6 Gal 1 expression remains high on plasma blasts and germinal center B cells following viral infection. To determine the *in vivo* role of the loss of this enzyme during viral infection, we infected ST6Gal I null mice with influenza A/HK x31. We demonstrate that loss of ST6Gal 1 expression results in similar infectivity in the lung at day 3, but significantly reduced influenza-specific IgM and IgG levels in the serum, as well as significantly reduced antibody-forming cells (AFCs) in the MedLN and spleen in the acute phase of influenza infection. By day 100 post-infection, despite similar overall levels of influenza-specific antibody in the serum, defects persist in the memory B cell compartment. These studies suggest that loss of ST6Gal 1 expression significantly impairs the viral specific B cell immune response and that differential expression of surface carbohydrates may impact the programming of memory B cells.

(147) **Galectin-8 Promotes Cytoskeletal Rearrangement in Trabecular Meshwork Cells through Activation of Rho Signaling**

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Primary Open Angle Glaucoma is a major blinding disease, characterized by elevated intraocular pressure due to insufficient outflow of aqueous-humor. The trabecular meshwork (TM) lining the outflow pathway modulates aqueous outflow facility. TM cell adhesion, cell-matrix interactions and Rho signaling in TM cells are thought to play key roles in the regulation of aqueous outflow. Here, we demonstrate that Galectin–8 (Gal8) modulates Rho signaling in TM cells. Specifically, we show that Gal8 is expressed in the TM and that TM cells adhere to and spread on Gal8-coated wells but not on galectin-1 or galectin-3-coated wells. The adhesion of TM cells to Gal8-coated wells is abolished by β-lactose, a competing sugar, but not by sucrose, a non-competing sugar. Experiments using glycan arrays revealed that Gal8 has a high affinity for a number of glycans including Galβ1-3GalNAc and NeuAcα2-3Galβ1-3GalNac and 3-O'-sulfo-Galβ1-4GlcNAc for which Galectins-1 and-3 have low affinity. Affinity chromatography using Gal8-columns identified α3β1, α5β1 and αvβ1 integrins as major countereceptors of Gal8 in TM cells. A function-blocking anti-β1 integrin antibody inhibited TM cell-adhesion to Gal8-coated wells. Cell spreading on Gal8 was associated with the accumulation of phosphorylated myosin light chain and the formation of stress fibers that was inhibited by the Rho inhibitor, C3 transferase, and by the Rho-kinase inhibitor, Y27632. Together, these findings present a novel function for Gal8 in activating Rho signaling in TM cells that may allow Gal8 to participate in the regulation of aqueous outflow facility. Support: NIH: EY 015168 (NP), GM62116 (JP); Research to Prevent Blindness

(148) **Mesenchymal Stem Cells: Metabolic Labeling and Detection of Glycoprotein Markers of Differentiation**

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Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into a variety of cell types including bone, adipose, and cartilage. Although MSCs express a number of glycosylated cell surface markers, they are not specific to MSCs, and are phenotypically heterogeneous depending upon tissue source and species. The goal of this study is to characterize changes in cell surface glycoprotein expression during MSC differentiation and to identify glycoprotein markers unique to differentiated cell types. Here we apply a metabolic labeling approach for the detection of glycoprotein markers before and during MSC differentiation into three different cell types, bone, adipose, and cartilage. The two-step labeling method involves the metabolic incorporation of unnatural azido-modified sugars into protein glycans and subsequent ligation with fluorescent azide-reactive detection probes utilizing the copper (I)-catalyzed cycloaddition reaction between azides and alkynes, or "click" chemistry. The glycoprotein detection reaction is highly sensitive, selective, and is performed under standard protein biochemistry conditions. Metabolic labeling of O-linked and sialic acid-containing glycoproteins was accomplished by feeding cells unnatural tetraacetylated azido-modified sugar precursors, GalNAz and ManNAz, respectively, for 72 hours prior to harvesting the cells. After labeling, the cells were lysed, and protein extracts were reacted with fluorescent alkyne detection probes. Our results demonstrate highly efficient labeling of O-linked glycoproteins and sialic acid containing glycoproteins in all cell types, as determined by gel electrophoresis and fluorescence imaging. Significant changes in cell surface glycoprotein profiles are seen upon differentiation into different cell types. Key glycoprotein markers are being identified by mass spectrometry.

(149) **Glycomics Analysis of Murine Stem Cells**

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Glycans attached to proteins and lipids impart a variety of biologically important and physiologically relevant functions like cell-cell interactions, adhesions, differentiation, lymphocyte homing, sperm-egg recognition, host-pathogen recognition, etc. Hence, complete structural analysis is crucial in understanding the chemistry and biology of glycans. In recent years, it has been shown that mass spectrometric strategies coupled with permethylation are highly sensitive tools for glycomics analysis of small quantities of tissues and cells. Here, we present a glycomic analysis of undifferentiated D3 mouse embryonic stem cells (mES). N-Glycans from D3 mES cells contained predominantly high mannose structures together with core fucosylated complex glycans. The abundance of bi-antennary structures was significantly high compared to tri- and tetra-antennary glycans within the complex N-glycans. Key ligands present on the termini of N-glycans were LeX/A along with sialyl (NeuAc and NeuGc) lactose and Gal-α-Gal structures. O-Glycans from D3 mES cells encompassed both Core 1 and Core 2 structures which were capped with NeuAc, LeX and Gal-α-Gal. Further glycomics studies are in progress with differentiated and knock out ES cell lines from mouse and human.

(150) **Roles for O-Fucose Glycans in Notch Signaling**

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The Notch signaling pathway regulates cell fate choices during both vertebrate and invertebrate developement. Notch is modified by a protein O-fucosyltransferase-1 (Pofut1) that attaches fucose to

Ser or Thr in EGF domains and by Fringe, a βeta;3N-acetylglucosaminyltransferase (βeta;3GlcNAcT) that transfers GlcNAc to O-fucose in EGF repeats. Notch1 EGF repeat 12 (EGF12) in the ligand binding domain is modified with O-fucose. In co-culture Notch signaling assays Notch1 with the mutation T466A, which precludes the addition of O-fucose to EGF12, has markedly reduced signaling. We have shown that this is due to the loss of fucose rather than the amino acid change. Thus, replacing Ala with Ser at aa 466 restores O-fucosylation and the signaling activity of Notch1. Restored signaling may be due to the addition of O-fucose or elongation with GlcNAc, Gal and sialic acid initiated by the action of Fringe. Lunatic fringe positively regulates Delta-1 induced Notch1 signaling and negatively regulates Jagged-1 induced Notch1 signaling. Previously we showed that the presence of Gal on O-fucose glycans and βeta;4galactosyltransferase-1 are necessary for Fringe to modulate Jagged-1-induced signaling. Using several different Chinese hasmer ovary (CHO) cell glycoslylation mutants in the co-culture Notch signaling assay, we now show that βeta;4galactosyltransferase-1 is required for potentiating Notch1 signaling induced by Delta1. Experiments in progress aim to identify mechanisms by which O-fucose glycans modulate ligand-induced Notch1 signaling.

(151) **O-Fucosylation of Thrombospondin Type I Repeats Is Essential for Normal Gastrulation in Mouse Embryos**

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Thrombospondin type 1 repeat (TSR) superfamily members play roles in cellular functions such as cell motility and inhibition of angiogenesis. Protein O-fucosyltransferase 2 (Pofut2) is predicted to add O-fucose to TSRs of this diverse family of proteins. By analogy to O-fucose on EGF repeats in the Notch family of receptors, we predict that O-fucosylation of TSRs will be important for protein maturation and/or function. To determine the function of TSR O-fucosylation, we characterized the effects of a gene trap disruption in Pofut2. Here we show that Pofut2 is ubiquitously expressed in early post implantation mouse embryos and is essential for normal gastrulation. Mouse embryos lacking Pofut2 activity died by mid-gestation with the abnormal phenotype appearing after onset of gastrulation (E6.5). Histological analysis and characterization of gene expression in mutant embryos suggests that both extra-embryonic and embryonic cell types are present, yet the distribution and organization of embryonic tissues is abnormal. The embryo appears constricted at the embryonic/extra-embryonic ectoderm border, and extra-embryonic ectoderm appears abnormal. Most striking is the apparent abundance of mesoderm in the embryo at the expense of embryonic ectoderm. Despite abundance of mesoderm, extra-embryonic tissues normally comprised of mesoderm, such as amnion, chorion and allantois, are absent. These results suggest that loss of Pofut2 promotes epithelial to mesenchymal transition, and provides evidence that O-fucosylation of TSRs normally restricts the differentiation of mesoderm to posterior primitive streak. This work was supported by CA12307101 to RSH and GM5396407 to BCH.

(152) **Genes Contributing to Synthesis of Heparan Sulfate in *Drosophila***

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Heparan sulfate (HS) proteoglycans play important roles in the control of cell growth and pattern formation of tissues during developmental processes. HS consist of the linkage tetrasaccharide and the subsequent repeating disaccharide units with various degree of sulfation. In *Drosophila*, the genes contributing to synthesis of repeating disaccharide units in HS such as *ttv*, *sotv*, *botv*, *Hs6st*, *Hs3st-B*, *Hs2st*, and *Paps* (*PAPS synthetase*) have already been reported; however, there are several unknown genes contributing to synthesis of HS such as the genes synthesizing the linkage tetrasaccharide and transporting the PAPS, which is a donor substrate of sulfotransferases. We isolated *β4GalT7*, *β3GalTII*, *PAPST1* (*sll*), and *dPAPST2*, determined their substrate specificity, and performed their functional analysis by using RNAi mutant flies. The knockdown of *β4GalT7*, *sll*, and *dPAPST2* in whole body by using *Act5C-GAL4* caused lethality or semi-lethality; it demonstrated that these genes are essential for the viability of the fly. *β3GalTII* exhibited genetic interactions in the wing with *β4GalT7* and in the eye with seven genes that encode enzymes contributing to the synthesis of HS. *dPAPST2* also displayed genetic interactions in the wing with *β4GalT7* and in the eye with four genes contributing to the synthesis of HS. Finally, *sll* genetically interacted with *dPAPST2* in the eye. Moreover, the knockdown of *β3GalTII* resulted in the decreased amounts of HS proteoglycans and the abnormal distribution of extracellular Wg. These results indicate that *β4GalT7*, *β3GalTII*, *sll*, and *dPAPST2* contribute to synthesis of HS *in vitro* and *in vivo*.

(153) **O-Glycan Expression and Function in Tubulogenesis**

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Carbohydrates on secreted and cell-surface molecules have been assumed to have profound effects on cell-cell interactions, adhesion and signaling events during eukaryotic development. Our group studies mucin-type O-linked glycosylation, which is an evolutionarily conserved protein modification initiated by the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase enzyme family. Recent studies from our group have demonstrated one member of this family in Drosophila (pgant35A) is essential for viability during multiple stages of development, especially tracheal tube formation during embryogenesis. Using lectins and carbohydrate-specific antibodies, the diversity of O-glycan patterns in developing tissues could be visualized by confocal microscopy. Notably, O-glycans are highly expressed on the apical surface of a number of ectodermally derived tissues such as the salivary glands, developing gut and tracheal system, suggesting roles for O-glycans in cell polarity and tube formation common to these organs. Recent studies of pgant35A mutants by immunostaining using organelle markers revealed altered patterns of intracellular trafficking. Ultrastructural analysis by transmission electronic microscopy showed irregular apical membranes and loss of the ladder-like septate junction structures. These results suggest that mucin-type O-linked glycosylation is important for the maintenance of proper apical composition of the tracheal system by influencing apical delivery and luminal deposition of proteins/glycoproteins. Our current work focuses on the identification of O-linked glycoproteins present in these organ systems in an effort to understand the role O-glycans play in diverse processes in both vertebrate and invertebrate development.

(154) **Glycoform-Focused Reverse Genomics (GFRG) in Spermatogenesis**

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Alpha-mannosidase II (MII) and alpha-mannosidase IIx (MX) are key enzymes that catalyze the first step in conversion of hybrid to complex-type N-glycan in the Golgi apparatus. Fukuda and our group previously reported that the MX KO mice are infertile and identified a unique N-glycan that plays an important role for spermatogenesis matured by germ cell-Sertoli cell adhesion (1). We also elucidated the minor but unusual N-glycan structures in MII/MX double KO mice using a developed systematic glycomic approach (2).

In this study we further assessed detailed testis glycome of both the MX KO mice and the wild type based on recently developed glycoblotting technique (3). Our analysis revealed the quantitative testis glycome including both neutral and sialylated N-glycans for the first time. A GlcNAc terminated tri-antennary and fucosylated N-Glycan, of which expression significantly decreased in MX KO testis, was regarded as a tag to identify a cluster of candidate proteins crucial for spermatogenesis and we could successfully identify a few glycopeptides from the tryptic digests of whole glycoproteins. Thus differentially expressed N-glycan between the multiple states can be focused as a tag to rationalize in developing focused proteomics/genomics, therefore we are proposing to call the method to glycoform –focused reverse genomics (GFRG). A technical aspect on purification and identification of targeted glycopeptides will be also discussed.

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(155) Proliferation- and Gene Expression-Analysis in GNE-Deficient Embryonic Stem Cells

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Sialic acids are widely expressed as terminal carbohydrates on glycoconjugates of eukaryotic cells. They are involved in a variety of cellular functions, such as cell adhesion or signal recognition. The key enzyme of sialic acid biosynthesis is the bifunctional UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE), which catalyzes the first two steps of sialic acid biosynthesis in the cytosol.

Previously, we have shown that inactivation of the GNE by gene targeting causes early embryonic lethality in mice, whereas heterozygous GNE-deficient mice are vital [1], although the overall sialylation is reduced by 25% in these mice [2]. Furthermore, we have identified GNE-interacting proteins, which are not related with its enzymatic activity [3].

In this study, we compared I. the proliferation and II. the gene expression profile of GNE-deficient embryonic stem cells with GNE-expressing embryonic stem cells in the absence or presence of the sialic acid precursor ManNAc. We found that the proliferation is directly correlated with GNE-expression and the cellular sialic acid concentration. In addition we present a detailed gene expression profile of GNE-deficient embryonic stem cells.

(156) Gangliosides Govern Signaling Networks Activated in Human Embryonic Cells by Moderate Strength Static Magnetic Field (SMF) Exposure

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This report describes the critical role of the two-way conversion between LacCer and ganglioside GM3 in governing signaling networks that respond to magnetic exposure. In this work, human embryoid body derived (hEBD) cells were exposed to moderate strength (~0.2 T) static magnetic fields (SMFs) for up to 6 days and resulting changes to gene expression were analyzed by global mRNA profiling. Evaluation of the microarray results with the Ingenuity Pathway Analysis software tool identified nine signaling networks that responded to SMF exposure. To validate the relevance of the microarray analysis, a series of experiments were performed centered on the inflammatory cytokine interleukin-6 (IL-6). The short-term (over one day) activation of IL-6 involved coordinate up-regulation of toll-like receptor 4 (TLR4) in a 'feed-forward' manner that enhanced both factors. Accompanying the TLR4 and IL-6 responses were concomitant changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 levels by as much as 70%. We show that these changes to gangliosides provide a controlling mechanism overlaid on the TLR4 and IL-6 signaling responses that augment pathway activation in the short term and attenuate the global cellular responses to SMF over longer exposure periods. These results form a framework that describes how the effects of magnetic exposure are transduced from the most-plausible molecular-level 'biosensor' (lipid microdomains) to phenotypic and behavioral responses that include neural differentiation. Finally, it is noteworthy that the connections between IL-6 and GM3 were maintained in the absence of SMF, establishing that gangliosides play a general role in regulating interleukin signaling.

(157) Characterization of N-LINKED Glycans on the Drosophila Sialyltransferase Protein, DSiaT by Mass Spectrometry

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The aim of the present study is to determine the structure and positions of glycosylation sites on the Drosophila sialyltransferase protein, DSiaT. The DSiaT represents the first characterized sialyltransferase in the protostome lineage of animals. This sialyltransferase is closely related to the ST6Gal family of vertebrate sialyltransferases, which indicates that DSiaT may represent the most evolutionary ancient type of matazoan sialyltransferases. The DSiaT-Protein A fusion protein has been expressed in Drosophila S2 culture cells and purified as described earlier (Koles et al. 2004, JBC 279: 4346-4357).

The DSiaT from a Coomassie Blue stained polyacrylamide gel were excised and then performed tryptic digestion in gel. The N-linked oligosaccharides were released from the glycopeptides extracted from gel pieces. The released N-glycans are permethylated and then analyzed by both MALDI-MS and NSI-MS/MS. To capture all glycans, automated MS/MS (total ion mapping) was also performed. MALDI-MS spectrum revealed that the main glycan of DSiaT has a fucosylated high-mannnose structure, Man3GlcNAc2Fuc1 (m/z = 1345). Another glycan with a high-mannose structure, Man3GlcNAc2 (m/z = 1171) was also detected as a minor component. In addition, the minor ions observed in the NSI-MS spectrum included more than ten glycans with high mannose structures. The all glycans found in NSI-MS spectrum were confirmed by NSI-MS/MS. The sites of N-linked glycosylation will also be determined using LC-MS analysis of the 18O labeling of asparagines residue prepared from the DSiaT glycoprotein.

(158) Differential Roles of Galectin-1 and Galectin-3 in Regulating Leukocyte Viability and Cytokine Secretion

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Galectin-1 (Gal-1) and galectin-3 (Gal-3) exhibit profound but unique immunomodulatory activities in animals but the molecular mechanisms are poorly understood. Early studies suggested that Gal-1 inhibits leukocyte function by inducing apoptotic cell death and removal, but recent studies show that some galectins induce exposure of the common death signal phosphatidylserine (PS) independently of apoptosis. Here we report that Gal-3, but not Gal-1, induces both PS exposure and apoptosis in primary activated human T cells, whereas both Gal-1 and Gal-3 induce PS exposure in neutrophils in the absence of cell death. Gal-1 and Gal-3 bind differently to the surfaces of T cells and only Gal-3 mobilizes intracellular Ca2+ in these cells. While Gal-1 does not alter T cell viability, it induces IL-10 production and attenuates interferon gamma (IFN-γ) production in activated T cells, suggesting the mechanism for Gal-1-mediated immunosuppression in vivo. These studies demonstrate that Gal-1 and Gal-3 induce differential responses in T cells and neutrophils, and identify the first factor, Gal-3, capable of inducing PS exposure with or without accompanying apoptosis in different leukocytes, thus providing a possible mechanism for galectin-mediated immunomodulation in vivo.

(159) Notch Signaling in Mouse Spermatogenesis

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Multiple components of the Notch signaling pathway have been previously shown to be expressed during spermatogenesis. Notch signaling is critical for cell fate determination, cell growth and development in metazoans. Notch receptors are a family of single transmembrane glycoproteins containing 29-36 EGF repeats in their extracellular domain. Many of these EGF repeats can be modified with O-fucose by protein O-fucosyltransferase-1 (Pofut1). This O-fucose may be extended with N-acetylglucosamine (GlcNAc), galactose and sialic acid. Targeted mutation of the mouse Pofut1 gene leads to embryonic lethality at ~E9.5 and a phenotype typical of mutants lacking downstream effectors that are required for signaling through all four Notch receptors. To investigate roles for Notch signaling in spermatogenesis, we have ablated the mouse Pofut1 gene using Cre recombinase transgenes expressed at different stages of mouse spermatogenesis. These mice were fertile and litters were of normal size. They produced sperm of wild type morphology and motility. There were no detectable histologic changes in testis cellular structures, nor changes in apoptosis by TUNEL assay. A Sertoli cell Cre recombinase transgenic mouse line was used to inactivate Pofut1 in mouse Sertoli cells. Those mice were also viable and fertile, but gave slightly decreased litter sizes and their body weight was somewhat lower than littermates. Thus, it appears that Notch signaling plays, at most, a very subtle role in mouse spermatogenesis. This work was supported by NIH grant RO1 30645 to PS.

(160) Mammalian N-Glycan Branching Protects against Innate Immune Self-Recognition and Inflammation in Autoimmune Disease Pathogenesis

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Autoimmune diseases are prevalent and often life-threatening syndromes yet the pathogenic triggers and mechanisms involved remain mostly unresolved. Protein asparagine linked- (N-) glycosylation produces N-glycan structures that significantly differ among the extracellular compartments of evolutionarily divergent organisms. Microbes and lower eukaryotes express cell surface and extracellular glycans bearing multiple exposed mannose linkages, unlike the complex N-glycan forms that predominate among vertebrates. Alpha-mannosidase-II (αM-II) deficiency diminishes complex-type N-glycan branching producing primitive N-glycans bearing multiple mannose linkage termini, and induces an autoimmune disease in mice similar to human Systemic Lupus Erythematosus. We find that disease pathogenesis leading to glomerulonephritis and kidney failure is non-hematopoietic in origin, independent of complement C3 and the adaptive immune system, mitigated by intravenous administration of immunoglobulin-G, and linked with recruitment and activation of the innate immune system in the kidney. Endogenous extracellular N-glycans produced in αM-II deficiency contain mannose-dependent ligands for innate immune lectin receptors that stimulate mesangial cells to produce pro-inflammatory cytokines, including monocyte-chemoattractant protein-1. αM-II deficiency abolishes the phylogenic basis of this glycomic non-self mannose-dependent recognition mechanism, resulting in the recruitment and activation of macrophages coincident with substantial iNOS induction, glomerulosclerosis, and autoimmune markers including renal dysfunction.. Mammalian N-glycan branching safeguards against the formation of an endogenous immunologic signal of non-self that provokes chronic inflammation in the pathogenesis of autoimmune disease.

(161) Roles for Mucin O-Glycans in Oogenesis and Fertilization

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To identify biological functions for O-glycans of oocyte glycoproteins, we have used oocyte-specific deletion of the *C1galt1* gene. The *C1galt1* gene encodes T-synthase, the enzyme that transfers Gal to O-GalNAc to generate core 1 and 2 O-glycans. Embryos lacking this activity die in utero at E13.5. However, functions for T-synthase during oogenesis or at fertilization could not be deduced due to the presence of maternal C1galt1 transcripts in oocytes. Females homozygous for a floxed *C1galt1* gene with a ZP3Cre transgene, which expresses Cre recombinase specifically in the oocyte at the beginning of oogenesis, were fertile. Thus eggs lacking T-synthase were fertilized. In addition, T-synthase null blastocysts developed in a timely fashion through blastogenesis, implantation and gastrulation to die at E13.5. However, females lacking T-synthase in their oocytes produced more eggs and had increased fertility. Ovarian weights and follicle numbers were greater but follicular apoptosis was unchanged. The number of follicles that enter the growing pool was unchanged as deletion occurs after recruitment, suggesting that increased fertility resulted from attenuated follicle development. In addition, T-synthase mutant ovaries contained numerous multiple-oocyte follicles (MOFs) rarely seen in controls. The MOFs appeared to form by adjacent, predominantly preantral, follicle joining - a new mechanism for MOF generation. MOFs were not however, the reason for increased fertility. These results identify novel roles for glycoprotein(s) from the oocyte as suppressor(s) of fertility and regulator(s) of follicular integrity in the mouse. This work was supported by NIH grant RO1 30645 to PS

(162) Vascular Endothelial Growth Factor ($VEGF_{165}$) Failed to Revert the Anti-proliferative Action of Tunicamycin in Capillary Endothelial Cells

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Capillary endothelial cell proliferation and differentiation, i.e., angiogenesis is a critical step in tumor growth and metastasis. Our laboratory has been studying the dynamic relationship between protein N-glycosylation and angiogenesis using an *in vitro* model of a non-transformed capillary endothelial cell line. To understand

the molecular process, we have used Tunicamycin, a potent N-glycosylation inhibitor. Initial results indicated cell cycle arrest in G1 and induction of apoptosis upon tunicamycin treatment. We, therefore, asked if tunicamycin-induced apoptosis could be reversed by VEGF since VEGF has been proposed to support the angiogenic switch. In our study we have observed that $VEGF_{165}$ (1 ng/ml) stimulated the cell proliferation in a time-dependent manner justifying the presence of active VEGF receptors on the cell surface. The level of total VEGF receptor-1 (Flk-1) expression remained same throughout but maximum receptor activation was observed within 3 hours of growth. In addition, these cells also expressed VEGF of varying molecular sizes. 2D-immuno Western blotting detected multiple VEGF species. When $VEGF_{165}$ (1 ng/ml) was added along with tunicamycin (1 μg/ml) and monitored the cell growth we observed the following: (i) the cell growth was reduced to almost one-half within 48 hours of tunicamycin treatment but VEGF failed to rescue the growth; and (ii) cellular VEGF expression was increased after 3 hours of treatment with no significant changes at other time points. We, therefore, conclude that VEGF is unable to neutralize the anti-angiogenic action of tunicamycin. Supported by grants: NIH U54-CA096297 and Susan G. Komen Breast Cancer Foundation BCTR5820.

(163) **Characterization of Mutant GM3 Synthase Deficient Human Fibroblasts**

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Increasing recognition of important roles of gangliosides in normal and abnormal cell function has motivated intense study of the consequences of modifications of cellular ganglioside content. The recent identification of a kindred with an autosomal recessive defect, a point mutation in the gene for GM3 synthase (Simpson et al, Nature Genetics 36:1225-9, 2004), offers a first opportunity to probe these questions in non-transformed human cells.

We established cultures from skin fibroblasts of three patients homozygous for the GM3 synthase point mutation, confirmed the molecular defect, and found a >95% reduction in ganglioside content (0.8±0.1 versus 12.7±0.8 nmol LBSA/107cells, normal fibroblasts). Activation of an alternate ganglioside synthesis pathway (e.g., the 0 pathway) was not detected. Cell morphology, by light microscopy, was normal.

However, fibroblast proliferation and migration assessed under stringent conditions--1% serum or suboptimal (0.5-2 ng/ml) EGF concentration--were altered; proliferation (cell count) was only 20%, and migration (wound scratch assay) 40%, of that of normal fibroblasts. While EGFR content was normal (Western blot), EGFR receptor activation was reduced by 50% from that of control fibroblasts, and EGF-induced phosphorylation of Rac/Rho, associated with cell migration, was completely absent.

We conclude that ganglioside depletion in these human mutant fibroblasts is nearly complete, and that it significantly reduces EGF-induced cell proliferation and migration, and EGF-related cell signaling.

(164) **Development of Mass Spectral Methods for Mapping O-Fucose and O-Glucose Modifications on Notch Receptors**

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Notch receptors play essential roles in cell fate decisions during development of metazoans. The extracellular domain of Notch contains up to 36 tandem epidermal growth factor-like (EGF) repeats, each of which is characterized by being approximately 40 amino acids in length and containing a 6-cysteine motif that forms three disulfide bonds. Many of the EGF repeats of Notch contain consensus sequences for O-fucosylation or O-glucosylation. Several recent studies demonstrate that the O-fucose and O-glucose modifications are essential for Notch function. It is therefore critical to identify where modifications occur and what structures exist at each site, to further our understanding of the mechanism by which O-glycosylation modulates Notch functioning. We have successfully mapped several sites of O-glycosylation on mouse Notch1 using site-directed mutagenesis, metabolic radiolabeling, and traditional oligosaccharide analysis. LC-ESI-MS/MS has proven itself to be a very effective, rapid, and powerful alternative tool for mapping O-glycosylation along proteins in recent years. We have expressed and purified the extracellular domains of mouse and Drosophila Notch proteins, subjected them to reduction/alkylation and trypsin digestion, and analyzed the resulting peptides via LC-ESI-MS/MS on an ion trap mass spectrometer. Numerous sites of O-glycosylation have been successfully mapped using this approach. We are also currently developing mass spectral methods for analysis of the structures of O-fucose and O-glucose glycans after release from Notch by alkali-induced β-elimination. Using these techniques, we are examining O-glycosylation of mouse Notch proteins 1-4, as well as Drosophila Notch. This work was supported by NIH grant GM61126.

(165) **O-Linked Glycan Diversity in the *Drosophila* Embryo**

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Appropriate glycoprotein O-glycosylation is essential for normal development and tissue function in multicellular organisms. Genetic, biochemical, and pathophysiologic studies in vertebrate and non-vertebrate species have repeatedly demonstrated the importance of O-linked glycans, including O-Man, O-Fuc, O-GlcNAc, O-Glc, as well as O-GalNAc initiated mucin-type structures. To comprehensively assess the developmental and functional impact of altered O-glycosylation, we have optimized methodology for analyzing the full complement of O-linked glycans in *Drosophila* embryos. Through multi-dimensional mass spectrometric analysis of permethylated glycans, following release from glycoprotein by reductive β-elimination, we detect both novel and previously reported O-glycans. The Core 1 mucin-type disaccharide (Galβ1,3GalNAc) is the predominant glycan in the total profile. HexNAcitol, Hexitol, xylosylated Hexitol, and branching extension of Core 1 with HexNAc (to generate Core 2 glycans) are also evident. After Galβ1,3GalNAc, the next most prevalent glycans are a mixture of novel, isobaric, linear and branched forms of a glucuronyl Core 1 disaccharide. Other less prevalent glycans are also extended with HexA, including Core 2 and O-Fuc structures. Although the expected disaccharide product of the Fringe glycosyltransferase (GlcNAcβ1,3Fucitol) is barely detectable in whole embryos, MS^n fragmentation and exoglycosidase sensitivity define a more prevalent, novel glucuronyl trisaccharide as GlcNAcβ1,3(GlcAβ1,4)Fucitol. Therefore, as described for Notch protein modification in vertebrates, Fringe-extended O-Fuc in *Drosophila* is a substrate for further elaboration. Full characterization of the *Drosophila* O-linked glycome, coupled with analysis of tissue- and stage-specific expression in wild-type and relevant mutant backgrounds, now provides an enriched context for assessing specific glycan functions.

(166) **Novel and Diverse Substrates for a Lectin Family of Ubiquitin Ligases**

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A significant fraction of all glycoproteins are misfolded and must be degraded. The endoplasmic reticulum-associated degradation (ERAD) pathway provides a quality control mechanism to eliminate these misfolded proteins from the ER. One marker used by the ER to identify misfolded glycoproteins is the presence of a trimmed, high-mannose (Man5-8GlcNAc2) glycan. This high-mannose tag serves as the signal for retrotranslocation into the cytosol, and subsequent targeted destruction by the ubiquitin proteasome pathway. Recently, two members -- FBXO2 and FBXO6 -- of a five-member F-box protein family were shown to bind high mannose glycans and participate in ERAD. All five proteins contain a conserved substrate-binding F-box associated (FBA) domain predicted to bind high mannose glycans. This FBA family is the only group of F-box ubiquitin subunits known to specifically target glycosylated substrates. Using lectin blots, glycan chip analysis, immobilized glycoprotein pulldowns, and site directed mutagenesis, we show that except for FBXO44 (which does not bind glycans) each family member has a unique specificity for glycosylated substrates. Members of this family were found to bind high mannose glycans, complex glycoproteins, and sulfated glycans, suggesting that this family plays a more diverse role in glycoprotein homeostasis than originally thought.

(167) **Identification and Characterization of Mouse Cosmc-2**

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Mucin-type O-glycans are mostly core 1 structures. Core 1 β3-galactosyltransferase (T-synthase) is responsible for synthesizing the core 1 disaccharide Galβ3GalNAcα-Ser/Thr (also called T-antigen). Mammalian T-synthase requires a specific molecular chaperone Cosmc (Core 1 β3-galactosyltransferase Specific Molecular Chaperone), which assists in its folding in vivo. Human Cosmc is mapped to Xq24; the murine ortholog of Cosmc, mCosmc, is also encoded by a single exon gene localized on the X chromosome. Recently, we identified a mCosmc homolog, which we designated mouse Cosmc-2 (mCosmc-2). mCosmc-2 is a single exon gene on chromosome 5, and is predicted to encode a 318 amino acid type-II putative membrane protein with 76% identity to mCosmc. mCosmc-2 can restore both human and mouse T-synthase activity when co-expressed in insect cells, which lack a Cosmc ortholog. Interestingly, while mCosmc is expressed highly and ubiquitously in all tissues, except for the testes, mCosmc-2 is restricted and highly expressed mainly in testes. Moreover, mouse T-synthase was expressed coordinately with mCosmc in most tissues, except for testes, in which the transcript level of T-synthase is relatively much higher. These data indicate that mCosmc-2 may replace mCosmc function in testes, or may have another biological function, such as chaperoning another enzyme. Roles of both mCosmc and mCosmc-2 *in vivo* are under investigation using gene targeting approaches. [Supported by NIH Grant GM068559 to RDC.]

(168) **The Cytoplasmic and Hydrophobic Domain of Rat Endomannosidase Are Required for its Efficient Golgi Localization**

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Golgi-localized endomannosidase represents an alternate glucosidase independent glucose-trimming pathway. Using a protease protection assay we demonstrated that endomannosidase is a type II membrane protein, containing a short cytoplasmic domain, a transmembrane domain and a large luminal domain. Its putative 25 amino acids signal sequence is sufficient for Golgi retention of secretory α1-antitrypsin in clone 9 hepatocytes or CHO cells and enables maturation of its N-linked oligosaccharides as demonstrated by acquiring Endo H resistance. Green fluorescent protein fused to the endomannosidase signal sequence exhibited a Golgi localization whereas shortening the length of the transmembrane domain prevented it indicating that the hydrophobic domain of endomannosidase is primary responsible for its Golgi retention. Any mutations within the cytoplasmic domain of endomannosidase, altering the three consecutive positively charged amino acids resulted in ER localization of the enzyme in CHO cells. In contrast, endomannosidase possessing either three lysines at this position, or a truncated cytoplasmic domain containing only three arginines exhibited a Golgi localization. Thus, the positively charged amino acids within the cytoplasmic domain of endomannosidase seems to be important for ER export. Conclusively, both, the cytoplasmic domain and the hydrophobic domain of endomannosidase are necessary for efficient Golgi localization of the enzyme.

(169) **Mass Spectrometric Characterization of *N*-linked Glycans of a Kappa Light Chain from a Patient Diagnosed with Primary Systemic Amyloidosis**

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Primary systemic amyloidosis is a protein misfolding disease characterized by extracellular deposits of amyloidogenic light chains (LCs) in various organs and tissues. Previous studies have shown that some post-translational modifications of immunoglobulin LCs, such as glycosylation and proteolysis, may play a key role in contributing to fibril formation by destabilizing the folding state of these proteins. In this study, we used a mass spectrometry based method to investigate a glycosylated kappa I light chain isolated from the urine of a patient diagnosed with primary amyloidosis and to determine the glycosylation profile of this LC protein, which was purified using dialysis and gel filtration chromatography. The presence of glycosylation was tested using a Molecular Probes glycoprotein gel stain kit and further demonstrated by the reduction of molecular mass of the intact LC by about 2 kDa after removal of the sugar moiety with PNGase F. To determine the glycosylation site, the LC was subjected to Asp-N digestion, and the product was fractionated by RP-HPLC and subsequently analyzed using MALDI-MS; the fractions containing the glycopeptides were treated with PNGase F and ESI-MS/MS analyses of the deglycosylated peptides identified the glycosylation site as Asn70. The released glycans were permethylated and analyzed by ESI-MS/MS. The MS results showed that the light chain contained a sialylated, core fucosylated triantennary chain as the major component, and some minor forms of triantennary and biantennary chains. Additional experiments will be performed to fully characterize the structures of these *N*-linked glycans from the amyloidogenic LC. Acknowledgements: NIH P41RR10888, P01HL68705.

(170) **Studies on the Purification and Hyrdolyzation of Fucoidan from Laminaria Japonica**

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The fucoidan was extracted from Laminaria japonica and then purified by D301R anion-exchange chromatography resin.Three fractions of F-0, F-1 and F-2 were obtained and the yields were 41.3%, 22.5% and 10.6% respectively. Fractions of F-0 and F-1 contained relatively low sulfate ester, which were 8.2% and 11.7% separately, and F-2 was a fraction with relatively high sulfate ester (26.5%). The findings determined by spectrophotometric method illustrated that the fucoidan contents of F-0, F-1 and F-2 were 78.7%, 86.3%, 91.2% respectively. The molecular weights estimated by Sephadex G-200 chromatography were 139KD and

143KD for F-1 and F-2 respectively. The extracted fucoidan was hydrolyzed at different temperatures by hydrochloric acid of different concentrations when hydroxyl radical, superoxide radical and DPPH system were applied for measuring the scavenging activities. The variables of hydrochloric acid hydrolyzing were then optimized. The findings indicated that the hydrolysates made by 0.03M HCl hydrolyzing at 80◎ for 3h had the highest scavenging activities and the average degree of the oligosacchride polymerization produced was 7 analyzed by MALDI-TOF. The low sulfated fraction F-1 had higher hydroxyl radical scavenging activity than F-2. F-1 and F-2 had no obvious different effects on scavenging activities of superoxide radical and DPPH. The hydrolysates had the most strong scavenging activities of hydroxyl radical, superoxide radical and DPPH when compared with those of F-1 and F-2 fractions.

(171) **Cell Surface Expression of a Heparan Sulfate Epitope Regulated by Sulfs, Extracellular Endosulfatases**

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Heparan sulfates (HS) are constituent of proteoglycans and are found on the cell surface and in the extacellular matrix. HS bind a diversity of protein ligands and thus can modulate cell signaling. The state of sulfation in glucosamines and uronic acids within the chains influences their binding activity. We have previously cloned and characterized two extracellular endoglucosamine 6-sulfatases, Sulf-1 and Sulf-2, in mice and humans. The Sulfs selectively liberate the 6-O sulfate groups on glucosamines present in N, 6-O and 2-O trisulfated disaccharides of HS and heparin. To clarify whether the Sulfs act on cell surface HS and remove 6-O sulfates, we transfected Sulf cDNAs into Chinese hamster ovary (CHO) cells and performed flow cytometric analysis with anti-HS antibodies. We utilized RB4CD12, an anti-HS phage display antibody that recognizes the trisulfated disaccharide structure. The RB4CD12 epitope was endogenously expressed on the cell surface of CHO cells. The level of cell surface expression of the epitope was greatly diminished in cells transfected with human Sulf-1 or Sulf-2 cDNA. The epitopes of 10E4 and HepSS-1 (monoclonal anti-HS antibodies that recognize N-sulfated disaccharide domains) were retained in these cells. The effects of the Sulfs on the RB4CD12 epitope were also seen in transfected HEK293 cells and HeLa cells. These data provide new strategies for cell surface remodeling of subdomains in HS.

(172) **Studies on the Extracting Technologies and Purification of Fucoidan from Sargassum Thunbergii**

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Abstract The fucoidan from Sargassum thunbergii was isolated by both hot water and enzyme hydrolyzing by taking the yield of extracted fucoidan, the content of sulfate radical and the content of polysaccharides as indicators. The orthogonal tests were designed to optimize the variable factors. The findings showed that the optimized factors for extracting fucoidan by hot water were temperature 98◎, pH6, time 5h, the amount of 15 times water of sample added. The yield of fucoidan extracted was 3.05% and the content of sulfate radical was 0.87%. The optimized factors for enzyme hydrolyzing were composite enzymes added 1.7%, temperature 45◎, pH 5.5 and hydrolyzing time 60 minutes. The yield of fucoidan extracted was 3.6%, in which the content of sulfate radical was 1.1%. The extracted fucoidan by enzyme hydrolyzing was then purified by anion-exchange chromatography DEAE－52 and five fractions named F1, F2, F3, F4 and F5 were obtained, in which the content of sulfate radical were 0, 3.94%, 6.57%, 12.60%, 38.08% respectively, and the recoveries of polysaccharides were 1.58%, 4.45%, 4.08%, 16.15%, 6.83% respectively. F1 had no sulfate radical content and was not the constituent of fucoidan. F2、F3、F4、F5 were further purified by Sephadex G–200. Six peaks were appeared, which were named as f1、f2、f3、f4、f5、f6. f2 and f4 were probably coagulates of fucoidan. The molecular weights of other four fractions were 40.5 kDa, 71.3 kDa, 132.5 kDa, 79.9kDa respectively.

(173) **Conformational Analysis of a Dermatan Sulfate - Derived Tetrasaccharide by NMR, Molecular Modeling and Residual Dipolar Coupling**

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The solution conformation of a dermatan-derived tetrasaccharide: ΔHexA-(1-3)-GalNAc4S-β-(1-4)-IdoA-α-(1-3)-red-GalNAc4S (S is a sulfate group) has been explored by means of NMR spectroscopy, especially NOE-based conformational analysis. The tetrasaccharide was present as four species, two of them are chemically different by the anomeric orientation of the reducing 2-deoxy-2-acetamido-galactose (red-GalNAc) residue, while the other two are originated by different conformations of the iduronic acid (IdoA) unit. The two α-β interconverting anomers were in a 0.6:1 ratio. Ring conformations have been defined by the analysis of 3JHH-coupling constants and inter-residual NOE contacts. Both 2-deoxy-2-acetamido-galactose residues (GalNAc) were found in the 4C1 chair conformation, the unsaturated uronic acid (ΔHex A) adopts a very major half-chair 1H2 conformation, while the IdoA residue stands either in the 1C4 chair or in the 2S0 skewed boat geometries, in a 4:1 ratio. There is a moderate flexibility of Φ and Ψ torsions as suggested by nuclear overhauser effect (NOE), molecular modeling (MM) and molecular dynamics (MD) studies. This fact has been further investigated by residual dipolar couplings (RDCs). One bond C-H RDCs (1DCH) and long range H-H (3DHH) RDCs were measured for the tetrasaccharide in a phage solution and interpreted in combination with restrained MD simulation. The RDC-derived data substantially confirmed the validity of the conformer distribution resulting from the NOE-derived simulations, but allowed an improved definition of the conformational behavior of the oligosaccharides in solution. In summary, the data showed a moderate flexibility of the four tetrasaccharide species at the central glycosidic linkage.

(174) **An LC/MS Platform for Glycomics of Glycosaminoglycans in Mammalian Organ Tissues**

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Glycosaminoglycans (GAGs) are linear sugar polymers covalently bound to a core protein. They are abundant on the cell surface and in the extracellular matrix, serving a wide range of functions. The structure of GAGs exhibits a great degree of polydispersity in composition, chain length, sulfation, acetylation and epimerization pattern. Glycomic studies of GAGs in different tissues of different species aim to understand how the structures and quantities of GAGs are related to aging, development, mutation and specific diseases. Mass spectrometry is a powerful tool for structural analysis of GAGs. Its use, however, depends on the availability of a general method for extraction of GAGs from tissue. We developed such a glycomic platform consisting of extracting GAGs from various rat tissues, and subjecting the GAGs to on-line LC/MS and LC/MS/MS analysis. The extraction consists of sequential chondroitin and heparin sulfate lyase digestion and weak

ion (DEAE) exchange workup. The LC/MS utilizes amide-80 as chromatography and ESI-QIT as mass detector. We have demonstrated that LC/MS and LC/MS/MS analysis of exhaustive digested of both chondroitin sulfate (CS) and heparan sulfate (HS) from these tissues give detailed information of the disaccharide composition of these GAGs and their differences among different tissues. An improved capillary electrophoresis method was also developed to serve as an orthogonal approach for disaccharide analysis and quantification. This platform will be useful for glycomic studies of GAGs in tissues directly harvested from mammals.

(175) **Tissue – Based Glycomics using Amide-HILIC LC-Tandem MS**

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Homeostasis of connective tissues depends on the maintenance of an extracellular matrix, consisting of an integrated assembly of collagens, glycoproteins, proteoglycans and glycosaminoglycans (GAGs). To define the functional roles of glycan expression during disease formation it is necessary to determine their patterns of expression as a function of tissue location. Isomeric chondroitin sulfate (CS) glycoforms differing in position and degree of sulfation and uronic acid epimerization play specific and distinct functional roles during development and disease onset. This work profiles the CS epitopes expressed by different joint tissues as a function of age and osteoarthritis. This analysis enables simultaneous profiling of the expression of saturated non-reducing end, linker region, and delta-unsaturated interior oligosaccharide domains of the CS chains among the different joint tissues. The results generate an unparalleled level of detail in profiling structural changes to connective tissue-derived GAGs during disease and development.

Sulfated GAGs were extracted from papain-digested connective tissue samples using a streamlined multi-step procedure. Oligosaccharides were partially depolymerized followed by derivatization with 2-anthranilic-3,4,5,6-d_4 acid. Derivatized tissue samples were cleaned, spiked with standard CSA-d_0-2AA, and subjected to an online amide-HILIC-LC-MS/MS platform using an Esquire 3000 ion trap. A combination of MS and tandem MS analysis was employed for detailed structural characterization.

Longer oligosaccharides may contain differing domains of sulfation and epimerization that may play crucial roles in development and disease processes. Characterization of the actual sulfation and epimerization positions within differing domains is also investigated using a high resolution, high sensitivity Orbitrap mass spectrometer.

(176) **Changes in Glycosylation Patterns and Gel Forming Mucins in Patients with Dry Eye Syndrome**

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Dry eye is a clinically defined disease that affects as much as 30% of the ophthalmologist patients. It can be categorized according to different clinical probes; these tests do not provide real information about the quality of the tear film, nor concentrations of different proteins in the tear film. MUC5AC and MUC2 are mucins that are implicated in the maintenance of the stability of the tear film, which is lost in dry eye syndromes. Purpose: To identify gel-forming mucin concentrations and glycosylation patterns of tear proteins of with patients dry eye syndrome. Methods: Patients were clinically classified in 7 groups of dry eye syndrome. Tear simples were obtained of both eyes and performed a quantification of protein concentration according to Lowry modified method. ELISA for MUC5AC and MUC2 and lectin modified ELISA with Sambucus nigra agglutinin (SNA), Maackia amurensis agglutinin (MAA), Amaranthus caudatus lectin (ACL) were performed. Results: 65 patients were included. Secondary Sjögren group of patients showed the greatest concentration of MUC5AC and MUC2 compared with the other groups ($p < 0.05$), and also the lowest concentration of protein identified with SNA and the highest identified with MAA ($p < 0.05$). The lowest total protein concentration was obtained in the group with evaporative dry eye.($p < 0.05$). Conclusions: Patients with secondary Sjögren disease showed a high MUC5AC and MUC2 concentration and up-regulation of syalic acid in alfa2,3 position with down regulation of syalic acid in alfa2-6 position in total protein

(177) **Sensitive Quantitation of Isoglobotriaosylceramide in the Presence of Isobaric Components using Electrospray Ionization-Ion Trap Mass Spectrometry**

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Isoglobotriaosylceramide (iGb3; Galα1-3Galβ1-4Glcβ1-1Cer) is a stimulatory antigen for a unique type of T cell, Natural Killer T cells. Produced in the lysosomal compartment by mammalian antigen presenting cells, iGb3 is one of the few clearly identified carbohydrate ligands for biological receptors. A major source of glycoconjugate structural diversity arises from the possibility of forming different linkages between the same monosaccharide units. Globotriaosylceramide (Gb3; Galα1-4Galβ1-4Glcβ1-1Cer) exists as a natural isomer for iGb3, and both isomers are frequently found together in mixtures of glycosphingolipids extracted from mammalian cell membranes. Discriminating these isomers has been feasible using monoclonal antibodies raised against specific carbohydrate epitopes, or by unambiguous structural characterization, which requires relatively large amounts of pure compounds isolated from grams, or tens of grams, of biological samples. However, the precise detection of iGb3 from small amounts of biological samples, where it may be mixed with Gb3 present in much higher abundance, is a pre-requisite for answering further important biological questions such as stimulation of NKT cells. Here we describe a specific and sensitive method based on ion trap mass spectrometry to discriminate iGb3 from Gb3. We also demonstrate its application to quantifying the amount of iGb3 in a prototype antigen presenting cell, rat RBL-CD1d cells, using a chemically synthesized short N-acyl chain iGb3 as internal standard. This methodology should have wide implications for functional glycosphingolipidomics of immune cells and glycosphingolipid biomarker analysis.

(178) **A Glycomics Platform for the Analysis of Permethylated Oligosaccharide Alditols**

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This communication reports the development of an LC/MS platform for the analysis of permethylated oligosaccharide alditols that, for the first time, demonstrates routine online oligosaccharide isomer separation prior to introduction into the mass spectrometer. The method leverages a high resolution liquid chromatography system with the superior fragmentation pattern characteristics of permethylated oligosaccharide alditols that are dissociated under low-energy collision conditions. The oligosaccharides are analyzed using quadrupole orthogonal time-of-flight (QoTOF) instrumentation and up to pseudo MS3 mass spectrometry. Glycoforms, including isomers, are readily identified and their

structures assigned. The isomer-specific spectra include highly informative cross-ring and elimination fragments, branch position specific signatures and glycosidic bond fragments, thus facilitating linkage, branch and sequence assignment. The method is sensitive and can be applied using as little as 40 fmol of derivatized oligosaccharide. Because permethylation renders oligosaccharides nearly chemically equivalent in the mass spectrometer, the method is semi-quantitative and, in this regard, is comparable to methods reported using high field NMR and capillary electrophoresis. The nature of many of the important questions in glycomics is such that sample material is often extremely limited, thus necessitating the development of highly sensitive methods for rigorous structural assignment of the oligosaccharides in complex mixtures. The glycomics platform presented here fulfills these criteria and should lead to more facile glycomics analyses.

(179) **Enrichment and Mass Detection of Glycans using Fluorous Affinity Tags**

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Because the details of carbohydrate structures cannot be comprehensively assessed at the level of intact glycoproteins, they are released by enzymatic or chemical methods and separated from the reaction mixture. Isolation and purification of the product glycans can be challenging due to the complexity and the limited quantities of biological samples. In this report, we describe a new strategy to trap glycans selectively on the basis of fluorous affinity technology. From complex mixtures, released glycans were coupled with F13-aniline (Fluorous Technologies, Pittsburgh, PA) and reduced in situ using 1M NaBCNH3. For neutral glycans, the reaction was complete in an hour at 85 degree, and for acidic glycans, mild condition was used to avoid sialic acid hydrolysis (60 degree for three hours). The conjugate was passed through a single F-SPE cartridge, the F-tag glycans were trapped and eluted selectively in narrow fractions (acidic glycans: 50% MeOH/H2O, and neutral glycans: 80% MeOH/H2O). This method has been successfully carried out on three model glycoproteins: bovine ribonuclease B (neutral glycans), chicken ovabumin (neutral glycans), and bovine fetuin (acidic glycans). With as little as 1 microgram of ribonuclease B, all five high mannose glycans peaks (Man5 to Man9) were evident in MALDI-MS profile.

(180) **Sialyloligosaccharide Expression in Human Milk**

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[1]Massachusetts General Hosp & Harvard Medical Schl, Boston, MA; [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [3]Instituto Nacional de Sciencias Medicas y Nutricio, Mexico City, Mx

Sialyloligosaccharides and fucosyloligosaccharides are major constituents of human milk. Fucosyloligosaccharide expression in milk varies over lactation and among individuals, and this variation relates to disease risk in breastfed infants. The present study measures expression of 12 major sialyloligosaccharides in the milk of 30 individuals from the US (87% of European ancestry) for the duration of their lactation (up to 76 weeks). The sialyloligosaccharides were resolved by capillary electrophoresis with a running buffer of aqueous 200 mM NaH2PO4 pH 7.05, containing 100 mM SDS made 45% (v/v) with methanol, with detection at 205 nm. The 12 major milk oligosaccharide standards were separated and quantified in a single 35-minute run. The total of these 12 sialyloligosaccharide concentrations were highest in the first few weeks of lactation (1-8 g/L [median 2.5] in the first week) with the median declining to less than 1 g/L after 12 weeks of lactation. At 4 weeks, the major oligosaccharides were DSLNT (0.5 g/L), 6'-SL (0.3 g/L), MSMFLNnH (0.16 g/L), MSMFLNH I and DSMFLNH (each at 0.1 g/L). MSLNnH, SLNFP II, 3'-S-3-FL, and SLNT a, b, and c were at lower concentrations. Individual oligosaccharides were expressed the highest at the onset of lactation, with less expression thereafter, with the exception of DSLNT and 3'-SL, which have increases in expression later in lactation. Individuals varied in expression of milk sialyloligosaccharides. One could now test whether pathogens that bind sialic acid-containing glycans on host mucosal surfaces are inhibited by specific human milk sialyloligosaccharides.

(181) **Online Tool for Predicting Glycan Structures from Glycosyltransferase Expression Data**

Mariko Yokota[1]; Akitsugu Suga[2]; Kosuke Hashimoto[2]; Kiyoko F. Aoki-Kinoshita[1]; Minoru Kanehisa[2]
[1]Soka University, Tokyo, Japan; [2]Kyoto University, Kyoto, Japan

Previously, a method to predict glycan structures from microarray expression data of glycosyltransferases was developed. This method was based on the calculation of the co-occurrence of glycosidic linkages among the glycan structures in the KEGG GLYCAN database. However, this method was limited as it used binary values dependent upon a threshold placed on the expression data. It also calculated the co-occurrence scores using all of the available glycan structures. Thus, it was later improved to incorporate the actual expression values into the scoring method (Suga, A., Yamanishi, Y., Hashimoto, K., Goto, S., and Kanehisa, M.; An improved scoring scheme for predicting glycan structures from gene expressison data. Genome Informatics 18(1), in press (2007)). In order to enable researchers to actually utilize this improved method, we have developed an online tool which is freely available using RINGS (Resource for INformatics of Glycomes at Soka), at http://rings.t.soka.ac.jp. The input to this tool can be in the form of accession numbers, NCBI Gene IDs or glycosidic bond information along with their corresponding expression values, and the output is a list of glycan structures sorted according to their scores. The output structures are displayed and linked to their annotation information as available in KEGG. Future work entails the improvement of this prediction method and evaluation against MS data corresponding to the expression data.

(182) **Progress toward Qualitative Discernment between Carbohydrate Sulfate and Phosphate Esters**

Parastoo Azadi[1]; Michael A Madson[2]
[1]Complex Carbohydrate Research Center, Athens, GA; [2]JC & Associate, Sunnyvale, CA

There is a lack of simple methods available to distinguish between carbohydrate sulfate and phosphate esters. Typically MS and MSn spectrometry are performed to attempt discernment between the isobaric derivatives. We have attempted a simple chemical/ms scheme to delineate between the two carbohydrate esters. We use borohydride and borodeuteride reduction of ammonium salts of the esters and examine their ms spectra. Standards, galactose-6-sulafte and glucose-6-phosphate, are made into ammonium salts and then reduced with borohydride and borodeuteride separately and mass spectra obtained. We have found evidence for single insertion of hydride and deuteride for both phophorylated and sulfated sugar. We are attempting to find conditions for double hydride/deuteride insertion for sulfate ester. Their spectra are reported and progress toward this approach will be noted.

(183) **N-Glycoproteomics - An Automated Workflow Approach**

Risto Renkonen[1]; Hannu Peltoniemi[2]; Ilja Ritamo[3]; Sakari Joenvaara[2]
[1]Haartman institute, University of Helsinki, Helsinki, Finland; [2]Medicel Ltd, Helsinki, Finland; [3]Finnish Red Cross Blood Service, Helsinki, Finland

Posttranslational modifications, such as glycan decorations, can dictate protein functions and thus have crucial importance in life

sciences. We developed an automated in silico workflow to analyse mixtures of native glycopeptides with tandem mass spectrometry and in silico workflow including database-related amino acid sequence identification followed by glycan analysis, and target-decoy filtering. We first verified this approach with 150 serotransferrin glycopeptide spectra where our automated workflow generated 10^5 putative interpretations from > 10^9 theoretical glycopeptides. After scoring all these 62 out of original 150 glycopeptide spectra obtained validated interpretation with concomitant amino acid sequences, glycan compositions and structures. Up to 12 different glycans at the same site as well as three different glycosylation sites were observed. When applying this method to human plasma specimen we could identify 80 glycopeptides with their glycan compositions or structures. Instead of weeks and months of interpretation work of mass spectrometry files our automated workflow can be executed in few hours and provide information concomitantly from both the amino acid and glycan moieties of intact glycopeptides in mixtures.

(184) **Structural Analysis of N- and O-Linked Glycans of N-Acetylglucosaminyltransferase Va (GnT-Va) $^{-/-}$ and Wild Type Mouse Brain Tissue**

Jin Kyu Lee; Hua-Bei Guo; Jae Min Lim; Lance Wells; Michael Pierce
University of Georgia, Athens, Georgia

N-acetylglucosaminyltransferase Va (GnT-Va) synthesizes a multiantennary branch on N-linked glycans, the branch containing GlcNAcβ(1,6)Man. Many studies suggested that increased β(1,6) branch is associated positively with invasiveness. Mice (GnT-Va$^{-/-}$ or Mgat5 $^{-/-}$) that lack GnT-Va expression have been used to study the effects of eliminating GnT-Va activity on tumor progression. When crossed with mice that express the polyoma middle T-antigen under control of the mouse mammary tumor virus promoter, the rate of metastasis of the mammary tumors was significantly reduced in the GnT-Va$^{-/-}$. This study provided strong evidence that changes in β(1,6) branching of N-linked glycans due to altered GnT-Va activity affect carcinoma progression *in vivo*. Guo *et al.* showed that mouse embryo fibroblasts from GnT-Va$^{-/-}$ mice have an increased rate of calcium-dependent cadherin-mediated cell-cell adhesion compared to with MEF from GnT-Va$^{+/-}$ littermates. However, structural analysis of glycans from wild and GnT-Va$^{-/-}$ mice have not been performed. Brain tissues from GnT-Va$^{-/-}$ and wild type mouse were isolated and de-lipidated as described by Aoki *et al.*. N-linked glycans were released by PNGase-F after trypsin digestion. O-linked glycans were released by β-elimination. To facilitate analysis by mass spectrometry, portions of released oligosaccharide mixture were permethylated. The permethylated glycans were infused directly into a linear ion trap mass spectrometer (LTQ). The total ion mapping (TIM) protocol was used for quantitation of glycans. The results from these studies will determine if there are effects on the expression of glycans other than those N-linked glycans with GlcNAcβ(1,6)Man.

(185) **Homogeneous UDP Detection: Applications in Glycobiology**

Thomas Zielinski; Andrew Kopp; Thane Westermeyer; Matt Staeben
BellBrook Labs, Madison, WI

UDP-glycosyltransferases (UGTs) are of interest as therapeutic and anti-microbial targets as well as for their role in drug metabolism. UGTs catalyze the transfer of several sugars to a wide spectrum of acceptor substrates including small molecules and proteins, and for this reason they have proven to be challenging high-throughput screening targets. However all UGTs generate UDP in stochiometric amounts with the glycosylated product, and this has enabled the development of a robust, generic HTS assay. The Transcreener UGT Far Red Assay relies on a competitive fluorescence polarization immunoassay for detecting UDP, and thus enables facile screening of any UGT, regardless of its acceptor substrate. The assay uses a far red tracer which minimizes compound interference and light scatter, resulting in robust detection of diverse UGTs in the most demanding HTS applications. The reliance of the assay on UDP detection is especially enabling when identifying substrates for uncharacterized UDP-glycosyltransferases. Here the utility of this assay to screen UGTs in a drug discovery HTS environment is demonstrated.

(186) **Mass Spectrometric Profiling of the Mesenchymal Stem Cell N-Glycome**

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Anne Olonen[2]; Maria Blomqvist[2]; Jari Natunen[2]; Anita Laitinen[1]; Sari Tiitinen[1]; Ulla Impola[1]; Jarmo Laine[1]; Petri Lehenkari[3]; Juhani Saarinen[2]; Leena Valmu[1]
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Mesenchymal stem cells are fibroblast-like adult multipotent progenitor cells that can be isolated from various sources such as bone marrow or cord blood. Mesenchymal stem cells are capable of differentiating into mesenchymal cell types like osteoblasts, chondroblasts and adipocytes. Stem cells hold an enormous therapeutic potential in regenerative medicine. However, before stem cells can be used in the clinical practice, there is a need for methods to thoroughly characterize them, to distinguish them from other cells, and to control variation within and between different cell lines. A glycomic approach to study stem cells provides an ideal platform to solve these issues. In this study, N-glycans from human bone marrow mesenchymal stem cells were analyzed by MALDI-TOF mass spectrometry. Exoglycosidase digestions were carried out to further characterize terminal epitopes. In addition, carbohydrate epitopes were studied by immunofluorescent staining to support the mass spectrometric data. More than one hundred glycan signals were detected for both mesenchymal stem cells and cells undergone osteogenic differentiation. Of these some signals were characteristic of stem cells and decreased upon differentiation, whereas other signals became more prominent upon differentiation. In conclusion, mesenchymal stem cells have a characteristic N-glycan profile that changes upon differentiation. The information on the stem cell glycome can be used to evaluate the differentiation stage of stem cells and to develop new stem cell markers (e.g. for antibody development) as well as to study the interactions of stem cells with their niches and thus develop improved *in vitro* culture systems.

(187) **GlycomeDB: A Carbohydrate Structure Metadatabase**

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Carbohydrates are the third major class of biological macromolecules, besides proteins and DNA. But in contrast to the other classes, neither a comprehensive database for carbohydrate structures, nor a universal structure encoding for computational purposes exists. After the cessation of funding for the Complex Carbohydrate Structure Database (CCSDB, often referred to as CarbBank) in 1997, several initiatives developed independent databases with partially overlapping foci. For each database, a proprietary encoding scheme for residues and topology of the structures was designed. As a result it is virtually impossible to get an overview of all deposited structures, and to compare the contents of the various databases.

We have analysed all of the public available databases and defined a sequence format based on XML called GlycoCT that is capable

of encoding all structural features of carbohydrate sequences. We have implemented procedures which downloads the structures of CFG, KEGG, GLYCOSCIENCES.de, BCSDB and CarbBank and translates the different structure notations to GlycoCT. Based on these data we have created a new database called GlycomeDB (www.glycome-db.org) containing all structures, their taxonomical annotations and references to the original databases.

During this data integration effort we have encountered inconsistencies in all public databases, which we have discussed in multiple feedback rounds with the responsible curators. Furthermore users are able to search GlycomeDB by different structural query options via a webportal. The results guide the user to the original databases, where they can profit from the diverse specific areas of expertise.

(188) **Progress toward Qualitative Discernment between Carbohydrate Sulfate and Phosphate Esters**

Parastoo Azadi[1]; Seongha Park[1]; Michael A Madson[2]
[1]*Complex Carbohydrate Research Center, Athens, GA;* [2]*JC & Associate, Sunnyvale , CA*

There is a lack of simple methods available to distinguish between carbohydrate sulfate and phosphate esters. Typically MS and MSn spectrometry are performed to attempt discernment between the isobaric derivatives. We have attempted a simple chemical/ms scheme to delineate between the two carbohydrate esters. We use borohydride and borodeuteride reduction of ammonium salts of the esters and examine their ms spectra. Standards, galactose-6-sulafte and glucose-6-phosphate, are made into ammonium salts and then reduced with borohydride and borodeuteride separately and their mass spectra are obtained. We have found evidence for single insertion of hydride and deuteride for both phophorylated and sulfated sugars. We are attempting to find conditions for double hydride/deuteride insertion for sulfate ester. Their spectra are reported and progress toward this approach will be noted.

(189) **Glycosylation Patterns in *Caenorhabditis elegans* Mutants Resistant to *Microbacterium nematophilum* Infection**

Elizabeth Palaima[1]; Maria Gravato-Nobre[2]; Jonathan Hodgkin[2]; John F. Cipollo[3]; Catherine E. Costello[1]
[1]*Boston University Medical Campus, Boston, MA;* [2]*University of Oxford, Oxford, United Kingdom;* [3]*Food and Drug Administration, Bethesda, MD*

The initial events of bacterial infection often involve pathogen recognition of host glycoconjugates. These interactions are instrumental to the development of strategies to target bacterial adhesion events. The nematode *Caenorhabditis elegans (C. elegans)* provides a model to study carbohydrate dependent host-pathogen interactions.

The coryneform bacteria *Microbacterium nematophilum (M. nematophilum)* causes infection in the C. elegans leading to an innate immune response characterized by a distinct swelling of the cuticle (Cipollo, J.F., Awad, A.M., et al. 2004, Gravato-Nobre, M.J., Nicholas, H.R., et al. 2005). In a genetic screen of *C. elegans* almost all **b**acterially **uns**wollen or *bus* mutants were resistant to *M. nematophilum* infection, including *bus-2*. The mutant *bus-2*, which bears a lesion in a predicted core-1 galactosyltransferase, was chosen as a model of carbohydrate dependent pathogen interaction. The current study aims to investigate the basis of bacterial resistance by examination of the *bus-2* mutant compared to the wild type N2 strain. Preliminary mass spectrometric analysis indicates that, compared to N2 Parental strain, *bus-2* has a change in relative abundance in some *O*-glycans. This work was supported by NIH grants RR10888 and RR15942.

Cipollo, J.F., Awad, A.M., et al. (2004) *srf-3*, a mutant of *Caenorhabditis elegans*, resistant to bacterial infection and to biofilm binding, is deficient in glycoconjugates. J Biol Chem, 279, 52893-52903.

Gravato-Nobre, M.J., Nicholas, H.R., et al. (2005) Multiple genes affect sensitivity of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. Genetics, 171, 1033-1045.

(190) **Strategies for LC/MS/MS Characterization of Highly Sulfated Glycoaminoglycans**

Nancy M. Leymarie; Hisham Naimy; Gregory Staples; Catherine E. Costello; Joseph Zaia
Boston University School of Medicine, Boston, MA

Heparins are the most highly sulfated glycosaminoglycan class, with 2-3 sulfate groups per disaccharide unit. Heparan sulfate (HS) is more structurally variable than heparin, with more abundant acetylated disaccharide repeats, and a greater range of sulfation. Mammalian chondroitin/dermatan sulfates (CS/DS) typically have 1 sulfate per disaccharide unit. In order to be effective for structural analysis, a tandem mass spectrometry method must generate cleavage of the oligosaccharide backbone while avoiding non-informative losses of sulfate groups. Tandem MS of CS/DS classes produces highly informative glycosidic bond cleavages. Heparins are considerably more challenging due to the high density of sulfation.

The goal of this work is to develop a method for on-line LC-tandem mass spectrometry of all GAG classes. Practically this entails balancing the charge states observed with on-line chromatography with the need to produce abundant backbone cleavages. An LC/MS/MS method has been developed to produce structural assignments of heparin and heparin sulfate compounds. The LC/MS/MS employs capillary amide-silica hydrophilic interaction chromatography online with a high resolution hybrid ion trap-Orbitrap mass spectrometer. The high sensitivity, high resolution and multiple MS/MS capabilities of the LTQ Orbitrap are ideally suited to elucidate the structural intricacies of these compounds. Heparin libraries (degree of polymerization 6 and 8) made from partial lyase digestion of porcine intestinal mucosa heparin were used to highlight the capabilities of the method.

(191) **LC/MS Analysis of Glycosaminoglycans from the Model Organism *Caenorhabditis elegans***

Gregory O. Staples[1]; Nancy Leymarie[1]; Michael J. Bowman[1]; Catherine E. Costello[1]; John F. Cipollo[2]; Joseph Zaia[1]
[1]*Boston University, Boston, MA;*
[2]*Food and Drug Administration, Bethesda, MD*

The soil-dwelling nematode *C. elegans* is known to express glycosaminoglycans (GAGs) that have an essential role in a still growing list of life processes such as cytokinesis, embryogenesis, and neuronal migration. Interestingly, the organism produces variably sulfated heparan sulfate (HS) at extremely low levels as compared to completely unmodified chondroitin sulfate (CS). Understanding the structure-function relationship of GAGs in *C. elegans* requires glycan analysis beyond the level of disaccharides, a task for which mass spectrometry (MS) is particularly suited, but the low levels of HS expressed in the organism present considerable analytical challenges. Chief among these is the separation of high levels of background matrix from the expressed GAGs. The work introduced here demonstrates both an extraction technique and LC/MS platform for *C. elegans* GAGs that is successful for both CS and HS. Unsulfated chondroitin (ΔDi-OS), as well as a variety of sulfated and nonsulfated HS disaccharides (ΔHS-IVA, ΔHS-IS, ΔHS-IVS, ΔHS-IIA/IIIA, and ΔHS-IIS/IIIS) were detected as proof of principle. The system is also successful for analyzing HS oligosaccharides from partial lyase digests, and work is ongoing to analyze such samples from *C. elegans* tissue.

The ability to purify and analyze starting material in the nanogram of analyte per milligram of tissue range shows promise for oligosaccharide analysis from this important model organism. This work was supported by NIH grants P41 RR10888 and R01 HL74197.

(192) **N-Acetyl-βeta-D-Hexosaminidase as a Potential Factor in Gestational Diabetes Mellitus Etiopathogenesis**

Danuta Dudzik; Wieslaw Zarzycki; Malgorzata Knas; Malgorzata Borzym-Kluczyk; Maria Gorska; Krzysztof Zwierz
Medical University of Bialystok, Bialystok, Poland

Gestational Diabetes Mellitus (GDM) is associated with a wide range of complications both to the mother and fetus. Identification of early risk markers may result in improved understanding of disease pathogenesis and identification of potential ways of intervention. N-acetyl-βeta-D-hexosaminidase (HEX), catalyzes the removal of N-acetylglucosamine or N-acetylgalactosamine residues from the non-reducing end of glycoconjugates oligosaccharide chains. C-peptide is a by-product of normal insulin production which role has been not fully recognized. It is associated to be a prognostic factor for Diabetes Mellitus.

The aim of our investigation was to determine the changes in HEX activity and C-peptide in GDM.

The blood serum of 10 pregnant women with diagnosed GDM, 10 women 3 months after GDM, and 10 pregnant healthy women, as a control group. 75-g OGTT (140-180 mg/dl), HbA1c, BMI and blood pressure were estimated. HEX activity (pKat/kg protein) was measured by the Chatteriee method modified by Zwierz. C-peptide (ng/ml) was determined by C-PEP-EASIA Kit. We observed a statistically significant increase of HEX activity in GDM patients compared to the healthy pregnant women (p= 0,000046) and a statistically significant decrease of HEX activity after 3 months after the GDM period (p=0,000000). A statistically significant increase was also found between the C-peptide concentrations in patients with non complicated pregnancies and patients with GDM (p=0,027). There were no statistical changes in C-peptide concentrations between the GDM group and 3 months after the GDM period. Our preliminary study might suggest the potential role of HEX in the GDM etiopathogenesis.

(193) **High-throughput Permethylation with 96-Well Plate Format for Glycan Analysis**

Pilsoo Kang; Yehia Mechref; Milos V. Novotny
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Although mass-spectrometric (MS) analysis of native glycans is routinely employed, to analyze permethylated glycans offers several advantages, including stabilization of the sialic acid residues, enhanced sensitivity, and easier tandem MS interpretation. Additionally, permethylation permits the simultaneous analysis of both acidic and neutral glycans in the positive MS mode, allowing a simultaneous quantitative comparison of their abundances.

We have previously introduced a solid-phase permethylation approach (Kang, P. et al. Rapid Commun. Mass Spectrom. 2005, 19, 3421-3428) as a highly efficient procedure for derivatization at the trace levels of glycans extracted from typical biological samples. Recently, this approach was extended to a spin-column format, which is yet faster and simpler. Another extension of the solid-phase permethylation involves the utility of a 96-well plate in which the sodium hydroxide beads are packed in each well. Permethylation using a 96-well plate is as efficient as spin-column format, yet it allows more samples to be permethylated simultaneously. In this procedure, the samples were permethylated through a 96-well plate arrangement, while the permethylated glycans are extracted by a C18 solid-phase packing for a high-throughput analysis. The 96-well plate permethylation method utilizes the same parameters as those used in the spin-column procedure, with the extraction step being optimized. A solid-phase permethylation through the 96-well permethylation format, when coupled with a MALDI/TOF-MS measurement, features both high sensitivity and high throughput. The utility of this approach will be demonstrated for glycomic profiling of human blood serum collected from different healthy and diseased subjects.

(194) **Glyco-Biomarker Identification with a New Glycoproteome Algorithm, The Glycodetector**

Masaya Ono; Setsuo Hirohashi; Tesshi Yamada
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Introduction: We developed a new glycoproteome algorithm, Glycodetector, which automatically identifies the parent peptide masses with glycoforms based on their MSMS spectra. 2DICAL (2-Dimensional Image Converted Analysis of Liquid chromatography and mass spectrometry) displays tryptic peptides in a two-dimensional plane, with axes of m/z values and retention time (Ono, Mol Cell Proteomics, 6:479, 2007). We analyzed 125 serum samples in order to identify alterations of the glycoforms in the serum glycoproteins of endometrial cancer patients.

Materials and Methods: Four commercial preparations of CEA were diluted to concentrations of 500 fmol and 5 pmol. Serum samples were obtained from 125 individuals (40 endometrial cancer patients, 30 healthy controls, 30 uterine myoma patients, and 25 uterine sarcoma patients), and the 12 most abundant proteins were removed with ProteomeLab IgY-12 SC affinity columns (Beckman Coulter). The samples were digested with trypsin and analyzed by LCMS and LCMS/MS measurements.

Results: Glycodetector identified five N-linked glycosylated peptides derived from CEA, and differences in glycoforms were detected among the four CEA preparations. There were 771 peptide peaks that were diagnostic of patients with endometrial cancer, uterine myoma, uterine sarcoma, and the controls. Glycodetector selected 68 of the 771 peaks as peptides with glycoforms, and 23 of the peptides were identified by a Mascot search.

Conclusion: Glycodetector identifies peptides with glycoforms, and 2DICAL analyzes differences in glycosylation and differences in the quantity of glycoproteins. The combination of Glycodetector and 2DICAL is an effective tool for detecting biomarkers based upon alterations in the glycoforms of glycoproteins.

(195) **A Chip-Based Amide-HILIC LC/MS Platform for Glycosaminoglycan Glycomics**

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A Chip-based Amide-HILIC LC/MS Platform for Glycosaminoglycan Glycomics

Improved analytical techniques are needed to facilitate investigations into the functional roles of glycosaminoglycans (GAGs) in biological systems. GAGs are linear carbohydrates with domains that vary in the extent of sulfation, acetylation and uronic acid epimerization. It is of particular importance to determine spatial and temporal variations of GAG domain structures in biological tissues. Mass spectrometry is effective for analysis of GAGs. In order to analyze GAGs from tissue, it must be used in conjunction with an on-line separation system. The purpose of the separation system is both to remove components that inhibit GAG ionization and to enable the analysis of very complex mixtures.

This work presents amide-silica hydrophilic interaction chromatography (HILIC) in a chip-based format for LC/MS of heparin, heparan sulfate and chondroitin/dermatan sulfate GAGs. The chip interface enables robust performance in the negative ion mode that is essential for GAGs and other acidic glycan classes. The built-in trapping cartridge reduces background from the biological tissue matrix. The HILIC chromatography separates bases on a combination of chain length, hydrophobic acetate groups and acidic sulfate groups. In summary, chip based amide-HILIC LC/MS is an enabling technology for GAG glycomics.

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(196) **The Stability of N-Glycans of Protonated Glycopeptide Ions in Gas Phase**

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Mass spectrometry (MS) of glycopeptides is becoming important in the glycoproteomics, since it enables high-throughput and sensitive elucidation of the key structural issues. While protonated species are usually analyzed in the MS of glycopeptides, fragmentation behaviors of the glycan moiety of glycopeptides in gas phase have not been studied in sufficient details. Herein, protonated molecules of tryptic glycopeptides bearing an N-linked biantennary oligosaccharide were generated by electrospray ionization (ESI) and subjected to collision-induced dissociation (CID) in an ion trap mass spectrometer.

For the glycopeptides containing a single (C-terminal) basic amino acid residue, the dissociation at the glycosidic linkage between antenna GlcNAc and trimannosyl core was prominent in triple-charged precursors than in double-charged ones. This was probably due to localization of surplus/mobile protons at the GlcNAc nitrogen. Next, to examine the lability of different fucosyl linkages, the glycopeptides bearing antenna α1,3- (Lewis) or core α1,6-fucose were analyzed. The antenna 1,3-fucose was dissociated by lower collision energy compared with the core 1,6-fucose. Paradoxically, the dissociation of terminal Gal-GlcNAc linked to mannose occurred at the lower collision energy for core 1,6-fucosylated glycopeptides than antenna 1,3-fucosylated ones, irrespective of their peptide backbone sequences. This finding suggested a difference in the cross-section between these glycopeptide isomers in gas phase.

In conclusion, the glycan profiling at a specific glycosylation site can be performed in terms of the signal intensities of glycopeptide ions in the ESI mass spectrum, and the fragmentation behavior in MS/MS is affected by the peptide backbone sequences.

(197) **Development of Novel Glycan Inhibitors of Tumor Metastasis**

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A large number of deaths from epithelial tumors of the breast, prostate, lung, ovary, liver, and kidney are caused by metastatic spread of the primary tumor. Unfortunately, there are currently no drugs on the market that directly target cancer cells in the process of spreading (metastasis). The tumor antigen, sialyl Lexis X (sLe^X) is a glycan expressed on the surface of epithelial tumors that correlates with poor prognosis, aggressive metastasis and death. In both human and animal studies sLe^X expression can drive aggressive metastatic behavior. Thus, developing a pharmacological approach to inhibit sLe^X on tumor cells could improve patient survival. We have rationally designed peracetylated GlcNAcβ3Galβ-O-Naphthalenemethanol (ZP103); a novel glycan inhibitor of sLe^X biosynthesis. ZP103 mimics the terminal O-linked substrate for sLe^X formation and specifically inhibits the formation of sLe^X structures that cap the O-linked carbohydrates without inhibiting protein glycosylation in general. In tumor cells, ZP103 inhibits the formation of sLe^X in the 10-50 μM range (Brown et al. 2003 J. Biol. Chem. 278:23352; Fuster et al. 2003 Cancer Res. 63: 2775). Systemic administration of ZP103 potently blocks tumor metastasis in mouse models (Brown et al. 2006 Clin.Cancer Res. 12:2894). Ongoing studies include pharmacology and toxicology in addition to high throughput screening to identify novel drug candidates.

(198) **Using an Enzymatic Combinatorial Approach to Identify Novel Anticoagulant Heparan Sulfate Structures**

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Heparan sulfate (HS) represents a major class of glycans that perform central physiological functions. Emerging HS and glycosaminoglycan microarray techniques offer a sophisticated tool to interrogate the structure and function relationship to develop novel therapeutic agents. Availability of the polysaccharides with specific sulfation patterns has been a limiting factor, impeding the accuracy and range for the studies of HS glycomics. Although organic synthesis provided structurally defined oligosaccharides, these compounds are primarily smaller than hexasaccharides, which may not fully represent the biological functions of polysaccharides with the size of 100 to 400 saccharide units. Here, we present a study aimed at developing an enzyme-based approach to synthesize a polysaccharide library with different sulfation patterns. A total of four sulfotransferases and one epimerase are involved in the biosynthesis of HS. Using different combination of the biosynthetic enzymes, we synthesized eight polysaccharides with different repeating units. Screening the synthesized polysaccharides, we discovered that polysaccharides without iduronic acid residue displayed strong binding affinity and high anti-Xa and anti-IIa activities. Further, this polysaccharide has no activity in promoting cell proliferation, providing the evidence to synthesize a functionally specific anticoagulant polysaccharide. The enzyme-base synthetic approach could become a general method for discovering new HS structures with unique biological functions.

(199) **Multiplexed Stable Isotopic Labeling of Carbohydrates and Quantitative Analysis by Mass Spectrometry**

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The increasing interest in glycomics has led to the widespread use of mass spectrometry to determine the carbohydrate components expressed in biological systems. However, the quantification of carbohydrates within different samples remains a daunting task, due to instrument and sample variability. Herein, we show custom-synthesized multiplexed stable isotope labeled tags that have broad applicability for the simultaneous

quantitation of four samples during the same mass spectrometry experiment. Results exploring the utility of multiplexed stable isotopic labeling from a variety of sources have been demonstrated using nanospray ionization and normal-phase capillary LC/MS systems.

A stable isotope-labeled tag in four forms (+0,+4,+8,+12) was synthesized for the purpose of labeling the reducing end of glycans.(Bowman, M.J. and Zaia, J. 2007) Glycosaminoglycans from the chondroitin sulfate proteoglycan class and pharmaceutical low-molecular weight heparins were partially depolymerized using enzymes to form oligosaccharide distributions. Oligosaccharides

were labeled with tetraplex stable isotope-containing tags by reductive amination. In addition, N-linked glycans derived from α-1-acid glycoprotein from four species were also multiplex-labeled and analyzed.

The abundances of ions in the MS mode serve to quantify compositions from a given sample in relative amounts to each of the other three samples. These results, along with tandem MS data, further demonstrate the principle of quantitation by multiplex analysis of carbohydrate using stable isotope tags.

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Bowman, M.J. and Zaia, J. (2007) Tags for the stable isotopic labeling of carbohydrates and quantitative analysis by mass spectrometry. Anal Chem, 79, 5777-5784.

(200) **The Regulation of Immunological Functions by Poly-N-Acetyl-Lactosamine Glycan**

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To study the role of poly-N-acetyl-lactosamine (polylactosamine) on immune systems, we generated mice lacking β1,3-N-acetylglucosaminyl transferase 2 (β3GnT2) gene which code the strongest polylactosamine synthetase. Resting B cells from β3GnT2 deficient mice showed augmented proliferative response and enhanced protein-tyrosine phsophorylation upon anti-IgM stimulation. T cells from β3GnT2 deficient mice also showed augmented proliferative response and increased Ca2+ influx upon stimulation with anti-CD3 and anti-CD28. To reveal the molecular basis of augmented immunological responses in β3GnT2 deficient mice, we investigated lymphoid surface antigens carrying polylactosamine. Lymphocyte surface glycoproteins from wild type and deficiency mice were biotinylated and immune precipitated with specific antibodies. Then, biotinylated glycoproteins were subjected to immunoblot analysis or to lectin array analsysis. Among the molecules tested, we found that polylactosamine was carried on CD19 in B cells and CD28 in T cells from wild type mice, whereas polylactosamine was not observed on these molecules in β3GnT2 deficient mice. It is known that both CD19 and CD28 molecules work as a positive regulator for BCR and TCR, respectively. These results strongly suggest that polylactosamine on glycoproteins regulate immune reaction by interfering non specific binding to BCR or TCR to elevate threshold.

This work was supported by New Energy and Industrial Technology Development Organization (NEDO) in Japan under the Ministry of Economy Trade, and Industry (METI).

(201) **Heterologous Expression of Rat α2,6 Sialyltransferase in *Pichia Pastoris* for Structural and Functional Studies**

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ST6Gal1 is one of the most studied enzymes of the CAZy Family 29 (GT29) glycosyltransferases. The enzyme has been shown to modulate CD22-mediated immune responses through the synthesis of its trisaccharide ligand product, Siaα2-6Galβ1-4GlcNAc (Sia6LacNAc). Despite such a critical role in immune function, no structural data is yet available for ST6Gal1 or any other CAZy GT29 family enzyme. A major limitation in the structural analysis of glycosylation enzymes, including ST6Gal1, is the large-scale expression and purification of the glycosylated enzymes in forms compatible with structural analysis.

We have successfully generated recombinant *Pichia* expression constructs using a modular approach to encode secreted forms of sialyltransferases. A construct containing the ST6Gal1 coding region was prepared in the *Pichia* vector pPIC-Z-αC and transformed into Pichia host strains. Controlled fermentation was used for expression of recombinant ST6Gal1 and the media composition and fermentation conditions were optimized in order to maximize recovery and the ^{15}N labeling of recombinant enzyme. HSQC spectra of uniformly ^{15}N labeled ST6Gal1 indicated that heterogeneous glycosylation may be a limiting factor for generation of a homogeneous enzyme preparation for NMR and X-ray crystallography. Glycan heterogeneity was subsequently eliminated by treating recombinant ST6Gal1 with Endo H followed by chromatography over ConA-Sepharose. Further characterization of deglycosylated ST6Gal1 by NMR indicates a flexible enzyme partially stabilized by substrate binding. A multiple isotope labeling strategy (^{2}H, ^{13}C and ^{15}N) is underway focused on NMR resonance assignment and eventual structural determination of the recombinant enzyme (Supported by NIH grant RR005351).

(202) **Identification of Lewis^{x}-Containing Granulocyte Ligands for the Endothelial Cell Scavenger Receptor C-Type Lectin**

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The scavenger receptor C-type lectin (SRCL), found on endothelial cells, is a trimeric transmembrane protein in which a cluster of C-type carbohydrate-recognition domains is projected from the cell surface at the end of an elongated stalk. The carbohydrate-recognition domains show unusually high selectivity for glycans terminating in Lewis^{x} or Lewis^{a} trisaccharides. Potential roles of SRCL are being investigated using fragments of the protein as tools to identify target glycoprotein ligands and to characterise the pattern of receptor expression. Glycoproteins bearing Lewis^{x}-containing glycans have been isolated from granulocytes using an engineered version of mannose-binding protein. Ligands for SRCL have been detected in the resulting pool of glycoproteins with radiolabelled SRCL. Two sets of candidate SRCL ligands have been identified by mass spectrometry and immunoblotting: one set, at approximately 175 kDa, consists of CD11b, ICAM-3 and/or CEACAM-1; the second set, at approximately 80 kDa, consists of CD18, matrix metalloproteases 8/9 and/or CEACAM-6/8. A similar approach demonstrated that SRCL binds to tumour-cell CD98. Polyclonal antibodies specific for human SRCL, generated using expressed carbohydrate-recognition domain as immunogen and for affinity purification, have been used to screen a tissue micro-array by immunohistochemistry. The results suggest that SRCL is expressed on subsets of endothelial cells and that it may also appear on some non-endothelial cell types. Expression of SRCL on endothelial cells, combined with the identification of glycoprotein ligands on granulocytes and tumour cells, suggests that this receptor may play a role in interaction of circulating cells with the vascular endothelium.

(203) **The Role of Galectin-3 in Experimentally Infected Mice with *Rhodococcus Equi***

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Galectin-3 is implicated in the fine tuning of innate immunity. Rhodococcus equi, an intracellular bacterial pathogen, causes severe granulomatous bronchopneumonia in young horses and immunocompromised humans. In the present study we investigate the role of galectin-3 in the innate resistance mechanism against R. equi infection. The bacterial challenge of galectin-3 deficient mice (gal3-/-) and their wild-type counterpart revealed that the LD50 for the gal3-/- was about 7 times higher than that for the gal3+/+ mice. When challenged with a lethal dose, gal3-/- mice showed higher survival rate and lower CFU recovering from liver and spleen, compared to gal3+/+ mice. The wild-type mice presented severe inflammation, which was accompanied by an increase in galectin-3 expression and followed by necrosis of hepatocytes. *In vitro* R. equi infection of peritoneal macrophages revealed that bacterial replication was lower, while the transcript levels of IL-12, IL-1β, IL-6, IL-10, TLR2, and MyD88 were higher in gal3-/- cells compared to gal3+/+ cells. Furthermore, the higher amounts of IL-1β produced by gal3-/- macrophages were correlated with the higher IL-1β serum levels detected in infected gal3-/- mice. Therefore galectin-3 may exert a regulatory role in innate immunity by augmenting IL-1β production and also affecting the TLR/MyD88 signaling pathway in macrophages. Because IL-1 signaling is required for the containment of infections by intracellular microorganisms, we postulate that the high levels of IL-1β may contribute to the enhanced resistance of gal3-/- mice to R. equi infection.

(204) **Presence of a Non-Human Sialic Acid on Biotherapeutic Products: Implications for Pharmacokinetics, Efficacy and Immunogenicity**

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Most currently approved biotherapeutic products glycoproteins such as clotting factors, hormones, growth factors and monoclonal antibodies are produced via recombinant expression. These recombinant N- and/or O-glycosylated biotherapeutics are typically produced in non-human mammalian cell lines with the ability to mimic the native glycosylation of the protein. However, non-human mammalian cells also produce the sialic acid N-glycolylneuraminic acid (Neu5Gc), which is absent in humans due to a human-specific deletion in the human CMP-Neu5Ac hydroxylase (CMAH) gene. Furthermore, cultured cells can take up and metabolically incoporate Neu5Gc from animal-derived sera and serum replacement products. Meanwhile, we have recently found that normal humans express a polyclonal and variable spectrum of circulating anti-Neu5Gc antibodies, sometimes present at high levels. We have now used ELISA, Western-Blotting and HPLC methods to confirm and extend the prior finding that many glycoprotein biotherapeutic products contain varying amounts of covalently bound Neu5Gc. Furthermore, we show that anti-Neu5Gc antibodies isolated from normal human serum can interact with such products in a Neu5Gc-specific manner *in vitro*. We are now using mice with a human-like defect in Neu5Gc synthesis to study the possibility that the presence of Neu5Gc on biotherapeutic agents can alter their pharmacokinetics, efficacy or immunogenicity, when recipients have pre-existing circulating anti-Neu5Gc antibodies. Such studies may help elucidate some previously unexplained reactions of human patients against biotherapeutic drugs.

(205) **A Systems Biology Approach for Decoding the Function of N-Glycans on Glycoprotein Receptors**

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Embryogenesis, tissue repair and adaptive immunity involve developmental sequences of cell proliferation followed by differentiation and cell cycle arrest. Growth factors and other cytokines bind glycoprotein receptors to stimulate growth or arrest signaling. The net response depends on the availability of both ligands and receptors. The number of N-glycans (n), a distinct feature of each glycoprotein sequence, cooperates with physical properties of the Golgi pathway to regulate surface levels of receptors. The Golgi pathway is ultrasensitive to hexosamine flux for the production of tri- and tetra-antennary N-glycans, which bind to galectins and form a molecular lattice that opposes glycoprotein endocytosis. Glycoproteins with few N-glycans (e.g. TβR, CTLA-4, GLUT4) exhibit enhanced cell surface expression with switch-like responses to increasing hexosamine concentration, whereas glycoproteins with high numbers of N-glycans (e.g. EGFR, IGFR, FGFR, PDGFR) exhibit hyperbolic responses. Computational and experimental data reveal that these features allow nutrient flux stimulated by growth-promoting high n receptors to drive arrest/differentiation programs by increasing surface levels of low n glycoproteins. By utiltizing microarray analysis and RNAi knockdown, we have identified GlcNAc-sensitive pathways on a global scale that confirm our model and directly affect growth/arrest regulation, complex N-glycan processing, and constitutive endocytosis. Our results reveal a mechanism for metabolic regulation of cellular transition between growth and arrest in mammals arising from apparent co-evolution of N-glycan number and biosynthesis.

(206) **Specific Enrichment and Identification of Azide Modified Glycoproteins using the Staudinger Ligation**

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Among more than 100 types of post-translational modifications, glycosylation is the most common with several diseases being associated with the aberrant form. It is for these reasons that the development of methods for the study of glycosylation is becoming increasingly important. Sialic acid expression on cell surface glycoproteins and the modification of intracellular proteins by O-linked b-N-acetylglucosamine (O-GlcNAc) are significant disease state post-translational modifications. Cell surface sialic acid is known to mediate a variety of cellular interactions during cell development, differentiation and tumor progression. It has been shown that enhanced sialyltransferase (ST) activity, which results in hypersialylation, is implicated in up-regulation of metastatic potential. The O-GlcNAc modification affects cell transcription, translation, and signaling. Several studies have shown the possible role O-GlcNAc plays in neurodegenerative diseases and diabetes. In this study the enrichment capabilities of the Staudinger ligation were used to examine the incorporation and presentation of sialic acid and O-GlcNAc. The Staudinger ligation is a reaction between an azide functionality and a phosphine derivative to yield an amide bond. Incorporating azide-modified mannose, a sialic acid precursor, and azide-modified N-acetylglucosamine, cell culture can produce modified glycoproteins. FLAG-phosphine, used for its immunochemical versatility, acts as a probe for the capture of the azide-modified glycoproteins. By exploiting these techniques, sialic acid and O-GlcNAc modified glycoproteins were identified by combining immunoprecipitation and LC-MS/MS. To our knowledge, this is the first reported instance of the utilization of a modified Staudinger ligation to enrich for modified

glycoconjugates, making available a powerful new tool for glycoprotein analysis.

(207) Oxime-linked Neoglycolipids - Powerful tools for Recognition Studies of Glucan-Binding Proteins in Microarrays

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Neoglycolipid (NGL) technology is the basis of an advanced microarray platform for assigning oligosaccharide ligands of carbohydrate-binding proteins[1-3]. The NGL-based microarrays currently include over 300 robotically-arrayed sequence-defined saccharide probes. These encompass NGLs derived from natural and synthetic oligosaccharides as well as glycolipids, and are expanding in number and structural diversity. As a complement to 'conventional' NGLs, which are prepared by reductive-amination, we have recently described a novel type of NGLs prepared by oxime-ligation with ring-closed monosaccharide cores[4]. A major advantage of oxime-linked NGLs is in presentation of short oligosaccharides and *N*-glycans for recognition by proteins that require non-reduced cyclic monosaccharide cores[4]. We have demonstrated by STD NMR studies[4] the specific recognition of the ring-closed form of an oxime-linked sugar by a lectin. In this communication we will focus on a further advantage of the oxime-ligation method, namely the efficient preparation of NGLs from certain oligosaccharides that are difficult to conjugate by reductive-amination. We will describe oxime-linked NGLs prepared from oligosaccharides of fungal and bacterial glucans with differing glucose linkages and chain lengths, and their recognition studies in microarrays with several glucan-binding proteins including carbohydrate-binding modules of bacterial glucan hydrolases. (Supported by MRC and UK Research Councils' Basic Technology Grant 'Glycoarrays')

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(208) Engineering Sugar-Binding Proteins to Detect Tumor-Associated Glycans

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Changes in glycosylation on tumour cells include increased expression of galactose-containing epitopes such as Lewis^x and Lewis^a antigens. We have modified rat mannose-binding protein (MBP) so that it shows specificity for galactose-terminated oligosaccharides. Glycan array analysis demonstrates that galactose-binding MBP (GalMBP) shows preference for structures with adjacent terminal galactose and fucose residues, such as the Lewis^x and Lewis^a trisaccharides. GalMBP binds to MCF7 breast cancer cells with 200-fold greater affinity than native MBP. Probing blots of gels of MCF7 membranes led to the identification of two predominant bands that are bound by radiolabelled GalMBP. These glycoprotein ligands have been purified on immobilised GalMBP columns. Analysis by mass spectrometry revealed that the ligands are forms of the heavy chain of CD98 (CD98hc), a cell-surface glycoprotein expressed in many cancerous cells. Recognition of purified CD98hc from MCF7 cells by anti-Lewis^x antibodies confirms the presence of the Lewis^x antigen and glycan analysis by mass spectrometry demonstrated that CD98hc contains heavily fucosylated glycans, including Lewis^a, Lewis^b, Lewis^x and Lewis^y structures. Parallel analysis of the Hodgkin Reed-Sternberg cell line L428 indicated that CD98hc is also the principal carrier of Lewis^x structures recognised by anti-CD15 antibodies that are used diagnostically for detection of Hodgkin's lymphoma. Thus, GalMBP provides a powerful tool for the characterisation of glycoproteins bearing tumour-associated glycans. The results demonstrate that specifically glycosylated CD98hc is a common tumour surface marker.

(209) Identification of the Major N-Glycosylated Glycoproteins from the Fruit-Fly Brain

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Glycosylation is thought to play an important role in the development and function of the nervous system, given the immense number of highly specific synaptic connections in the brain. Even though a large number of glycoproteins have been studied in the CNS of many organisms, very little information is available about the functional role of their N- or O-glycans.

In an attempt to fill this gap, we have initiated a large-scale identification of N-glycosylated glycoproteins and their N-glycan attachment sites from the fruit-fly brain.

Using a combination of lectin-affinity chromatography steps and LC-MS/MS analysis we identified a total of 205 glycoproteins carrying at least one N-linked carbohydrate chain and revealed their 307 N-glycan attachment sites. The size of the resulting dataset furthermore allowed the statistical characterization of amino acid distribution around the N-linked glycosylation sites. N-glycans, which were released and analyzed separately, were found to be dominated by oligo- and paucimannosidic structures, although a monoantennary sialylated glycan was also detected. The wealth of information gathered in this study should significantly facilitate future genetic and molecular approaches addressing the role of N-glycosylation in the CNS of Drosophila.

(210) Analysis of the Anticoagulant Potential of Glycosaminoglycans Expressed in the Different Human Umbilical Vein Wall Layers

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Sulfated glycosaminoglycans are expressed throughout the vessel wall and there is evidence that they might participate in the control of blood coagulation. To investigate the dynamics of glycosaminoglycan expression and anticoagulant potential throughout the vessel wall layers, a bulk of sulfated glycosaminoglycan was extracted from umbilical veins. These veins were divided in three groups: intact veins, containing the three layers, intima, media and adventitia (IMAL), veins without the intima (MAL) and veins with only the adventita layer (AL). We hypothesized that the intima layer, which is in direct contact with blood, would have glycosaminoglycans with higher anticoagulant activity than glycans from the other two layers. Our results reveal that IMAL veins have a higher proportion of heparan sulfate (32%) compared to MAL and AL veins (20% both), suggesting that the expression of this glycan is concentrated in the intima layer. Surprisingly, there was no difference in anticoagulant activity of heparan sulfate extracted from all three groups. Analysis of the disaccharide composition of heparan sulfate showed that IMAL samples had higher proportion of non-sulfated disaccharide units (54%) than MAL and AL samples (40% both). This suggests a less sulfated heparan sulfate expressed by the intima layer. In

conclusion, the heparan sulfate expressed in the umbilical vein wall has the same anticoagulant activity despite its localization, although it is concentrated in the intima layer. The difference in sulfation pattern indicates some specificity in the intima layer heparan sulfate expression, but it cannot be directly related to the anticoagulant potential of these molecules.

(211) New Method for Lectin Labeling using Sugar Tethered Catalyst

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Because sugar-binding proteins, so called lectins, play important roles in many biological phenomena, the lectin selective labeling should be useful for investigating the details of the lectin-involved biological processes as well as for providing molecular tools for analysis of saccharides and these derivatives. We describe herein a new strategy for lectin selective labeling based on an acyl transfer reaction directed by ligand-tethered DMAP (4-dimethylaminopyridine). DMAP is a talented acyl transfer catalyst which can activate an acyl ester for transferring it to a nucleophilic residue. To dictate the acyl transfer reaction to a lectin of interest, we connected the DMAP to a saccharide ligand specific to a target lectin. It was clearly demonstrated by biochemical analyses that the target-selective labeling of Congerin II, an animal lectin having the selective affinity for Lactose/LacNAc, was achieved in the presence of Lac-tethered DMAPs and acyl donors containing probes such as fluorescent molecules or biotin. Conventional peptide mapping experiments using HPLC and tandem mass-mass analysis revealed that the acyl transfer reaction site-specifically occurred at Tyr 51 of Cong II. This strategy was successfully extended to other lectin by changing the ligand part of ligand-tethered DMAP. We also demonstrated that this labeling method is applicable not only to a purified lectin in test tubes, but also to crude mixtures such as E. coli lysates or a homogenized mammalian tissue samples expressing Congerin.

(212) Structural Features of *Trichomonas vaginalis* LPG that Regulate Inflammatory Responses in Reproductive Tract Epithelial Cells

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The parasitic protozoan *Trichomonas vaginalis* (TV) causes one of the most common non-viral sexually transmitted infections worldwide. The infection has been linked to increased incidence of HIV transmission, inflammation and other complications related to women's reproductive health. Lipophophoglycan (LPG) is the predominant glycosylphophatidyl inositol (GPI)-anchored molecule present on the surface of the parasites. We have shown that TV-LPG (but not the LPG of bovine parasite *Tritrichomonas foetus*) upregulates expression of inflammatory mediators (IL-8, MIP-3□) by human female lower reproductive tract epithelial cells and extended our studies by defining the biochemical nature and novel biological functions of LPG. Endo-β-galactosidase treatment of LPG and PI-core released oligosaccharides ranging from 2.5-6 glucose units. These fractions and those released by mild acid hydrolysis were analyzed by Glyco-FACE, HPAE-PAD and tandem MS, and the results indicated the presence of GalN, Rha, Gal, GlcN, Glc, and Xyl, including lactosamine repeats. MALDI-TOF MS of mild acid-released LPG PI-core showed signals in the region *m/z* 8700-9300. MALDI-TOF MS analysis of the PI-core after endo-β-galactosidase treatment is underway. We have determined that the PI-core of LPG is more active in inducing the inflammatory responses in the human lower female reproductive tract epithelial cells. We also showed that β-galacoside-binding protein galectin-1 binds to *T. vaginalis* parasites and to its LPG and PI-core. The detailed biochemical analyses of LPG and its PI-core are essential for understanding the mechanisms involved in *T. vaginalis*-induced immune regulation. Acknowledgements: NIH NICHD R21 HD054451 (RF) and NCRR P41 RR010888 and S10 RR015942 (CEC).

(213) Expression of Glycosilates Antigens in Breast Cancer

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The breast cancer is the most common carcinoma in women that they present some alteration in the mammary gland. It has been detected that in most of the cells of carcinomas there is an incomplete synthesis of the oligosaccharides chains, forming less complex structures like antigens T (the Galβ1,3GalNAcα1-O-Ser/Thr) and Tn (GalNAcα1-O-Ser/Thr). These antigens are associated with the uncontrolled growth of the cells. In addition the expression to oligosacarídicas structures related to the Sialil antigen Lewis x, can be responsible for the evasion of the immune system on the part of the cells that express them. These changes in the glycosylation can be studied lectins that are proteins able to recognize carbohydrates of specific and reversible way. In these work we used immnunohistochemistry we lectin to evaluate changes in glycosylation in fibroadenoma and breast cancer samples. Lectins from: Griffonia (Bandeiraea) simplicifolia, Dolichos biflorus, Artocarpus integrifolia and Amaranthus leucocarpus recognized showed different pattern in fibroadenoma and breast cancer sample suggesting, changes in the expression of T and Tn antigens during the development of breast cancer.

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(214) A Human-Specific Mechanism for Dietary Exacerbation of Atherosclerosis

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Sialic acids are present at the terminal positions of glycans that coat the surfaces of all vertebrate cells. The two most common mammalian sialic acids are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). CMP-N-acetylneuraminic Acid Hydroxylase, the enzyme responsible for Neu5Gc biosynthesis, was inactivated during human evolution. However humans are not completely Neu5Gc deficient. Previous work has shown that free Neu5Gc can be metabolically incorporated into human glycans *in vitro*, as well as into the human body via oral intake. Interestingly, adult humans express varying and sometimes high levels of polyclonal anti-Neu5Gc antibodies. Atherosclerosis is a central pathology of cardiovascular disease, characterized by unresolved inflammation of the vascular wall. We hypothesized that the combination of dietary Neu5Gc incorporation into vascular endothelium and circulating anti-Neu5Gc antibodies contributes to the inflammatory state exacerbating atherosclerosis in humans. Immunohistochemistry of adult human aorta autopsy samples showed strong endothelial expression of Neu5Gc. We modeled this finding *in vitro* by loading cultured human endothelial cells with Neu5Gc or Neu5Ac. Exposure to human sera with high anti-Neu5Gc reactivity resulted in Neu5Gc-dependent antibody deposition, classical complement deposition, immediate P-selectin presentation, and long-term E-selectin expression. TNF-α, a potentiator of inflammation enhanced anti-Neu5Gc antibody reactivity, suggesting a feed forward mechanism for anti-Neu5Gc-

dependent inflammation in a pro-inflammatory paradigm. In conclusion, our hypothesis may substantiate the atherogenic potential of Neu5Gc-rich foods e.g., red meat and dairy products, and explain the unusual frequency and severity of life-threatening clinical manifestations of atherosclerosis in humans as opposed to other closely related mammals.

(215) Increased Frequency of Non-Sulfated Sialyl Lewis X on High Endothelial Venule-Like Vessels in Gastric MALT Lymphoma

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The majority of primary gastric lymphomas is B cell lymphoma of mucosa-associated lymphoid tissue (MALT) type. It is widely accepted that chronic infection of *Helicobacter pylori* leads to generation of *H. pylori*-reactive T cells, which, in turn, activate a polyclonal population of B cells. With time, a monoclonal but T cell-dependent population of proliferating B cells emerges. If untreated, genetic mutations accumulate in these proliferating B cells, and they eventually become T cell-independent. We previously showed that peripheral lymph node addressin (PNAd)-expressing high endothelial venule (HEV)-like vessels are induced in *H. pylori*-associated chronic gastritis, and that the progression of chronic inflammation is highly correlated with the occurrence of PNAd-expressing HEV-like vessels (Kobayashi *et al*., Proc Natl Acad Sci USA **101:** 17807-17812, 2004). These results indicate that at inflammatory sites, lymphocyte recruitment is partly facilitated by PNAd. However, precise analysis of gastric MALT lymphoma in association with HEV-like vessels has not yet been done. We performed immunohistochemical analysis of biopsied specimens with gastric MALT lymphoma, and found that the number of MECA-79-positive HEV-like vessels in gastric MALT lymphoma was smaller than that in severe chronic gastritis. Moreover, while only a small number of MECA-79-positive HEV-like vessels were detected, HECA-452-positive HEV-like vessels were frequently observed. These results provide a potential histological marker to distinguish MALT lymphoma and severe chronic gastritis. This work was supported by Grant-in-Aid for Young Scientists B-1879240 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by NIH grant PO1 CA 71932.

(216) Preferential Glycosylation of MAdCAM-1 on HEV-Like Vessels in the Active Phase of Ulcerative Colitis

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Ulceratve colitis (UC) is a chronic inflammatory disorder affecting the colonic mucosa, and a diffuse lymphocyte infiltrate in the lamina propria is almost universally present. Two molecular interactions are implicated in the initial step of lymphocyte homing, i.e., one is via L-selectin-PNAd, and the other is via α4β7 integrin-MAdCAM-1. We previously showed that PNAd-expressing HEV-like vessels are preferentially induced in the active phase of UC (Suzawa *et al*. Am J Gastroenterol, 2007); however, the role of MAdCAM-1 was not considered. To clarify the participation of MAdCAM-1 in the lymphocyte homing in UC, 44 biopsy specimens composed of active (N=32) and remission phases (N=12) of UC were subjected to immunostaining for MECA-79, MAdCAM-1 and CD34, and those immunostained sections were quantitatively analyzed. We found that the percentage of MECA-79$^+$ vessels among MAdCAM-1$^+$ vessels in active phase is greater than that seen in remission phase with high statistical significance (P=0.0009), though the percentages of MAdCAM-1$^+$ vessels among CD34$^+$ vessels do not differ between these two phases. Laser confocal immunofluorescence microscopy analysis demonstrated not all, but some fractions of MAdCAM-1$^+$ vessels are also positive for MECA-79. These data as a whole suggest that MAdCAM-1$^+$ vessels themselves could not effectively recruit lymphocytes, and glycosylation of MAdCAM-1 (addition of PNAd carbohydrates) is prerequisite for lymphocyte homing in the active phase of UC. This work was supported by Grant-in-Aid for Young Scientists B-1879240 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by NIH grant PO1 CA 71932.

(217) Caveolin-1 via CD147 Glycosylation Affects the Lymphatic Metastasis of Murine Hepatocarcinoma Cell Lines

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Caveolin-1 (Cav-1) is a major component protein of caveolae and a potential promoting metastasis gene. CD147 is a plasma membrane glycoprotein, enriched on the surface of many malignant tumor cells. As a result of heterogeneous N-glycosylation, CD147 exists in a highly glycosylated form, HG-CD147 (~ 40-60 kDa) and lowly glycosylated form, LG-CD147 ~32 kDa) as well. HG-CD147 was thought to be associated with matrix metalloproteinase (MMP) production on the surface of many malignant tumor cells. Our previous studies indicated that caveolin-1 was highly expressed in the HcaF and HcaP mouse hepatocarcinoma cell lines, which have high and low metastatic potential in the lymph nodes, respectively, but was not expressed in Hepa1-6 mouse hepatocarcinoma cell lines without lymphatic metastasis potential. In this study we showed the ratio of HG-CD147/LG-CD147 protein expression on HcaF and HcaP was much higher than that on Hepa1-6 cells. Further, silencing Cav-1 expression by siRNA in Hca-F cells suppressed the conversion of LG-CD147 to HG-CD147, down-regulated MMP-11 expression and decreased invasion capability of Hca-F cells. Conversely, a stable high express on of Cav-1 in Hepa1-6/Cav-1 cell caused to an increased proportion of HG-CD147 relative to LG-CD147, increased production of MMP-11 and a higher invasive capability. These results suggest that caveolin-1 via CD147 glycosylation plays a crucial role in tumor lymphatic metastasis and might represent a new potential target for gene therapy. This work was supported by grants from NSFC (No.30470400 and 30670466, to J. Zhang)

(218) O-GlcNacylation of Phospholamban Induces Cardiac Dysfunction

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Cardiac type sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2a) plays a major role in cardiac muscle contractility. Phospholamban (PLN) regulates the function via its Ser^{16}-phosphorylation. Since it has been proposed that the Ser/Thr residues on cytoplasmic and nuclear proteins are modified by O-linked N-acetylglucosamine (O-GlcNAc), we examined the effect of O-GlcNAc modification (O-GlcNAcylation) on the function of PLN. In rat adult cardiomyocytes trated with O-(2-acetamid-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate (PUGNAc), O-GlcNAcylation of PLN was significantly increased compared to non-treated cardiomyocytes. Simultaneously, Ser^{16}-phosphorylation and pentamer formation of PLN were reduced.

The same results were observed in heart homogenates from diabetic rats. The measurement of SERCA activity showed that the inhibitory function of PLN was increased in PUGNAc-treated cardiomyocytes, resulting in a lower peak and a prolonged relaxation time in mechanical properties and a lower peak and a prolonged decay time in the profiles of calcium transient. These data suggest that PLN is O-GlcNAcylated, resulting in the deterioration of cardiac muscle function. This might define a novel mechanism by which PLN regulation of SERCA2a is altered under conditions where O-GlcNAcylation is increased, such as those occurring in diabetes.

(219) **Glycomic Mapping of O- and N-Linked Glycans from Major Rat Sublingual Mucin**

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Carbohydrate moieties of salivary mucins play various roles in life processes, especially as a microbial trapping agent. While structural details of the salivary O-glycans from several mammalian sources are well studied, very few information is currently available for the corresponding N-glycans. The existence of N-glycans alongside the O-glycans on mucin isolated from rat sublingual gland has previously been implicated by total glycosyl compositional analysis but the respective structural data are both lacking. The advent of facile glycomic mapping and sequencing methods by mass spectrometry (MS) has enabled a structural reinvestigation into many previously unsolved issues. For the first time, high energy collision induced dissociation (CID) MALDI-MS/MS as implemented on a TOF/TOF instrument was applied to permethyl derivatives of mucin type O-glycans and N-glycans, from which the linkage specific fragmentation pattern could be established. The predominant O-glycans carried on the rat sublingual mucin were defined as sialylated core 3 and 4 types whereas the N-glycans were determined to be non-bisected hybrid types similarly carrying a sialylated type II chain. The masking effect of terminal sialylation on the tight binding of rat sublingual mucin to Galβ1→4GlcNAc specific lectins and three oligomannose specific lectins were critically demonstrated in this study.

(220) **Cancer Vaccine Delivery System using Oligomannose Coated Liposomes**

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We recently established a novel drug delivery system (DDS) using oligomannose-coated liposomes (OMLs) which are probably taken up by macrophages (Mφ) to carry anti-cancer drugs to milky spots known as a preferential metastatic site of gastric cancers (Ikehara Y. et al. Cancer Res. 66(17): 8740-8, 2006). In the present study, we applied this intraperitoneal DDS for systemic cancer immunotherapy employing ovalbumin (OVA) as a model antigen. The cells taking up the OMLs containing FITC-OVA injected into the peritoneal cavity were confirmed to be Mφ, as they showed adhesive characteristics and expressed F4/80 and CD11b almost exclusively. Peritoneal Mφ also took up bare OVA directly to the same extent as OML-enclosed OVA (OML-OVA), as it is a highly mannosilated protein. The Mφ taking up OML-OVA, however, could activate OVA-specific CD8+ (from OT-I: H-2Kb/OVA257-264-specific) and CD4+ (from OT-II: H-2Ab/OVA323-339-specific) T cells much more effectively *in vitro* than those taking up bare OVA. Furthermore, only the mice pre-immunized with OML-OVA rejected E.G7-OVA (OVA-transfected EL4) but not EL4. These results indicate that the OMLs can also be used as an effective antigen delivery system for cancer immunotherapy activating both CTL and Th subsets. Supported by the Industrial Technology Research Grant Program (04A01548a) from NEDO and in part by PROBRAIN.

(221) **Profiling the Peptide Substrate Specificities of the UDP-Gal: Glycoprotein-α-GalNAc β3 Galactosyltransferase (Core 1 T) using Oriented Random Peptide Substrates**

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Mucin-type protein O-glycosylation is initiated by addition of α-GalNAc to Ser/Thr residues to the peptide core catalyzed by a family of polypeptide N-α-acetylgalactosaminyltransferases (ppGalNAcTs). A common substitution of GalNAc is the addition of β-Gal, forming the Core 1 structure (β-Gal(1-3)-α-GalNAc-O-Ser/Thr). The extent that peptide sequence modulates this step is poorly understood; however, peptide sequence and neighboring glycosylation effects have been implicated (Eur.J.Biochem. *221*, 1039(1994), J.Biol.Chem. *277*, 7736(2002), Biochemistry *43*, 4137(2004)). Towards systematically addressing the role of peptide sequence on the specificity of the Core 1 transferase, we utilized the random peptide: GAGAXXXX(T-O-α-GalNAc)XXXXAGAGK, (X = G,A,P,V,I,F,Y,E,D,N,S,R,K,H) as a substrate. The Core 1 glycosylated product was isolated on an immobilized PNA (*Arachis hypogaea*) lectin column and analyzed by Edman amino acid sequencing as performed previously for characterizing ppGalNAc T specificity (J.Biol.Chem. *281*, 23403(2006)). Interestingly, significant enhancements are observed for Phe and Tyr at the +3 and +4 positions relative to the site of glycosylation while smaller enhancements for Gly at the +1 position was observed. Weaker enhancements for Glu and Asp were also detected at the -1 position in agreement with previous studies. Overall, the Core 1 T shows fewer peptide residue preferences compared to the ppGalANc T's. Nevertheless, the Core 1 T displays a number of unique preferences which suggests that Core 1 glycosylation may indeed be directed in some instances towards specific protein targets. Supported by NIH-NCI RO1 CA-78834.

(222) **Biological Activities of the Lectin from Sea Mussel *Crenomytilus Grayanus***

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A lectin, Crenomytilus grayanus (CGL), was purified from sea mussel C. grayanus, which showed GalNAc/Gal specificity. Molecular weight of CGL obtained was determined by SDS-PAGE to be 18000. The hemagglutinating activity of CGL was independent of the divalent cation Ca2+. CGL displays mitogenic effect on BALB/c splenocytes. CGL also inhibited angiotensin converting enzyme (ACE). The IC50 value was 6.82mg/mL. The peristalsis of rabbit ileum was increased by CGL.

(223) **Specificity of Anti-Glycan Monoclonal Antibodies Assessed on a Glycan Microarray**

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Numerous studies have been carried out to understand the biological roles of carbohydrates and how changes in carbohydrate expression contribute to the cell functions and diseases. Glycan-specific antibodies have been used as tools to monitor the expression of carbohydrate antigens in cell differentiation and disease. However, the degree to which the specificity of an individual antibody has been checked for cross-reactivity with related structures is tied with its unique history, and assays used for such analysis are not standardized to readily allow comparisons. To provide a reference for detailed comparisons of anti-glycan antibodies, the Consortium for Functional Glycomics (CFG) has set a strategic objective to assess the specificities of glycan specific antibodies widely used by the research community. Analysis is performed on the CFG glycan microarray containing over 320 glycans representing major classes of terminal structure motifs found on glycoconjugates. The specificities of over 40 anti-glycan monoclonal antibodies have been conducted and the results are deposited to the CFG database (wwwfunctional glycomics.org). Antibodies to Lewis epitopes and their sialyl/sulfo derivatives (7LE,T-174, 2-25LE,T-218,73-30, P12, H18A, F3, AH6, SH1, 2D3, KM231, 2H-5, KM93, FH6, G72), blood group antigens (CLCP, 2C9, HH4, HH6, BE2, TH5) and tumor-associated antigens (B35.1, HH8, 1E3, 5F4, 3E8, 3F1,TKH2) were tested, each in several concentrations. Results show that antibodies vary considerably in their crossreactivity to glycan structures related to their primary specificity. We acknowledge the generosity of investigators and commercial suppliers for providing antibodies for this work. (Supported by NIGMS grant GM62116).

(224) **SCFA-Monosaccharide Analogs: New Tools to Deconvolute the Influence of HDACi and NF-κB Over Invasion in Metastatic Breast Cancer Cells**

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Our laboratory has recently shown that the regiospecific attachment of n-butyrate to a monosaccharide 'scaffold' controls several biologic properties of the resulting short chain fatty acid (SCFA)-ManNAc hybrid molecules. In this presentation, we take advantage of analogs with 'histone deacetylase inhibitor (HDACi)-only' activity (exemplified by per-butyrated D-mannose) and matched counterparts (represented by per-butyrated N-acetyl-D-mannosamine, ManNAc) with combined HDAC/NF-κB inhibition to explore the impact of HDACi on NF-κB target genes implicated in metastasis. In our first experiments, the 'HDACi-only' analogs were exploited to uncover counteracting molecular-level changes in metastatic MDA-MB-231 cells that included the increased transcription of pro-MMP-9 and MUC1 together with the offsetting down-regulation of CXCR4 mRNA and a decrease in active MMP-9. These counterbalancing factors resulted in no net change in the invasiveness of treated cells. By contrast the second group of analogs – which demonstrated herein have dual inhibition of HDAC and NF-κB –uniformly inhibited investigated pro-invasive oncogenes and substantially decreased invasion. Mechanistically, the ability of the second set of compounds to inhibit pro-invasive genes, in particular MUC1 that proved to be a critical determinant of whole cell behavior, was linked to a 'built in' capacity – uniquely present in amino sugar analogs with an N-acetyl group at the C-2 position and an ester-linked n-butyrate group at the C-6 position – of these analogs to combine NF-κB inhibition with HDACi activity. In ongoing work, microarray analysis has provided further mechanistic insight into the action of these analogs and animal studies are underway to demonstrate *in vivo* efficacy.

(225) **Beta3GlcNAc-T5 Induction in Gastric Epithelial Cells by Helicobacter Pylori Leads to Expression of Sialyl-Lewis X, the Ligand for SabA Adhesin**

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A long-term infection by Helicobacter pylori (Hp) is associated with gastric carcinoma. Hp binding/interaction with host cells alters the host's gene expression. Expression of the inflammation-associated sialyl-Lex antigen in the gastric epithelium is induced during persistent Hp infection, suggesting that Hp may trigger the host tissue to retailor the gastric mucosal glycosylation patterns to a more favorable environment for its adhesion. Hp has been shown to adhere to sialylated glycoconjugates during chronic inflammation. The biosynthesis of complex carbohydrate structures that may be altered by Hp, namely those that may function as ligands for bacterial adhesins (BabA, SabA) remains largely unknown. We evaluated host gene expression in response to Hp infection.

Hp induced significant alterations in 168 of the 1031 genes tested. The most virulent Hp strain led to altered expression of glycosylation-related genes, including the increased expression of b3GlcNAc-T5, a glycosyltransferase involved in the synthesis of Lewis determinants. Further evaluation of a panel of different Hp strains showed that b3GlcNAc-T5 overexpression was elicited specifically by virulent cagPAI+ strains. b3GlcNAc-T5 overexpression in stably-transfected MKN45 and AGS gastric cell lines leads to increased expression of sialyl-Lex antigen.

In conclusion, our results show that highly pathogenic Hp strains induce b3GlcNAc-T5. Overexpression of this enzyme leads to sialyl-Lex formation suggesting that this can be the mechanism by which Hp modulates the synthesis of the SabA adhesin ligand, essential to strengthen epithelial attachment and achieve successful colonization.

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(226) **Basic Residues in the Polysialyltransferases are Critical for NCAM Polysialylation but not Enzyme Activity**

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Polysialic acid (PSA) is a developmentally regulated glycan that negatively modulates cell adhesion and is critical for a variety of important processes including brain development and neuronal regeneration. The neural cell adhesion molecule, NCAM, is one of only a few polysialylated mammalian proteins. We hypothesize that polysialylation is a protein-specific modification that requires an initial protein-protein interaction between polysialyltransferase (polyST) and substrate. The first fibronectin type III repeat (FN1) of NCAM is required for polysialylation of the N-glycans on the adjacent Ig5 domain. Mutation of a FN1 acidic surface patch reduced or abolished NCAM polysialylation, and suggested that it may be part of a polyST recognition region. With this in mind, we searched for a complementary basic region in the polySTs. We have identified a polybasic region (PBR) in the polySTs (71-105 in PST and 86-120 in STX) that is distinct from the polysialyltransferase domain (PSTD) identified by Nakata et al. (Glycoconj. J. (2006) 23, 423-436). Alanine replacement of Arg82 and Arg93 in the PST PBR or the homologous residues in the STX

PBR (Arg97 and Lys108) dramatically reduced NCAM polysialylation while not impacting enzyme localization or activity (autopolysialylation). Interestingly, replacement of each basic residue with the alternate basic residue (Arg->Lys or Lys->Arg) had no effect on NCAM polysialylation suggesting that the charge of the residue was critical for function. Our results demonstrate that these basic residues in PST and STX are critical for NCAM polysialylation and suggest that they may participate in polyST-NCAM interactions.

(227) **Role of Gangliosides in *Paracoccidioides brasiliensis* Adhesion to Human Fibroblasts**

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In order to better understand the initial steps of *Paracoccidioides brasiliensis* infection in humans, binding of yeast forms to lung fibroblasts and its Triton-X100 insoluble fraction was studied. By SDS-PAGE and immunoblotting, it was verified that the major extracellular protein present in the human fibroblasts is the fibronectin, and it may have a central role in *P. brasiliensis* adhesion. Since human lung fibroblasts express mainly GM1, GM2 and GM3 when these cells were incubated with anti-GM3 monoclonal antibody or cholera toxin B subunit (which binds specifically to GM1) a significant inhibition of fungal adhesion was observed, 35% and 33% respectively. Binding of GM1 to yeast forms of *P. brasiliensis* was confirmed by immunofluorescence using cholera toxin B subunit conjugated to Alexa Fluor, no reactivity was observed in control experiments using *Candida albicans*. It was also demonstrated that *P. brasiliensis* binds to plates coated with galactosylceramide, lactosylceramide, trihexosylceramide, GM1, GM3, GD3 and GD1a, suggesting that binding of *P. brasiliensis* to human lung fibroblasts may involve glycosphingolipids presenting at non-reducing end residues of βgalactose or neuraminic acidα2-3galactoseβ1. Conversely, no binding was detected when plates were adsorbed with glycosphingolipids which contain terminal residue of β-N-Acetylgalactosamine residue, such as globoside, GM2 and asialo-GM2. Taken together, these results strongly suggest that gangliosides GM3 and GM1 associated with lipid rafts in human lung fibroblasts are involved in binding and/or infection by *P. brasiliensis*.

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(228) **Genetically Altered Mice with Different Glycosyltransferase Deficiencies Show Different Expression of Lewis antigens in the Gastric Mucosa**

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Glycosyltransferases controlling different glycosylation steps: O-glycan branching (Core2-GlcNAcT), terminal H-type 1 fucosylation (FUT2), and terminal alpha2,3sialylation (ST3Gal-IV) are important in the biosynthesis of Lewis antigens in the gastric mucosa.

This study characterizes the expression of glycans in mice knock-out for the enzymes involved in these steps. These models can contribute for better understanding the mechanisms controlling the biosynthesis of those glycan structures and can be further used for Helicobacter pylori infection studies.

The FUT2 null mice gastric mucosa show no staining of the Fuc alpha1-2 specific UEA-I lectin. The type1 chain Lea and Leb antigens were expressed in similar levels among all mice excepting the FUT2 null mice which has no expression of Leb and higher levels of Lea. Similar patterns of expression of the type2 Lewis antigens were observed apart from the FUT2 null mice which showed a weaker expression of the Ley antigen.

The FUT2 null mice lack the alpha-1,2fucosyltransferase gene responsible for the transfer of fucose in an alpha-1,2 linkage to form the terminal H type-1 structure which explains the complete lack of expression of Leb. This observation is consistent with the increased expression of Lea in the gastric mucosa of these mice. The lower expression of Ley observed in the FUT2 null mice suggest that this enzyme is also playing a role in the terminal fucosylation of type2 structures.

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(229) **Masking as a Mode of Regulation of Functional Activity of Galectins**

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It is well known that *cis*-interaction prevents siglecs binding to the ligands expressed on other cells. We address the question whether the *cis*-masking can regulate functional activity of galectins. To this end an experimental model was developed where galectins from various species (human galectins-1, -2, -3, rat galectin-2, mouse galectin-3) were loaded on cells (normally galectin free) and probed with multivalent glycocongugates. In parallel, specificity was studied in solid phase assay based on inhibition of asialofetuin /galectin interaction. Dramatic difference was observed i) for sulfated disaccharides 3-O-Su-Galβ1→4(3)GlcNAc: high affinity to human galectin-1 (but not to galectin-3) in solid phase assay and absence of binding in cell assay; ii) for oligolactosamines LN3'LN, LNnT and LN_3 that displayed high affinity to human galectins-2 and-3 and did not bind to the same rodent analogs, however in solid-phase assay oligolactosamine-binding pattern of rodent galectins was similar to human one. Generally, cell-coated galectins demonstrated more pronounced selectivity. To address the question whether this selectivity is a result of lectins masking, galectin-loaded cells were treated by β- or α-galactosidase and probed with probes displayed low affinity to galectins. β;- and not α-degalactosylation of galectin-loaded cells significantly improved binding of 3-O-Su-Galβ1-3GlcNAc to galectin-1, Gal1β-4GlcNAc to all tested galectins and LN_3 - to rodent galectins-2 and -3. We concluded that galectins can be masked on cell surface by β-galactosides and this probably preventing less specific interaction with multitude galactosylated glycans. (The work is supported by the grant of Russian Foundation for Basic Research N 04-04-49689 and 07-04-00969).

(230) **Glycan Variation on Serum Mucins in Pancreatic Cancer**

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The carbohydrate structures on certain mucins are frequently altered in pancreatic cancer patients. A characterization of the variation in those structures over many cancer patients could be used to assess their diagnostic value and to identify possible functional relationships with disease. Using antibody arrays with lectin detection, we characterized certain glycan levels of several mucins (MUC1, MUC5ac, MUC16) in the sera of pancreatic cancer patients (n = 23) and control subjects (n = 23). Probing the proteins with over 30 different glycan binding proteins revealed high variability in glycan structures between people and molecules.

MUC1 and MUC5ac showed increased T- and Tn-antigen, lactosamine, and Lewis blood group structures, and MUC16 showed increased Lewis blood group structures in a subset of patients. Glycan detection greatly improved the ability to discriminate the cancer sera from control sera, relative to detecting the glycoprotein alone. At a threshold defining an 87% specificity (3/23 control samples above the threshold), 7/23 cancer patients (30% sensitivity) showed an elevation in the MUC5ac core protein, but 23/23 cancer patients (100% sensitivity) showed an elevation in WGA binding on MUC5ac. A similar improvement was found by detecting the CA 19-9 epitope on the MUC1 core protein. These data provide the first look at the variability in glycan structures between people and molecules and have revealed previously unknown associations with cancer. These results also validate the concept of using glycan detection for improved biomarker accuracy and highlight the advantages of this technology for that research.

(231) **Comparative Analysis of Specificity of Human and Rodent Galectins-2 and -3 in Cell Composition**

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The known approaches for study galectin specificity are based on artificial assays poorly reflecting complicated situation on cell surface. The aim of this work was to study carbohydrate specificity of galectins in cell composition isolated from various species and compare the binding profile with the data of solid phase assay. To this end an experimental model was developed where human or rodent galectins -2 or -3 were loaded on normally galectin free cells and probed with multivalent glycoconjugates. In parallel, specificity was studied in solid phase assay based on binding of galectins to asialofetuin. Trisaccharide Galα-Galβ1-4GlcNAc (Galα3'LN) was the most potent ligand for human and rodent galectins -2 and -3. Dramatic difference was observed for linear and branched oligolactosamines: high affinity to human galectins and absence of binding to rodent galectins in cell assay; in solid-phase assay oligolactosamine-binding profile of rodent galectins was similar to the human one. Further, i)cells treated with β- and not α-galactosidase before galectin loading lose the ability to bind galectins; ii) treatment of cells by inhibitor of N-glycosylation and not O-glycosylation lead to pronounced decrease of galectins binding to cells. iii) β-degalactosylation of galectin-loaded cells significantly improved binding of LN_3 to rodent galectins. We concluded that galectins utilize N-glycans with β-galactosides termini for anchoring to cell surface and rodent galectins to an even greater degree than human ones can be masked on cell surface by *cis*-ligands. (The work is supported by the grant of Russian Foundation for Basic Research 07-04-00969).

(232) **Tandem Mass Spectrometry Fragmentation of Glycans by Multiple Approaches**

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Glycans and glycoconjugates have been analyzed by energetic electron capture and photodissociation experiments for ions generated by electrospray (ESI) or matrix-assisted laser desorption/ionization (MALDI) in a custom-built Fourier transform ion cyclotron resonance mass spectrometer (FTICR MS), and the results are being compared to collisionally-activated decomposition (CAD) spectra obtained by the FTMS and by LTQ-Orbitrap and quadrupole orthogonal time-of-flight (QoTOF) MS [1-4]. Dissociation of glycosidic bonds under CAD and photodissociation produced primarily B and Y ions whereas C and Z ions were prominent in the "hot" electron capture dissociation (ECD) spectra. A-type cross-ring cleavages were observed in the CAD spectra; complementary A- and X-type pairs were generated by ECD. More abundant internal fragments resulted from CAD compared to ECD. Electron detachment dissociation (EDD) of acidic glycans in the negative mode produced more extensive cross-ring cleavages than CAD. Sequential photodissociation and CAD steps achieved more complete coverage of structural details in glycoconjugates. Procedures that employed multiple fragmentation steps provided information on branched structures that enabled differentiation of isomeric structures. The fragmentation patterns are highly reproducible and can be used in a complementary manner to determine the structures of unknown glycans and glycoconjugates.

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(233) **Dendritic Cells in Mice with Deficiency in Sialyltransferases ST3Gal1 and ST6Gal1**

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The immune importance of the sialyltransferases ST3Gal1 and ST6Gal1 are increasingly evident. ST6Gal1 knockout (KO) mice have an impaired B lymphocyte proliferation and ST3Gal1-KO mice have an almost absence of peripheral T lymphocyte CD8b- subtype. Dendritic cells (DCs) are known for their coordinating role in the immune system, as they endocyte, process and present foreign antigens to T lymphocytes. Besides the known ST3Gal1 and ST6Gal1-KO mice lymphocyte malfunction, their DCs haven't been characterised. We have previously observed that the expression of sialylated O- and N-glycans increases during human DCs differentiation, probably due to the increased expression of ST3Gal1 and ST6Gal1 genes. These features anticipate a role for these STs in DC immunobiology. To investigate this, we begin to analyse DCs from ST6Gal1 and ST3Gal1-KO mice. We examined the DC's subtypes in blood, lymph nodes and spleen and the major distinction was observed for ST3Gal1-KO mice, which exhibited increased levels ($p<0,01$) of DCs expressing B220+ plasmacytoid marker and an almost absence ($p<0,05$) of blood DCs expressing CD8α+ lymphoid marker. Since we previously observed that human DC endocytosis was affected by neuraminidase treatment, we also studied the endocytic capacity of bone marrow derived DCs. Preliminary results revealed a slight altered endocytic capacity of both KO mice DC's and further investigations are envisaged to elucidate the affected endocytosis mechanism. Nevertheless, our results support the hypothesis that ST6Gal1 and ST3Gal1 modulate DC functionality; and probably their deficiency, in DCs, is involved in the lymphocyte malfunction observed in the respective KO mice.

(234) **Glycoconjugates of novel Metastatic and Non-Metastatic Brain Tumor Cells**

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The absence of *in vivo* metastatic/invasive models has hindered development of new therapies. Glioblastomas are highly invasive

brain tumors that can metastasize if given access to extraneural sites. We describe three new autochthonously arising brain tumors in the inbred VM mouse strain. The VM-M2 and VM-M3 tumors were highly invasive when grown orthotopically in brain and metastasize to multiple organ systems (liver, kidney, spleen, and lung) when given access to extraneural tissue. The VM-NM1 tumor, though highly malignant, was neither invasive nor metastatic. Gangliosides and gene expression in the cultured cell lines from the VM tumors were compared with those of cultured astrocyte and macrophage (RAW 264.7) cell lines. Gangliosides in the metastatic VM-M2 and VM-M3 cells were similar to those of the RAW cells and consisted mostly of GM2, GM1, and GD1a with undetectable levels of GM3. In contrast, GM3 was a major ganglioside in non-metastatic VM-NM1 tumor cells and astrocytes. The neutral glycolipid, asialo-GM1, and the macrophage/microglial genes (CD11b, Iba1, F4/80, CD68, CD45, and CXCR4), were expressed in the VM-M2 and VM-M3 tumor cells and in RAW, but were not expressed in the VM-NM1 tumor cells or in astrocytes. In contrast, the non-invasive VM-NM1 cells expressed properties of neural stem cells including nestin and ganglioside GD3. The VM-M2 and VM-M3 cells, in contrast to the AC and VM-NM1 cells, express Lac-di-NAc and HNK-1 epitopes that participate in cell invasion. The results indicate that glycoconjugates can distinguish metastatic from non-metastatic cells and may be targets for novel therapies.

(235) **Ganglioside GM3 Inhibits the Pro-Angiogenic Effects of Vascular Endothelial Growth Factor and Ganglioside GD1a**

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Gangliosides are sialic acid-containing glycosphingolipids that have long been associated with tumor malignancy and metastasis. Mounting evidence suggests that gangliosides also modulate tumor angiogenesis. Tumor cells shed gangliosides into the microenvironment, which then produce both autocrine and paracrine effects on tumor cells and tumor associated host cells. We previously showed that genetic changes in the ratio of the simple monosialo-ganglioside, GM3, to the complex disialo-ganglioside, GD1a, modulated angiogenesis in mouse brain tumors. In this study, we show that GM3 counteracts the pro-angiogenic effects of vascular endothelial growth factor (VEGF) and GD1a. GM3 significantly inhibited the action of VEGF and GD1a on the proliferation of human umbilical vein endothelial cells (HUVEC), and inhibited the migration of HUVEC towards VEGF as a chemoattractant. Enrichment of added GM3 in the HUVEC membrane also reduced phosphorylation of VEGF receptor 2 (VEGFR-2) and downstream Akt. Moreover, GM3 significantly reduced the pro-angiogenic effects of GD1a in the *in vivo* Matrigel plug assay. Pharmacological inhibition of GM3 biosynthesis with glucosyl transferase inhibitor, N-butyldeoxynojirimycin (NB-DNJ), significantly increased HUVEC proliferation and VEGFR-2 and Akt phosphorylation. We conclude that GM3 has anti-angiogenic action and will have therapeutic potential for reducing tumor angiogenesis.

(236) **Glycan Profiling of Native Proteins Captured by Antibody Arrays**

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Glycosylation is an important determinant of protein function, and glycans offer promise as sensitive and specific serum biomarkers for prostate and other cancers. Glycan detection on antibody arrays is a novel approach to high-throughput glycan analysis. In this method, antibodies immobilized in an array capture specific proteins of interest from complex samples; changes in the glycosylation of captured proteins are detected using biotinylated lectins. Since capture antibodies are themselves glycosylated, we have chemically derivatized our capture antibodies to inhibit binding of lectins to glycosylated capture Abs.

Glycan detection using antibody arrays is ideally suited for examining the glycosylation of multiple specific proteins in complex samples like serum and plasma because it first concentrates serum proteins using antigen-specific, high-affinity monoclonal antibodies and then detects particular glycans on those captured proteins using specific biotinylated lectins. This approach enables glycosylation measurements of critical, lower abundance glycoproteins present in serum and cell lysates. The detection and characterization of lower abundance glycoproteins in complex samples can be extremely difficult using other high-throughput methods such as lectin arrays.

Additionally, we have coupled glycan detection using antibody arrays to multiplexed protein expression assays to distinguish changes in the amount of glycan per protein molecule from changes in the underlying protein concentration.

Preliminary data from clinical samples was obtained using a 40-plex antibody array. We compared results from prostate cancer patient sera (Stage II, III and IV) with sera from patients diagnosed with benign prostatic hyperplasia, breast, colon, rectal, and lung cancer, as well as normal controls.

(237) ***Leishmania (Leishmania) Amazonensis* Glycolipids and Sphingolipids Membrane Organization. Role in *Leishmania* Infectivity**

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Leishmania are parasites that cause a number of human tropical and sub-tropical diseases. *Leishmania* have a digenetic life cycle comprising promastigote forms which replicate in the midgut of sandflies and are transmitted to mammals, and amastigote forms present intracellularly in the mammal host's macrophages. Our group characterized glyco(sphingo)lipids expressed in amastigote and promastigote forms of *Leishmania (Leishmania) amazonensis* employing immunochemical and structural analysis. Monoclonal antibodies directed to glycosphingolipids (GSLs) of amastigote forms of *L. (L.) amazonensis*, inhibited up 80% of macrophage invasion by amastigotes, indicating that GSLs, at the parasite surface, are relevant in mediating binding of *L. (L.) amazonensis* amastigotes to macrophages. On the other hand, no GSLs are expressed in promastigotes, glycosylinositolphospholipids (GIPLs) are the major glycolipids expressed in the parasite surface. It was demonstrated that GSLs in amastigotes, and GIPLs and IPC in promastigotes are organized in lipid rafts, as shown by the presence of low density membranes, resistant to treatment with 1% Triton X-100 at 4 ˚C, after ultracentrifugation in sucrose gradient. Since sterols are present in high concentration in these lipid rafts, parasites were treated with 20 mM or 40 mM of methyl-β-cyclodextrin (sterol binding reagent) and their infectivity analyzed. In these condition no alteration of parasite viability was observed, but a significant inhibition of the macrophage infectivity was detected (about 65% for amastigotes, and 60% for promastigotes). These data indicate that GSLs/GIPLs organization in lipids rafts are important for *L. (L.) amastigotes* infectivity.

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(238) **Naive Regulatory Human T Lymphocytes Subset in Peripheral Blood Expressed O-Glycosidically Linked Glycans Recognized by Amaranthus Leucocarpus Lectin**

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Lectins are excellent tools for oligosaccharides characterization, for isolation of cellular populations and are currently used to evaluate the immune status of patients. Surface cellular glycosylation pattern has been involved in development, maturation, and immune regulation. In this study, we characterized a subpopulation of CD4+ T cells of human Peripheral blood recognized by Amaranthus leucocarpus lectin (ALL, specific for T and Tn antigens). ALL recognized CD25+ FOXP3+ CD45RA+ CCR7+ T cells. The cytokine expression in non-stimulated CD4+ ALL+ T cells were positive to intracellular staining against IL-10>IL-4>TGF-b>IFN-g. We observed significant increased frequency of CD4+CD25+FOXP3+ALL- in stimulated than in non-stimulated cells (p=0.007), while the frequency of CD4+CD25+FOXP3+ALL+ was increased 2 times in stimulated cells than in non-stimulated cells (p=0.036). Indicating that T cell mediated process, are accompanied by a programmed remodelling of cell surface glycans. Taken together Amaranthus leucocarpus lectin recognizes a subset of human natural regulatory T CD4+ CD25+ FOXP3+ lymphocytes in peripheral blood with naïve phenotype that were able to produce cytokines related to immune-regulation.

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(239) Characterization of Lectin from Helianthus Tuberosus L. and Effects of Crude Extract Proteins on Rabbit-Isolated Ileum

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A lectin from Helianthus tuberosus L. was purified and its effects of crude extract proteins on rabbit-isolated ileum was investigated.DEAD-Sephacel and Sephadex G-200 were used to purified the lectin; SDS-PAGE and MALDI-TOF were used to obtain the molecular mass of the lectin;SDS-PAGE showed that the protein is about 17.6 kDa, MALDI-TOF mass spectrometry showed the exact molecular mass was 15.379 kDa. The lectin was found to agglutinate native and trypsinized rabbit erythrocytes. The hemagglutination activity was inhibited by heparin, PSM and albumin. The hemagglutination activity was stable below 50°C and was depend on the presence of divalent cations Ca2+. The crude extract protein could act stimulation effect on rabbit-isolated ileum, and was dose-dependent. When the protein was treated with trypsin or pepsin, respectively, the products could also stimulate ileum.There are huge differences between this purified lectin and the known Heltuba from Helianthus tuberosus L.. The crude extract protein could act stimulation effect on rabbit-isolated ileum no matter treated with or without trypsin or pepsin.

(240) Antigen-Lewis X Conjugate Elicits Potent Immune Responses in Mice

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Fucosylated glycans on various pathogens are known to promote Th2 immunity. We have shown earlier that Le^x-BSA (BSA modified with 11 Le^x) elicited in mice much higher levels of BSA-specific IgE and IgG1, increased levels of Th2 cytokines and reduced level of a Th1 cytokine than mice sensitized with BSA. We showed that the capability for such immunomodulatory effect resided in dendritic cells. Free oligosaccharides, Le^x, produced similar but smaller effects. *In vitro* studies using human monocyte-derived DCs (MDDCs) and mouse BMDCs indicated that the inhibition of LPS-induced proflammatory cytokine production by Lex may be via DC-SIGN, since this effect was abrogated by the addition of anti-DC-SIGN Abs or mannan.

Typical plant, insect, and food allergens often contain alpha-(1-3)-fucosylated glycans. Using a solid-phase binding assay, we showed that MOXF3 (a truncated glycan from bromelain that contains an alpha-1,3-linked fucose and xylose)-BSA and three purified allergens, Bermuda grass pollen allergen (BG60), Der p2 (house dust mite) and Cor a11 (hazelnut), bound to both DC-SIGN and L-SIGN in a saturable fashion, which was inhibitable by L-fucose and Le^x, but not by N-acetyl-lactosamine. Functionally, purified allergens could induce TNF-α production in human MDDCs and this induction was inhibited by anti-DC-SIGN Abs. Interestingly, although DC-SIGN is known to bind both Le^x- and mannose-containing glycans, Man51-BSA showed much lower Th2-promoting effect, despite its strong binding to DC-SIGN.

(241) Structural and Biochemical Aspects of the Incorporation of O-GalNAc into alpha-Dystroglycan

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The glycoprotein alpha-dystroglycan (alpha-DG) has generated much interest because of its presence as a key component of the dystrophin glycoprotein complex, particularly in organizing the extracellular matrix in muscle tissue, its rare feature of alpha-O-linked mannosyl protein glycosylation, and the relationship of defects in its glycosylation with muscular dystrophy pathologies. The sequential glycosylation steps, with initial O-mannosylation on some of the S and T residues followed by addition of GalNAcs and extension of glycans, provide an interesting system to probe relative conformational effects of the two modes of glycosylation and factors effecting selectivity of polypeptide GalNAc transferase enzymes. To investigate these, a series of mannosylated glycopeptides based on amino acid sequences from the central mucin-like portion of alpha-DG have been prepared. These provide well defined models for characterizing their properties as enzyme substrates and for their conformational study with NMR methods. Glycosylation mapping in the native glycoprotein indicates that O-Man and O-GalNAc residues occur in close proximity, and we are evaluating the ability of several ppGalNAc transferases to install GalNAcs in analogous glycopeptide contexts. In conformational analysis, we are refining the structure of mannosylated glycopeptides and comparing the results with an analogous construct with GalNAc instead of mannose. This combined effort is revealing the relative roles of the two substitutions in fostering the extended structure of the glycoprotein backbone in α-DG, and contributing to the understanding of structure-function relationships in the processing of the glycoprotein and in its ultimate properties.

(242) Construction and Structural Characterization of Versatile Lactosaminoglycan-Related Compound Library for the Synthesis of Complex Glycoconjugates

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Lactosaminoglycans (poly-*N*-acetyllactosamine) are cell surface carbohydrates composed of the disaccharide unit *N*-acetyllactosamine, and function as a variety of signal molecules by altering structures in cases of cellular differentiation, cell-cell interaction, and malignant alterations. Mucin-type glycoproteins (mucins) having *O*-glycan and cell surface glycosphingolipids play a fundamental role in many biological processes including cell adhesion, signal transduction, and immune response. It has been well documented that a dynamic structural change in the lactosaminoglycan motif might be essential for modulating functions of glycoconjugates. Therefore synthetic glycoconjugates containing poly-*N*-acetyllactosamine could become nice tools both for investigating the basic structure-function relationship of those and for drug discovery research. Here we present an efficient procedure for the construction and precise structural characterization of a lactosaminoglycan-related compound library based on the unique substrate specificity and properties of a bacterial β1,3-GlcNAcT (*Neisseria meningitidis* LgtA) as a key extension enzyme. Combined use of LgtA and human β1,4-GalT allowed for the synthesis of various building blocks having different size and structures of poly-*N*-acetyllactosamine chains from some Fmoc-Ser/Thr derivatives. These building blocks could be used to the construction of novel MUC1-related glycopeptides on the basis of solid-phase peptide synthesis under microwave irradiation. In addition, it should be emphasized that the present strategy can be employed for the synthesis of glycosphingolipids, even though the procedures are carried out on the ceramide mimic polymer supports. The glycosphingolipid mimetic polymer indicated growth inhibition effect against *Helicobacter pylori*.

(243) Role of N-glycolylneuraminic Acid in Feline and Canine Parvovirus infections

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Feline panleukopenia virus (FPV) has been known for many years as the cause of serious diseases in cats, and canine parvovirus (CPV, which is more than 99 % identical to FPV), emerged as a pathogen of dogs in the 1970's. While these viruses require the transferrin receptor for initiating infection, additional mechanisms might exist. Indeed, despite being extremely small viruses with a high mutation rate, the ability to hemagglutinate red blood cells (RBCs) is a highly conserved function of all CPV and FPV isolates. We therefore investigated the hemagglutinating and sialic acid binding properties of CPV and FPV. RBCs from a wide range of species were tested. Hemagglutination was stronger at 4°C than at 37°C. Both CPV and FPV hemagglutinated at pH 6.2 but only FPV did at pH 7.2. Moreover, it appeared that the non-human sialic acid N-glycolylneuraminic acid (Neu5Gc) was necessary for hemagglutination. This specificity was also corroborated by adhesion and flow cytometry experiments using cells in which surface Neu5Gc levels were modulated by feeding the sialic acid. Virus binding to cells required Neu5Gc on the surface and was abolished by neuraminidase treatment. However, binding was not affected by switching sialic acid linkages from alpha2-3 to alpha2-6, by over-expressing ST6Gal-I in CHO cells fed with Neu5Gc. We are currently studying the role of Neu5Gc binding for infectivity *in vitro* and *in vivo*.

(244) Over Expression of Complex Mucin-Type Glycans in Invasive Cervical Cancer

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Invasive cervical cancer constituted 9.8% of cancers worldwide and represented the first causes of death by cancer in Mexico. Glycosylation has been suggested to participate in carcinogenesis and metastasis. In this work we analyzed O-glycosylation in invasive cervical cancer by histochemistry using lectins specific for O-glycosidically linked glycans in biopsy from cervical cancer in stage IIB. Our results showed diminution in the sialic acid expression in a2-3 and a2-6 linkage, and increased expression of T and Tn antigens; however, the lectin Machaerocereus eruca (MEA, specific for glycans in complex mucin) showed increased recognition of tumoral cells and tumoral tissue stroma, healthy cervical tissue was negative for MEA, the treatment with trypsin inhibited all recognition of MEA. Interaction of MEA lectin was inhibited only with stomach mucin. The receptor for MEA from cancer cervical lysate is a 21 kDa glycoprotein as demonstrated by WB; suggesting that over expression of mucin type glycans in cervical cancer favor invasion and probably immune evasion.

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(245) Cell Surface Carbohydrates Attached to alpha-Dystroglycan Function as Tumor Suppressor

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Binding of laminin to carbohydrates specifically expressed on alpha-dystroglycan is essential for epithelial adhesion and tissue organization. Indeed, impairment in laminin-alpha-dystroglycan interaction results in muscular dystrophy, which is defective in muscle development. Biosynthesis of specific carbohydrates on alpha-dystroglycan is directed by a unique putative glycosyltransferase, Large (Nat Genet 28:151-154, 2001). Large has two functional domains, one of which has similarity to i-antigen extension enzyme cloned by us (PNAS USA 94: 14294-14299, 1997). While Large is thought to be a glycosyltransferase, its function is not fully known. Our studies show that i-enzyme collaborates with Large to form glycans in alpha-dystroglycan. We found that the expression of i-enzyme is critical for forming this unique glycan, whose amount is inversely correlated to malignancy of breast and prostate carcinoma cells. PC-3 prostate cancer cells expressing a negligible amount of i-enzyme invades much more than high expresser. Moreover, the low expresser formed larger tumors in the prostate and formed more metastasis in neighboring lymph nodes in the mouse. Forced expression of i-enzyme reversed both invasive and malignant property. The high expresser adheres to laminin more strongly than the low expresser, although they grow similarly. These results demonstrate that carbohydrates recognized by laminin on alpha-dystroglycan function as tumor suppressor, and increase of this unique carbohydrate may lead to a new therapy for treatment of carcinoma. Studies are now in progress to determine the structure of the carbohydrates essential for laminin binding. This work was supported by NIH grants CA48737 and PO1 CA71932.

(246) Increased Core 3 Structure Suppresses Tumor Formation and Metastasis in Prostate Cancer through the Dysfunction of beta 1 Integrin

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The core 3 structure of mucin-type O-glycans is formed by adding N-acetylglucosamine on GalNAcα-Ser/Thr (T-antigen) structure by β1,3-N-acetyglucosaminytransferase 6 (β3GnT-6), which is decreased in tumor cells. In this study, we first examined whether core 3 structure is involved in prostate tumor formation and metastasis. The second is to reveal the molecular mechanism how core 3 structure modulates the tumorigenesis. To understand these aspects, we transfected β3GnT-6 stably into PC-3 and LnCa-P prostate cancer cells. Core 3 overexpressed cells showed decreased proliferation activity, and apoptotic cells were detected only in core 3 transfectants. When inoculated in the prostate of nude mice, the size of tumor and metastasis to lymph node was decreased in core 3 transfectants. Decreased migration activity on collagen type IV, fibronectin, and laminin was also shown in core 3 transfectants, as well as invasive activity was decreased on core 3 transfectants. Beta 1 integrin is involved in adhesion to these molecules, and invasiveness of PC-3 cells was inhibited by functional blocking anti-beta 1 integrin antibody. In addition, maturation of beta 1 integrin and heterodimerization between alpha 2 and beta 1 integrin is decreased in core 3 transfectants. These results suggest that increased core 3 structure can reduce beta 1 integrin-mediated cell adhesion, thereby induces the loss of malignancy by prostate cancer cells.

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(247) **Regiospecific Attachment of Short Chain Fatty Acid (SCFA) Groups to 'Core' Amino Sugars Decouples Metabolic Flux from Toxicity**

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Hybrid molecules composed of short chain fatty acids (SCFAs) ester-linked to monosaccharide scaffolds generally have been thought to elicit biological responses from the activity of either the SCFA or the sugar moiety after cellular uptake and hydrolysis by non-specific esterases. In this report, by attaching n-butyrate to N-acetyl-D-mannosamine (ManNAc) to form the per-acylated analog 'Bu4ManNAc' (1) and partially-acylated derivatives 1,3,4-O-Bu3ManNAc (2) and 3,4,6-O-Bu3ManNAc (3), we demonstrate that unique biological responses can be obtained from specific structural features of the parent molecules. For example, 2 supported a high level of flux through the sialic acid pathway without attendant growth inhibition or toxicity whereas 3 induced apoptosis efficiently. We then demonstrated that these findings were general for SCFA-hexosamine analogs by showing the structure-activity relationships extended to various 'core' sugars (i.e., ManNAc and GlcNAc) and SCFAs (i.e., acetate, propionate, butyrate, and valerate). Moreover, by combining the highly-active SCFA butyrate with the less active SCFA acetate on the same monosaccharide scaffold, cellular uptake, 'anti-cancer' responses, and metabolic flux could be controlled with even greater precision. In summary, this study established SCFA-ManNAc hybrid molecules as a versatile platform for selectively controlling distinctive biological activities (which includes the inhibition of oncogenes implicated in metastatic cancer, described in detail in an accompanying poster from our laboratory by Campbell and co-athors) through the regiospecific placement of the SCFA moiety on a sugar scaffold and thereby provides increased flexibility for emerging metabolic oligosaccharide engineering (MOE) technologies.

(248) **Fungal Glycolipids as Infectivity Factors and Potential Targets for Development of New Therapeutic Strategies**

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Studies carried out in our laboratory delineated a complete glycolipid profile of *Paracoccidioides brasiliensis* and these results prompted us to perform a systematic study and detailed characterization of glycoinositolphosphorylceramides (GIPCs), from others dimorphic/pathogenic or opportunistic fungi. These studies revealed a remarkable structural diversity of these glycoconjugates among these microorganisms. Novel glycan structures, not found in mammals, built up on the monosaccharide cores GlcNα1-2Ins, Manα1-2Ins, or Manα1-6Ins, were found to be expressed differently among several fungal species. Thus, while *Saccharomyces cerevisiae* and a wide variety of mycophatogens express GIPCs bearing Manα2IPC, only *Sporothrix schenckii* present all three carbohydrate cores described above. Recently, more complex structures, built up on cores GlcNα1-2Ins, and Manα1-2Ins, have been found in *Aspergillus fumigatus*. Glucosylceramide was also detected in all fungi analyzed (except for *S. cerevisiae*), whereas galactosylceramide was detected only in *A. fumigatus* and yeast forms of *S. schenckii*. The biological meaning of the dimorphism of monohexosylceramides is still unknown, the wide range of ceramide and glycosyl portion can be a crucial factor for these fungi to adapt to an ample variety of substratum and growth conditions, as well as in infectivity processes. Studies carried out in our laboratory provided an overview of the remarkable diversity of fungal GIPCs and monohexosylceramides on several pathogenic/opportunistic fungi. It will be discussed the use of novel fungal glycan structures as targets for highly specific/sensitive immunoassays as well as key glycosyltransferases not present in mammals as potential targets for more efficient therapies of systemic mycosis.

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(249) **Water Soluble Synthetic Glycolipids**

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A series of glycolipids ranging from 2 to 80 sugar residues were successfully created. These synthesised glycolipids consist of three functional components: a bi-lipid tail which enables the molecule to insert in cell membranes, a solubilisation linker that contributes to water solubility of the molecule and a carbohydrate head group. These synthetic glycolipids were shown to dissolve in water or cell culture media and spontaneously and harmlessly incorporate into cell membranes of red cells, cultured cells and embryos. By controlling conditions we were able to create "designer" cells expressing finite and controlled levels of normal and novel carbohydrate determinants.

Specialised human and animal red cells bearing A, B, H, acquired B antigen (deacetylated A antigen), Lewis, and the animal linear-B antigen were created and shown to be valuable diagnostic tools. Synthetic glycolipid modified cultured cells and embryos bearing functional carbohydrates including hylauronic acid as a glycolipid were shown to support cellular adhesion.

With the potential to change the carbohydrate head to virtually an unlimited range of molecules and the ability of these molecules to harmlessly insert into cell membranes, this technology is expected to have many new diagnostic and therapeutic applications.

(250) **Unique Glycan Recognition by Human Galectins-1, -2, and -3**

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Human galectins have functionally divergent roles, although most of the members of the galectin family bind weakly to the simple disaccharide lactose (Galβ1-4Glc). To assess galectin-glycan interactions in more detail, we explored the binding of several important galectins (Gal-1, Gal-2, and Gal-3) on a glycan microarray containing hundreds of structurally diverse glycans. All three galectins exhibited unique glycan binding characteristics. Only Gal-1 and Gal-2 bound complex-type N-glycans and extended core 1 O-glycans with high affinity, while Gal-2 and Gal-3, but not Gal-1, bound A and B blood group antigens. Gal-2 failed to recognize any sialylated glycans regardless of linkage, whereas Gal-1 and Gal-3 bound α2-3, but not α2-6 sialylated glycans. All galectins showed higher binding to sulfated glycans relative to unsulfated ones. Each galectin exhibited higher binding for glycans with poly-N-acetyllactosamine (PL) sequences (Galβ1-4GlcNAc)n when compared to Nacetyllactosamine (Galβ1-4GlcNAc) in the microarray. However, only Gal-3 preferred PL when assessed by solution-based surface plasmon resonance. Removal of the terminal galactose residue in PL abrogated its recognition by Gal-1 and Gal-2 while having no substantial effect on Gal-3 recognition, demonstrating that Gal-3 recognizes internal Nacetyllactosamine units. These results provide novel insights into the functional constraints of glycan recognition by each galectin and underscore the basis for differences in biological activity.

(251) **A Role for P1-Promoter Derived Pool of ST6Gal-1 in Regulating Allergic Inflammation**

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Recently we demonstrated a role for ST6Gal-1 generated by the liver-specific promoter, P1, in the regulating acute neutrophilic inflammation, circulating neutrophil homeostasis, and in replenishment of granulocyte numbers. Mutant mice with a global ST6Gal-1 deficiency by inactivation of the coding region (Siat1-null) or a partial ST6Gal-1 deficiency from ablation of the P1 promoter (Siat1dP1) have exaggerated thioglycollate-elicited peritonitis that is to a large part attributable to greater sensitivity to G-CSF and elevated bone marrow granulopoiesis. Here we report an additional role of the P1-derived ST6Gal-1 pool in modulating experimental allergic inflammation. Using the ovalbumin model of allergic respiratory inflammation, we show that both ST6Gal-1 deficient mice have significantly greater acute pulmonary eosinophilic inflammation on the basis of inflammatory cell numbers recovered from bronchial-alveolar lavage and in the degree of elevated plasma Th2 cytokines when compared to wild-type mice. The degree of excess airway inflammation was identical between Siat1-null and Siat1dP1 mice, implicating the P1-derived pool of ST6Gal-1 in this process. Excess eosinophilia is at least in part the result of an elevated capacity for eosinophil production, and the bone marrows from both mutant mice, when compared to wild-type mice, have greater numbers of eosinophil progenitors when assessed by colony-forming assays performed on semi-solid media in the presence of IL-5. Finally, in wild-type animals , induction of airway inflammation by allergen provocation led to a significant reduction in circulatory ST6Gal-1 levels, further implicating the hepatically-produced, P1-mediated pool of ST6Gal-1 as a mediator of regulating eosinophil production and the development of allergic inflammation.

(252) **Real Glycome Profiling using Lectin Microarray**

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Mammalian cells in nature are covered with a dense and complex array of glycans called the glycocalyx, which comprises various forms of glycoconjugates (the glycome). The cellular glycome has been assumed to vary between cell types, stages of development, and during malignant transformation. The functional consequences of the glycome changes are likely to be recognized by endogenous lectins that regulate a myriad of cellular processes. Recently, a technique called lectin microarray was developed for rapid profiling of glycosylation, although its use was mainly restricted to glycoproteins, unable to profile the cellular glycome. Here we present a simple and sensitive procedure for direct analysis of the mammalian cell-surface glycome. Fluorescent-labeled live cells were applied in situ to the established lectin microarray consisting of 43 immobilized lectins with distinctive binding specificities. After washing, bound cells were directly detected by an evanescent-field fluorescence scanner in a liquid phase without fixing and permeabilization. The results demonstrate that the developed method is capable of profiling global changes of "the real glycome", providing a snap shot of the cellular glycome.

(253) **Role of Galectin-3 and Mucin-Type O-Glycans in Breast Cancer and its Metastasis to Brain**

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Galectin-3 has been implicated in tumor progression. We demonstrated immunohistochemically that Galectin-3 was negative in normal breast tissue, but it was highly increased in breast cancer and in metastatic tissues to brain; similarly, histochemistry with mucin-specific lectins showed increased recognition in breast tumor and metastasis with Machaerocereus eruca agglutinin (Fuα 1,2 (GalNAcα1,3) Galß1,4 in complex mucin) but not for Amaranthus leucocarpus and Arachis hypogaea lectins (GalNAc/Gal specific). Mucin-type glycans and Galectin-3 colocalized in breast cancer and metastasis, but not in normal tissue, suggesting upregulated biosynthesis of complex O-glycosidically linked glycans and Galectin-3 favor breast cancer progression and brain metastasis.

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(254) **Characterization of E-Cadherin Glycosylation in Canine Mammary Carcinoma**

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E-cadherin is a transmembrane protein that could be post-translationally modified by glycosylation. Little is known about the pattern of E-cadherin glycosylation and its role in the stability of adherens-junctions and in the process of malignancy.

The aim of this study was to characterize the pattern of E-cadherin glycosylation in canine mammary carcinoma.

Canine mammary carcinoma cell line (CMT-U27) was used for immunofluorescence labelling of carbohydrate antigens. Immunoprecipitation studies of E-cadherin followed by Western-blot analyses were performed using lectins (Sambucus nigra agglutinin, SNA; Maackia amurensis lectin, MAL-I; Phaseolus vulgaris agglutinin, PHA-L; Galanthus nivalis agglutinin, GNA) and specific monoclonal antibodies for the detection of carbohydrate antigens.

Evaluation of expression of carbohydrate antigens in the CMT-U27 cell line showed expression of Sialyl-Lex, Lea and Lex. Immunoprecipitation of E-cadherin and Western-blot studies showed positivity to alpha-2,6 linked sialic acid (SNA) and alpha-2,3 linked sialic acid (MAL-I) at 120 KDa band that correspond to E-cadherin. The presence of high mannose type oligosaccharides was ascertained in E-cadherin of CMT-U27 cell line as indicated by positive reaction with GNA. The specific reaction with PHA-L indicated the existence of GlcNAcB1,6-branched complex type glycans on E-cadherin. No detection of Sialyl-Lex, Lea and Lex antigens were observed on 120 KDa range.

Structural characterization of E-cadherin glycans are being performed by MALDI-MS.

These results indicate that canine E-cadherin from CMT-U27 cell line is glycosylated with high mannose type glycans as well as B1,6-branched oligosaccharides and contain terminal alpha-2,6 and alpha-2,3 linked sialic acid residues.

(255) Design and Synthesis, and Evaluation of Sialidase Inhibitors Based on Enzymatic Dynamism

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Potent and specific sialidase inhibitors are attractive target to cure infective diseases, because guest sialidases have a key role in the process of many infections such as cholera, trypanosomiasis, and influenza. Most reported sialidase inhibitors represented by Tamiflu and Relenza are designed from static information of X-ray crystallographic analysis. Although these compounds are effective for influenza, reports of side-effect and resistance viruses proved the potential demand of other compounds developed from other strategies. Dynamic structural changes of proteins are attractive target to develop novel inhibitor. Mechanism-based labeling of protein is a useful tool to reveal dynamics of protein structure and function. Here we report a novel mechanism-based fluorescent labeling reagent designed for sialidase, and its application to characterize the nucleophiles involved in enzymatic activities of *Vibrio cholerae* sialidase and *Trypanosoma cruzi* trans-sialidase. MALDI-TOF/TOF MS analysis revealed that a pair of Asp and Arg residues was specifically labeled by the reagent in common. Interestingly, these labeled sites allocated far from the active site reported by crystallographic analysis. This unprecedented finding suggests that unrevealed dynamic nature of substrate recognition and/or catalytic action may exist. Then we designed basic structures for novel inhibitors to interfere the motion of the sialidase with rigid substituents. Then we synthesized compound's libraries having various rigid substituents using Sharpless's Click reaction. As a result, most potent inhibitors for each sialidase among known inhibitors were founded.

(256) β-1,6-Glucan Stimulates Fungal Phagocytosis by Human Neutrophils

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Innate immunity depends upon recognition of surface features common to a broad group of pathogens. In gram negative bacteria, lipopolysaccharide recognition by immune cells leads to inflammation. In fungi, the glucose polymer β-glucan has been implicated in fungal recognition. Fungal walls have two kinds of β-glucan: β-1,3-glucan and β-1,6-glucan. The predominance of β-1,3-glucan in the wall and its recognition by macrophages has led to the presumption that β-1,3-glucan is the key immunological determinant for both macrophages and neutrophils. Here we show that in human neutrophils, β-1,6-glucan mediates engulfment, production of reactive oxygen species, and expression of HSPs more efficiently than β-1,3-glucan. Neutrophils rapidly ingest beads coated with β-1,6-glucan, while ignoring those coated with β-1,3-glucan. Complement factors C3b/C3d are deposited on β-1,6-glucan more readily than on β-1,3-glucan, recognized by CR3. β-1,6-glucan is also important for efficient engulfment of *Candida albicans*. These unique stimulatory effects could have useful medical applications.

(257) An Efficient Strategy for the Exploration of Specific Inhibitors of Sialyltransferases

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The characteristic compositions detected in glycoprotein of transformed cells is often caused by alteration of the Sialyltransferase (siaT) activities, as shown in human colorectal cancer, breast carcinoma, and so on. It seems likely that some specific inhibitors of the siaTs can become nice tools for developing new type of therapeutic reagents against abovementioned diseases as well as for basic insight into molecular mechanism of these important glycan synthetic enzymes.

To establish rapid and efficient process for the development of potent inhibitors, we planned the construction of novel approach by combining use of large compound library and high throughput screening system. Here we employed an efficient "click chemistry" between two CMP-NANA-based precursors bearing an azide group at C-9 or C-5 position and commercially available alkynes to give rise to over 70 novel compounds library.

When the aminooxy-functionalized peptides (aoWR reagent) as isotope-labeled and highly sensitive tag moiety were conjugated with the designated acceptor substrate of siaTs, the enzyme-mediated sialylation in the presence of CMP-NANA can be monitored quantitatively by means of common MALDI-TOF mass spectrometry. It was clearly demonstrated by using α-2,3 (rat) and α-2,6 (rat) siaTs that the present protocol allows for high throughput screening of the inhibitory activity by newly synthesized compounds library. Our results revealed that some of the new derivatives exhibited specific and strong inhibitory effect on the one of the two siaTs with extremely high selectivity.

(258) Studies of the Interaction between PGIP and PG using SPR and Differential Proteolysis/MS

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The enzymes produced by fungi that degrade pectin play crucial roles in both agriculture and industry. While pectin degrading enzymes are industrially beneficial and their production represents a multi-billion dollar industry, agricultural crop loss due to phytopathogenic fungi is a worldwide problem. Many fungi use

endopolygalacturonases (EPGs) to hydrolyze the cell wall polysaccharide homogalacturonan as one of the first steps in invasion. Plant defense mechanisms directed toward EPGs have evolved that utilize polygalacturonase-inhibiting proteins (PGIPs) to alter the activity of the EPG. The mode of action of a particular fungal EPG and its inhibition by PGIPs may be critical factors in determining whether the fungus is a viable pathogen. At the same time, the interaction between EPGs and PGIPs can be affected by different factors such as pH and substrate. We are using surface plasmon resonance to study the specificity and kinetics of the interactions between bean polygalacturonase-inhibiting protein (PGIP) and different fungal endopolygalacturonases (EPGs) in the presence or absence of homogalacturan. Results show that the presence of substrate has a moderate to strong effect on the EPG/PGIP interaction, and that the level of that effect is dependent on the exact EPG/PGIP pairing. Differential proteolysis/MS experiments have allowed us to identify the probable sites of EPG/PGIP interaction.

(259) **Hyaluronic Acid N-Deacetylase Revealed**

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Hyaluronic acid (HA) *N*-deacetylase, the enzyme that removes *N*-bonded acetyl (−$COCH_3$) groups from skin HA in the 7th decade of life (Longas et al., 1987, *Carbohydr. Res.* 159:127-136), is specific for *N*-bonded acetyl moieties of D-GlcNAc and D-GalNAc where C_1 is β-linked (Longas et al., 2003, *BioMacromolecules* 4:189-192). This work reports a partial characterization of HA *N*-deacetylase in skin homogenate of 76.5 ± 1.12 year-old subjects, using fractional precipitation with $(NH_4)_2SO_4$, and polyacrylamide gel electrophoresis, under denaturing and nondenaturing conditions in 1- and 2-dimensions. Enzyme activity was established after incubation of each protein-containing gel (protein-gel) with a 5% (w/v) polyacrylamide gel containing 0.013% (w/v) HA (HA-gel). The protein-gel and the HA-gel were superimposed, and incubated at 37°C for 12 hr in a moist chamber of 0.05 M $(NH_4)_2SO_4$ buffer (pH 7.86). The protein-gel was stained for protein in Coomassie blue, and the HA-gel was stained for white fluorescence in 0.01 % (w/v) Calcofluor white M2R/0.50 M NaCl. After the sandwich reaction, the HA-gel that reacted with nondenatured homogenate revealed three fluorescent bands, but the one that reacted with denatured homogenate revealed one.

The white fluorescence on the HA-gels should originate from *N*-deacetylated HA (Trudel et al.,1990, *Anal. Biochem.* 189:249-253). Therefore, the data show that HA-*N*-deacetylase(s) is composed of three isozymes of apparent molecular weights 79,000; 72,000; and 63,000 Daltons, with the smallest one retaining biologic activity after denaturation.

(260) **Glycosylation of CB Subunit of Crotoxin Facilitates its Phospholipase Activity: A Possible Role of C-Mannosylation**

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Crotoxin is the major toxic component of the South American rattlesnake Crotalus durissus venom. It is composed of two different subunits, CA called crotapotin and CB, a phospholipase A2 with low toxicity and high enzymatic activity. In order to determine the influence of glycans on the phospholipase activity of the CB subunit, purified samples treated or not with PNGase-F or neuraminidase, were submitted to an indirect phospholipase kinetic assay. The treatment with PNGase-F resulted in a decrease of enzymatic activity, as compared to native CB subunit. The 15 kDa apparent molecular mass of CB, determined by SDS-PAGE, is not affected by neuraminidase treatment but decreases to 10 kDa after PNGase-F treatment, suggesting that the molecule contains non-sialylated glycans. Potential sites of N-glycosylation and C-mannosylation are predictable by using the NetNGlyc 1.0 and NetCGlyc 1.0 softwares, respectively, and only C-mannosylation putative sites were found in the CB molecule. On the basis of the CB structural similarity with the Agkistrotoxin, which is a phospholipase A2-type presynaptic neurotoxin from Agkistrodon halys pallas, the three-dimensional structure of the CB was modeled by using Swiss-PDBViewer. The homology model indicates that two putative sites of C-mannosylation are located close to the CB catalytic site. We postulate that CB glycans facilitate its phospholipase activity and we are presently looking for unequivocal demonstration that CB is C-mannosylated. These observations reinforce the idea previously proposed by us and other groups that glycosylation exerts a relevant role on the biological activities of snake venom toxins.

(261) **Structural Insights into the Mechanism of pH-Dependent Ligand Binding by the 46 kDa Cation-Dependent Mannose 6-Phosphate Receptor**

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The 46 kDa cation-dependent MPR (CD-MPR) is a key component of the lysosomal enzyme targeting system that binds newly synthesized mannose 6-phosphate (Man-6-P)-containing acid hydrolases in the Golgi and transports them to the endosomal/lysosomal system. The interaction between the MPRs and Man-6-P-containing ligands is sensitive to pH: the homodimeric CD-MPR binds lysosomal enzymes optimally in the pH environment of the trans Golgi network (~pH 6.5) and releases its cargo in acidic endosomal compartments (< pH 5.5) and at the cell surface (pH 7.4). Divalent cations have also been shown to modulate receptor activity by enhancing binding affinities. Our previous crystallographic studies have shown that at pH 6.5, the CD-MPR bound to Man-6-P adopts a significantly different quaternary conformation than the CD-MPR in a ligand-unbound state, a finding that represents an unusual observation among known lectin structures. To gain a better understanding of how the CD-MPR carries out its cellular functions, we have obtained additional crystal structures representative of the various environments encountered by the receptor: CD-MPR bound to Man-6-P in the presence and absence of Mn2+ at pH 6.5, bound to a terminally phosphorylated trisaccharide (P-Man(α1,2)Man(α1,2)Man-O-(CH2)8 COOMe) at pH 6.5, unbound at pH 4.8, and bound to the non-phosphorylated trimannoside oligosaccharide from a crystallographic neighbor at pH 7.4. A detailed comparison of the available CD-MPR structures reveals the positional invariability of specific binding pocket residues and implicates inter-monomer contact(s) as regulators of pH-dependent carbohydrate binding.

(262) **Conjugation of Guanidinoneomycin to High Molecular Weight Cargo Facilitates Delivery into Cells through a Heparan Sulfate Dependent Pathway**

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We recently described a derivative of neomycin, called guanidinoneomycin, in which all of the amino groups of the aminoglycoside were converted to positively charged guanidinium

groups (Elson-Schwab et al. (2007) J. Biol. Chem. 282:13585). A biotinylated form of guanidinoneomycin was prepared and conjugated to streptavidin-phycoerythrin-Cy5. Incubation of wildtype and mutant Chinese hamster ovary cells with the derivative at low concentration (nanomolar) showed binding and uptake in a heparan sulfate-dependent fashion. Clathrin dependent internalization occurred and internalized compound was found in stable endosomal vesicles. Delivery occurred at transporter concentrations where no cellular toxicity was observed and was entirely dependent on cell surface heparan sulfate. Conjugation of guanidinoneomycin to saporin, a ribosome inactivating agent, demonstrated cytoxicity, directly showing that some of the conjugate escaped intracellular vesicles and gained access to the cytoplasm. Application of the compound to tumor cells revealed striking differences in sensitivity, but little variation in the extent of binding. These findings suggest that the nature of the receptor may determine the capacity to internalize guanidinoneomycin and its ultimate destination inside the cell. Further studies are underway to determine the intracellular fate of the internalized compound and the identity of the relevant proteoglycan receptors.

(263) Alternative Strategies for Analysis of Fungal Glycolipids and their Interactions

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Fungi are receiving attention because they are major pathogens of agricultural plants and important opportunistic pathogens of humans. The susceptibility to infection by opportunistic and other fungal pathogens has risen due to an increase in the population of immunocompromised persons such as HIV infected, cancer, organ and tissue transplant and critically ill patients. The fungal sphingolipid biosynthetic pathway incorporates the enzyme inositol phosphorylceramide (IPC) synthase, which catalyzes the transfer of inositol-1-phosphate to C-1 hydroxy of ceramide to produce IPC. Further addition of sugars to IPC yields a variety of complex sphingolipids called glycosylinositol phosphoryl ceramides (GIPCs). Since this step is characteristic of fungi (and other non-mammalian species), it is targeted for development of new anti-fungal drugs. Our goal is to characterize these glycosphingolipids (GSLs) in order to aid functional and immunological studies. We have previously demonstrated the utilization of the enzyme sphingolipid ceramide N-deacylase (SCDase) for the deacylation of the ceramide moiety of GIPCs and their subsequent derivatization using amine reactive fluorocarbon-based tags. In this study we utilize various naturally isolated fungal GIPCs to generate fluorocarbon derivatives that are compatible with MS strategies. We will show how these can aid characterization of the unique pattern of expression of glycans in GIPCs of various fungal species. We will also discuss the use of these derivatives for microarray screening with selected carbohydrate binding proteins (CBPs) in studies directed towards development of immunodiagnostic strategies.

(264) LUCA2, A Glycotope Specific Antibody, Modulates PMN Activity through CD11b

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Active inflammation is characterized by the activation, migration, and accumulation of polymorphonuclear leukocytes (neutrophil, PMN). The integrin αMβ2 (CD11b/CD18, C3b, Mac-1) is the predominant adhesive ligand on human PMN and plays a central role in PMN activation, adhesion and migration across endothelial and epithelial barriers, phagocytosis of opsonized particles, and granule release. More recently, CD11b/CD18, specifically the Lewis x antigen displayed by CD11b/CD18, was described to be the key component in PMN/dendritic cell (DC) interaction. In an attempt to isolate monoclonal antibodies useful for the modulation of active inflammation, we screened a panel of antibodies for binding to PMN and identified LUCA2, an antibody raised against a lung tumor stem cell, as a PMN activity-modulating antibody. In initial screens, LUCA2 was culled out for its ability to cause spontaneous PMN activation and degranulation. Through biochemical studies, the LUCA2 binding antigen on PMN was identified to be CD11b. With further studies, it was discovered LUCA2/CD11b binding was N-glycanase sensitive. Finally, LUCA2 was demonstrated to interact directly with BSA conjugated Lewis a and Lewis x antigen, the interaction with Lewis x being the more robust. Studying LUCA2 modulation of CD11b/CD18 directly, it was discovered that LUCA2 increased CD11b/CD18 function and accentuated CD11b/CD18 mediated adhesion of the colonic epithelial cell line T84. Taken together, LUCA2 an antibody which modulates CD11b/CD18 function through a purely carbohydrate epitope.

(265) Structural Basis for Recognition of Breast and Colon Cancer Carbohydrate Epitopes by Helix Pomatia Lectin

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Helix pomatia agglutinin (HPA) is a N-acetylgalactosamine (GalNAc) binding lectin found in the albumen gland of the roman snail. This lectin that has been used extensively in histopathology since its binding to tissue sections from breast and colon cancers is correlated with the worst prognosis for the patients. The lectin recognizes α-D-N-acetylgalactosamine (αGalNAc) containing epitopes which are only present in cancer cell lines having a high likelihood to undergo metastasis, such as the HT29 cancer colon cell line. Several breast cancer cell lines have also been shown to be labeled, although IGROV1, an ovarian cancer cell line, is not.

The high resolution crystal structure of the lectin complexed with GalNAc demonstrated a new fold with hexameric arrangement. The atomic basis for specificity for GalNAc could be unraveled and correlated with a particular network of hydrogen bonds. Other crystal structures were obtained in complex with two GalNAc containing epitopes associated with cancer, the Tn (αGalNAc-Ser) and Forssman (αGalNAc1-3GalNAc) antigens. A histidine residue makes hydrophobic contact with the aglycon, rationalizing the preference for GalNAc bearing an additional sugar or amino acid in the αposition. These structures provide the molecular basis for the use of HPA in metastasis research.

(266) Design and Syntheses of 'Photo-Sugars' and their Utility in Glycobiology

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Cell surface glycoproteins interact with other proteins or glycproteins to mediate cell-cell adhesion events, binding of soluble ligands, and cell-pathogen interactions. Despite the critical roles of these interactions, identification of glycoproteins' binding partners remains challenging, due to the transient and low-affinity nature of carbohydrate-mediated interactions.

One of the most established chemical approaches to detect interactions of molecules is photo-affinity labeling. This approach is so powerful that even weak interactions can be detected as covalently crosslinked adducts like 'snapshots'. However, difficulties in synthesis sometimes prevent easy access to photo-activatable compounds. We have designed and synthesized some simple 'photo-sugars' that can be activated by UV light. These photo-sugars are expected to be incorporated into biosynthetic

pathway and utilized by glycosyltransferases to form complex glycoproteins. Proof-of-concept results of *in vitro* and in situ photo-crosslinking studies will be presented including detection of the CD22 homo-dimer on the cell surface.

(267) **Structure and Function of O-glycosylated Tandem Repeats from Candida albicans ALS Adhesins**

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TR repeats of the Candida albicans Als proteins are conserved beta-branch-rich 36-residue sequences that are present in all known alleles of the proteins. They are heavily O-glycosylated. Cellular assays show that the repeats have cell-cell adherence activity. Circular dichroism spectroscopy shows beta-sheet-rich spectra for this region of soluble Als5p and for a synthetic 36 residue peptide. These repeats contribute to aggregation of cells that express Als proteins. Ab initio modeling with ROSETTA and LINUS both generated consistent fold structures of three-stranded beta sheets in all tested repeats, whereas models of randomized sequences of the same composition did not converge to similar structures. Modeling of O-glycosylations revealed a consistent pattern of surface modifications, with exposed hydrophobic regions of the surface surrounded by glycosylation sites. Such sites are "classic" subunit interaction surfaces, and suggest a pattern of promiscuous interactions between any TR domains in any Als proteins expressed on cell surfaces.

(268) **Domain 5 of the Cation-Independent Mannose 6-Phosphate Receptor Preferentially Binds Phosphodiesters (Man-P-GlcNAc)**

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The 300 kDa cation-independent mannose 6-phosphate receptor (CI-MPR) and the 46 kDa cation-dependent MPR (CD-MPR) are key components of the lysosomal enzyme targeting system that bind newly synthesized mannose 6-phosphate (Man-6-P)-containing acid hydrolases and divert them from the secretory pathway. Previous studies have mapped two high affinity Man-6-P binding sites of the CI-MPR to domains 1-3 and 9, and one low affinity site to domain 5 within its 15-domain extracytoplasmic region. A structure-based sequence alignment predicts that domain 5 contains the four conserved residues (Gln, Arg, Glu, Tyr) identified as essential for Man-6-P binding by the CD-MPR and domains 1-3 and 9 of the CI-MPR. Here we show by surface plasmon resonance (SPR) analyses of constructs containing single amino acid substitutions that these conserved residues (Gln-644, Arg-687, Glu-709, Tyr-714) are critical for carbohydrate recognition by domain 5. Furthermore, the N-glycosylation site at position 711 of domain 5, which is predicted to be located near the binding pocket, has no influence on carbohydrate binding affinity. Endogenous ligands for the MPRs that contain solely phosphomonoesters (Man-6-P) or phosphodiesters (Man-P-GlcNAc) were generated by treating the lysosomal enzyme, acid alpha-glucosidase (GAA), with recombinant GlcNAc-phosphotransferase and uncovering enzyme. SPR analyses using these modified GAAs demonstrate that unlike the CD-MPR or domain 9 of the CI-MPR, domain 5 exhibits a 14- to 18-fold higher affinity for Man-P-GlcNAc than Man-6-P, implicating this region of the receptor in targeting phosphodiester-containing lysosomal enzymes to the lysosome.

(269) **Design and Synthesis of Novel Glycolipids: Potentially Useful Surfactants for the Isolation and Crystallization of Membrane Proteins**

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Integral membrane proteins (IMPs) play vital roles in many cellular functions and are important targets for drug development. Understanding of their function is often circumscribed by the lack of structural information due to difficulty in obtaining suitable crystals for the X-ray diffraction. Synthetic glycolipids are useful for the extraction and crystallization of membrane proteins. Choice of the right detergent has been the key to success in membrane protein solubilization and crystallization. The number and structural variation of the commercially available surfactants are limited.

Driven by the need to synthesize structurally diverse glycolipids, we have designed and synthesized several novel glycolipids (1 – 4) utilizing the Click Chemistry. Reaction of the commercially available peracetylated glycosyl azides with n-alkyl propargyl ether in the presence of Cu(I) as catalyst followed by Zemplen de-O-acetylation led to the regioselective formation of 1 in quantitative yield. Reaction of the known 2,3,4-tri-O-acetyl-1,6-diazido-D-glucopyranose with two equiv. of n-alkyl propargyl ether under similar conditions followed by deprotection afforded, in 61 % overall yield, 2 that represents a novel class of reverse Bolaamphiphiles. The third design represents a structural hybrid of glycosylasparagine and glycolipid stitched by a 1,2,3-triazole ring obtained by the reaction of N-glycosylazidoacetamide with n-alkyl propargyl ether in good yields. The scheme for the synthesis of 4, alkoxyacetamido sugar, involves the reaction of 4,6-isopropylidine-2,3-di-O-benzyl-β-D-glucopyranosylchloroacetamide with long chain alcohol. Details of the work will be presented.

(270) **Structure, Specificity and Affinity or BclA, a Soluble Mannose-Binding Lectin from Burkholderia Cenocepacia**

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Chronic colonization of the lungs by opportunist bacteria such as Pseudomonas aeruginosa and members of the Burkholderia cepacia complex (BCC) is the major cause of morbidity and mortality among cystic fibrosis (CF) patients. Pa-IIL (lecB gene) a soluble lectin from P. aeruginosa, has been the subject of much interest because of its very strong affinity for fucose. Orthologs have been identified in the opportunist bacteria Ralstonia solanacearum, Chromobacterium violaceum and Burkholderia of BCC. The genome of J2315 strain of B. cenocepacia, responsible for epidemia in CF centers, contain three genes that code for proteins with PA-IIL domains.

BclA (Burkholderia cenocepacia lectin A) was cloned in E. coli and the recombinant protein was obtained. Printed Glycan arrays performed by the Consortium for Functional Glycomics (CFG) demonstrated that BclA has a strict specificity for oligosaccharide with a terminal α-mannose residue. Thermodynamics of the interaction of BclA with α-methyl-mannoside (αMeMan) demonstrates a dissociation constant (Kd) of 2.75 10-6 M. The protein has been crystallized and the structure of the complex with αMeMan was solved at 1.7Å resolution. The lectin forms a homodimer with one binding site per monomer, each one containing two calcium ions and one sugar ligand. Crystallographic structures of BclA complexed with Manα1-3Man and Manα1-3(Manα1-6)Man have also been obtained and the latter one displays interesting cross-linking of neighboring lectins by the trisaccharide.

(271) **Galectin-3 Association to Cell Surface Mucins Is Necessary to Maintain Mucosal Barrier Function**

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Maintenance of the mucosal barrier is critical to prevent damage and infection of wet surfaced epithelia. At the ocular surface, disruption of this barrier, clinically evidenced by rose bengal dye staining, is associated with a number of pathologies. Galectins are ß-galactoside-binding proteins that may participate in the stabilization of mucosal barriers by interacting with carbohydrates on the cell surface glycocalyx. Using glycogene-chip microarrays, we found that galectin-3 and the cell surface-associated mucin MUC1 were among the most highly expressed genes in human conjunctiva. Cell surface biotinylation and confocal microscopy on corneal and conjunctival biopsies, and human corneal-limbal epithelial (HCLE) cells, demonstrated membranous localization of galectin-3 predominantly on apical and subapical cells. By contrast, low levels of galectin-3 were found in tear secretions. MUC1 and MUC16 from HCLE cells effectively eluted from galectin-3 affinity columns after competitive inhibition with ß-lactose, indicating that cell surface mucins are counter-receptors for galectin-3 in a galactose-dependent interaction. In functional assays, incubation of HCLE cells with ß-lactose, but not with sucrose and maltose, resulted in transient uptake of rose bengal. In these experiments, galectin-3 significantly decreased at the cell surface after addition of ß-lactose, concomitant to an increase in culture media. Incubation of mouse corneas with ß-lactose resulted in the appearance of punctate epithelial staining after rose bengal administration, as compared to control disaccharides. These results indicate that cell surface mucins are counter-receptors for galectin-3 and that alteration of this association may contribute to the pathological disruption of the mucosal barrier. Support: NIHEY014847, EY03306, EY07088, GM62116.

(272) **Characterization of Glycosphingolipids from Saccharomyces Cerevisiae by NMR Spectroscopy and Electrospray Mass Spectrometry**

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Glycosphingolipids (GSLs) play important roles in many fundamental biological processes. Glycosylinositol phosphorylceramides (GIPCs) are characteristic GSLs of fungi which are not found in mammals. The GIPCs of the hemiascomycete fungus Saccharomyces cerevisiae are inositol-phosphorylceramide (IPC), mannose-inositol-phosphorylceramide (MIPC) and mannose-di-(inositol-phosphoryl)-ceramide (M(IP)2C). Although the polar headgroups of animal and fungal GSLs are fundamentally different, the pathways for ceramide biosynthesis have many common features, with key homologous genes shared between them. For this reason, S. cerevisiae, a widely used laboratory model, is considered relevant for sphingolipid functional interactions in all eukaryotes. Recent reports also imply that fungal GIPCs are important targets for the antifungal action of plant defensins. In order to study intermolecular interactions of S. cerevisiae GIPCs, it is crucial to know their structures in detail. However, while the structures of S. cerevisiae GIPCs are often assumed to be completely established, detailed data precisely specifying the primary linkage sequence of the polar groups of MIPC and M(IP)2C have never been published. As a preliminary step in planned studies of GIPC interactions, we have completed the detailed structural characterization of MIPC and M(IP)2C from S. cerevisiae using NMR spectroscopy and positive mode electrospray ionization mass spectrometry (ESI-MS). Results presented here showed that multistep MSn analysis of Li+ adducts yields structurally informative fragment ions which are useful for unambiguous sequencing of M(IP)2C structure. Together with NMR analysis, the linkages in MIPC and M(IP)2C are clearly established.

(273) **Investigation of Sialo-Linkage in the Human Respiratory Tract Epithelial Cells using Mass Spectrometry**

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SA (α 2,3) Gal linkage, the putative avian influenza H5N1 virus receptor, has been found in the human lower respiratory tract rather than the upper tract by the lectin binding experiment. The absence of SA (α 2,3) Gal linkage in the human upper respiratory tract was attributed to the reason of poor "human-to-human" virus transmission. In contrast to this notion, avian H5N1 viruses can replicate efficiently in the human upper respiratory tissues according to a recent report.To investigate the exact sialic acid linkage nature in the human respiratory tract, two model cell lines Detriot562 and A549 (ATCC Inc, Manassas, VA) were selected as the representative of upper and lower respiratory cell lines, respectively. A mass spectrometry method has been used to differentiate α 2,3 and α 2,6 linkages involving MSn fragmentation and chemical modification of sialic acid group. Purified N-glycans were reacted with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in methanol. α 2-6 sialic acids were converted quantitatively into their methyl esters whereas the α 2-3 sialic acids form lactones with a characteristic mass difference of (-32) units. This method has been successfully tested with sialo-glycans from bovine fetuin, providing a straightforward resolution of the isomeric SA-Gal linkages. Many sialo-glycans has been detected in both human cell lines, α2-3 linkage was found both in A549 and Detroit 562 cell lines, so this explained well why avian H5N1 viruses can replicate in the human upper respiratory tissues.

(274) **Molecular Modeling of Family GT8 Glycosyltransferases**

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GTs have been classified into families on the basis of amino acid sequence similarities in the CAZy database (http://afmb.cnrs-mrs.fr/CAZY). To date, the database comprises around 30,000 known and putative GT sequences that have been divided into 89 families. Large differences are observed among GT families. Some of them are polyspecific and comprise a huge number of sequences from various sources and whose functions are widespread whereas others are monospecific and contain only a few sequences. For the many putative GT genes, which arose from the systematic sequencing programmes of various genomes, and which fall within these polyspecific families, it is often difficult to assign a precise function to these genes on the basis of sequence similarities. For those families, we are wondering if the knowledge of the 3D-structure could help to decipher the substrate specificities of the many putative GT sequences.

The large polyspecific GT8 family was considered as an interesting case study because (i) it comprises two 3D-representatives catalyzing very different reactions (LgtC, an α4-GalT from Neisseria meningitidis, and the rabbit glycogenin, a self-glucosylating protein), (ii) it includes sequences from prokaryotic and eukaryotic species which exhibit very different enzyme functions, and (iii) it also comprises a high number (approx. 40) of non-annotated Arabidopsis thaliana ORFs. We took advantage of the structural data, to further explore the sequence determinants along the polypeptide chains that confer substrate specificities and

to tentatively assign a function for the many Arabidopsis hypothetical proteins.

(275) Glycomics Analysis of HIV-1 Glycoforms: Insights to Glycan Mediated Immune Suppression

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The possibility exists that the carbohydrate sequences associated with gp120, the major surface glycoprotein of HIV-1, act as functional groups that enable this glycoprotein to manifest immunomodulatory effects in AIDS patients. Consistent with this hypothesis, gp120 preparations derived from a variety of different human lymphoblastoid cell lines modulate many different immune responses *in vitro*, whereas gp120 glycoforms associated with CHO cells do not. Bisecting type oligosaccharides are the predominant complex type N-glycans expressed in gp120 from human H9 lymphoblastoid cells. HEK293 cells readily add unusual lacdiNAc and fucosylated lacdiNAc sequences to transfected glycoproteins. These sequences are associated with schistosomes and glycodelin-A, an immunomodulatory glycoprotein expressed in the decidua during pregnancy. The major goal of this study was to determine if gp120 synthesized in HEK293 cells would also acquire lacdiNAc and fucosylated lacdiNAc sequences. If so, then such glycoforms might display different immunomodulatory effects compared to the lymphocyte associated glycoforms. Structural analyses were performed on N-glycans of gp120 synthesised in HEK293 cells. These studies indicate the presence of bi-, tri-, and tetraantennary N-glycans of which approximately 47% are bisected. There were only very marginal amounts of lacdiNAc antennae and no detectable fucosylated lacdiNAc sequences. These findings indicate that gp120 preferentially acquires bisecting type N-glycans in cell types that normally express low levels of this sequence, suggesting that gp120 has a low Km for human GnT III. Thus gp120 may acquire bisecting type N-glycans in many different human cell types, perhaps contributing to the deleterious effects of HIV-1 in patients with AIDS.

(276) Compositional Analysis of Glycosaminoglycans using Quantitative Glycan Reductive Isotope Labels

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To facilitate quantitative disaccharide analysis of glycosaminoglycans, we utilized Glycan Reductive Isotope Labels (GRIL) for tagging the reducing end of oligosaccharides with stable isotopes. Because different isotope tags have no effect on chromatographic retention times but can be discriminated by a mass detector, differentially isotope-tagged samples can be compared simultaneously by liquid chromatography/mass spectrometry (LC/MS) and quantified by admixture with known amounts of standards. Our studies of model organisms confirm previously published results for chondroitin sulfate from a number of different species but our analysis of Chinese hamster ovary cells detected a disaccharide derived from dermatan sulfate not previously reported in this cell line. Our studies also show that Hydra vulgaris and Drosophila melanogaster produce heparan sulfate with a high content of unsubstituted glucosamine residues compared to heparan sulfate from cultured cells, mice, and Caenorhabditis elegans. In addition to providing a more reliable and sensitive means of compositional disaccharide anaylysis for heparan and chondroitin sulfates, GRIL-LC/MS can also be used to perform strucural analysis of keratan sulfate to determine the content of sulfated galactose and N-acetylglucosamine residues. Since tagged disaccharides elute at higher organic buffer concentrations, better ionization of and detection of analytes was achieved. We used this effect to size hyaluronic acid oligosaccharides by comparing the ratio of reducing end (saturated) disaccharide to internal (unsaturated) disaccharides produced after complete enzymatic depolymerization. We also developed a new shorthand nomenclature for all glycosaminoglycan disaccharides (Disaccharide Structure Code, DSC) as a way to compare compositions and describe linear sequences.

(277) Molecular Characterization of Flu-Receptor – Hemagglutinin Interactions. Computational Prediction of HA Specificity

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Hemagglutinin mediates attachment to and entry of influenza virus into host cells by binding to sialic acid receptors at the cell surface. Human influenza viruses preferentially bind to sialic acid linked to galactose by α-2,6 linkages; the main type found on the epithelial cells of the human upper respiratory tract. Avian viruses tend to bind to α-2,3 linkages that are found predominantly on avian intestinal epithelium. All influenza A viruses that have infected mammals emerged as some point from avian species. Changes in the amino acid sequence of HA can alter the sialic acid specificity of influenza viruses, with the change of one or two amino acids being sufficient to change the receptor binding specificity and affect interspecies transmission barriers.

We report computational simulations using the GLYCAM force field of human and avian receptor – HA complexes, based on structural data for the human 1934 H1 influenza strain. The theoretical methods correctly identify this H1 hemagglutinin as selective for human α-2,6 linkages and provide insight into the origin of the affinity differences, but also indicate limitations of current simulational methods.

(278) Role of Sialic Acid in Optimizing the Catalytic Activity of BpSP-I, a New Thrombin-Like from Bothrops Pauloensis Snake Venom

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We have previously reported the thrombin-like activity of a glycoprotein (BjussuSP-I) isolated from the B. jararacussu venom. N-glycans close to the catalytic triad of BjussuSP-I have been showed to optimize the thrombin-like activity of the molecule. In the present study we report a novel N-glycosylated protein venom endowed of thrombin-like activity, denoted BpSP-I, which was isolated from Bothrops pauloensis snake venom. The isolation of such serine-protease was held through three chromatographic steps (CM-Sepharose, Benzamidine-Sepharose and RP-HPLC). A single 36 kDa band was provided by BpSP-I SDS-PAGE analysis. The apparent MM of BpSP-I has decreased to 32 and 27 kDa following neuraminidase or PNGase-F treatment, respectively, indicating that the molecule contains sialic acid and N-glycans. The BpSP-I enzymatically treated samples were assayed for thrombin activity. The fibrin clot formation of was equally reduced in both, deglycosylated and desialylated forms, suggesting an important

role for sialic acid in improving serine-protease activity. The relevance of N-glycan and sialic acid in the BpSP-I thermostability and resistance to acid and alkali pH conditions has been evaluated through a coagulation kinetic assay. In such denaturing conditions, both deglycosylated and asialo forms of BpSP-I have decreased more drastically the thrombine-like activity than the native enzyme. Taken together, our results point out the relevance of sialic acid and N-glycan moieties in the serine-protease activity of BpSP-I, and their role in protecting BpSP-I from thermal, acid, and alkali denaturing.

(279) The Glycome of *Schistosoma Mansoni* Egg and Cercarial Secretions

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Interaction of pathogen-derived glycan constituents by carbohydrate binding proteins on cells of the host immune system is an important step in the mounting of an immune response during an infection by a pathogen. To better understand this protein-glycan interaction during an infection by the parasitic helminth *S. mansoni*, we have carried out a glycomic analysis of the N- and O-glycans derived from glycoproteins in *S. mansoni* egg and cercarial secretions. Our results show that *S. mansoni* secrete glycoproteins with glycosylation patterns that are complex and stage-specific. Core xylosylation and difucosylation were dominant features in cercarial and egg secretions respectively. O-glycan core structures from cercarial secretions primarily consisted of the core sequence Galβ1-->3(Galβ1-->6)GalNAc. In contrast, egg-secreted O-glycans predominantly carried the mucin-type core 1 (Galβ1-->3GalNAc) and 2 (Galβ1-->3 (GlcNAcβ1-->6)GalNAc) structures. Additionally, we identified a novel O-glycan core in both secretions in which a Gal residue is linked to the protein. High levels of terminal fucosylation were detected in N- and O-glycans and include stage-specific patterns (Jang-Lee, J. *et al* 2007). Glycan structures identified in this study are potential ligands for carbohydrate binding proteins on cells of the host immune system and could therefore represent one of the crucial determinants for successful parasite establishment.

(280) Analysis of Isomeric Oligosaccharide Mixtures by Sequential Mass Spectrometry

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Mass spectrometry has become the methodology of choice for analysis of oligosaccharide structure. This can take the form of MS profiles to obtain glycan compositions, tandem mass spectrometry to obtain structural information, or sequential mass spectrometry, to obtain comprehensive structural details. In addition, mass spectrometry is frequently coupled with liquid chromatography to simplify the sample that is presented to the mass spectrometer. We demonstrate here that isomeric oligosaccharide structures can be identified and determined without prior chromatographic separation by using sequential mass spectrometry. This technique was applied to resolve the isomeric mixtures from human IgG which would be difficult or impossible to identify by MS or MS/MS methods alone. N-linked glycans were released enzymatically, reduced, and permethylated. The MSn spectra were collected with an LTQ equipped with a nanospray source. Data were submitted to OSCAR, a glycan topology assignment algorithm. Complicating the identification of isomeric oligosaccharides is the fact that oligosaccharides are built from a relatively small set of monomer types. This produces the potential that similar neutral losses may arise from different structures. In addition, low-abundance isomers are frequently obscured by more intense ions, and this is demonstrated in several examples of isomeric mixtures from human IgG glycans. These results show that sequential mass spectrometry can be successfully used to distinguish isomeric structures from a mixture without prior chromatographic separation. Moreover, we also show that MS/MS or MS3 analysis may not be sufficient to properly characterize these structures.

(281) Protein Scaffolds for High-Affinity CD22 Ligands

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CD22 is a B cell-restricted sialic acid-binding immunoglobulin-like lectin (Siglec) whose function as a regulator of B cell receptor signaling is modulated by glycan ligands bearing the terminal sequence NeuAcα2-6Gal. CD22 ligands are low affinity (Kd = ~0.2 mM), but abundant expression of these glycans on the B cell surface masks the ligand-binding site. Previously we found that a polymeric display of high-affinity CD22 ligands, 9-biphenylcarbonyl-NeuAcα2-6Galβ1-4GlcNAc (BPC-NeuAc), with a valency of 500, but not 15, bound to CD22 on native B cells. To explore probes with intermediate valency, but with defined structural rigidity, two viral capsids, cowpea mosaic virus (CPMV) and bacteriophage Qβ, were evaluated as CD22 probes. CPMV capsids contain 60 copies each of a small and large subunit protein and have been decorated with up to 150 copies of BPC-NeuAc by chemo-enzymatic synthesis. These capsids demonstrate sufficient avidity to bind to native B cells. Similarly, Qβ, with 180 asymmetric units, has been derivatized with up to 470 BPC-NeuAc ligands per capsid, and also bind to native B cells. In an alternative approach we have used decavalent anti-nitrophenol (NP) IgM antibody to display hetero-bi-functional ligands comprised of a CD22-ligand coupled to the antigen NP. In this approach the protein scaffold participates in ligand driven assembly of multivalent IgM-CD22 complexes on the surface of native B cells. To pursue the flexibility of this approach, we are evaluating ligands with different spacers and anti-NP antibodies with lower oligomeric states (e.g. bivalent anti-NP IgG and tetravalent anti-NP IgA).

(283) Differential Effects of Bovine Kidney and Porcine Intestinal Heparan Sulphate on Breast Cancer Cell Adhesion and Migration

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Cell adhesion and migration are important parameters that determine cancer prognosis. Overexpression of heparan sulphate, a sulphated glycosaminoglycan, has been correlated with poor clinical outcome in breast cancer. To investigate the roles of different heparan sulphate species in regulating tumour cell adhesion and migration, we cultured MCF-7 breast cancer cells on collagen I or fibronectin in the presence of chlorate, a competitive inhibitor of glycosaminoglycan sulphation. Chlorate treatment resulted in an increase in cell adhesion and focal adhesion complex formation, and reduced cancer cell migration. This was accompanied by upregulated expressions of focal adhesion kinase and paxillin mRNA transcripts and proteins, as determined by real-time RT-PCR and immunohistochemistry respectively. The effect of chlorate treatment in increasing cancer cell adhesion was blocked by administration of either bovine kidney or porcine intestinal heparan sulphate. Porcine intestinal heparan sulphate was also effective in preventing the chlorate-induced inhibition of cell migration. In contrast, cancer cells exposed to chlorate plus bovine

kidney heparan sulphate showed an even greater reduction in the speed of migration compared to cells treated with chlorate alone. The structures of bovine kidney and porcine intestinal heparan sulphate differ in their degree and pattern of sulphation. Taken together, these results show that different heparan sulphate species have differential effects on breast cancer cell adhesion and migration.

(284) **Methodology and Characterization of Non-Covalent Protein Bound Heparin Oligosaccharides by Mass Spectrometry**

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Glycoaminoglycans (GAGs); heparin, heparan sulfate (HS), dermatan and chondroitin sulfate are involved in important physiological and pathological processes such as regulation of enzymes, signaling molecules in response to cellular damage (wounding, infection, tumorigenesis), as well as bacterial infection via direct interaction with intra- and inter-cellular proteins. Over the past decade, an increasing number of proteins have been discovered which interact with GAGs. The goal of this work is to elucidate the mechanisms whereby cellular responses to growth factor stimuli are modulated through expressed GAG structures. This work describes a high sensitivity LC-MS based binding assay method for determining compositions of GAG oligosaccharides binding proteins.

The method uses an amide-silica HILIC column coupled with a quadrupole time-of-flight mass spectrometer. A model system for a heparin binding protein has been used, namely Antithrombin III (ATIII). Heparin libraries made from a partial heparin lyase digestion of porcine intestinal mucosa heparin (degree of polymerization, dp, 6 and 8) were first characterized and quantified by LC-MS. The different libraries were then incubated with ATIII and the bound fractions were analyzed by the same method. The amide HILIC LC/MS method enables the screening and quantitative analysis of a library of heparin/HS oligosaccharide for epitopes that are able to bind ATIII. Furthermore, the binding specificity was confirmed using non-binding GAG controls. The bound dp6-dp8 heparin fraction had enhanced Factor Xa inhibitory activity.

(285) **X-Ray Crystallography of JAA-F11 Antibody to TF-Ag and Molecular Modeling to Improve Peptide Mimics of TF-Ag**

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Thomsen Friedenreich antigen (TF-Ag) is a carbohydrate antigen found on many types of tumors, including breast, prostate, bladder and ovarian. Passive transfer of JAA-F11, a monoclonal anti-TF-Ag antibody, has been shown to block metastasis and provide a survival advantage in mice with metastatic breast cancer. Some cancer patients have an immune response to TF-Ag and this correlates with a better prognosis. Creation of an immune response in the patient to TF-Ag has clinical potential, however, it is difficult to form this response because carbohydrate antigens form a less effective T-cell independent response whereas, peptide antigens evoke a more effective T-cell dependent immune response process, causing isotype switching, affinity maturation and memory development. Immunization with peptide mimics of TF-Ag created an immune response to TF-Ag. In order to improve the response, the spatial arrangement of JAA-F11 was studied by X-ray crystallography in order to predict which amino acids were involved in binding to the peptide mimics. JAA-F11 has been purified, crystallized and the structure has been determined at a resolution of 2.1Å on which, molecular modeling with the peptides with this crystal structure is underway to understand the binding in order to improve the peptides. Peptide sequences with alterations will be synthesized to determine effect of changes on binding. This research has the potential to develop a novel vaccine against breast cancer.

(286) **Quantifiable Fluorescent Glycan Microarray**

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Glycan microarray expansion is limited by the difficulties encountered in generating complex glycan structures using chemo/enzymatic methods. We used the fluorescent linker, 2,6-diaminopyridine (DAP), to derivatize free, reducing glycans covalently attached to epoxy-activated glass slides. Non-contact printing was performed using a Piezorray printer from Perkin Elmer where the average spot volume is 0.333 nL. The array was validated using biotinylated lectins detected with cyanine 5-streptavidin.

We explored printing efficiency of LNFIII-DAP on NHS- and epoxy-derivatized slides with Aleuria aeurantia lectin (AAL). Clear AAL binding signals were obtained on NHS-derivatized slides when LNFIII-DAP was printed at ~16 µM, but with epoxy-derivatized slides, we detected binding at 4 µM. Since DAP is fluorescent, we quantified different Glycan-DAP derivatives (GDAPs) on the glass slides before and after blocking. We calculated a 30-45% printing efficiency on epoxy-activated slides. The ability to quantify the "on-slide" amounts of glycan derivative permits analysis of GBP binding as a function of both glycan on the slide and GBP concentration. Data from these analyses showed that AAL binding to LNFIII has a Kd ~22 nM when measured on the glycan array; in solution the Kd is in the mM range. These results are consistent with the high avidity of a multivalent glycan presentation on the array.

The GDAP microarray as an excellent platform for natural glycan array development by facilitating the separation and immobilization of small amount of glycans from natural sources and for determining binding parameters of glycan binding proteins (GBPs).

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(287) **Uncovering Unique N-linked Structural Isomers in Cancer via MSn disassembly**

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Molecular glycosylation is involved in key developmental roles including cell differentiation, innate immunity and signal transduction. Equally as demonstrable are numerous aspects of tumor development, from cellular proliferation to angiogenesis and metastasis. Glycosyltransferase expression, localization, and activity are the basis for the glycodynamics associated with tumor transformation and progression. Modulation in such criteria lead to a variety of generalized transformations in cancer such as altered branching of N-linked and O-linked glycans, oligosaccharide truncation, and disturbances in sialylation and fucosylation. A disease state shift in glycosyltransferase activities promote glycan structural changes as shown by mass spectrometry (MS). Shifts in glycosyltransferase activity may also lead to unique structural isomers undetectable by MS glycomic profiling (e.g. MALDI). Using cultured cells from spontaneous VM mouse brain tumors we demonstrate a straightforward approach utilizing sample permethylation and ion-trap MS^n disassembly that detects

previously uncharacterized isomeric structures specific to murine astrocytoma. To date, this approach has uncovered 18 individual neutral glycan molecular compositions that upon subsequent MS^n disassembly each composition demonstrates at least two unique structural isomers specific to murine astrocytoma. Most structural isomers were not detected following MS^2 and required multiple ($MS^{n>2}$) stages for detailed structural elucidation.

(288) **C-Termini of both Alg13 and Alg14 Proteins Are Required for Formation of the Alg13/Alg14 Complex**

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In most eukaryotes, the second step of N-linked glycosylation at ER membrane is catalyzed by a novel UDP-N-acetylglucosamine transferase which is comprised of two subunits, Alg13 and Alg14. Formation of Alg13/14 complex is crucial for enzyme activity and regulates the N-link glycosylation in eukaryotic cells. We have undertaken a structural and functional analysis of Alg13 and 14 by using yeast cell as a model for understanding the complex formation of this unique and important eukaryotic glycosyltransferase. Deletion analyses identified three regions essential for enzyme activity and two of them are directly required for the formation of Alg13/14 complex. Mutant Alg13 protein lacking an N-terminal region is unstable, while protein lacking C-terminal region is stable, but fail to interact with Alg14. Last three C-terminal hydrophobic amino acids of Alg14 have been demonstrated important for maintaining the integrity of Alg13/14 complex. A series of chimera proteins were also made between the yeast and human Alg13 and then applied for analyzing the specificity of complex formation. Human Alg13 with the yeast predicted C-terminal α helices region fails to form an active complex with human Alg14, while yeast Alg13 with the human C-terminus works normally. A point mutation exchanging the third C-terminal Amino acid between human (Arginine) and yeast (Isoleucine) Alg14 damaged the activity of human Alg13/14 complex but have no effects on the yeast complex. Taken together, our results suggest that both C-termini of Alg13 and 14 proteins are involved in the formation of an active complex and determine its specificity.