ABSTRACTS SUBMITTED FOR THE 2006 MEETING OF THE SOCIETY FOR GLYCOBIOLOGY

November 15–19, 2006
Universal City, California

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PROGRAM OVERVIEW

Wednesday, November 15	8:30 am – 12:30 pm	**SATELLITE SYMPOSIUM I: NEW TECHNOLOGIES FOR GLYCOMICS** Organized by Richard Cummings, *Emory University* and Michael Pierce, *University of Georgia*
	1:30 pm – 5:00 pm	**SATELLITE SYMPOSIUM II: THERAPEUTIC RECOMBINANT GLYCOPROTEINS – PRODUCTION, PURIFICATION AND ANALYTICAL METHODS** Organized by Joseph Siemiatkoski, *Biogen Idec* and Shekar Ganesa, *Genzyme*
	7:00 – 7:10 pm	**CONFERENCE OPENING** Opening Remarks: **Linda G. Baum,** *President, Society for Glycobiology*
	7:10 – 9:00 pm	**SESSION I: GLYCAN – MEDIATED CELLULAR SIGNALING**
	9:00 – 10:00 pm	**WELCOME RECEPTION**
Thursday, November 16	8:30 – 10:20 am	**SESSION II: GLYCAN BIOSYNTHESIS, PROCESSING AND RECOGNITION**
	10:45 am – 12:35 pm	**SESSION III: DEVELOPMENTAL GLYCOBIOLOGY**
	2:00 – 4:00 pm	**POSTERS and EXHIBITS**
	4:00 – 6:10 pm	**SESSION IV: NOVEL ANALYTICAL TECHNIQUES – SYNTHESIS, VALENCY AND ARRAYS**
Friday, November 17	8:30 – 10:00 am	**SESSION V: GLYCANS IN NEUROBIOLOGY – DEVELOPMENT AND FUNCTION**
	10:30 am – 12:20 pm	**SESSION VI: GLYCANS IN MICROBIAL PATHOGEN-HOST INTERACTIONS**
	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.** *Ticket required. Extra tickets for guests may be ordered.*
Saturday, November 18	8:30 – 9:45 am	**SESSION VII: GLYCANS IN CANCER – PROGNOSIS TO THERAPY**
	10:15 am – 12:30 pm	**SESSION VIII: GLYCAN CONTROL OF LEUKOCYTE MIGRATION AND FUNCTION IN INFLAMMATION**
	2:00 – 4:00 pm	**POSTERS and EXHIBITS**
	4:00 – 5:40 pm	**SESSION IX: GLYCANS IN IMMUNE DEVELOPMENT AND FUNCTION**
Sunday, November 19	8:30 am – 2:30 pm	**MEETING OF THE CONSORTIUM FOR FUNCTIONAL GLYCOMICS** Attendance is open to all interested scientists and is free of charge. Heading into the next funding period, the CFG plans to focus Participating Investigator (PI) meetings on the biology of glycan-protein interactions. This first PI meeting of the new funding period will focus on "Glycan binding proteins: Biological insights from glycan microarrays" and will include presentations by PIs using CFG resources. Attendees will also be updated on progress at the CFG during the last year, including the glycomics initiative and updates to the databases. New initiatives for the coming years will be presented. There will be an opportunity for PIs to provide feedback on the generation of resources and on initiatives such as the creation of a reagent bank. The agenda can be found at http://glycomics.scripps.edu/PI2006Agenda.pdf Please notify Anna Crie (annacrie@scripps.edu) of your intention to attend on or before October 7, 2006.

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WEDNESDAY, NOVEMBER 15
8:30 AM – 12:30 PM
SATELLITE SYMPOSIUM I: NEW TECHNOLOGIES FOR GLYCOMICS, *Grand Ballroom*
Organized by Richard Cummings, *Emory University* and Michael Pierce, *University of Georgia*
Each talk will be 20 minutes with 5 minutes for questions

TBA, Cathy Costello, *Boston University School of Medicine, Boston, MA*

New Methods for Glycoproteomics, Ron Orlando, *CCRC, University of Georgia, Athens, GA*

MS-Based Quantitative Glycan Mapping in Biomedical Investigations, Milos Novotny, *National Center for Glycomics and Glycoproteomics, Indiana University, Bloomington, IN*

Working towards Comprehensive, High-throughput Strategies for Comparative Glycosphingolipidomics, Steve Levery, *Univ. of New Hampshire, Durham, NH*

Applications and Development of Glycan Microarrays, David Smith, *Emory University, Atlanta, GA*

Oligosaccharide Microarrays to Decipher the Glyco Code, Ten Feizi, Imperial College London, Middlesex, UK,

TBA, Ola Blixt *The Scrips Research Institute, La Jolla, CA*

Synthetic GPI Glycan Microarrays as Tool for Anti-toxin Malaria Vaccine, Faustin Kamena, *Swiss Federal Institute of Technology, Zurich, Switzerland*

WEDNESDAY, NOVEMBER 15
1:30 – 5:00 PM
SATELLITE SYMPOSIUM II: THERAPEUTIC RECOMBINANT GLYCOPROTEINS – PRODUCTION, PURIFICATION AND ANALYTICAL METHODS, *Grand Ballroom*
Organized by Shekar Ganesa, *Genzyme* and Joseph Siemiatkoski, *Biogen Idec*

Evaluation of Expression Systems for the Production of Mannose-terminated Glucocerebrosidase, Scott Van Patten, *Genzyme Corporation, Framingham, MA*

Comparison of Glycosylation for Hybridoma and CHO Derived mAb, Jennifer Liu, *Amgen Inc., Thousand Oaks, CA*

Characterization of Carbohydrate Profiles of Monoclonal Antibodies Derived from NS0 Cell Lines, Wenjun Mo, *Medimmune Inc., Gaithersburg, MD*

Recombinant Glycoproteins: Analyzing the Icing on the Cake, Parastoo Azadi, *Complex Carbohydrate Research Center, Athens, GA*

Unique Glycation of a Recombinant Humanized Antibody, Boyan Zhang, *Genentech Inc., San Francisco, CA*

Improved Methods for the Analysis of N- and O-Linked Oligosaccharides from Glycoproteins, Michael Madson, *Dionex Corporation, Sunnyvale, CA*

Carbohydrate Analysis by Capillary Electrophoresis: A Biotechnology Perspective, Oscar Salas-Solano, *Genentech Inc., San Francisco, CA*

High-throughput Screening of Monoclonal Antibody Glycosylation using LC/MS, James Carroll, *Pfizer Global R&D, St. Louis, MO*

Assessing Glycoprotein O-linked Oligosaccharide Composition, Structure and Distribution by ESI-MS, John Briggs, *Genentech Inc., San Francisco, CA*

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WEDNESDAY, NOVEMBER 15
7:10 – 9:00 PM
SESSION I
GLYCAN – MEDIATED CELLULAR SIGNALING, *Grand Ballroom*
Chair: Jim Dennis, *University of Toronto*

Time		Abstract Number
7:10	**Decoding the Structure-Activity Relationships of Glycosaminoglycans in the Brain**; Linda C. Hsieh-Wilson; *Caltech and HHMI, Pasadena, CA*	17
7:30	**Inflammatory Signaling by the C-type Lectin Recpetor Dectin-1**; David M. Underhill; *Cedars-Sinai Medical Center, Los Angeles, CA*	2
7:50	**Deciphering the 'O-GlcNAc Code': Lessons from C. elegans and Human disease**; John A. Hanover; *NIDDK, National Institutes of Health, Bethesda, MD*	3
8:10	**CD33 type Siglecs are Degraded by the SOCS3 via ECS E3 Ligase**; Jim Johnston; *Queen's University Belfast, Belfast, Northern Irelan*	4
8:30	**Modulation of Normal Signaling by Shed Tumor Gangliosides.**; Stephan Ladisch; *Children's National Medical Center, Washington, DC*	5
8:50	**N-glycan Processing Integrates Cellular Responsiveness to Extracellular Cues in Cancer Progression And T Cell Activation**; Ken Lau[1]; Emily A. Partridge[1]; Pamela Cheung[1]; Cristina I. Silvescu[2]; Ani Grigorian[3]; Vernon N. Reinhold[2]; Michael Demetriou[3]; James W. Dennis[1]; *[1]Mount Sinai Hospital, Toronto, Canada; [2]University of New Hampshire, Durham, NH; [3]University of California, Irvine, CA*	6
8:55	**N-Acetylglucosaminyltransferase III Antagonizes N-Acetylglucosaminyltransferase V on Alpha3beta1 Integrin-Mediated Cell Migration**; Yanyang Zhao[1]; Jianguo Gu[1]; Takatoshi Nakagawa[1]; Akihiro Kondo[1]; Nana Kawasaki[2]; Eiji Miyoshi[1]; Naoyuki Taniguchi[1]; *[1]Osaka University Graduate School of Medicine, Osaka, Japan; [2]National Institute of Health Sciences, Tokyo, Japan*	7

9:00 – 10:00 PM
WELCOME RECEPTION

THURSDAY, NOVEMBER 16
8:30 – 10:20 AM
SESSION II
GLYCAN BIOSYNTHESIS, PROCESSING AND RECOGNITION, *Grand Ballroom*
Chair: Karen Colley, *University of Illinois*

Time		Abstract Number
8:30	**A Key Enzyme in Protein N-glycosylation: Oligosaccharyl Transferase**; Manasi Chavan[1]; Guangtao Li[1]; Zhiqiang Chen[2]; Huilin Li[2]; Hermann Schindelin[3]; William J. Lennarz[1]; *[1]Stony Brook University, Stony Brook, NY; [2]Brookhaven National Laboratory, Upton, NY; [3]Würzburg University, Würzburg, Germany*	8
8:50	**Structural Snapshots of the Mannose 6-Phosphate Receptors**; Nancy M. Dahms; *Medical College of Wisconsin, Milwaukee, WI*	9
9:10	**The "Lipid-linked Oligosaccharide/CDG-I/ER Stress Response" Triad**; Mark A. Lehrman; *UT-Southwestern Medical Center, Dallas, TX*	10
9:30	**Requirement of Fatty Acid Remodeling for Raft-association of GPI-anchored Proteins.**; Taroh Kinoshita[1]; Yuko Tashima[1]; Toshiaki Houjou[2]; Morihisa Fujita[3]; Takehiko Yoko-o[3]; Yoshifumi Jigami[3]; Ryo Taguchi[2]; Yusuke Maeda[1]; *[1]Osaka University, Osaka, Japan; [2]University of Tokyo, Tokyo, Japan; [3]Natl Inst of Adv Indust Sci and Technol, Tsukuba, Japan*	11
9:50	**Glycan Biosynthesis, Processing and Recognition - invited talk**; Jonathan Weissman; *UCSF, San Francisco, CA*	12
10:10	**A DHHC protein regulates activity and subcellular transport of GalNAc transferase B in Drosophila melanogaster**; Anita Stolz[1]; Benjamin Kraft[1]; Manfred Wuhrer[2]; Cornelis H. Hokke[2]; Rita Gerardy-Schahn[1]; Hans Bakker[1]; *[1]Medizinische Hochschule Hannover, Zelluläre Chemie, Hannover, Germany; [2]Leiden University Medical Centre, Parasitology, Leiden, The Netherlands*	13
10:15	**The Mammalian and *Drosophila* Orthologous UDP-GalNAc: polypeptide α-N-acetylgalactosaminyltransferases (ppGalNAc-Ts) T1 and T2 Possess Highly Conserved Peptide Substrate Specificities**; Thomas A. Gerken[1]; Oliver Jamison[1]; Kelly G. Ten Hagen[2]; *[1]Case Western Reserve Univ., Cleveland, OH; [2]National Institutes of Health, NIDCR, Bethesda, MD*	14

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THURSDAY, NOVEMBER 16
10:45 AM – 12:35 PM
SESSION III
DEVELOPMENTAL GLYCOBIOLOGY, *Grand Ballroom*
Chair: Jeff Esko, *UCSD*

Time **Abstract Number**

10:45 **Roles for O-Fucose and Pofut1 in Notch Signaling in Mammals.**; Pamela Stanley; Changhui Ge; Mark Stahl; Kazuhide Uemura; Shaolin Shi; *Albert Einstein College Medicine, New York, NY* ····· 15

11:05 **Mind the Gap! Glyco-therapies for Enteric Protein Loss**; Hudson H Freeze; *The Burnham Institute for Medical Research, La Jolla, CA* ····· 16

11:25 **O-Glycosylation of Cysteine-Knot Motifs**; Robert S. Haltiwanger; Malgosia Dlugosz; Yi Luo; Kelvin Luther; Aleksandra Nita-Lazar; Nadia Rana; Hideyuki Takeuchi; Bernadette C. Holdener; *Stony Brook University, Stony Brook, NY*····· 18

11:45 **Analysis of the Conserved Oligomeric Golgi (COG) Complex**; Monty Krieger; *MIT, Cambridge, MA*····· 19

12:05 **Developmental Regulation of HSPG Synthesis during Drosophila Embryogenesis**; Douglas Bornemann; Sangbin Park; Rahul Warrior; *UC Irvine, Irvine, CA*····· 20

12:25 **Unlike Mammalian GRIFIN, the Zebrafish Homologue (DrGRIFIN) may Represent a Functional Carbohydrate-Binding Galectin**; Hafiz Ahmed; Gerardo R. Vasta; *Center of Marine Biotechnology, UMBI, Baltimore, MD* ····· 21

12:30 **A Mucin-type O-Glycosyltransferase is required during Multiple Stages of Drosophila Development**; E Tian; Kelly G. Ten Hagen; *NIDCR, NIH, Bethesda, MD*····· 22

2:00 – 4:00 PM
POSTER SESSION I, *Studio Suites I - IV*

THURSDAY, NOVEMBER 16
4:00 – 6:10 PM
SESSION IV
NOVEL ANALYTICAL TECHNIQUES – SYNTHESIS, VALENCY AND ARRAYS, *Grand Ballroom*
Chair: Anne Dell, *Imperial College, London*

Time **Abstract Number**

4:00 **Lectins Bind to Multivalent Glycoproteins with a Large Gradient of Binding Constants**; Curtis F. Brewer; Tarun K. Dam; *Albert Einstein College of Medicine, Bronx, NY*····· 23

4:20 **Using Glycodendrimers to Study Protein-carbohydrate Interactions**; Mary J Cloninger; *Montana State University, Bozeman, MT*····· 24

4:40 **Automated Oligosaccharide Synthesis and the Direct Formation of Carbohydrate Microarrays**; Nicola L. Pohl; *Iowa State University, Ames, IA* ····· 25

5:00 **Structure/thermodynamic Relationship in Lectin/glycan Interaction. Strategies for High Affinity Binding**; Anne Imberty; *CERMAV-CNRS, Grenoble, France* ····· 26

5:20 **Specificity of Glycosaminoglycan Binding to CCR2 Chemokines: Significance of Sulfation Binding Sites**; Julie Leary; *University of California, Davis, CA* ····· 27

5:40 **Influenza HA Structure and Receptor Binding using the Glycan Microarray**; Ian A. Wilson; James Stevens; Ola Blixt; James Paulson; *The Scripps Research Institute, La Jolla, CA*····· 28

6:00 **Heterobivalent Ligands: A Versatile Approach to Ligand Induced Protein Aggregation Exemplified by the Structure-based Design of Shiga Toxin Antagonists**; Pavel Kitov; David Bundle; *University of Alberta, Edmonton, Alberta* ····· 29

6:05 **New Mass Spectrometry Tools for Glycosaminoglycans Analysis**; Bérangère Tissot[1]; Stuart M. Haslam[1]; Howard R. Morris[1]; Jeremy E. Turnbull[2]; Andrew K. Powell[2]; Zheng-liang Zhi[2]; John T. Gallagher[3]; Christopher J. Robinson[3]; Anne Dell[1]; *[1]Imperial College, London, UK; [2]University of Liverpool, Liverpool, UK; [3]University of Manchester, Manchester, UK* ····· 30

FRIDAY, NOVEMBER 17
8:30 – 10:00 AM
SESSION V
GLYCANS IN NEUROBIOLOGY – DEVELOPMENT AND FUNCTION, *Grand Ballroom*
Chair: Ron Schnaar, *Johns Hopkins University*

Time **Abstract Number**

8:30 **Sialoglycans Regulate Axon Regeneration after Central Nervous System Injury – the Therapeutic Potential of Sialidase**; Andrea Mountney[1]; Lynda J.S. Yang[2]; Matthew R. Zahner[1]; Ileana Lorenzini[1]; Katarina Vajn[1]; Lawrence P. Schramm[1]; Ronald L. Schnaar[1]; *[1]The Johns Hopkins School of Medicine, Baltimore, MD; [2]University of Michigan, Ann Arbor, MI*····· 31

8:50 **β1,3-N-acetylglucosaminyltransferase 1 is required for Axon Pathfinding by Sensory Neurons.**; Gary Schwarting; Tim Henion; *Univ of Massachusetts Medical Sch - Shriver Center, Waltham, MA*····· 32

9:10 **The LARGE Glycosyltransferase Family**; Jane E Hewitt; *University of Nottingham, Nottingham, UK*····· 33

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9:30 **Role of Glypican-1 in Brain Development;** Yi-Huei Linda Jen, Michele Musacchio, and Arthur D. Lander; *Dept. of Developmental & Cell Biology, Univ. of California, Irvine, CA* ····· 34

9:50 **N-Acetylmannosamine Treatment Rescues a Mouse Model of Hereditary Inclusion Body Myopathy**; Marjan Huizing[1]; Riko Klootwijk[1]; Belinda Galeano[1]; Irini Manoli[1]; Mao-Sen Sun[1]; Carla Ciccone[1]; Daniel Darvish[2]; Donna Krasnewich[1]; William A Gahl[1]; *[1]NIH, NHGRI, Bethesda, MD; [2]HIBM Research Group, Encino, CA* ····· 35

9:55 **GnT-Vb Expression Increases O-Mannosyl-linked HNK-1 Epitope Leading to Changes in Neuronal Cell Adhesion and Migration**; Karen L. Abbott[1]; Karolyn Troupe[1]; Rick T. Matthews[2]; Michael Pierce[1]; *[1]Complex Carbohydrate Research Center, UGA, Athens, GA; [2]Yale University, New Haven, CT* ····· 36

FRIDAY, NOVEMBER 17
10:30 AM – 12:20 PM
SESSION VI
GLYCANS IN MICROBIAL PATHOGEN-HOST INTERACTIONS, *Grand Ballroom*
Chair: Sam Turco, *University of Kentucky*

Time **Abstract Number**

10:30 **Modulation of the Host Immune Response by Schistosome Glycoconjugates**; Ellis Van Liempt[1]; Sandra Meyer[2]; Sandra J. Van Vliet[1]; Anneke Engering[1]; Boris Tefsen[1]; Caroline M.W. Van Stijn[1]; Rudolf Geyer[2]; Yvette Van Kooyk[1]; Irma Van Die[1]; *[1]VU University Medical Center, Amsterdam, the Netherlands; [2]Justus Liebig University, Giessen, Germany* ····· 37

10:50 **Role of M. tuberculosis Cell Wall Carbohydrates in Host Adaptation**; Larry S. Schlesinger; *The Ohio State University, Columbus, OH* ····· 38

11:10 **The Glycobioloy of Nipah Virus Entry**; Benhur Lee; *University of California, Los Angeles, Los Angeles, CA* ····· 39

11:30 **Immune Recognition of Candida Albicans: The Taste of a Fungus**; Neil Gow; *University of Aberdeen, Aberdeen, U.K.* ····· 40

11:50 **Arenaviruses Mimic the Molecular Mechanism of Receptor Recognition used by alpha-Dystroglycans's Host-Derived Ligands**; Jillian M. Rojek[1]; Kevin P. Campbell[2]; Stefan Kunz[1]; *[1]The Scripps Research Institute, L a Jolla, CA; [2]Howard Hughes Medical Institute University of Iowa, Iowa City, IA* ····· 41

12:10 **Functional Glycoproteomic Analysis of *Caenorhabditis elegans* Interaction with Bacterial Pathogens**; Jenny Tan; Hui Shi; Harry Schachter; *Hospital for Sick Children, Toronto, Canada* ····· 42

12:15 **Inhibition of *Helicobacter pylori* binding by Lewis b or Sialyl-Lewis x Carrying Recombinant Mucin-type Proteins Produced by Glyco-engineered CHO Cells**; Anki Gustafsson[1]; Jining Liu[1]; Rolf Sjöström[2]; Håkan Yildirim[1]; Elke Schweda[1]; Michael E. Breimer[3]; Thomas Borén[2]; Jan Holgersson[1]; *[1]Karolinska Institutet, Stockholm, Sweden; [2]Umeå University, Umeå, Sweden; [3]Sahlgrenska University Hospital, Göteborg, Sweden* ····· 44

2:00 – 4:00 PM
POSTER SESSION II, *Studio Suites I - IV*

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

4:30 – 5:30 PM
KARL MEYER LECTURE, *Grand Ballroom*
Emerging Roles of O-GlcNAc as a Nutrient/Stress Sensor Globally Regulating Signaling, Transcription, and Protein Turnover.; Gerald W. Hart; Chad Slawson; Mike Housley; Quira Zeidan; Stephen Whelan; Wagner Dias; Win Cheung; Kaoru Sakabe; Pui Butkinaree; Kyoungsook Park; Shino Shimoji; Zihao Wang; John Bullen; *Johns Hopkins Medical School, Baltimo*re, MD 1

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

SATURDAY, NOVEMBER 18
8:30 AM – 9:45 AM
SESSION VII
GLYCANS IN CANCER – PROGNOSIS TO THERAPY, *Grand Ballroom*
Chair: Joe Lau, *Roswell Park Cancer Institute*

Time **Abstract Number**

8:30 **On the Role of Galectin-3 in Cancer Metastasis**; Avraham Raz; *Wayne State University, Karmanos Cancer Institute, Detroit, MI* ····· 45

8:50 **The role of Glycosphingolipid Gb3 in Colon Cancer Invasiveness**; Olga Kovbasnjuk[1]; Rakhilya Murtazina[1]; Oksana Gutsal[1]; Anne Kane[2]; Mark Donowitz[1]; *[1]Johns Hopkins School of Medicine, Baltimore, MD; [2]Tufts New England Medical Center, Boston, MA* ····· 46

9:10 **Glycans in Cancer – Prognosis to Therapy, Invited Talk**; Steve Rosen; *UCSF, San Francisco, CA* ····· 47

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9:30 **Development and Characterization of Peptide Mimics of TF-Antigen**; Jamie Heimburg[1]; Adel Almogren[1]; Sue Morey[1]; Olga V. Glinskii[2]; Virginia H. Huxley[2]; Vladislav V. Glinsky[2]; Rene Roy[3]; Richard Cheng[1]; Kate Rittenhouse-Olson[1]; *[1]University at Buffalo, Buffalo, NY; [2]University of Missouri, Columbia, MO; [3]University of Quebec at Montreal, Montreal, Canada* ····· 48

9:35 **Expression of Tn and SialylTn Antigens in Human Tumor Cell Lines Raised from Mutation in Molecular Chaperone Cosmc**; Tongzhong Ju[1]; Grainger Lenneau[2]; Tripti Gautam[2]; Yingchun Wang[1]; Doris Benbrook[2]; Marie H. Hanigan[2]; Richard D. Cummings[1]; *[1]Emory University School of Medicine, Atlanta, Georgia; [2]The University of Oklahoma health Sciences Center, Oklahoma City, Oklahoma* ····· 49

9:40 **Glycoprotoemic Changes In Human Blood Serum Associated with Breast Cancer**; Yehia Mechref[1]; Milan Madera[2]; Benjamin Mann[2]; Iveta Klouckova[2]; Milos V. Novotny[1]; *[1]National Center for Glycomics and Glycoproteomics, Bloomington, IN; [2]Dept of Chemistry, Indiana Universtiy, Bloomington, IN* ····· 50

SATURDAY, NOVEMBER 18
10:15 AM – 12:30 PM
SESSION VIII
GLYCAN CONTROL OF LEUKOCYTE MIGRATION AND FUNCTION IN INFLAMMATION, *Grand Ballroom*
Chair: Robert Sackstein, *Harvard University*

Time **Abstract Number**

10:15 **Mechanisms of Cell Adhesion through Selectin-glycan Interactions Under Flow**; Rodger McEver; *Oklahoma Medical Research Foundation, Oklahoma City, OK* ····· 51

10:35 **Transcriptional Basis for Selectin Ligand Expression by Th1 Cells**; Geoffrey S. Kansas; *Northwestern Medical School, Chicago, IL* ····· 52

10:55 **6-Sulfo sialyl Lewis X on both *N*- and *O*-Glycans Play Critical Roles as L-Selectin Ligands**; Minoru Fukuda; *Bunham Institute for Medical Research, La Jolla, CA* ····· 53

11:15 **Sialylation-dependent Regulation of α4β1 Integrin Receptors**; Alencia V Woodard-Grice; Alexis C McBrayer; Susan L Bellis; *University of Alabama at Birmingham, Birmingham, AL* ····· 54

11:20 **Platelets generate inflammatory and angiogenic fragments of hyaluronan**; Carol de la Motte[1]; Julie Nigro[1]; Amit Vasanji[1]; Hyunjin Rho[1]; Sudip Bandyopadhyay[1]; Robert Stern[2]; *[1]Cleveland Clinic, Cleveland, OH; [2]UC San Francisco, San Francisco , CA* ····· 55

SATURDAY, NOVEMBER 18
4:00 – 5:40 PM
SESSION IX
GLYCANS IN IMMUNE DEVELOPMENT AND FUNCTION, *Grand Ballroom*
Chair: Linda Baum, *UCLA*

Time **Abstract Number**

4:00 **NKT Cells Recognize Different Types of Bacterial Glycolipids**; Mitchell Kronenberg[1]; Emmanuel Tupin[1]; Yuki Kinjo[1]; Douglass Wu[2]; Masakazu Fujio[2]; Moriya Tsuji[3]; Timothy Sellati[4]; Dirk Zajonc[2]; Ian Wilson[2]; Chi-huey Wong[2]; *[1]La Jolla Inst. Allergy & Immunol, La Jolla, CA; [2]Scripps Research Institute, La Jolla, CA; [3]Rockefeller University, New York, NY; [4]Albany Medical College, Albany , NY* ····· 56

4:20 **Mammalian N-Glycosylation Inhibits Innate Immune Mechanisms that Induce and Mediate Autoimmune Disease**; Ryan S. Green; Jamey D. Marth; *Howard Hughes Medical Institute, UCSD, La Jolla, CA* ····· 57

4:40 **Dendritic Cells and the Recognition of Glycan Structures to Mediate Cellular Communication and Immune Responses**; Yvette van Kooyk; *Molecular Cellbiology and Immunology, VUmc, Amsterdam, the Netherlands* ····· 58

5:00 **Glycans in Immune Development and Function, Invited Talk**; Carrie Miceli; *UCLA, Los Angeles, CA* ····· 59

5:20 **Role of Protein Mannosylation in Linking Innate and Adaptive Immune Responses to Fungi**; Stuart M Levitz; *UMass Medical Center, Worcester, MA* ····· 60

THURSDAY, NOVEMBER 16
2:00 – 4:00 PM
POSTER SESSION I, *Studio Suites I - IV*

Topics:
GLYCAN – MEDIATED CELLULAR SIGNALING
GLYCAN BIOSYNTHESIS, PROCESSING AND RECOGNITION

These posters should be set up on either Wednesday from 1 – 10 pm or on Thursday from 7:00 – 8:00 am and removed by 6:30 pm on Thursday

Topic: GLYCAN – MEDIATED CELLULAR SIGNALING

Poster Number **Abstract Number**

1 **N-Glycan Processing Integrates Cellular Responsiveness to Extracellular Cues in Cancer Progression and T Cell Activation**; Ken Lau[1]; Emily A. Partridge[1]; Pamela Cheung[1]; Cristina I. Silvescu[2]; Ani Grigorian[3]; Vernon N. Reinhold[2]; Michael Demetriou[3]; James W. Dennis[1]; *[1]Mount Sinai Hospital, Toronto, Canada; [2]University of New Hampshire, Durham, NH; [3]University of California, Irvine, CA* ····· 6

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2 **N-Acetylglucosaminyltransferase III Antagonizes N-Acetylglucosaminyltransferase V On Alpha3beta1 Integrin-Mediated Cell Migration**; Yanyang Zhao[1]; Jianguo Gu[1]; Takatoshi Nakagawa[1]; Akihiro Kondo[1]; Nana Kawasaki[2]; Eiji Miyoshi[1]; Naoyuki Taniguchi[1]; *[1]Osaka University Graduate School of Medicine, Osaka, Japan; [2]National Institute of Health Sciences, Tokyo, Japan* ····· 7

3 **Calpain Mediated Cytoskeletal Cleavage during Galectin-1 Induced T-Cell Death**; Mabel Pang; James He; Linda G. Baum; *UCLA, Los Angeles, CA* ····· 61

4 **Interaction of GM3 with N-linked GlcNAc of Epidermal Growth Factor Receptor (EGFR) Inhibits EGFR Tyrosine Kinase**; Seon-Joo Yoon[1]; Kenichi Nakayama[2]; Toshiyuki Hikita[1]; Kazuko Handa[1]; Sen-itiroh Hakomori[1]; *[1]Pacific Northwest Res Ins, and Univ. of Washington, Seattle, WA; [2]Institute of General Industrial Research, Takamatsu, Kagawa , Japan* ····· 62

5 **Structural Features of Galectins that Regulate Receptor Recognition and Intracellular Signaling in T Cell Death**; Shuguang Bi*; Lesley Earl*; Michael Smith; Linda G. Baum; *UCLA School of Medicine, Los Angeles, CA*··· 63

6 **Structure and Biological Significance of *Trichomonas vaginalis* LPG**; B.N. Singh[1]; John J. Lucas[1]; Gary R. Hayes[1]; Ulf Sommer[2]; Catherine E. Costello[2]; Raina N. Fichorova[3]; *[1]SUNY Upstate Medical University, Syracuse,, NY; [2]Boston University School of Medicine, Boston, MA; [3]Brigham and Women's Hospital, Boston, MA* ····· 64

7 **Dietary and Genetic Control of Pancreatic Beta-Cell Glucose Transporter Glycosylation Promotes Insulin Secretion in Suppressing the Pathogenesis of Type-2 Diabetes**; Kazuaki Ohtsubo; Shinji Takamatsu; Jamey D. Marth; *Howard Hughes Medical Institute/ UC, San Diego, La Jolla, CA* ····· 65

8 **A Putative Role for the Involvement of β-N-Acetylglucoseaminyltransferase (OGT) in Membrane Associated Signaling**; Simon Amzalleg; Daniel Fishman; Jacob Gopas; Shira Elhyany-Amzalleg; Shraga Segal; *Ben Gurion University of the Negev, Beer-Sheva, ISRAEL*····· 66

9 **The Research on the Antioxidant and Hypoglycemic Activity of Polysaccharide from Tea**; Zhi Yu[1]; Yun Zhang[1]; Dejiang Ni[2]; *[1]Huazhong Agricultural University, WUHAN, HUBEI CHINA; [2]Key Lab of Horticultural Plant Biology of Ministry, WUHAN, HUBEI CHINA* ····· 67

10 **Does the Cytoplasmic/Nuclear Tobacco Lectin Bind to Endogenous N-glycans?**; Nausicaa Lannoo[1]; Els Van Pamel[1]; Richard Alvarez[2]; Willy J Peumans[1]; Els JM Van Damme[1]; *[1]Ghent University, Gent, Belgium; [2]University of Oklahoma, Oklahoma, USA*····· 68

11 **Tissue-specific Roles for GNE in Cell Growth**; zhiyun wang; Zhonghui Sun; Kevin J Yarema; *Johns Hopkins University, Baltimore, MD*····· 69

12 **The Influence of Gestational Diabetes Mellitus on N-acetyl-β-D-hexosaminidase Activity in the Blood Serum.**; Danuta Dudzik; Malgorzata Knas; Wieslaw Zarzycki; Malgorzata Borzym-Kluczyk; Krzysztof Zwierz; *Medical University, Bialystok, Poland*····· 70

13 **Modeling a CDG-Ib Intestine in vitro: Silencing phosphomannose Isomerase Predisposes for Intestinal Epithelial Protein Leakage**; Lars Bode; Gabriella Settergren; Hudson H Freeze; *Burnham Institute for Medical Research, La Jolla, CA* ····· 71

14 **Ganglioside GM2/GM3 Inhibits HGF-induced cMet Activation and Cell Motility in Bladder Epithelial Cells, through Functional Organization of Components in Glycosynapse**; Adriane R. Todeschini,; Kazuko Handa; Sen-itiroh Hakomori; *Pacific Northwest Res Ins and Univ. of Washington, Seattle, WA*····· 72

15 **Cell Signaling Mediated by Carbohydrate-to-carbohydrate Interaction: GM3 Binds to EGFR via N-Linked Oligosaccharide and Regulates Autophosphorylation.**; Nagako Kawashima[1]; Seon-Joo Yoon[2]; Sen-itiroh Hakomori[2]; Ken-ichi Nakayama[1]; *[1]AIST, Takamatsu, Japan; [2]Pacific Northwest Research Institute, Seattle, WA*····· 73

16 **Core fucosylation is Crucial for the Function of Growth Factor Receptor(s)**; Xiangchun Wang[1]; Jianguo Gu[2]; Eiji Miyoshi[1]; Naoyuki Taniguchi[1]; *[1]Osaka University, Suita, Osaka, Japan; [2]Tohoku Pharmaceutical University, Sendai, Miyagi,Japan*····· 74

17 **Structural and Functional Differences between Human and Non-Human Cell Expressed Human Cytokines and Growth Factors**; Kate Liddell; Denese Marks; Hui Jiang; Nicole Wilson; Teresa Domagala; Linda Crofts; Glenn Pilkington; Greg Russell-Jones; *Apollo Cytokine Research, Sydney, Australia* ····· 75

18 **Bacterial Symbionts Induce a *fut2*-dependent Fucosylated Niche on Colonic Epithelium Via a TLR-4 Sentinel That Activates ERK and JNK Signaling**; David S. Newburg[1]; Di Meng[1]; Cheryl Young[1]; Amy Baker[1]; Susan L. Tonkonogy[2]; R. Balfour Sartor[3]; W. Allan Walker[1]; N. Nanda Nanthakumar[1]; *[1]Massachusetts General Hospital, Boston, MA; [2]North Carolina State University, Raleigh, NC; [3]University of North Carolina, Chapel Hill, NC*····· 76

19 **Disruption of O-GlcNAc Cycling Mimics Diabetes Mellitus in *C. elegans***; Michele E. Forsythe; Dona C. Love; Eun Ju Kim; Brooke C. Lazarus; William Prinz; Gilbert Ashwell; Michael W. Krause; John A. Hanover; *National Institutes of Health, Bethesda, MD*····· 77

20 **Heparan Sulfate Proteoglycan Modulation by Inflammation**; Arthur R. Ayers; April Reimers; Derek Erstad; Bengt Phung; *Albertson College of Idaho, Caldwell, Idaho* ····· 78

21 **Non-anticoagulant 2,3-de-O-sulfated Heparin as a Potential Novel Therapeutic for Patients with Protein-Losing Enteropathy**; Lars Bode[1]; Robert J Linhardt[2]; Hudson H Freeze[1]; *[1]Burnham Institute for Medical Research, La Jolla, CA; [2]Rensselaer Polytechnic Institute, Troy, NY*····· 79

22 **A Small Molecule Neutralizing Agent for Heparan Sulfate**; Manuela Schuksz[1]; Jillian R. Brown[1]; David P. Ditto[1]; Omai B. Garner[1]; Brett E. Crawford[2]; Charles A. Glass[2]; Jeffrey D. Esko[1]; *[1]University of California, San Diego, La Jolla, CA; [2]Zacharon Pharmaceuticals, Inc., La Jolla, CA*····· 80

23 **Modulation of the Secreted and Membrane Glycoproteome of Adipocytes via the Induction of Insulin Resistance**; Jae-Min Lim; Kazuhiro Aoki; Michael Tiemeyer; Dorothy B. Hausman; Lance Wells; *University of Georgia, Athens, GA* ····· 81

24 **Elevation of Intracellular Glycosylation, O-GlcNAc, Attenuates the Anti-Apoptotic Effect of Insulin in CHO-IR Cells**; Chin Fen Teo; Enas Gad El-Karim; Lance Wells; *University of Georgia, Athens, GA*····· 82

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THURSDAY, POSTER SESSION I
GLYCAN BIOSYNTHESIS, PROCESSING AND RECOGNITION

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89 **Site-Mapping and Glycan Characterization of Functional Alpha-Dystroglycan**; Sana Hashmi[1]; Stephanie Hammond[1]; Jae-Min Lim[1]; Kazuhiro Aoki[1]; Mindy Perlman[1]; Gerardo Gutierrez-Sanchez[1]; James Wheeler[1]; James M. Ervasti[2]; Carl Bergmann[1]; Michael Tiemeyer[1]; Lance Wells[1]; *[1]University of Georgia, Athens, GA; [2]University of Minnesota, Minneapolis, MN* ····· 145

90 **Glycoprotein Labeling and Detection: Novel Click Chemistry-based Applications for Gel Electrophoresis, Flow Cytometry, and Fluorescence Microscopy**; Brian J Agnew; Nancy Ahnert; Suzanne Buck; Scott Clarke; Courtenay Hart; Kapil Kumar; Tamara Nyberg; *Molecular Probes-Invitrogen, Eugene, OR* ····· 146

91 **Profiling of Polysaccharide–Receptor Interaction with Recombinant Innate Immunity Receptor-Fc Fusion Proteins**; Shih-Chin Cheng[1]; Wen-Bin Yang[2]; See-Wen Chin[1]; Chun-Cheng Lin[2]; Chun-Hung Lin[2]; Yu-Ju Chen[2]; Po-Chiao Lin[2]; Ming-Fung Wu[1]; Chi-Huey Wong[2]; Shie-Liang Hsieh[1]; *[1]National Yang-Ming University, Taipei, Taiwan; [2]Genomics Research Center, Academia Sinica, Taipei, Taiwan* ····· 147

FRIDAY, NOVEMBER 17
2:00 – 4:00 PM
POSTER SESSION II, *Studio Suites I - IV*

Topics:
DEVELOPMENTAL GLYCOBIOLOGY
NOVEL ANALYTICAL TECHNIQUES – SYNTHESIS, VALENCY AND ARRAYS
GLYCANS IN MICROBIAL PATHOGEN-HOST INTERACTIONS

These posters should be set up from 7:30 – 8:30 am on Friday and removed by 6:30 pm on Friday

Topic: DEVELOPMENTAL GLYCOBIOLOGY

Poster Number **Abstract Number**

1 **Unlike Mammalian GRIFIN, the Zebrafish Homologue (DrGRIFIN) May Represent a Functional Carbohydrate-Binding Galectin**; Hafiz Ahmed; Gerardo R. Vasta; *Center of Marine Biotechnology, UMBI, Baltimore, MD* ····· 21

2 **A Mucin-Type O-Glycosyltransferase is required during Multiple Stages of Drosophila Development**; E Tian; Kelly G. Ten Hagen; *NIDCR, NIH, Bethesda, MD* ····· 22

3 **The Studies on the Extracting Technologies and Purification of Fucoidan from Laminaria japonica**; Qiukuan Wang[2]; Yunhai He[1]; Ting Zhang[1]; Xingju Yu[2]; *[1]Dalian Fisheries University, Dalian, P.R.China; [2]Dalian Institute of Chemical Physics, Dalian, P.R.China* ····· 148

4 **A Zebrafish Model for Mucolipidosis II**; Heather R Flanagan-Steet[1]; Richard A Steet[1]; Stuart Kornfeld[2]; *[1]University of Georgia, Athens, GA; [2]Washington University School of Medicine, Saint Louis, MO* ····· 149

5 **A New Mutation that Alters Tissue-specific Expression of N-Linked Glycans in the *Drosophila* Embryo**; Sarah R. Baas; Mary Sharrow; Megan Middleton; Nicole Price; Kazuhiro Aoki; Jae-Min Lim; Lance Wells; Michael Tiemeyer; *Complex Carbohydrate Research Center, UGA, Athens, GA* ····· 150

6 **Identification and Characterization of a Novel *Drosophila* 3' -Phosphoadenosine 5' -Phosphosulfate Transporter**; Emi Goda[1]; Shin Kamiyama[2]; Takaaki Uno[1]; Hideki Yoshida[1]; Morio Ueyama[1]; Akiko Kinoshita-Toyoda[3]; Hidenao Toyoda[3]; Ryu Ueda[4]; Shoko Nishihara[1]; *[1]Soka University, CREST, Hachioji, Japan; [2]Soka University, NEDO, Hachioji, Japan; [3]Chiba University, CREST, Chiba, Japan; [4]NIG, CREST, Mishima, Japan* ····· 151

7 **Role of O-Glycosylation in Quality Control of Notch Folding**; Nadia A. Rana; Aleksandra Nita-Lazar; Yi Luo; Robert S. Haltiwanger; *Stony Brook University, Stony Brook, NY* ····· 152

8 **Dissecting the Biological Role of Mucin Type O-Glycosylation using RNA Interference in Drosophila Cell Culture**; Liping Zhang; Kelly G. Ten Hagen; *NIDCR, National Institutes of Health, Bethesda, MD* ····· 153

9 **In vivo Functional Studies of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases In Drosophila melanogaster**; Ying Zhang; Kelly G. Ten Hagen; *National Institutes of Health, Bethesda, MD* ····· 154

10 **Glycotranscriptome Analysis during Differentiation of Murine Embryonic Stem Cells Assayed by High-throughput Real-time RT-PCR**; Alison Nairn; Kyle Harris; M. Kulik; Stephen Dalton; J. Michael Pierce; Kelley W. Moremen; *University of Georgia, Athens, GA* ····· 155

11 **Glycomic Analysis of Oligosaccharides that Bind Sperm**; Emily D. Collins; David J Miller; *University of Illinois at Urbana-Champaign, Urbana, IL* ····· 156

12 **Immunological Studies of Plant Cell Wall Glycome Dynamics**; Michael G. Hahn; *University of Georgia, Athens, GA* ····· 157

13 **Isolation of Glycoproteins from *Caenorhabditis elegans* by Lectin Affinity Chromatography**; Nívea Maria Rocha Macedo[1]; Emerson Soares Bernardes[1]; Marcos A C Oliveira[1]; Carlos E Winter[2]; Maria Cristina Roque-Barreira[1]; José César Rosa[1]; *[1]University of Sao Paulo - FMRP, Ribeirao Preto, Sao Paulo; [2]University of Sao Paulo - ICB, Sao Paulo, Sao Paulo* ····· 158

14 **Role of Skp1 Prolyl Hydroxylation and Glycosylation in Oxygen-Dependent Development in Dictyostelium**; Christopher M. West; Zhuo A. Wang; Hanke van der Wel; Ira J. Blader; *University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104* ····· 159

15 **Knock-down of Galectin-1-like Proteins in Zebrafish (*Danio rerio*) Reveals a Muscle and Heart Developmental Phenotype**; Hafiz Ahmed; Gerardo R. Vasta; *Center of Marine Biotechnology, UMBI, Baltimore, MD* ····· 160

16 **The N-Glycome of Human Embryonic Stem Cells**; Tero Satomaa[1]; Annamari Heiskanen[1]; Milla Mikkola[2]; Cia Olsson[2]; Maria Blomqvist[1]; Taina Jaatinen[3]; Jari Helin[1]; Jari Natunen[1]; Timo Tuuri[4]; Timo Otonkoski[5]; Juhani Saarinen[1]; Jarmo Laine[3]; *[1]Glykos Finland Ltd., Helsinki, Finland; [2]University of Helsinki, Helsinki, Finland;*

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FRIDAY, POSTER SESSION II
NOVEL ANALYTICAL TECHNIQUES – SYNTHESIS, VALENCY AND ARRAYS

Poster Number **Abstract Number**

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36 **Structural Characterisation of Permethylated Glycans using MALDI-TOF/TOF**; Jihye Jang Lee; Bérangère Tissot; Maria Panico; Howard R. Morris; Anne Dell; Stuart M. Haslam; *Imperial College, London, United Kingdom* ····· 179

37 **Pathogen Antigens Probed by On-Cell Solution NMR**; Hugo F. Azurmendi[1]; Lauren Wrightson[1]; Loc B. Trinh[2]; Joseph Shiloach[2]; Darón I. Freedberg[1]; *[1]CBER/FDA, Bethesda, MD; [2]NIDDK/NIH, Bethesda, MD* ····· 180

38 **Comparative Glycomics of Connective Tissue Glycosaminoglycans using Mass Spectrometry**; Alicia M. Hitchcock; Catherine E. Costello; Joseph Zaia; *Boston University School of Medicine, Boston, MA* ····· 181

39 **Development of New Fluorocarbon-based HPLC-MS Methods for Glycosylinositol Phosphorylceramide Analysis**; Emma A. Arigi; Yunsen Li; Steven B. Levery; *University of New Hampshire, Durham, NH* ····· 182

40 **A Novel Glycoproteomic Approach for the Complete Characterization of Glycopeptides from Complex Biological Mixtures**; James A. Atwood[1]; Zuzheng Luo[1]; Brent Weatherly[2]; Barry Boyes[1]; Ron Orlando[1]; *[1]Complex Carbohydrate Research Center, Athens, GA; [2]BioInquire LLC, Athens, GA* ····· 183

41 **Sialoside Analog Arrays for Identifying High Affinity Analogs of Siglec Ligands**; Ola Blixt; Shoufa Han; Julia Hoffmann; Liang Liao; Ying Zeng; James C. Paulson; *The Scripps Research Institute, La Jolla, CA* ····· 184

42 **Combined use of Hydrazide Functionalized Polymer and Sequential Tag Exchange; a General Protocol with *Glycoblotting* for Functional Glycomics (Part 2).**; Jun-ichi Furukawa[1]; Yoshiaki Miura[1]; Hiromitsu Kuramoto[2]; Hideyuki Shimaoka[2]; Masaki Kurogochi[1]; Mika Nakano[3]; Yasuro Shinohara[1]; Shin-Ichiro Nishimura[1]; *[1]JST Project Team, Hokkaido University, Sapporo, Japan; [2]Sumitomo Bakelite Co. Ltd, Tokyo, Japan; [3]Shionogi & Co. Ltd., Osaka, Japan* ····· 185

43 **Glycomic Sequencing of Complex Glycans from Glycosphingolipids by High Energy CID MS/MS and Validation of Linkage Specific Fragmentation Characteristics**; Yao-Yun Fan; Shin-Yi Yu; Sz-Wei Wu; Kay-Hooi Khoo; *Institute of Biological Chemistry, Taipei, Taiwan,R.O.C* ····· 186

44 **Further Improvement of the System for Evanescent-field Fluorescence-assisted Lectin Microarray**; Noboru Uchiyama; Atsushi Kuno; Jun Hirabayashi; *AIST, Tsukuba, Ibaraki* ····· 187

45 **Molecular Cloning of Two Distinct Sialyltransferases, α-2,3- and α-2,6-Sialyltransferases, from a Marine Bacterium.**; Hitomi Kajiwara; Masako Ichikawa; Hiroshi Tsukamoto; Yoshimitsu Takakura; Takeshi Yamamoto; *Glycotechnology Business Unit, Japan Tobacco Inc., 700 Higashibara, Iwata, Shizuoka, Japan* ····· 188

46 **Molecular Cloning and Characterization of α-2,6-Sialyltransfease from Vibrionaceae Photobacterium sp. JT-ISH-224**; Masako Ichikawa; Hitomi Kajiwara; Hiroshi Tsukamoto; Yoshimitsu Takakura; Takeshi Yamamoto; *Glycotechnology Business Unit, Japan Tobacco Inc., 700 Higashibara, Iwata, Shizuoka, Japan* ····· 189

47 **Neoglycolipids Prepared via Oxime-ligation for Microarray Analysis of Carbohydrate-Protein Interactions**; Yan Liu[1]; Wengang Chai[1]; Paul R. Crocker[2]; Helen M.I. osborn[3]; Ten Feizi[1]; *[1]Imperial College London, Harrow, UK; [2]Dundee University, Dundee, UK; [3]University of Reading, Reading, UK* ····· 190

48 **Electrospray Ion Mobility Spectrometry of Isomeric Carbohydrates**; Joseph Zaia[1]; Iain Campuzano[2]; Kevin Giles[2]; Robert Bateman[2]; Compson Keith[2]; Catherine E. Costello[1]; *[1]Boston University, Boston, MA; [2]Waters Corporation, Manchester, UK* ····· 191

49 **A Survey of Siglec Binding Preferences using Carbohydrate Microarrays**; Maria Asuncion Campanero-Rhodes [1]; Robert A Childs [1]; Wengang Chai [1]; Mark S Stoll[1]; Paul R Crocker [2]; Ten Feizi [1]; *[1]Imperial College, London, UK; [2]University of Dundee, Dundee, UK* ····· 192

50 **Comparative Glycoproteomics of the Trypanosoma Cruzi Lifecycle**; Ron Orlando[1]; James A. Atwood[1]; Todd Minning[2]; Arthur Nuccio[1]; Daniel B. Weatherly[2]; Rick Tarleton[2]; *[1]CCRC/UGA, Athens, GA; [2]CTEGD/UGA, Athens, ga* ····· 193

51 **Glycan Array and Structural Analysis of AAV Capsid – receptor Binding Specificity**; Mavis Agbandje-McKenna[1]; Edward B Miller[1]; Michael DiMattia[1]; Brittney Gurda-Whitaker[1]; Lakshmanan Govindasamy[1]; Robert McKenna[1]; Nicholas Muzyczka[1]; Sergei Zolotukhin[1]; Richard A Alvarez[2]; Ola Blixt[3]; James C Paulson[3]; *[1]University of Florida, Gainesville, Florida; [2]University of Oklahoma Health Science Center, Oklahoma City, Oklahoma; [3]The Scripps Research Institute, La Jolla, California* ····· 194

52 **Mass Spectrometry of Fluorocarbon-Modified Glycosphingolipids. Potential for Comprehensive High-Throughput Structural Profiling**; Yunsen Li; Emma Arigi; Steven B. Levery; *University of New Hampshire, Durham, NH* 195

53 **Microwave Assisted Glycoprotein Labeling and Detection.**; Kapil Kumar; Tamara Nyberg; Courtenay Hart; Nancy Ahnert; Brian Agnew; *Molecular Probes - Invitrogen, Eugene, Oregon* ····· 196

54 **Novel Isotope Coded Affinity Tag for Quantitative Mass Spectrometry of Glycans**; Baoyun xia[2]; Christa Feasley[1]; Goverdhan P. Sachdev[1]; Richard D Cummings[2]; *[1]The University of Oklahoma health Sciences Center, Oklahoma City, OK; [2]Emory University School of Medicine, Atlanta, GA* ····· 197

55 **Characterization of Unknown Oligosaccharides in Glycoprotein and Milk**; Michael A. Madson; Srinivasa Rao; Chris Pohl; *Dionex Corporation, Sunnyvale, CA* ····· 198

FRIDAY, POSTER SESSION II
GLYCANS IN MICROBIAL PATHOGEN-HOST INTERACTIONS

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56 **Functional Glycoproteomic Analysis of *Caenorhabditis elegans* Interaction with Bacterial Pathogens**; Jenny Tan; Hui Shi; Harry Schachter; *Hospital for Sick Children, Toronto, Canada* ····· 42

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SATURDAY, NOVEMBER 18
2:00 – 4:00 PM
POSTER SESSION III, *Studio Suites I - IV*

Topics:
GLYCANS IN NEUROBIOLOGY – DEVELOPMENT AND FUNCTION
GLYCANS IN CANCER – PROGNOSIS TO THERAPY
GLYCAN CONTROL OF LEUKOCYTE MIGRATION AND FUNCTION IN INFLAMMATION
GLYCANS IN IMMUNE DEVELOPMENT AND FUNCTION

These posters should be set up from 7:30 – 8:30 am on Saturday and removed at 4:00 pm on Saturday

Topic: GLYCANS IN NEUROBIOLOGY – DEVELOPMENT AND FUNCTION

Poster Number **Abstract Number**

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5 **Characterization of N-linked Glycans on the Prion Glycoprotein (PrPc) by Mass Spectrometry**; Parastoo Azadi[1]; Mayumi Ishihara[1]; Chaoyang Li[2]; Man-Sun Sy[2]; *[1]Complex Carbohydrate Research Center, Athens , GA; [2]2Case Western Reserve University School of Medicin, Cleveland, OH* ····· 230

6 **Neural Differentiation of Human Stem Cells *via* Metabolic Sialic Acid Engineering**; Prabhani U. Atukorale; Srinivasa-Gopalan Sampathkumar; Mark B. Jones; Adrienne V. Li; Anshu Sarje; Andrew Lewis; Pao-Lin Che; Kevin J. Yarema; *The Johns Hopkins University, Baltimore, MD* ····· 231

7 **Polysialic Acid Determines Cell Fate of Neural Precursor Cells in Mouse Brain Development**; Kiyohiko Angata[1]; Barbara Ranscht[1]; Alexey Terskikh[1]; Jamey D. Marth[2]; Minoru Fukuda[1]; *[1]Burnham Institute for Medical Research, La Jolla, CA; [2]HHMI, University of California San Diego, La Jolla, CA* ····· 232

8 **Dietary Ganglioside and Neurochemistry in the Developing Rat**; Meghan B. Watson; Tom Clandinin; *Alberta Institute for Human Nutrition, Edmonton, Canada* ····· 233

9 **Neural Expression of β4GalNAcTA is Required for Normal Crawling Behavior in *Drosophila*;** Nicola Haines; Bryan A. Stewart*; University of Toronto, Mississauga, Canada* ····· *234*

10 **Global Expression Analysis of Glycoconjugates in Rat Central Nervous System using Lectin Histochemistry**; Miti Shah; Michelle Kilcoyne; Diane Hagner; Sergei Svarovsky; Ranu Jung; Lokesh Joshi*; The Biodesign Institute at ASU, Tempe, Arizona, USA* ····· *235*

11 **The Expression and Function of *Drosophila* sialyltransferase in the Central Nervous System**; Elena Repnikova; Kate Koles; Jared Pitts; Haiwen Li; Jennifer Shaffer; Vlad Panin; *Texas A&M University, College Station, TX* ····· 236

12 **A Structural Role for O-GalNAc Protein Glycosylation in alpha-Dystroglycan**; Mian Liu[1]; Andrew Borgert[1]; Kelly Ten Hagen[2]; George Barany[1]; David Live[1]; *[1]Univeristy of Minnesota, Minneapolis, MN; [2]National Institutes of Health, Bethesda, MD* ····· 237

13 **Lewis X Glycan Decreases Neural Progenitor Proliferation by Preventing FGF-2 Binding**; Pascal M. Lanctot[1]; Andrew R. Willhoite[2]; Jasodhara Ray[2]; Ajit Varki[1]; Fred H. Gage[2]; *[1]University of California, San Diego, La Jolla, California; [2]Salk Institute, La Jolla, California* ····· 238

SATURDAY, POSTER SESSION III
GLYCANS IN CANCER – PROGNOSIS TO THERAPY

Poster Number **Abstract Number**

14 **Development and Characterization of Peptide Mimics of TF-Antigen**; Jamie Heimburg[1]; Adel Almogren[1]; Sue Morey[1]; Olga V. Glinskii[2]; Virginia H. Huxley[2]; Vladislav V. Glinsky[2]; Rene Roy[3]; Richard Cheng[1]; Kate Rittenhouse-Olson[1]; *[1]University at Buffalo, Buffalo, NY; [2]University of Missouri, Columbia, MO; [3]University of Quebec at Montreal, Montreal, Canada* ····· 48

15 **Expression of Tn and SialylTn Antigens in Human Tumor Cell Lines Raised from Mutation in Molecular Chaperone Cosmc**; Tongzhong Ju[1]; Grainger Lenneau[2]; Tripti Gautam[2]; Yingchun Wang[1]; Doris Benbrook[2]; Marie H. Hanigan[2]; Richard D. Cummings[1]; *[1]Emory University School of Medicine, Atlanta, Georgia; [2]The University of Oklahoma health Sciences Center, Oklahoma City, Oklahoma* ····· 49

16 **Glycoprotoemic Changes In Human Blood Serum Associated with Breast Cancer**; Yehia Mechref[1]; Milan Madera[2]; Benjamin Mann[2]; Iveta Klouckova[2]; Milos V. Novotny[1]; *[1]National Center for Glycomics and Glycoproteomics, Bloomington, IN; [2]Dept of Chemistry, Indiana Universtiy, Bloomington, IN* ····· 50

17 **Glycosidase Activity and Lysotracker Staining Pattern in Breast Cancer Cell Lines Compared with a Normal Breast Cell Line**; Kushen Ramessur; Pamela Greenwell; Miriam V Dwek; *University of Westminster, London, UK* ····· 239

18 **Carboxylated N-glycans in Inflammation-mediated Colon Cancer**; Geetha Srikrishna[1]; Nissi Varki[2]; Hudson H. Freeze[1]; *[1]The Burnham Institute for Medical Research, La Jolla, CA; [2]University of California, San Diego, La Jolla, CA* ····· 240

19 **Enzymatic Large-scale Synthesis of MUC6-Tn Glycoproteins for Anti-tumor Vaccination**; Teresa Freire; Richard Lo-Man; Claude Leclerc; Sylvie Bay; *Institut Pasteur, Paris, France* ····· 241

20 **A Glycomic Approach to Drug Resistance: Direct Interaction between Cisplatin and *N*-glycans**; Hiroaki Nakagawa[1]; Shinji Hayashi[2]; Shigeaki Abe[1]; Noriko Nagahori[1]; Kenji Monde[1]; Miki Ichikawa-Ohira[2]; Hirosato Kondo[3]; Shin-Ichi Akiyama[4]; Kisaburo Deguchi[1]; Akira Nakagawara[2]; Shin-Ichiro Nishimura[1]; *[1]Hokkaido University, Sapporo, Japan; [2]Chiba Cancer Research Institute, Chiba, Japan; [3]Shionogi & Co., Ltd., Osaka, Japan; [4]Kagoshima University, Kagoshima, Japan* ····· 242

21 **Mgat5 Specific ShRNA Suppress the Growth of Mammary Adenocarcinoma Cells in vivo and Stimulating Th1 Cells Activation**; Xiao-Lian Zhang; DongQing Li; *Wuhan University School of Medicine, Wuhan, P R China* ····· 243

22 **Antitumor Activity of a Novel Lectin from the Alga Dasa villosa**; D T Li[1]; Z H Zhang[1]; Li Zhong[1]; Z Y Zhang[1]; T J Cui[1]; D M Wang[2]; W Li[1]; *[1]Dalian Fisheries University, Dalian, China; [2]Dalian Medical University, Dalian, China* ····· 244

23 ***N*-Glycans Correlate 5-FU Resistance in Colon 26 and Derived Cell Lines**; Jun Hamaguchi[1]; Hiroaki Nakagawa[1]; Masato Takahashi[1]; Takeaki Kudo[1]; Naoya Kamiyama[2]; Bailong Sun[1]; Yuji Sato[1]; Kisaburo Deguchi[1]; Satoru Todo[1]; Shin-Ichiro Nishimura[1]; *[1]Hokkaido University, Sapporo, Japan; [2]Asahikawa Medical College, Asahikawa, Japan* ····· 245

24 **A Novel Drug Delivery System. Carbohydrate Recognition-based and Controlled release System using Intraperitoneal Macrophages as a Cellular Vehicle**; Yuzuru Ikehara[1]; Toru Niwa[1]; Sanae Ikehara[1]; Le Biao[2];

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43 **Hetero-bifunctional CD22 Ligands Drive IgM Binding and Complement Killing of B Cells**; Shoufa Han[1]; Brian E. Collins[1]; Pavel I. Kitov[2]; Mary O'Reilly[1]; David R. Bundle[2]; James C. Paulson[1]; *[1]The Scripps Research Institute, La Jolla, CA; [2]University of Alberta, Edmonton AB, Canada* 265

44 **Nanoparticle-Based Sensing of Glycan-Lectin Interactions**; Joseph Wang; Yun Xiang; Zong Dai; Jared Gerlach; Jeffrey La Belle; Lokesh Joshi; *The Biodesign Institute at ASU, Tempe, Arizona* 266

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(1) **Emerging Roles of O-GlcNAc as a Nutrient/Stress Sensor Globally Regulating Signaling, Transcription, and Protein Turnover**

Gerald W. Hart; Chad Slawson; Mike Housley; Quira Zeidan; Stephen Whelan; Wagner Dias; Win Cheung; Kaoru Sakabe; Pui Butkinaree; Kyoungsook Park; Shino Shimoji; Zihao Wang; John Bullen
Johns Hopkins Medical School, Baltimore, MD

O-GlcNAc remained undetected until 1983, primarily because it generally does not affect gel electrophoresis, it is rapidly hydrolyzed upon cell damage, and it is extraordinarily difficult to detect by mass spectrometry. Recent proteomic studies have now identified over 400 O-GlcNAcylated proteins. In mammals and plants, O-GlcNAc is required for life. O-GlcNAc often competes with, and regulates phosphorylation at the same sites. Increased O-GlcNAcylation blocks insulin signaling, leading to insulin-resistance, a hallmark of diabetes. Increased O-GlcNAcylation underlies many aspects of 'glucose toxicity' in diabetes. Increased O-GlcNAc on the transcription factor FOXO1, accounts for the inappropriate increased gluconeogenesis in the liver of diabetics. Increased O-GlcNAcylation inhibits protein degradation both directly and by inactivation of the proteasome. O-GlcNAc regulates transcription, both positively and negatively, depending upon the promoter and transcription factors involved. O-GlcNAc also regulates translation and recent data suggest that at-least 15 ribosome proteins and translational factors are O-GlcNAcylated, including key components of the mTOR pathway. The O-GlcNAc Transferase forms transient complexes at the mid-body of dividing cells, which regulate cytokinesis. We hypothesize that dysregulation of the balance between O-GlcNAcylation and phosphorylation in signaling, transcription and cytoskeletal regulation, is directly underlying glucose toxicity in diabetes and hyperphosphorylation seen in neurodegenerative diseases. Supported by NIH grants HD13563, CA42486, DK61671, DK71280, and NIH contract N01-HV-28180. Dr. Hart 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.

(2) **Inflammatory Signaling by the C-type Lectin Receptor Dectin-1**

David M. Underhill
Cedars-Sinai Medical Center, Los Angeles, CA

Dectin-1 is a lectin receptor for beta-glucan that is important for innate recognition of fungi by macrophages and dendritic cells. This receptor triggers phagocytosis, activates production of antimicrobial reactive oxygen species, and collaborates with Toll-like receptor 2 to orchestrate cellular cytokine and chemokine production. While the mechanism of Toll-like receptor signaling shares much in common with cytokine receptor signaling, we have discovered that Dectin-1 signaling has similarities to antigen receptor signaling. Thus signaling pathways generally associated with acquired immunity, including the Src/Syk and NFAT (Nuclear Factor of Activated T cells) pathways, are activated in phagocytes upon detection of zymosan or yeast by Dectin-1. Microarray gene expression analysis reveals of set of genes that are specifically activated by Dectin-1 signaling, independent of Toll-like receptors.

(3) **Deciphering the 'O-GlcNAc code': Lessons from C. elegans and Human disease**

John A. Hanover
NIDDK, National Institutes of Health, Bethesda, MD

The Hexosamine Signaling Pathway leading to the reversible addition of O-GlcNAc to target proteins is 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. We have focused on the enzymes of O-GlcNAc cycling. Differentially targeted isoforms of O-GlcNAc transferase reside in mitochondria, nuclei and cytoplasm. We solved the X-Ray structure of the TPR domain of OGT that mediates the recognition of diverse O-GlcNAc targets though a mechanism similar to that used by importin α. Using recombinant forms of OGT and O-GlcNAcase, we found that O-GlcNAcase, YES tyrosine kinase and Tau are isoform-specific targets of OGT. We also carried out targeted mutagenesis studies of the catalytic domains of both OGT and the O-GlcNAcase. We showed that two isoforms encoded by the O-GlcNAcase gene are enzymatically active. Yet, a polymorphism associated with human type-2 diabetes maps to an intron in the gene disrupting isoform-specific splicing. We generated mouse knockout models targeting the mouse OGT and O-GlcNAcase genes to examine the relationship between O-GlcNAc metabolism and insulin signaling. To define the function of hexosamine signaling in a genetically amenable organism, we studied null alleles of OGT and the O-GlcNAcase (OGA) in Caenorhabditis elegans. These knockouts impact O-GlcNAc cycling, metabolism and dauer formation. Thus, O-GlcNAc cycling may ''fine-tune'' insulin-like signaling 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 and obesity.

(4) **CD33 type Siglecs are Degraded by the SOCS3 via ECS E3 ligase**

Jim Johnston
Queen's University Belfast, Belfast, Northern Ireland

CD33 is a member of the Sialic acid binding immunoglobulin-like lectin (Siglec) family of inhibitory receptors and a therapeutic target for acute myeloid leukaemia (AML). It contains a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM), which can recruit SHP-1 and SHP-2. How CD33 expression is regulated is unclear. Suppressor of cytokine signalling 3 (SOCS3) is expressed in response to cytokines, LPS and other PAMPs, and competes with SHP-1/2 binding to ITIMs of cytokine receptors thereby inhibiting signalling. In this study, using peptide pulldown experiments we find that SOCS3 can specifically bind to the phosphorylated ITIM of CD33. Additionally, following cross-linking SOCS3 can recruit the ECS E3 ligase resulting in accelerated proteasomal degradation of both CD33 and SOCS3. Our data suggests the tyrosine motifs in CD33 are not important for internalisation while they are required for degradation. Moreover, SOCS3 inhibited the CD33-induced block on cytokine-induced proliferation. This is the first receptor shown to be degraded by SOCS3 and where SOCS3 and its target protein are degraded concomitantly. Our findings clearly suggest that during an inflammatory response, the inhibitory receptor CD33 is lost by this mechanism. Moreover, this has important clinical implications as tumours expressing SOCS3 may be refractory to α-CD33 therapy.

(5) **Modulation of Normal Signaling by Shed Tumor Gangliosides**

Stephan Ladisch
Children's National Medical Center, Washington, DC

Aberrant tumor ganglioside metabolism, including substantial ganglioside shedding, characterizes a broad range of tumors, including neuroectodermal tumors such as melanoma, neuroblastoma, and lymphoma. Increased synthesis and shedding of these membrane glycosphingolipids, and their uptake by membranes of normal cells in the tumor microenvironment, has significant implications for tumor formation and progression. We have found that ganglioside enrichment of normal cell membranes results in significant upregulation of normal cell signaling. This is exemplified by enhanced fibroblast EGF receptor activation and downstream signaling, and enhanced vascular endothelial cell VEGF receptor activation and downstream signaling, in ganglioside-enriched normal cells. In turn, this caused enhancement of EGF-induced fibroblast proliferation and VEGF-induced vascular endothelial cell proliferation and migration. Interestingly, ganglioside enrichment of human umbilical vein vascular endothelial cells also caused even very low, normally barely stimulatory, VEGF concentrations to trigger robust VEGFR dimerization and autophosphorylation, downstream signaling, and cell proliferation and migration. By dramatically lowering the threshold for growth factor activation of contiguous normal stromal cells, shed tumor gangliosides may promote tumor progression by causing these normal cells to become increasingly autonomous from growth factor requirements, by a process that we term tumor-induced progression of the microenvironment (supported by NIH grants CA42361 and CA61010).

(6) **N-Glycan Processing Integrates Cellular Responsiveness to Extracellular Cues in Cancer Progression and T Cell Activation**

Ken Lau[1]; Emily A. Partridge[1]; Pamela Cheung[1]; Cristina I. Silvescu[2]; Ani Grigorian[3]; Vernon N. Reinhold[2]; Michael Demetriou[3]; James W. Dennis[1]
[1]Mount Sinai Hospital, Toronto, Canada; [2]University of New Hampshire, Durham, NH; [3]University of California, Irvine, CA

The Mgat5 deficiency suppresses cancer progression, and increases T cell sensitivity to auto-antigens. N-glycans on signaling receptors bind galectins at the cell surface, forming a molecular scaffold that opposes receptor loss due to constitutive endocytosis, thus regulating signal sensitivity (Partridge et al. Science 306:120, 2004). Here, we show that UDP-GlcNAc stimulates the biosynthesis of tri- and tetra-antennary N-glycans, the high-affinity galectin ligands, in a multistep ultrasensitive manner. This kinetics results from decreasing affinities for donor UDP-GlcNAc and concentrations of Mgat1, Mgat2, Mgat4 and Mgat5 enzymes, and Golgi transit time. However, glycoform variants increase exponentially with N-glycan chains per receptor, and computational simulations reveal that N-glycan multiplicity suppresses Golgi-mediated ultrasensitivity for surface receptor retention. Moreover, multiplicity is greater on growth-promoting receptor tyrosine kinases than receptors that mediate arrest/differentiation. Thus, sensitivities to growth and arrest cues can be concurrently but differentially regulated by N-glycan multiplicity and hexosamine titration of N-glycan processing. In practice,

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growth signaling in carcinoma cells and progression to the invasive phenotype is enhanced by titration of the hexosamine/N-glycan pathway and then restricted. This restriction is dependent on up-regulation of surface TGF-β receptors, which has low multiplicity. T cell activation by antigens provides a second example of rapid proliferation followed by arrest dependent on CTLA-4 (low multiplicity). We show that growth stimulates hexosamine/N-glycan activity and is required for subsequent up-regulation of surface TGF-β and CTLA-4 receptors by enhancing their association with galectins. Our results suggest that metabolic regulation of N-glycan processing can tune transitions between growth and differentiation/arrest.

(7) N-Acetylglucosaminyltransferase III Antagonizes N-Acetylglucosaminyltransferase V On Alpha3beta1 Integrin-Mediated Cell Migration

Yanyang Zhao[1]; Jianguo Gu[1]; Takatoshi Nakagawa[1]; Akihiro Kondo[1]; Nana Kawasaki[2]; Eiji Miyoshi[1]; Naoyuki Taniguchi[1]
[1]Osaka University Graduate School of Medicine, Osaka, Japan; [2]National Institute of Health Sciences, Tokyo, Japan

N-Acetylglucosaminyltransferase V (GnT-V) catalyzes the addition of beta1,6 GlcNAc-branching of N-glycans which contributes to metastasis. N-acetylglucosaminyltransferase III (GnT-III) catalyzes the formation of a bisecting GlcNAc structure in N-glycans, resulting in the suppression of metastasis. It has long been hypothesized that the suppression of GnT-V product formation by the action of GnT-III would also exist in vivo, which will consequently lead to the inhibition of biological functions of GnT-V. To test this, we draw a comparison among MKN45 cells, which were transfected with GnT-III, GnT-V, or both, respectively. We found that alpha3beta1 integrin-mediated cell migration on laminin 5 was greatly enhanced in the case of GnT-V transfectant. This enhanced cell migration was significantly blocked after the introduction of GnT-III. Consistently, an increase in bisected GlcNAc but a decrease in beta1,6 GlcNAc branched N-glycans on integrin alpha3 subunit was observed in the double transfectants of GnT-III and GnT-V. Conversely, GnT-III-knockdown resulted in increased migration on laminin 5, concomitant with an increase beta1,6 GlcNAc branched N-glycans on alpha3 subunit in CHP134 cells, a human neuroblastoma cell line. Therefore, in this study, the priority of GnT-III for the modification of alpha3 subunit may be an explanation for why GnT-III inhibits GnT-V-induced cell migration. Taken together, our results demonstrate for the first time that GnT-III and GnT-V can competitively modify the same target glycoprotein, furthermore positively or negatively regulate its biological functions.

(8) A Key Enzyme in Protein N-Glycosylation: Oligosaccharyl Transferase

Manasi Chavan[1]; Guangtao Li[1]; Zhiqiang Chen[2]; Huilin Li[2]; Hermann Schindelin[3]; William J. Lennarz[1]
[1]Stony Brook University, Stony Brook, NY; [2]Brookhaven National Laboratory, Upton, NY; [3]Würzburg University, Würzburg, Germany

Following initiation of translocation across the membrane of the endoplasmic reticulum via the translocon, polypeptide chains are N-glycosylated by the oligosaccharyl transferase (OT) enzyme complex. Translocation and N-glycosylation are concurrent events and would be expected to require juxtaposition of the translocon and the OT complex. We have studied the structure of the nine subunits of OT and how they interact with each other. In addition, to determine if any of the subunits of the OT complex and the translocon complex mediate interaction to generate a supercomplex, we initiated a systematic study using the split-ubiquitin approach. Interestingly, the OT subunit Stt3p was found to interact only with Sec61p, while another OT subunit, Ost4p, was found to interact with all three components of the translocon, Sec61p, Sbh1p and Sss1p. We were able to confirm these split-ubiquitin findings by a chemical cross-linking technique. Based on our findings, we conclude that the association of these two complexes is stabilized via multiple protein-protein contacts. Currently, we are working in collaboration with Dr. Huilin Li determine the structure of the OT complex by cryo electron microscopy and then, in collaboration with Hermann Schindelin, we hope to obtain a high resolution structure by X-ray crystallography. The next step will be to study the structure of the OT-translocon supercomplex.

(9) Structural Snapshots of the Mannose 6-Phosphate Receptors

Nancy M. Dahms
Medical College of Wisconsin, Milwaukee, WI

The biogenesis of lysosomes requires the correct sorting of >50 acid hydrolases from their site of synthesis in the endoplasmic reticulum to their final destination in lysosomes. The 46kDa cation-dependent mannose 6-phosphate receptor (CD-MPR) and the 300kDa cation-independent MPR (CI-MPR) divert these soluble enzymes from the secretory pathway by delivering their cargo bearing Man-6-P on N-glycans from the trans Golgi network (TGN) to endosomes. Man-6-P is generated in two steps: GlcNAc phosphodiester is formed in the cis-Golgi followed by removal of GlcNAc in the TGN to expose the phosphomonoester. The MPRs display optimal ligand binding at ~pH 6.4 and no detectable binding below pH 5, which is consistent with binding newly synthesized lysosomal enzymes in Golgi compartments and releasing these enzymes in the acidic environment of endosomes. Our biochemical and structural studies have begun to reveal the related, yet distinct, carbohydrate binding pocket architectures of these two receptors. To understand the mechanisms used to modulate ligand binding and release by the MPRs upon changes in pH and why the CD-MPR, but not the CI-MPR, is unable to bind ligand at the cell surface, we have obtained the crystal structure of the dimeric CD-MPR under different pH conditions. These structures, in addition to the crystal structure of a mutant CD-MPR that lacks a salt bridge between the two monomers of the dimeric molecule, reveal significant differences in their quaternary structure and implicates an important role for inter-monomer interactions in pH-dependent carbohydrate recognition. (Supported by NIH grant DK42667.)

(10) The "Lipid-linked Oligosaccharide/CDG-I/ER Stress Response" Triad

Mark A. Lehrman
UT-Southwestern Medical Center, Dallas, TX

Homeostasis of the endoplasmic reticulum (ER) requires efficient synthesis of the lipid-linked oligosaccharide (LLO) $Glc_3Man_9GlcNAc_2$-P-P-dolichol and transfer of its glycan to asparaginyl residues of nascent polypeptides. Disruption of this process, as occurs in the Type I Congenital Disorders Of Glycosylation (CDG-I), results in ER stress. Indeed, evidence exists for ER stress response activation in several CDG-I genotypes. Paradoxically, while serum glycoproteins in CDG-Ia patients with phosphomannomutase defects are hypoglycosylated, patients' cultured fibroblasts in physiological glucose produce $Glc_3Man_9GlcNAc_2$-P-P-dolichol and glycoproteins normally. Our laboratory is interested in how the ER stress response may stimulate the LLO pathway, both during normal ER homeostasis and as a compensatory mechanism in CDG-I. Three independent LLO biosynthesis-ER stress response regulatory systems have been identified. (i) In a manner well-known for the ER stress response, expression of key LLO biosynthesis genes is activated. (ii) PERK, an eIF2α kinase which attenuates translation in response to ER stress, adjusts polypeptide synthesis to compensate for reduced flux through the LLO pathway by sensing the resultant misfolded proteins. (iii) Hexose phosphates which are precursors of glycosyl donors are elevated by the ER stress response, apparently by altering glycogen metabolism. In summary, we find that the ER stress response stimulates the LLO pathway in a multifaceted, compensatory manner. This may explain the persistence of $Glc_3Man_9GlcNAc_2$-P-P-dolichol biosynthesis, with ER stress, in cultured CDG-Ia fibroblasts. The "LLO/CDG-I/ER stress response" triad also suggests novel strategies for therapeutic CDG-I intervention. Supported by grants GM38545 (NIH) and I-1168 (Welch Foundation).

(11) Requirement of Fatty Acid Remodeling for Raft-Association of GPI-Anchored Proteins

Taroh Kinoshita[1]; Yuko Tashima[1]; Toshiaki Houjou[2]; Morihisa Fujita[3]; Takehiko Yoko-o[3]; Yoshifumi Jigami[3]; Ryo Taguchi[2]; Yusuke Maeda[1]
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GPI-anchored proteins (GPI-APs) are concentrated in lipid rafts as revealed by their efficient recovery in detergent-resistant membrane (DRM) fraction. GPI-APs usually have two saturated fatty chains in the PI moiety, a requirement for stable association with the liquid ordered raft membrane. Here we report that GPI-APs with two saturated fatty chains are generated from those bearing an unsaturated chain by fatty acid remodeling. We previously reported CHO cells defective in PGAP2 gene that have decreased surface expression of GPI-APs. The PGAP2-defective cells generated lyso-GPI-APs that are then cleaved by unknown phospholipase D resulting in release of the soluble GPI-APs. In the present study, we isolated double mutant cells from the PGAP2-mutant CHO cells based on the recovery of cell surface expression of GPI-APs. We reasoned that an additional mutation in a factor involved in a step upstream to the PGAP2-mediated step might result in the surface expression of unremodeled GPI-APs. Mass-spectrometric analysis demonstrated that GPI-APs isolated from the double mutant CHO cells have unsaturated chains, such as 18:1 and 20:4 in the sn2 position whereas GPI-APs from wild-type CHO cells have exclusively 18:0 chain. We identified the gene responsible for the second mutation in the double mutant, termed PGAP3, which encoded a 320 amino acid membrane protein. We then assessed association of GPI-APs with lipid rafts. Recovery of unremodeled GPI-APs from the double mutant cells in the DRM fraction was very low. Therefore,

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fatty acid remodeling mediated by PGAP3 and PGAP2 is essential for raft-association of GPI-APs.

(12) **Glycan Biosynthesis, Processing and Recognition**

Jonathan Weissman
UCSF, San Francisco, CA

TBD

(13) **A DHHC Protein Regulates Activity and Subcellular Transport of GalNAc Transferase B in Drosophila melanogaster**

Anita Stolz[1]; Benjamin Kraft[1]; Manfred Wuhrer[2]; Cornelis H. Hokke[2]; Rita Gerardy-Schahn[1]; Hans Bakker[1]
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In mammals, the lacdiNAc (GalNAcβ1,4GlcNAc) glycotope is limited to a number of specific proteins.The epitope is more widespread in invertebrate glycoconjugates and an antigenic determinant in e.g. parasitic worms like Schistosoma mansoni. Accordingly, GalNAc transferase activity has been found in many invertebrates and cDNA clones encoding the activity have been isolated. Using an expression cloning strategy in CHO cells, we have cloned a drosophila GalNAc transferase (β4GalNAcTB), which, in contrast to the previously identified β4GalNAcTA (Haines et al. 2004), requires a multiple membrane spanning protein of the zinc finger DHHC domain family for activity. In an in vitro assay system we demonstrated that coexpression of the GalNAc transferase with the DHHC protein dramaticly increases activity. Under the assay conditions used, it exceeds even the activity of β4GalNAcTA, characterised to be a cofactor independent enzyme. Moreover, the characterisation of biosynthetic products has shown that the β4GalNAcTB together with the DHHC protein participate in the biosynthesis of glycosphingolipids. Using an RNAi approach, we demonstrated in Drosophila Schneider cells that transition of β4GalNAcTB from the ER to the Golgi apparatus depends on the DHHC protein. Accordingly, the knocking down of the DHHC protein in Drosophila Schneider cells leads to a drastic decrease of lacdiNAc structures on the cell surface. In contrast, localisation of β4GalNAcTA was unaffected.

In this work we present a new glycosyltransferase regulating protein with chaperone activity, which is involved in the localisation and activity of the β4GalNAc transferase in Drosophila melanogaster.

(14) **The Mammalian and *Drosophila* Orthologous UDP-GalNAc: Polypeptide α-N-Acetylgalactosaminyltransferases (ppGalNAc-Ts) T1 and T2 Possess Highly Conserved Peptide Substrate Specificities**

Thomas A. Gerken[1]; Oliver Jamison[1]; Kelly G. Ten Hagen[2]
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A large family of ppGalNAc-Ts catalyzes the first step of mucin-type protein O-glycosylation, transferring α-GalNAc to serine and threonine residues of polypeptide acceptors. The peptide substrate specificity and specific protein targets of the individual ppGalNAc-T family members remains poorly understood. Orthologues of several members are identifiable in *Drosophila, C. elegans* and other lower eukaryotes suggesting these transferases have evolutionarily conserved and biologically significant roles. Functional mutations in two isoforms are deleterious to man (ppGalNAc-T3) and the fly (pGANT35A). We have developed a series of oriented random peptide substrate libraries, based on the GAGAXXXTXXXAGAGK sequence motif (where X=randomized positions), for obtaining a comprehensive determination and quantification of the peptide substrate specificities of the mammalian ppGalNAc-T1 and -T2 isoforms (Gerken et. al. submitted). With these substrates, previously unknown features consistent with the X-ray crystal structures of the transferases have been obtained. We now report studies on the *Drosophila* orthologues of T1 and T2, pGANT5 and pGANT2, whose catalytic and ricin domains show 57% and 66% sequence identity with their mammalian counterparts. We find that ppGalNAc-T2 and pGANT2 have identical substrate specificities, in keeping with the high conservation of their putative peptide binding site residues (17 of 20 residues conserved). ppGalNAc-T1 and pGANT5 also display very similar specificities. However, since their putative peptide binding site residues are less conserved (12 of 20 residues) some differences are observed. These results suggest that the specificities of the orthologous transferases are highly conserved across species. (Supported by NIH-NCI grant RO1-CA-78834).

(15) **Roles for O-Fucose and Pofut1 in Notch Signaling in Mammals**

Pamela Stanley; Changhui Ge; Mark Stahl; Kazuhide Uemura; Shaolin Shi
Albert Einstein College Medicine, New York, NY

We are investigating mechanisms by which O-fucose glycans control Notch signaling in mammals. A single O-fucose site in EGF12 resides in the ligand binding domain of all Notch receptors. We have generated mice with a point mutation (T466A; Notch112f) in Notch1 that precludes the transfer of O-fucose to the ligand binding domain. Notch112f/12f mice are viable and fertile. Notch signaling and ligand binding are both reduced in mouse embryo fibroblasts and embryonic stem (ES) cells derived from Notch112f/12f mice. Defective Notch1 signaling was also observed in thymic T cells. T cell development in Notch112f/12f mice will be described. To investigate the consequences of more global changes in O-fucosylation of Notch receptors we have used ES cells lacking Pofut1 or Lec13 CHO cells which have low levels of GDP-fucose. Ligand-dependent signaling assays and soluble Notch ligand binding assays were used to show that Pofut1 null ES cells do not bind Notch ligands nor transduce Notch signals. However, endogenous Notch receptors are expressed at equivalent levels on Pofut1 null and wild type ES cells. Lec13 cells bind reduced amounts of Notch ligands, and are markedly defective in ligand-induced Notch signaling, although cell surface Notch receptor expression is also not significantly changed. The combined data provide strong evidence that mammalian Notch receptors, unlike Drosophila Notch, are not dependent on Pofut1 or O-fucose for cell surface expression, but require O-fucose in order to optimally bind Notch ligands and to undergo the activation steps that result in Notch signaling.

(16) **Mind the Gap! Glyco-Therapies for Enteric Protein Loss**

Hudson H Freeze
The Burnham Institute for Medical Research, La Jolla, CA

Environmental insults and genetic deficiencies can precipitate a life-threatening condition. In Congenital Disorders of Glycosylation (CDG), patients with portal hypertension develop enteric protein loss called protein-losing enteropathy (PLE) especially during stress, infection, or inflammation. Oral mannose supplements reverse PLE in phosphomannose isomerase-deficient CDG-Ib patients, but not in other types of CDG. Still other children with congenital heart defects but normal N-glycosylation, develop PLE following infections long after corrective Fontan surgery. For these patients, subcutaneous injections of anti-coagulant heparin relieve PLE by an unknown mechanism. In both diseases, heparan sulfate (HS) and its major carrier, syndecan 1 (Scd1), are lost only from the epithelial cells of the small intestine during PLE; they return when PLE subsides. We hypothesized that loss of Scd1 and/or HSPG, synergizes with increased pro-inflammatory cytokines and venous pressure to destabilize tight junctions and produce PLE. In a cellular model, we show that loss of Sdc1, HS, and N-glycosylation have additive effects on the cytokine-induced protein leakage. Mannose reverses the glycosylation effect in the CDG-Ib-like cells, and heparinoids prevent protein loss in both Sdc1- and HS-deficient cells by intercepting cytokines, TNFα and IFN&gamma. Scd1- or intestinal HS-deficient mice have elevated cytokine-induced protein leakage, which is eliminated with non-anticoagulant 2,3-O desulfated heparin. An adult CDG-Ib patient developed PLE and elected heparin over mannose therapy. Three months later, her PLE was completely gone. The results offer an explanation for heparin therapy and suggest that it may be useful for other CDG patients with PLE. (Support: R21 HL 078997)

(17) **Decoding the Structure-Activity Relationships of Glycosaminoglycans in the Brain**

Linda C. Hsieh-Wilson
Caltech and HHMI, Pasadena, CA

Chondroitin sulfate glycosaminoglycans are sulfated polysaccharides that have been implicated in neuronal development, spinal cord injury and long-term memory storage. We are developing chemical strategies that permit the first direct investigations into the structure-activity relationships of chondroitin sulfate and provide a powerful set of tools for understanding their physiological functions. Chondroitin sulfate oligosaccharides were assembled using a convergent, synthetic approach that permits installation of sulfate groups at precise positions along the carbohydrate backbone. Using these well-defined structures, we demonstrate that specific sulfation motifs function as molecular recognition elements for growth factors and modulate neuronal growth. Our results provide fundamental insights into the role of sulfation and evidence for a 'sulfation code,' whereby glycosaminoglycans encode functional information in a sequence-specific manner analogous to DNA, RNA and proteins.

(18) **O-Glycosylation of Cysteine-Knot Motifs**

Robert S. Haltiwanger; Malgosia Dlugosz; Yi Luo; Kelvin Luther; Aleksandra Nita-Lazar; Nadia Rana; Hideyuki Takeuchi; Bernadette C. Holdener
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Epidermal growth factor-like (EGF) repeats are small, cysteine-knot motifs with six conserved cysteines forming three disulfide bonds. EGF repeats containing the appropriate consensus sequences can be modified by O-fucose and O-glucose glycans. The Notch receptor contains multiple tandem EGF repeats decorated with both O-fucose and O-glucose. Elimination of the enzyme responsible for addition of O-fucose to Notch, protein O-fucosyltransferase 1 (Pofut1), results in embryonic lethality in mice with Notch-like phenotypes. Mutation of specific O-fucose or O-glucose sites also affects Notch activity in cell-based assays, suggesting that these modifications are essential for Notch function. Recently O-fucose was found in a different context, that of a thrombospondin type 1 repeat (TSR). Like EGF repeats, TSRs are small cysteine-knot motifs with six conserved cysteines forming three disulfide bonds, although in a distinct pattern from EGF repeats. Hofsteenge and coworkers demonstrated that TSRs from several proteins (thrombospondin, properdin, F-spondin) are modified with O-fucose glycans at a proposed consensus sequence. Over 40 TSR-containing proteins in the mouse genome contain this consensus. As a first step to probing biological function, we have identified the enzyme responsible for adding O-fucose to TSRs: protein O-fucosyltransferase 2 (Pofut2). Like Pofut1, Pofut2 appears to be ER localized and can distinguish between folded and unfolded TSRs. We have generated a mouse lacking functional Pofut2. Homozygotes display an embryonic lethal phenotype. We are currently examining these mice in more detail to more clearly define the phenotype. This work was supported by GM61126.

(19) **Analysis of the Conserved Oligomeric Golgi (COG) Complex**

Monty Krieger
MIT, Cambridge, MA

Multisubunit peripheral membrane protein complexes play important roles in Golgi-associated membrane trafficking and glycoconjugate processing. One of these is the Conserved Oligomeric Golgi (COG) complex, an eight-subunit (Cog1-8) peripheral Golgi protein involved in membrane trafficking and glycoconjugate synthesis. Defects in COG activity can cause abnormalities in glycoconjugate synthesis, intracellular protein sorting, and, in some cases, cell growth. Mammalian Cog1- or Cog2-null Chinese hamster ovary (CHO) cell mutants, the first COG mutants to be isolated and characterized, exhibit multiple abnormally dilated Golgi cisternae and pleiotropic defects in Golgi-associated glycosylation reactions affecting virtually all N-linked, O-linked and lipid-linked glycoconjugates. COG appears to participate in retrograde vesicular transport and is required to maintain normal Golgi structure and function; however, the precise molecular mechanism by which COG influences Golgi structure and function is unclear. COG mutations interfere with normal transport, distribution and/or stability of Golgi proteins associated with glycoconjugate synthesis and trafficking, and lead to failure of spermatogenesis in Drosophila melanogaster, misdirected migration of gonadal distal tip cells in Caenorhabditis elegans, and type II congenital disorders of glycosylation in humans. The use of somatic cell and other mutants to study COG structure and function will be discussed.

(20) **Developmental Regulation of HSPG Synthesis during Drosophila Embryogenesis**

Douglas Bornemann; Sangbin Park; Rahul Warrior
UC Irvine, Irvine, CA

In rosophila signaling by the BMP4 homolog Decapentaplegic (Dpp) is critical for patterning the dorsal region of the embryo and for cell fate specification and proliferation in wing imaginal discs. Mutations in the GAG chain polymerases Tout velou (Ttv) and Sister of tout velou (Sotv), impair signaling by Dpp, Hedgehog (Hh) and Wingless (Wg) in the wing disc, demonstrating a requirement for HSPGs in these growth factor pathways. Surprisingly, although Hh and Wnt signaling is compromised in embryos lacking HSPGs, BMP signaling is not affected. To understand the basis for these observations, we examined the temporal regulation of GAG chain addition to HSPG core proteins. We found that in Drosophila, GAG chain synthesis is under tight developmental control. Essentially no synthetic activity is detectable in the first three hours of embryogenesis with a rapid onset at about four hours following fertilization. The time period when the biosynthetic process is inactive correlates with the interval during which the Dp/BMP activity gradient is established, while the onset of GAG chain addition coincides with Hh and Wnt signaling-dependent patterning of the embryonic epidermis. We find that the timing of GAG chain addition is controlled at a post-transcriptional level through regulated translation of at least one of the GAG chain polymerases. Interestingly, this mechanism may be phylogenetically conserved, suggesting that regulation of GAG chain synthesis could represent an important strategy to differentially alter the activity of specific signaling pathways at unique stages during development.

(21) **Unlike Mammalian GRIFIN, the Zebrafish Homologue (DrGRIFIN) May Represent a Functional Carbohydrate-Binding Galectin**

Hafiz Ahmed; Gerardo R. Vasta
Center of Marine Biotechnology, UMBI, Baltimore, MD

Galectins, a family of ß-galactoside-binding proteins, participate in a variety of biological processes, such as early development, tissue organization, immune functions, tumor evasion and cancer metastasis. However, the detailed mechanisms of their biological roles still remain unclear. As many as fifteen galectins are known in mammals, excluding some galectin-like sequences such as lens crystalline protein GRIFIN (galectin related inter fiber protein) and the hematopoietic stem cell precursor, HSPC159. Although they have close similarity to galectin sequences, due to the lack of ligand (lactose) binding activity they are not considered to be members of the galectin family but possibly products of evolutionary co-option. We have identified a homologue of the GRIFIN in zebrafish (*Danio rerio*) (designated DrGRIFIN), which is also expressed in the eye, as evidenced from whole mount immunostaining of 48 hpf (hour post fertilization) embryos. As evidenced by RT-PCR, it is weakly expressed in the embryos as early as 21 hpf and strongly at all later stages tested [30 hpf and 2, 3, 4, 5, 6, and 7 dpf (days post fertilization)]. In adult zebrafish tissues, however, DrGRIFIN is also expressed in oocyte, brain, and intestine. Unlike the mammalian equivalent, DrGRIFIN contains all amino acids critical for ligand-binding, and thus, may represent a functional candidate relevant to developmental processes of the fish lens. (Supported by NIH Grant R01 GM070589-01 to GRV)

(22) **A Mucin-Type O-Glycosyltransferase is Required During Multiple Stages of Drosophila Development**

E Tian; Kelly G. Ten Hagen
NIDCR, NIH, Bethesda, MD

The UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase enzyme family is responsible for the first committed step in the synthesis of mucin-type O-glycans on protein substrates. Sequence and functional conservation exists between certain members of this family in mammals and the fruit fly, Drosophila melanogaster. One member in Drosophila (pgant35A) has been shown to be essential for viability. In an effort to elucidate the specific developmental stages and processes affected by this glycosyltransferase, we have analyzed pgant35A mutants in more detail. Results of genetic studies indicate that pgant35A is required at multiple stages of development; homozygous mutant animals derived from heterozygous parents arrest during embryogenesis (~25% of total homozygous mutants) and larval development (~30%). Those mutants that survive to pupation never proceed past pupal stage P4ii (early pupation). These results indicate that pgant35A is required to complete pupation and also plays a role during embryonic and larval development. Recent work has shown that pgant35A has strong maternal RNA expression in the embryo. To define when pgant35A is first required during development, we generated germline clones (which contain no wild-type maternal pgant35A transcripts in the oocytes). Homozygous maternal/zygotic (m/z) pgant35A mutants arrested during embryogenesis, clearly demonstrating a crucial role for this gene during embryonic development. Immunohistochemistry using various developmental markers and lectins was employed to elucidate the specific defects seen in these mutants. These studies demonstrate that pgant35A and O-linked glycosylation are required at multiple distinct stages of Drosophila development and provide insight into the role of the mammalian ortholog.

(23) **Lectins Bind to Multivalent Glycoproteins with a Large Gradient of Binding Constants**

Curtis F. Brewer; Tarun K. Dam
Albert Einstein College of Medicine, Bronx, NY

Recent studies show negative cooperativity in the isothermal titration microcalorimetry data of galectins-1, -2, -3, -4, -5, and -7, and truncated, monomer versions of galectins-3 and -5, binding to asialofetuin (ASF), a glycoprotein with nine LacNAc epitopes (Dam et al. 2005) Biochemistry 44, 12564-12571). Similar data have been shown for the binding of plant lectins to synthetic multivalent carbohydrates (Dam et al. (2002) Biochemistry 41, 1351-1358). Although the observed Ka values for ASF binding to the galectins and two truncated forms are only 50- to 80-fold greater than that of LacNAc, analysis of the data in terms of the relationship between the observed macroscopic free energy of binding and the decreasing microscopic free energies of binding of the epitopes shows that the first LacNAc epitope of ASF binds with approximately 6,000-fold higher affinity than the last epitope. Thus, the microscopic binding constants of the galectins to the first epitope(s) of ASF are in the nM range, with a gradient of decreasing binding constants of the remaining epitopes. The results indicate that the above galectins bind with fractional, high affinities to multivalent glycoproteins such as ASF,

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independent of the quaternary structures of the galectins. The results have important implications for the binding of lectins including galectins to multivalent carbohydrate receptors.

(24) **Using Glycodendrimers to Study Protein-Carbohydrate Interactions**

Mary J Cloninger
Montana State University, Bozeman, MT

Multivalent protein-carbohydrate interactions serve a critical function in many intercellular recognition events including the infection of host cells by viruses and bacteria and the adhesion and metastasis of cancer. A thorough understanding of the fundamental requirements of multivalent protein-carbohydrate interactions is essential if therapeutic agents are to be developed that rely on protein-carbohydrate interactions. Carbohydrate-containing polymers ranging from linear polymers to nanoparticles have been reported for the investigation and control of protein-carbohydrate interactions.

Dendrimers, highly branched, tree-like macromolecular compounds, are ideal frameworks on which to append carbohydrates. The sizes of dendrimers can be readily varied, and synthetic strategies designed to rapidly and reproducibly provide appropriate materials for protein and cellular assays have been developed. Assays with glycodendrimers bearing mixtures of low and high affinity carbohydrates presented in a variety of patterns suggest that the degree of lectin clustering and the affinity of the dendrimers for the lectins can be predictably attenuated. Dendrimers bearing mixtures of mannose, glucose, galactose were synthesized, and the binding of these dendrimers to lectins is described. In addition, dendrimers bearing increasing loadings of clusters of mannose groups were synthesized, and the binding of these dendrimers with lectins will be reported. Results of hemagglutination inhibition assays and surface plasmon resonance studies will be presented.

(25) **Automated Oligosaccharide Synthesis and the Direct Formation of Carbohydrate Microarrays**

Nicola L. Pohl
Iowa State University, Ames, IA

An understanding of biological responses to carbohydrates is vital for rational vaccine design as well as a range of other problems, but is currently limited in part because well-defined saccharide structures are difficult to access. This talk will discuss the latest developments in a practical new automated approach for the rapid synthesis of oligosaccharides that avoids the problems of standard solid-phase methods and how this alternate strategy is also integral for directly screening these synthetic carbohydrates for their biological activities.

(26) **Structure/Thermodynamic Relationship in Lectin/Glycan Interaction Strategies for High Affinity Binding**

Anne Imberty
CERMAV-CNRS, Grenoble, France

Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity. Although protein-carbohydrate interactions are characterized by low affinity, bacterial lectins use a variety of strategy to attain high affinity for specific binding to host carbohydrate

Lectin-carbohydrate interactions are generally characterised by a low affinity for monovalent ligands, a drawback balanced by multivalency that provides high avidity for substrates with several potential ligands available, such as complex glycans or cell surfaces. In general, a millimolar affinity is observed for lectin binding to monosaccharides. Better affinity is obtained for longer ligand, i.e. oligosaccharides, and the interactions are typified by a favourable enthalpy term, due to the high number of hydrogen bonds, that is offset by an unfavourable entropy contribution that has been attributed either to solvent rearrangement or to loss of ligand conformational flexibility.

Recent interest in bacterial lectins involved in pathogenesis and host recognition has been accompanied by thermodynamic characterisation that demonstrated much higher affinity than that observed for plant or animal lectins. Calcium–dependent lectins from opportunistic pathogens *Pseudomonas aeruginosa*, *Chromobacterium violaceaum* and *Ralstonia solanacearum* all display sub-micromolar range affinity towards their carbohydrate ligands. We used combined titration microcalorimetry and x-ray crystallography approaches to decipher the thermodynamical and structural basis for high affinity binding of bacterial lectins to host carbohydrates.

(27) **Specificity of Glycosaminoglycan Binding to CCR2 Chemokines: Significance of Sulfation Binding Sites**

Julie Leary
University of California, Davis, CA

Glycosaminoglycans are heterogenous mixtures of highly sulfated, negatively charged carbohydrates. These sulfated oligomers are known to interact with proteins, specifically chemokines, on the surface of the endothelium during chemotaxis. Limited information is available on the specificity, composition and isomeric structure of these GAG. In the studies presented herein, we have determined the composition and position of sulfation for GAG's from both heparan sulfate and heparin that are specific binding partners of various chemokines. Hydrophobic trapping and non-covalent complexation experiments were used to determine which specific GAG, from both heparin and heparan sulfate, show binding to CCR2 and CCR5 chemokines. Compositional analysis of the specific binders is undertaken using enzymatic digestion of the oligomers once they are removed from the protein-GAG complex through sequential salt washings. ESI-FTICR is used to analyze the non-covalent protein-carbohydrate complexes produced during incubation of the GAG with various chemokines. Modifications have been made to the instrument which allow for the measurement of complexes in the 250 kDa mass range. Mass spectrometry data is compared to isothermal calorimetry measurements both of which are in good agreement, thus substantiating the formation of the protein-ligand, and multimer proteins in solution. Both MS and MS/MS are used to identify specific GAG binding to the chemokine. Data from the heparan sulfate library was somewhat different from the heparin library which indicates additional specificity of GAG produced from heparin VS those from heparan sulfate.

(28) **Influenza HA Structure and Receptor Binding using the Glycan Microarray**

Ian A. Wilson; James Stevens; Ola Blixt; James Paulson
The Scripps Research Institute, La Jolla, CA

The 1918 influenza pandemic was by far the most deadly, resulting in ~50 million deaths worldwide. The crystal structure of the hemagglutinin (HA) from the 1918 virus revealed structural features maintained in avian HA's (1). For an avian virus to adapt to humans, the HA receptor binding site must change specificity from α2-3 (avian gut) to α2-6 (human respiratory tract) linked sialosides. We probed the fine specificity of the 1918 HA and determined only two changes are required to switch receptor specificity (2). A similar structural and receptor characterization of the HA from a highly pathogenic H5N1 virus showed that its structure most closely resembles the 1918 H1 HA rather than other HAs (3). H5 HA binds specifically to avian α2-3 linked sialosides and two different sets of mutations that interconvert avian and human receptor binding for H1 and H3 viruses do not provide a clear switch in specificity for the H5 HA. However, H3 mutations on this H5 background reduces α2-3 binding and increases interaction with biantennary α2-6 linked sialosides. Furthermore, these studies can detect fine differences in HA specificity, such as for extra sulfation, fucosylation or sialylation of the terminal trisaccharide.

(1) Stevens et al. (2004) Science 303, 1866-1870.

(2) Stevens et al. (2006) J. Mol. Biol. 355, 1143-1155.

(3) Stevens et al. (2006) Science 312, 404-410.

(29) **Heterobivalent Ligands: A Versatile Approach to Ligand Induced Protein Aggregation Exemplified by the Structure-Based Design of Shiga Toxin Antagonists**

Pavel Kitov; David Bundle
University of Alberta, Edmonton, Alberta

Recently, a novel approach for inhibition of multivalent receptors was suggested, which utilizes the supramolecular effect. The inhibitor, a low molecular weight, hetero-bifunctional compound mediates high avidity, supramolecular assembly between the target receptor and an endogenous multivalent protein. This concept of heterobivalent ligand induced aggregation has the potential for more general applications involving receptors on cell surfaces. One embodiment we envisioned is ligand mediated immunotargeting that employs antibody or mammalian lectins. This concept was successfully applied for immunotargeting clustered receptors on cells surface (see abstract by S. Han *et al*.). Here we report the design and activity of Shiga toxin (Stx) inhibitors that mediate specific aggregation of Stx with serum amyloid P component (SAP). Shiga toxins are major virulence factors in bacterial dysentery. SAP is an innate immune system serum protein that is implicated in amyloid formation. Structural information at atomic resolution for both proteins as complexes with their respective ligands was used to design very compact inhibitors. Efficient chemo-enzymatic synthesis was implemented to obtain the hetero-bifunctional ligands, containing Pk-trisaccharide fragment for recognition by Stx and cyclic pyruvate for binding to the SAP Ca^{2+}-dependent binding site. The inhibitory activity of the ligands was demonstrated to be strongly dependent on SAP concentration.

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Elaboration and further examples of this concept will be presented.

(30) **New Mass Spectrometry Tools for Glycosaminoglycans Analysis**

Bérangère Tissot[1]; Stuart M. Haslam[1]; Howard R. Morris[1]; Jeremy E. Turnbull[2]; Andrew K. Powell[2]; Zheng-liang Zhi[2]; John T. Gallagher[3]; Christopher J. Robinson[3]; Anne Dell[1]
[1]Imperial College, London, UK; [2]University of Liverpool, Liverpool, UK; [3]University of Manchester, Manchester, UK

The multidisciplinary UK Glycochips Consortium is devoted to the development of carbohydrate microarray technologies and associated analytical methodologies especially mass spectrometry. Among the various goals of this consortium, the production of glycosaminoglycan (GAG)-arrays is one of the most challenging. Protein-GAG interactions are amongst the most complicated to elucidate mainly because of the structural complexity of the glycan and the difficulty to analyse small amounts of highly sulphated sugars. Mass spectrometry is one of the most powerful techniques for heparin/HS structural characterisation and among the mass spectrometry methodologies electrospray-ionization (ESI) has been most successful, this method being also used for the analysis of protein/carbohydrate interaction. However this methodology is not suitable for on-chip detection and structural characterisation. On the other hand, Matrix Assisted Laser Desorption Ionization (MALDI) is the method of choice for on-chip analysis. Despite its sufficient mass precision, MALDI analysis of native HS samples remains difficult and limited because of the difficulty of ionising such highly-charged molecules. We demonstrated earlier the possibility to combine ESI-TOF, MALDI TOF and MALDI TOF/TOF analyses to partially characterise heparin oligosaccharides up to the dp8 level. We have now developed several new MALDI TOF and MALDI TOF/TOF methodologies. This includes improving the characterisation of native heparin oligosaccharides, enabling us to detect intact species carrying up to 9 sulphate groups. We have also analysed specific protein/heparin interaction using MALDI TOF. Furthermore, we applied these new methodologies to other nano-tools developed by the UK Glycochips Consortium such as gold nanoparticles.

(31) **Sialoglycans Regulate Axon Regeneration after Central Nervous System Injury – the Therapeutic Potential of Sialidase**

Andrea Mountney[1]; Lynda J.S. Yang[2]; Matthew R. Zahner[1]; Ileana Lorenzini[1]; Katarina Vajn[1]; Lawrence P. Schramm[1]; Ronald L. Schnaar[1]
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The adult central nervous system (CNS) is inhibitory for axon regeneration, limiting recovery from CNS injuries. This is due, in part, to endogenous axon regeneration inhibitors that accumulate at CNS injury sites, including myelin-associated glycoprotein (MAG, Siglec 4). MAG on residual myelin binds to sialoglycans on axons to halt regeneration. MAG inhibition of axon outgrowth from neurons cultured in vitro is reversed by sialidase treatment, suggesting the same treatment might enhance regeneration in vivo. We tested this in two animal models. In one, rat nerve roots were cut as they exited the spinal cord and a peripheral nerve graft was inserted. Sialidase was delivered to the graft site for 14 days via osmotic pump. Dye tracking was used to quantify spinal axon outgrowth. Sialidase-treated rats had 2.6-fold greater spinal axon outgrowth ($p < 0.005$) than did control rats (Yang, et al., Proc. Natl. Acad. Sci. USA 103, 11057 (2006)). In a second model, rats were subjected to spinal cord contusion, a common form of spinal cord injury in humans. Sialidase was delivered intrathecally for 14 days. Animals were monitored for motor behavior for 5 weeks, then were tested for renal sympathetic nerve activity in response to blood pressure changes, a circuit compromised by spinal cord injury. Preliminary results indicate a therapeutic benefit from sialidase delivery in this model. The results imply that axonal sialoglycans are required for inhibition of axon regeneration, and that sialidase may improve recovery from CNS injuries. Supp. by NIH grants NS046669 and HL16315.

(32) **β1,3-N-Acetylglucosaminyltransferase 1 is Required for Axon Pathfinding by Sensory Neurons**

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Neurons of different sensory modalities in the peripheral nervous system have a unique glycan profile that is distinct from CNS neurons. The mAb 1B2 recognizes terminal N-acetyllactosamine on mature olfactory sensory neurons (OSNs) in the olfactory epithelium but does not interact with neurons in the brain. The glycosyltransferase β3GnT1 plays an essential role in lactosamine synthesis. In situ hybridization reveals that β3GnT1 is expressed by OSNs beginning at early embryonic stages. β3GnT1-/- mice lose lactosamine expression on OSNs and have severely disorganized axonal projections. This defect is accompanied by increased neuronal cell death in neonatal mice followed by an increase in neurogenesis. These results are supported by analysis of β3GnT1-/- mice expressing tau-LacZ or -GFP with specific odorant receptor (OR) subsets. Some OR-specific axons initially target the appropriate region but fail to reach their final target and die postnatally. Other axons bypass their normal targets to extend into inappropriate regions of the CNS. The differential affect of β3GnT1 loss on targeting may reflect the heterogeneity of lactosamine expression inherent to individual neurons. We have also examined the expression of galectins capable of mediating cell-cell and cell-matrix interactions during development. Galectins-1 and -9 are both expressed in axon pathways of the developing olfactory system suggesting that galectin/lactosamine interactions may play important roles in axon guidance. In summary, these results suggest that lactosamine glycans are required for establishing sensory connections, and for subsequent survival and homeostasis of olfactory neurons. Supported by NIH grant DC00953.

(33) **The LARGE Glycosyltransferase Family**

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Abberrant glycosylation of α-dystroglycan (α-DG), a receptor for extracellular matrix proteins, is a major pathological mechanism underlying congenital muscular dystrophy (CMD). Most of these forms of CMD are also associated with abnormal neuronal migration in the CNS. Loss of α-DG glycosylation has also been implicated in cancer. Thus far, seven genes have been identified that are required for functional glycosylation of α-DG. This glycosylation pathway is conserved across vertebrates, including chicken and zebrafish. We have focused on the *LARGE* gene, which is mutated in the myodystrophy mouse; an animal model of CMD. Vertebrate genomes contain a closely-related paralogous gene (*LARGE2*) that arose from an ancestral gene duplication event. Over-expression of either LARGE or LARGE2 in cultured cells induces hyperglycosylation and functional ligand binding of α-DG, although this biochemical activity is not yet defined. We are interested in determining the functions of these two genes. During mouse embryogenesis, expression of *LARGE* is mostly confined to the developing nervious system. In contrast, *LARGE2* is widely expressed with high levels in the sub-epidermis, in endodermal components of organs including kidney, lung and trachea, and in ectodermal derivatives such as olfactory epithelium, whisker and tooth buds. Therefore, *LARGE2* may play an important role in the glycosylation of α-DG (and other as yet undefined targets) during tissue formation in embryogenesis.

(34) **Role of Glypican-1 in Brain Development**

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Cell surface heparan sulfate (HS) acts as a co-receptor for numerous growth factors, morphogens, adhesive proteins, and guidance molecules, and has shown to be essential for many events in nervous system development. The major core protein carriers of HS are the syndecans and glypicans. Although five of the six vertebrate glypicans are expressed during brain development, glypican 1 (GPC-1) appears very early, and remains to become the major HS proteoglycan of the adult brain. To investigate the function of this molecule, we created mice with a disruption of the first coding exon of the glypican-1 (GPC1) gene. We also obtained a gene-trap allele of GPC1. Both types of GPC1-/- mice are protein-null, viable, fertile, and show no gross anatomical abnormalities. Their brains are patterned normally, except for a marked reduction or absence of the anteriormost lobe of the cerebellum, but are noticeably small: GPC1-/- brains contain 20% fewer cells, and weigh ~17% less than wildtype brains. Body size, in contrast, is normal. Heterozygous brains display an intermediate phenotype. Mutant animals exhibit brain size reduction of this magnitude as early as embryonic day 9.5 (e9.5), but are indistinguishable from wildtype one day earlier. Phosphohistone H3 and TUNEL staining indicate that the reduction in cell number in GPC1 mutant mice is due to a decrease in proliferation within the neuroepithelium. These data demonstrate an important role for glypican-1, prior to the onset of neurogenesis, in determining brain size.

(35) **N-Acetylmannosamine Treatment Rescues A Mouse Model of Hereditary Inclusion Body Myopathy**

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HIBM is an adult onset, recessive neuromuscular disorder involving progressive muscle atrophy and weakness. This debilitating myopathy results from deficiency of UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), the rate-limiting enzyme of sialic acid (SA) biosynthesis. Decreased GNE activity

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impairs SA production, which may interfere with sialylation of muscle glycoproteins such as a-dystroglycan and PSA-NCAM. We created GNE knock-in mice mimicking the Persian-Jewish GNE mutation, M712T. Homozygous (-/-) mutant mice did not survive beyond postnatal day 3 (P3). At P2, GNE-epimerase activity in -/- skeletal muscle was 20% of normal, but histological examination showed no muscle pathology. Rather, the -/- kidneys had subcapsular hemorrhages, proteinuria, and signs of glomerular disease. As a treatment option, we administered N-acetylmannosamine (ManNAc), which resulted in survival beyond P3 in 43% of the -/- pups. Surviving -/- mice were smaller than their littermates, but appeared healthy otherwise. At P2, -/- mice that received ManNAc had less severe kidney hemorrhages, their muscle GNE-epimerase activities increased to 50% of normal, and brain tissues showed increased sialylation of PSA-NCAM. It remains unclear if the surviving -/- mice will develop a muscular pathology later in life. Taken together, survival of -/- mice, improved kidney pathology, increased GNE activity and increased sialylation of PSA-NCAM after ManNAc administration strongly supports consideration of a clinical trial of ManNAc for the myopathy of HIBM.

(36) **GnT-Vb Expression Increases O-Mannosyl-Linked HNK-1 Epitope Leading to Changes in Neuronal Cell Adhesion and Migration**

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O-mannosyl-linked glycans constitute a third of all brain O-linked glycoproteins, and yet very little is understood about their functions. Several congenital muscular dystrophies with central nervous system defects are caused by genetic disruptions in glycosyltransferases responsible for the synthesis of O-mannosyl glycans. The glycosyltransferase GnT-Vb, also known as GnT-IX, is expressed abundantly in the brain and testis and is proposed to be the enzyme that branches O-mannosyl-linked glycans. We have discovered that GnT-Vb and PomGnT1, enzymes involved in the O-mannosyl glycosylation pathway, play an active role in modulating integrin and laminin-dependent adhesion and migration of human neuronal cells. Migration of neuronal cells is a vital component of neural development and the HNK-1 antigen is expressed on migrating neurons in the developing nervous system. We present evidence that GnT-Vb expression increases the expression of O-mannosyl linked HNK-1 epitope in a human neuronal culture model. Our results identify GnT-Vb as a key regulator of signaling pathways that control neuronal cell adhesion and migration

(37) **Modulation of the Host Immune Response by Schistosome Glycoconjugates**

Ellis Van Liempt[1]; Sandra Meyer[2]; Sandra J. Van Vliet[1]; Anneke Engering[1]; Boris Tefsen[1]; Caroline M.W. Van Stijn[1]; Rudolf Geyer[2]; Yvette Van Kooyk[1]; Irma Van Die[1]
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Infection with the parasitic helminth *Schistosoma mansoni* is a major cause of suffering and death especially in tropical areas. *S. mansoni* generates a large array of glycoconjugates that play a critical role in the immunobiology of schistosomiasis. The early stage of infection is characterized by a T helper 1 (Th1) response. After egg-laying, this Th1 response switches towards Th2, driven by the highly glycosylated egg antigens. In the host, dendritic cells (DCs) trigger the onset of cellular immunity. They recognize invading pathogens via receptors such as Toll-like receptors (TLRs) and C-type lectins. DCs internalize antigens for presentation to T cells, and provide signals that direct naïve Th cells to differentiate into Th1, Th2 or T-regulatory cells.

Here we present an overview of our ongoing research that is aimed at understanding the molecular mechanisms by which parasite glycans interact with DCs to induce polarized T cell responses. We showed that glycoconjugates from different parasite stages, cercariae, adult worms and soluble egg antigens (SEA), differentially induce activation of human monocyte-derived immature DCs and modulate TLR-induced DC maturation and cytokine production. SEA is captured and internalized by DC through the C-type lectins DC-SIGN, MGL and the MR, and the antigens are targeted to MHC class II-positive compartments. Our data strongly suggest that interaction of C-type lectins with parasite glycans is important to regulate the T cell polarizing capacity of the DCs.

Van Die I and Cummings RD (2006). Glycans modulate immune responses in helminth infections and allergy. *Chem Immunol Allergy* 90: 91-112

(38) **Role of M. tuberculosis Cell Wall Carbohydrates in Host Adaptation**

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Tuberculosis causes tremendous morbidity and mortality in the world. Critical in establishment of M. tuberculosis (M.tb) infection are entry and survival in the macrophage. The M.tb cell envelope is heavily glycosylated with the abundant mannose-containing lipoglycans lipoarabinomannan (ManLAM), lipomannan, and phosphatidyl-myo-inositol mannosides which bind to C-type lectins expressed on macrophages and dendritic cells (DCs). The mannose receptor (MR) is a prototypic pattern recognition receptor and C-type lectin that mediates phagocytosis of virulent strains of M.tb by human macrophages. Ongoing studies in the laboratory are providing evidence that the nature of surface mannosylation of M.tb has a major impact on the ability of the bacterium to interact with C-type lectins and thereby modulate macrophage responses. The MR is highly expressed on alternatively activated alveolar macrophages, binds to mannose motifs of both host and microbial origin, and is located on the chromosome within a susceptibility locus for mycobacterial infection. Thus, M.tb may exploit the MR's role as a scavenger receptor to enter the phagocyte in a form of molecular mimicry. We use molecular, biochemical, and cell biology techniques to characterize the biosynthesis of cell envelope mannosylated lipoglycans from M.tb strains and determine their impact on the biology of M.tb-macrophage interactions. This information should enhance our knowledge of TB pathogenesis and also potentially identify new bacterial therapeutic targets since mannose metabolism has been shown to be essential for the survival of mycobacteria.

(39) **The Glycobioloy of Nipah virus Entry**

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Nipah virus (NiV) is a Priority Pathogen in the NIH Biodefense Research Agenda and can cause up to a 70% mortality rate. Our research focuses on the glycan structures on the Nipah viral envelope that modulate target cell binding and membrane fusion. Deletion of selected N-glycan sites results in a hyperfusogenic phenotype, or rather, in viruses that can enter cells more efficiently. Nevertheless, we suggest that the NiV maintains these glycan structures to "shield" the Nipah viral envelope from neutralizing antibodies. Our studies are generating selectively deglycosylated Envs that are more neutralization sensitive, and may lead to design of a more potent immunogen. In addition, we have previously shown that an innate immune system lectin, galectin-1, can bind to N-glycans on the Nipah fusion protein and potently inhibit virus-cell fusion. We now report a novel mechanism by which gal-1 mediates its viral inhibitory effect, and show that a specific N-glycan site on the Nipah virus fusion gives rise to the cognate glycan structure(s) bound by gal-1. These results show that NiV entry is modulated by the complex calculus arising from the positive and negative interactions between N-glycans on its envelope glycoprotein and various arms of the host immune system.

(40) **Immune Recognition of Candida Albicans: The Taste of a Fungus**

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The outer layer of the cell wall of Candida albicans is heavily enriched in glycosylated proteins that is the immediate point of contact and interaction with the human host. The inner cell wall layer contains the two structural polysaccharides, chitin and b–1,3 glucan to which the mannoproteins are attached. We constructed a series of mutant strains in selected glycosyl transferase genes that led to truncation of the C. albicans O- and N-linked mannans and used these to explore the role of the glycans on fungal pathogenesis. We then used a combination of defined mutants of the pathogen surface and in pathogen pattern recognition receptors along with receptor-blocking agents to explore how C. albicans is recognised by the innate immune system. Cytokine production by human mononuclear cells or murine macrophages was markedly reduced when stimulated by C. albicans mutants defective in mannosylation. Recognition of mannosyl residues was mediated by mannose receptor protein binding to N-linked mannosyl residues, and Toll-like receptor 4 binding to O -linked mannosyl residues. Residual cytokine production was mediated by recognition of b–1,3 glucan by the dectin-1/TLR2 receptor complex. In conclusion, recognition of C. albicans by monocytes/macrophages is mediated by three recognition systems each of which senses a specific layer of the C. albicans cell wall.

References: Hobson et al JBC 2004;279:39628; Bates et al JBC 2005;280:23408; Munro et al JBC 2005;280:1051; ; Bates et al J Biol Chem 2006;281:90; Netea et al 2006 J Clin Invest. 116(6), 1642-1650

(41) **Arenaviruses Mimic the Molecular Mechanism of Receptor Recognition Used by alpha-Dystroglycans's Host-Derived Ligands**

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The arenavirus Lassa fever virus (LFV) is the causative agent of a severe hemorrhagic fever in humans and infects over 300, 000 people annually causing several thousand deaths. The cellular receptor of LFV is alpha-dystroglycan (alpha-DG), a cell surface receptor that provides an essential molecular link between the extracellular matrix (ECM) and the cytoskeleton. Alpha-DG is subject to a complex pattern of O-glycosylation that is crucial for its function. We demonstrate that two specific glycan modifications, protein O-mannosylation and modification by the glycosyltransferase LARGE are critical for alpha-DG's function as a cellular receptor for LFV and other arenaviruses. Interestingly, over-expression of LARGE restores alpha-DG's arenavirus receptor function in O-mannosylation deficient cells, indicating that sugars attached by LARGE, but not the O-mannosyl glycans themselves, are crucial for virus binding. Together, our data demonstrate a striking similarity in the molecular details of alpha-DG binding between arenaviruses and ECM proteins, suggesting that the viruses evolved to mimic the highly conserved mechanism of receptor recognition used by alpha-DG's host-derived ligands. As a consequence, the glycoprotein (GP) of LFV efficiently competes with the interaction of alpha-DG with ECM proteins and interferes with the normal function of this important cellular receptor, contributing to virus-induced host cell dysfunction. We are identifying alpha-DG-associated host cell proteins whose interactions, activation states, and cellular localization are changed by LFV binding. Such cellular proteins represent likely candidates for viral targets critically involved in the pathogenesis of LFV infections in humans.

(42) Functional Glycoproteomic Analysis of *Caenorhabditis elegans* Interaction with Bacterial Pathogens

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Although many glycoproteins have been implicated in important physiological and pathological processes, many more remain to be investigated and new methods must be developed for analysis of glycoproteins on a global scale (functional glycoproteomics). Our work on *Caenorhabditis elegans* suggests a unique approach to the problem. *C.elegans* has three genes encoding active UDP-GlcNAc:α-D-mannoside β1,2-*N*-acetylglucosaminyltransferase I (GlcNAcTI) involved in *N*-glycan synthesis. In contrast to the developmental abnormalities of GlcNAcTI-null vertebrates and flies, GlcNAcTI-triple-null worms develop into apparently normal adults. Wild type worms fed *E.coli* OP50 on PGS, an enriched high osmolarity medium, are killed by infection. Wild type larvae are killed by a toxin secreted by *Pseudomonas aeruginosa* strain 14 on PGS. Analysis of three single-null, three double-null and the triple-null mutants shows dramatic differences in their responses to these pathogenic bacteria. The data suggest: (a) each GlcNAcTI isoenzyme plays a distinct role in the interaction of *C.elegans* with the bacteria; (b) there are differences in the sites and/or times of expression of each GlcNAcTI gene; (c) each isoenzyme acts on a different subset of protein targets. We have developed a mass spectrometric technique to determine the protein subsets that carry only oligomannose, only paucimannose and both oligo- and paucimannose *N*-glycans in each of the three double-null worms (each worm expresses only one of the three GlcNAcTI genes). Identification of these nine relatively small protein subsets will yield information on the functions of *N*-glycans in worm-pathogen interactions. (Funded by CIHR).

(43) Immunization with MIC1 and MIC4 Induces Protective Immunity Against Toxoplasma gondii

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Toxoplasma gondii is an obligate intracellular protozoan parasite that infects virtually all nucleated cells in warm-blooded animals. The host cell invasion is tightly coupled to the apical release of micronemal proteins (MIC). We have previously reported that MIC1 is a lectin that can be co-purified with MIC4 (MIC1/4 fraction) by affinity chromatography on immobilized lactose from tachyzoites antigen of the T. gondii RH strain. Immunization of C57BL/6 mice with MIC1/4 fraction induced high serum levels of IgG1 and IgG2b specific antibodies. MIC1/4-stimulated spleen cells from immunized mice produced IL-2, IL-12, IFN-g, IL-10, but not IL-4, suggesting the induction of a polarized Th1 type immune response. When orally challenged with 40 cysts of the ME49 strain, the immunized mice had 68% fewer brain cysts than the control mice. Immunization was associated with 80% survival of the mice challenged with 80 cysts, contrasting with 100% mortality of the non-immunized mice in the acute phase. The exit of the immunization procedure motivated us to produced MIC1 recombinant protein. The cDNA encoding MIC1 have been cloned in a plasmid containing a histidine tag in N-terminal fusion with the region encoding the protein. The bacterial expression vector were used to transform the Escherichia coli strain BL21(DE3) Rosetta. The obtained recombinant MIC1 was purified and showed preserved lectin activity toward D-lactose. When used in immunization protocols, recombinant MIC1 triggered protective immune response against murine toxoplasmosis. We envisage taking advantage of the recombinant MIC1 as a vaccinal preparation able to confer protection against congenital and acquired toxoplasmosis

(44) Inhibition of *Helicobacter pylori* binding by Lewis b or sialyl-Lewis x Carrying Recombinant Mucin-Type Proteins Produced by Glyco-Engineered CHO Cells

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Helicobacter pylori is a gram-negative bacterium capable of inducing peptic ulcer disease, gastric adenocarcinoma and gastric lymphoma. It binds to host epithelial cells via the carbohydrate epitopes Lewis (Le) ^{b} and sialyl-Le^{x} (SLe^{x}), which therefore are likely to be of major importance for *H. pylori* infection.

Mucins are glycoproteins with large numbers of O-linked glycans, and may be ideal scaffolds for multivalent presentation of carbohydrate epitopes with therapeutic potential.

Stable CHO transfectants were engineered to express a mucin-immunoglobulin fusion protein together with the core 3 GnT-VI, GalT-V, FUT-II and FUT-III glycosyltransferase genes to obtain Le^{b}, or the core 2 GnT-I and FUT-VII genes to obtain SLe^{x} on the mucin-type protein. The fusion proteins were analyzed by Western blotting using carbohydrate-specific antibodies, and their released and permethylated O-glycans characterized by ion-trap mass spectrometry.

PSGL-1/mIgG$_{2b}$ produced by CHO-PLe^{b} cells was strongly stained by an anti-Le^{b} antibody. Likewise, PSGL-1/mIgG$_{2b}$ produced by the CHO-PSLe^{x} clone stained strongly with an anti-sialyl-Le^{x} antibody. These reactivities persisted after PNGase F treatment. Mass spectrometry (MS) and MS/MS confirmed the presence of sequences consistent with Le^{b} on core 3 and SLe^{x} on core 2, respectively. Initial *in vitro* studies indicate that the inhibitory capacity of the Le^{b} or sLe^{x} carrying fusion proteins on *H. pylori* adhesion to Le^{b} or sialyl-diLe^{x}-HSA neoglycoconjugates, respectively, are in the nano- to picomolar concentrations. The ability of these recombinant mucins to inhibit *H. pylori* adhesion and infection will be investigated in a mouse model of *H. pylori* infection in the near future.

(45) On the Role of Galectin-3 in Cancer Metastasis

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Galectin-3, a member of the β-galactoside-binding gene family, is a multifunctional protein implicated in a variety of biological functions, including tumor cell adhesion, proliferation, differentiation, angiogenesis, cancer progression and metastasis. Recent studies revealed that intracellular galectin-3 exhibits the activity to suppress drug induced apoptosis and anoikis (apoptosis induced by the loss of cell anchorage) that contribute to cell survival. Resistance to apoptosis is essential for cancer cell survival and plays a role in tumor progression. Conversely, it was recently shown that tumor cells' secreted galectin-3 induces T-cells' apoptosis, thus playing a role in the immune escape mechanism during tumor progression through induction of apoptosis of cancer infiltrating T-cells.

I'll summarize recent evidence on the role of galectin-3 as an anti-apoptotic and/or pro-apoptotic factor in various cell types and discuss the recent understanding of the molecular mechanisms of galectin-3 role in apoptosis. I'll also discuss potential therapeutic directions for further analyses of this multifunctional protein and the clinical implications.

(46) The Role of Glycosphingolipid Gb3 in Colon Cancer Invasiveness

Olga Kovbasnjuk[1]; Rakhilya Murtazina[1]; Oksana Gutsal[1]; Anne Kane[2]; Mark Donowitz[1]
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The most devastating aspect of cancer is the emergence of metastases. Thus, identification of potentially metastatic cells among a tumor cell population and the underlying molecular changes that switch cells to a metastatic state are among the most important issues in cancer research. In these studies we showed that in the metastatic stage of human colon cancer the

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glycosphingolipid Gb3 is significantly upregulated. In addition, a sub-population of cells with a migratory phenotype that are greatly enriched in Gb3 and are highly invasive was identified in human colon cancer cell lines. Transfection of Gb3 synthase into non-cancerous epithelial cells lacking endogenous Gb3 induced the invasive cell phenotype, while Gb3 knock out by siRNA from the colon cancer epithelial cells expressing Gb3 inhibit cell invasiveness, demonstrating that Gb3 is necessary and sufficient for cell invasiveness. Gb3 serves as the receptor for Shiga toxin 1. Uptake of the non-catalytic B-subunit of Stx1 by human colon cancer cells expressing Gb3 caused apoptosis of these Gb3-positive invasive cells. Significant up-regulation of Gb3 in human metastatic colon cancer and in invasive cells in colon cancer cell models suggests that Gb3 could be a marker and potential therapeutic target in colon cancer cells primed to metastasize. The mechanisms of Gb3 expression in colon cancer cells are under investigation.

(47) **Glycans in Cancer – Prognosis to Therapy, Invited Talk**

Steve Rosen
UCSF, San Francisco, CA

TBD

(48) **Development and Characterization of Peptide Mimics of TF-Antigen**

Jamie Heimburg[1]; Adel Almogren[1]; Sue Morey[1]; Olga V. Glinskii[2]; Virginia H. Huxley[2]; Vladislav V. Glinsky[2]; Rene Roy[3]; Richard Cheng[1]; Kate Rittenhouse-Olson[1]
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Thomsen-Friedenreich Antigen (TF-Ag), a carbohydrate tumor-associated antigen, is highly surface-expressed on several types of tumor cells, contributing to cancer cell adhesion and metastasis to sites containing TF-Ag-binding lectins. A highly-specific IgG3 monoclonal antibody developed to TF-Ag interferes with TF-Ag binding to vascular endothelium, blocking a primary step in tumor metastasis. Since antibodies to surface antigens can also be cytotoxic, development of a vaccine that generates antibodies towards TF-Ag would be clinically valuable. Since carbohydrate antigens generate T cell independent responses, identifying TF-Ag peptide mimics may be useful in generating T cell dependent responses. Research has shown antibody development to saccharide antigens and memory in response to peptide mimic immunizations. Further, T cells primed by peptide mimics react with carbohydrate antigens producing cellular responses and MHC molecules can display glycopeptides. This leads us to hypothesize that vaccinations using unique peptide mimics of TF-Ag will generate immune responses to TF-Ag epitopes on tumor cells which will be clinically useful in active immunotherapy of many cancers.

In vitro experiments using JAA-F11 antibody to TF-Ag confirmed peptide mimicry and in vitro model systems demonstrated peptide mimics blocked rolling and stable adhesion of cancer cells to vascular endothelium. Current experiments utilize linear and multiple antigenic peptides for immunizations and serum analysis by ELISA for TF-Ag-reactive antibody production. Molecular modeling and X-ray crystallography of peptide mimics and JAA-F11 are currently underway. This approach could decrease tumor burden in cancer patients by specifically targeting TF-Ag positive cancer cells and aid in blocking new tumor formation.

(49) **Expression of Tn and SialylTn Antigens in Human Tumor Cell Lines Raised from Mutation in Molecular Chaperone Cosmc**

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Tn (GalNAcα-Ser/Thr) and SialylTn (STn, NeuAcα2,6GalNAcα-Ser/Thr) antigens are tumor-associated carbohydrate antigens (TACAs) expressed by 60~70% of human carcinomas. Normally, Tn antigen is modified by the Core 1 β3GalT (T-synthase) to form the Core 1 structure, Galβ1,3GalNAcα-Ser/Thr (T-antigen). Recently, we found that T-synthase activity requires Cosmc, the Core 1 β3GalT-specific molecular chaperone, which prevents aggregation/proteosomal degradation of T-synthase. Although it was reported that Tn/STn antigen expression in human tumors are associated with poor prognoses, the genetic basis for their expression is not known. Here we show that expression of Tn/STn antigen in human tumor cell lines results from acquired mutations in Cosmc. T-lymphoblast Jurkat cells, which have minimal T-synthase activity and express Tn/STn antigens, contain a mutated Cosmc with a T-deletion at 469bp. Coloretical carcinoma LSC cells that lack T-synthase activity and express Tn/STn antigens contain a mutated Cosmc gene with a T-insertion at 26bp. Colorectical carcinoma LS174T is a mixed population that contains both Tn/STn positive and negative cells. While the Tn/STn negative cells with a high T-synthase activity contain a normal Cosmc, the Tn/STn positive cells have minimal T-synthase activity and have an A-deletion at 482bp. All these mutations result in a loss of chaperoning function of Cosmc due to its open reading frame shift. Introducing the wild-type Cosmc into Jurkat cells and LSC cells not only restore the T-synthase activity, but also correct the structures of O-glycans on cell surface. These results demonstrate that expression of Tn and STn result from mutations in Cosmc.

(50) **Glycoprotoemic Changes In Human Blood Serum Associated with Breast Cancer**

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Analysis of glycoproteins in body fluids and tissues of mammals presents a significant analytical challenges, but it also has a significant potential in a search for disease biomarkers. The analytical challenge is partially due to the ubiquity of glcyosylation and a frequent presence of important glycoproteins in only trace quantities. For example, glycoproteins shed into the blood stream from cancerous cells could be measured to enable cancer diagnosis in early stages or follow the effectiveness of therapy. To facilitate such measurement, we have used a combination of immunafinity depletion, lectin chromatography and high-temperature reversed-phase LC fractionation, fllowed by LC-MS/MS analyses. The complexity of human blood serum sample has been reduced as follows: Immunoaffinity depletion was carried out on a MARS column, resulting in an efficient removal of the six most abundant proteins (albumin, anti-trypsin, haptoglobin, IgA, IgG, and transferrin). Glycoproteins present in the depleted sample were enriched using affinity chromatography employing four lectins with different specificities (Con A Canavalia ensiformis, SNA-I Sambucus nigra, UEA-I Ulex europaeus, PHA-L Phaseolus vulgaris) immobilized on agarose gels, further desalted and subjected to high-temperature reversed phase fractionation providing efficient separation and excellent recoveries. Collected fractions were subsequently digested and subjected to LC/MSMS analysis utilizing LTQ FT MS. The combination of methodologies focused on a substantial reduction of sample complexity and a targeted lectin affinity enrichment facilitated the assessment of glycoproteins changes associated with breast cancer. Human blood serum glycoproteomes isolated from both healthy individuals and cancer patients using this methodologies were compared.

(51) **Mechanisms of Cell Adhesion through Selectin-glycan Interactions Under Flow**

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Binding of selectins to cell-surface glyoconjugates enables leukocytes to tether to and roll on activated platelets and endothelial cells and on other leukocytes. The leukocyte mucin PSGL-1 mediates interactions with L-selectin on other leukocytes and with P-selectin on activated platelets and endothelial cells. However, P- and L-selectin differ in the kinetics, affinity, and mechanical properties with which they bind to PSGL-1. L-selectin also binds to peripheral node addressin (PNAd), a group of lymph node endothelial-cell mucins that present the recognition determinant 6-sulfo-sLex as a capping structure on O-glycans. L-selectin requires a threshold shear to enable leukocytes to tether to and roll on vascular surfaces. Transport mechanisms govern flow-enhanced tethering, whereas force governs flow-enhanced rolling by prolonging lifetimes of L-selectin-ligand complexes (catch bonds). Using selectin crystal structures, molecular dynamics simulations, site-directed mutagenesis, single-molecule force and kinetics experiments, Monte Carlo modeling,and flow-chamber adhesion studies, we found that eliminating a hydrogen bond to increase flexibility of a hinge between the lectin and EGF domains of L-selectin reduced the shear threshold for adhesion by two mechanisms. One affects on-rate by increasing tethering through greater rotational diffusion. The other affects off-rate by strengthening rolling through augmented catch bonds with longer lifetimes at smaller forces. By forcing open the hinge angle, ligand may slide across its interface with L-selectin to promote rebinding, thereby providing a mechanism for catch bonds. Thus, allosteric changes remote from the ligand-binding interface regulate both bond formation and dissociation.

(52) **Transcriptional Basis for Selectin Ligand Expression by Th1 Cells**

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Control of cell surface glycosylation is critical for various facets of effective immunity, including migration of effector leukocytes to sites of infection and inflammation via recognition of endothelial selectins. We are interested in

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deciphering the signaling and transcriptional mechanisms which underlie surface expression of glycans which function as selectin ligands on CD4+ T lymphocytes, and the relationship between these mechanisms and those controlling Ag-driven CD4 cell differentiation. Previous work by numerous investigators has shown that, in vitro, Th1 cells express substantially more selectin ligands than Th2 cells or unpolarized (Th0) cells. Separately, the importance of the transcription factors Stat4 and T-bet to Th1 differentiation has been well documented. Our previous work has documented a substantial defect in both E-selectin ligands and P-selectin ligands on activated Stat4-/- Th1 cells, due to a failure to induce both C2GlcNAcT-I and another glycosyltransferase. We have recently extended this analysis to show that T-bet-/- CD4 cells show a very similar phenotype, and we have identified ST3Gal-VI as a second enzyme whose induction in Th1 cells requires both Stat4 and T-bet. Thus, Th1 differentiation and Th1 cell expression of selectin ligands have a shared transcriptional basis. Our results also allow us to assign induction of glycosyltransferases responsible for selectin ligand biosynthesis to two broad categories: induction of FucT-VII and ST3Gal-IV occurs via TCR engagement, likely accounting for the low level of selectin ligands on unpolarized activated T cells, whereas induction of C2GlcNAcT-I and ST3Gal-VI in Th1 cells occurs via IL-12-triggered Stat4 activation and T-bet activity.

(53) 6-Sulfo Sialyl Lewis X on both *N*- and *O*-Glycans Play Critical Roles as L-Selectin Ligands

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Lymphocyte homing is mediated by specific interactions between L-selectin on lymphocytes and sulfated carbohydrate addressin expressed on high endothelial venules (HEV) of lymph nodes. We previously demonstrated that 6-sulfo sialyl Lewis X is present as capping structures on core 2 branch and/or core 1 extended *O*-glycans (Cell 105:957-969, 2001). Further two sulfotransferases were shown to cooperatively contribute to the synthesis of L-selectin ligands, and lymphocyte homing and contact hypersensitivity was reduced in the double knockout mouse (Nat Immunol 6:1096-1004 and 6:1105-1113, 2005). To determine the roles of 6-sulfo sialyl Lewis X on core 2 branch and extended core 1 structures, we have recently generated mutant mice deficient in both core 2 and core 1-extension enzymes. Surprisingly, the double deficient mice exhibited a significant remaining lymphocyte homing activity, although *O*-glycan containing GlyCAM-1 from the double deficient mice lack 6-sulfo sialyl Lewis X and does not support lymphocyte rolling at all. The remaining L-selectin ligand activity on HEV was judged to be on *N*-glycans as *N*-glycosidase abrogated L-selectin binding to HEV, *N*-glycan-specific E-PHA inhibited lymphocyte homing and binding to HEV, and 6-sulfo sialyl Lewis X was demonstrated on *N*-glycans. Using L-selectin-IgM chimera, one of the major counter-receptors in double deficient mice was identified as CD34. Interestingly, contact hypersensitivity was slightly compromised in the double deficient mice. These results suggest that *N*-glycan-based L-selectin ligands provide robust lymphocyte homing but L-selectin ligands on both *N*-and *O*-glycans are required for lymphocyte recruitment during inflammatory response. Supported by NIH grants CA71932 and CA48737.

(54) Sialylation-Dependent Regulation of α4β1 Integrin Receptors

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In response to inflammatory stimuli, monocytes become activated and simultaneously begin differentiating along the macrophage lineage. These processes are accompanied by the activation of multiple integrin receptors. The α4β1 integrin binds to VCAM-1 on the endothelium and thereby facilitates monocyte extravasation. We hypothesize that α4β1 activation is due, in part, to the synthesis of a β1 integrin species that lacks α2-6-linked sialic acid, a modification added by the ST6Gal I sialyltransferase. During in vitro differentiation of monocytes into macrophages, which can be induced by phorbol esters such as PMA, the expression of ST6Gal I is downregulated, which leads in turn to hyposialylation of the β1 integrin subunit. The expression of hyposialylated α4β1 receptors is temporally correlated with increased cell adhesion to VCAM-1. In addition, ST6Gal I downregulation, integrin hyposialylation, and VCAM-1 binding are all directed by a PKC/ras/ERK signaling cascade, a pathway known to be in involved in monocyte differentiation. Importantly, preventing the synthesis of hyposialylated integrins, via forced expression of ST6Gal I, blocks VCAM-1 binding. The mechanisms underlying ST6Gal I downregulation during monocyte differentiation are not yet understood, however our recent results suggest that ST6Gal I is cleaved by BACE1, a protease that has not previously been identified in monocytes/macrophages. We find that BACE1 is markedly upregulated during monocyte differentiation (via a PKC/ras/ERK signaling cascade), and the time course for BACE1 upregulation is in good agreement with the time required for loss of cellular ST6Gal I. Taken together, these results describe a novel mechanism for regulation of α4β1 integrins.

(55) Platelets Generate Inflammatory and Angiogenic Fragments of Hyaluronan

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Breakdown products of hyaluronan (HA) promote angiogenesis and wound healing. Size-dependent HA fragments induce pro-inflammatory and pro-angiogenic factors from macrophages, dendritic, endothelial and tumor cells, but the origin of such signaling-sized HA fragments are unknown. We find that endothelial cells generate HA cables in response to a TNF-α that are adhesive for leukocytes and other cells. We now find that platelets also bind to these HA structures. Platelets carry a specific degradative enzyme, hyaluronidase-2 (Hyal-2), that in vitro rapidly breaks down the HA cables preferentially to the proper signaling sized HA fragments. Hyal-1 the other major somatic hyaluronidase, degrades HA to very small oligomers, not known to be inflammatory. Platelets carry only Hyal-2 with no evidence for Hyal-1. Platelets thus break HA down to signaling-sized fragments, and unlike all other tissues, do not have the ability to degrade such fragments further. The data are consistent with a model of an inflammatory loop whereby perturbed small vessels bind platelets and leukocytes to HA cables, principally by CD44. The platelet Hyal-2 creates signaling sized breakdown products from the HA structures. The generated fragments in turn act locally on leukocytes to trigger synthesis of pro-inflammatory cytokines and chemokines, and stimulate endothelial cells to produce pro-angiogenic factors. The leukocytes generate TNF-α, that feed back on the endothelium to produce even more HA, thus perpetuating the cycle. Thus platelets are the origin of signal-sized HA fragments. These observations have implications for autoimmune disorders, for chronic inflammatory conditions, and the subsequent fibrotic response.

(56) NKT Cells Recognize Different Types of Bacterial Glycolipids

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Natural Killer T (NKT) cells are highly conserved T lymphocyte subpopulation. They express an invariant T cell antigen receptor (TCR) α chain and they recognize glycolipids presented by CD1d. We recently reported that the invariant TCR expressed by mouse and human NKT cells recognizes glycosphingolipids with α branched sugars purified from Sphingomonas bacteria. The structure of a Sphingomonas glycolipid bound to CD1d, determined by X ray crystallography, shows the lipid tails buried in the CD1d groove and the single galacturonic acid sugar exposed for TCR recognition. The in vivo NKT cell response to Sphingomonas is driven by TCR recognition and mice that lack NKT cells have reduced bacterial clearance. Sphingomonas are not highly pathogenic, however, and it remained unknown if NKT cells can recognize other classes of glycolipids derived from pathogenic microbes. We now show that mouse and human NKT cells recognize glycosyl diacylglycerols from Borrelia burgdorferi, which causes Lyme disease. Interestingly, the response to these compounds was highly dependent on the nature of the aliphatic chains, with the addition of a single unsaturated bond having an enormous influence on antigenic potency. NKT cells are activated during B. burgdorferi infection, and the glycolipid from B. burgdorferi stimulated NKT cell cytokine release. This response required TCR recognition and was independent of MyD88 activation of APC. These data provide evidence that NKT cells recognize diacylglycerol microbial glycolipids, which are more broadly distributed in pathogenic microbes than glycosphingolipids containing ceramide, and they suggest that this TCR-mediated recognition provides protection from microbial pathogens.

(57) Mammalian N-Glycosylation Inhibits Innate Immune Mechanisms that Induce and Mediate Autoimmune Disease

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Autoimmune diseases are a group of pathogenic syndromes that can engage both innate and adaptive immune systems in cellular activation responses that override normal mechanisms of self-tolerance. The interplay between innate and adaptive immunity in the initiation of autoimmune disease has been increasingly studied during the early phases of pathogenesis. Yet mammalian

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autoimmune diseases are thus far characterized as syndromes mediated by the adaptive immune system, and typically reflect pathogenic defects that can be transferred upon hematopoietic reconstitution by bone marrow grafts. In contrast, we find that the absence of the alpha-mannosidase-II (αM-II) enzyme induces an autoimmune disease diagnostic of systemic lupus erythematosus that originates from innate immune system activation by endogenous stimuli residing among radiation-resistant somatic cells. Hematopoietic reconstitution using αM-II null donors does not induce or transfer disease, nor does wild-type marrow inhibit disease pathogenesis. Remarkably, loss of the adaptive immune system in animals lacking both αM-II and RAG-1 amplifies tissue pathogenesis coincident with elevated macrophage recruitment, increased severity of glomerulonepthritis, and impaired kidney function. Intravenous IgG treatment attenuates macrophage recruitment and iNos induction while maintaining normal kidney function. αM-II deficiency interferes with mammalian N-glycan branching thereby exposing mannose residues at the cell surface and modulates endogenous mannose receptor expression, implicating this lectin-based innate immune recognition system in autoimmune disease pathogenesis. These findings imply that the evolutionary acquisition of complex N-glycan branching in vertebrates afforded the innate immune system the ability to distinguish glycomes of pathogenic organisms from host N-glycosylation in promoting mechanisms of self-tolerance.

(58) **Dendritic Cells and the Recognition of Glycan Structures to Mediate Cellular Communication and Immune Responses**

Yvette van Kooyk
Molecular Cellbiology and Immunology, VUmc, Amsterdam, the Netherlands

Dendritic cells (DCs) are specialized in the recognition of pathogens as well as self antigens and play a pivotal role in the control of immunity and tolerance. C-type lectin receptors expressed by DC are involved in the recognition and capture glycosylated self-antigens and pathogens. We have studied in great detail the function and the glycan specificity of the DC-specific C-type lectin DC-SIGN and MGL. DC-SIGN recognizes high mannose structure and Lewis antigens, whereas MGL recognizes GalNAc structures. Both C-type lectins are involved in pathogen and tumor cell uptake, which has been suggested to lead to immune escape.

To date little is know on the specificity of C-type lectins for self-glycoproteins. We observed that the expression of Lewis antigens on neutrophil regulates neutrophil DC interactions through DC-SIGN, illustrating an important function of C-type lectins to mediate cellular interactions with subsets of cells expressing specific glycans structures. More importantly, we recently identified that MGL expressed by DCs regulates effector T cell function by its interaction with CD45. Our results indicate that MGL binding negatively regulates TCR mediated signaling and cytokine responses. It is clear that our understanding of the diversity of C-type lectins being expressed on DC, as well as their carbohydrate specific recognition profile, opens a new area of molecular interactions mediated by protein-glycan interactions. This will further allow us to understand the complexity of 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.

(59) **Glycans in Immune Development and Function, Invited Talk**

Carrie Miceli
UCLA, Los Angeles, CA

TBD

(60) **Role of protein mannosylation in linking innate and adaptive immune responses to fungi**

Stuart M Levitz
UMass Medical Center, Worcester, MA

Cryptococcus neoformans is a major pathogen in persons with deficient T-cell immunity. The glucuronoxylomannan capsule confers virulence to the fungus. My laboratory has been studying C. neoformans mannoproteins (MP), a heterogeneous group of antigens that elicit cell-mediated immune responses in mice and humans. MP share a C-terminal serine/threonine-rich region, that is the site of heavy O-linked mannosylation, followed by a glycosylphosphatidylinositol (GPI) anchor that presumably serves as a cell wall attachment site. We have hypothesized that the extensive mannosylation plays an essential role in immune stimulation by targeting MP to mannose receptors (MR) on antigen-presenting cells (APC). Two MR, the macrophage mannose receptor (MMR) and dendritic cell-specific ICAM-3-grabing nonintegrin (DC-SIGN), were shown to bind MP. Conversely, MR blockade with mannosylated ligands reduced uptake of MP and inhibited T-cell activation. The immunodominant APC responsible for immune stimulation was shown to be dendritic cells (DC). The kinetics of MP capture by DC were rapid and dependent on MR. By confocal microscopy, intracellular MP colocalized with MHCII, MMR and DC-SIGN. Model vaccines containing an antigenic fragment of ovalbumin (OVA) have been engineered. Mannosylated OVA made in the yeast Pichia pastoris stimulated a greater MHCII-dependent CD4+ immune response compared with unglycosylated counterparts made using E. coli vectors. These studies suggest that DC provide the crucial link between innate and adaptive immune responses to C. neoformans via a process by which MR efficiently bind and internalize MP, leading to antigen presentation and the initiation of an effective T cell response.

(61) **Calpain Mediated Cytoskeletal Cleavage during Galectin-1 Induced T-Cell Death**

Mabel Pang; James He; Linda G. Baum
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Galectin-1 induces death of human and murine T cells and thymocytes. The galectin-1 T cell death pathway is distinct from other cell death pathways; however, intracellular signaling pathways and intracellular events in galectin-1 induced T cell death are not well understood. The goal of our studies is to define intracellular events critical in galectin-1 induced T cell death. Galectin-1 binds to several T cell surface glycoprotein receptors that regulate susceptibility to cell death, including CD45. CD45 is a large transmembrane glycoprotein; the extracellular domain is abundantly N- and O-glycosylated and binds galectin-1, and the intracellular domain binds the cytoskeletal linker protein fodrin that connects to the actin cytoskeleton. We have found that fodrin cleavage occurs during galectin-1 death of Jurkat T cells. Fodrin cleavage requires the action of mu-calpain, as a mu-calpain inhibitor blocks fodrin cleavage, while inhibitors of other calpains had no effect on fodrin cleavage. Fodrin fragments remain associated with CD45 after cleavage, and the role of CD45 in regulating susceptibility of fodrin to mu-calpain cleavage is addressed. Fodrin cleavage is not required for certain hallmarks of cell death, such as phosphatidylserine externalization, membrane permeability, release of mitochondrial effectors or DNA degradation, as mu-calpain inhibition did not affect these events. However, cytoskeletal breakdown may be critical for morphologic changes in dying cells, or engulfment of dying cells by phagocytes; we are currently examining these events.

(62) **Interaction of GM3 with N-Linked GlcNAc of Epidermal Growth Factor Receptor (EGFR) Inhibits EGFR Tyrosine Kinase**

Seon-Joo Yoon[1]; Kenichi Nakayama[2]; Toshiyuki Hikita[1]; Kazuko Handa[1]; Sen-itiroh Hakomori[1]
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Epidermal growth factor receptor (EGFR) plays an essential role in control of epithelial cell growth, particularly that of cancers. EGFR tyrosine kinase is known to be inhibited by GM3, but not other gangliosides (1), surrounding the receptor in microdomain. The inhibitory effect of GM3 on EGFR tyrosine kinase is now defined by interaction of N-linked glycan having multivalent GlcNAc termini with GM3, through carbohydrate-to-carbohydrate interaction (2), based on the following evidence: (i) EGFR band (molecular mass ~170 kDa), stained by its specific antibody, is also stained by mAb (J1) or lectin (GS-II), both directed to GlcNAc termini of N-linked glycan. (ii) The fraction separated by GS-II contained ~170 kDa band stained by anti-EGFR antibody, and bound specifically to GM3-liposomes or GM3-coated microspheres. (iii) GM3 inhibitory effect on EGFR tyrosine kinase was abrogated in vitro by co-incubation with glycan having multiple GlcNAc termini, and also in cell culture in situ incubated with the same glycan. (iv) Cells treated with 1□deoxymannojirimycin expressed high mannose-type, GlcNAc phospho-Man, or hybrid-type structure. (v) Cells treated with swainsonine expressed complex-type, in part with hybrid-type. Cells (iv) and (v) both displayed higher GM3-dependent inhibition of EGFR kinase than cells not treated with inhibitors. These findings indicate that the target of GM3 which inhibits EGF-induced EGFR tyrosine kinase is the N□linked glycan with terminal GlcNAc linked to EGFR.

References: (1) Bremer E, Schlessinger J, Hakomori S (1986) JBC 261: 2434-40. (2) Yoon S, Nakayama K, Takahashi N, et al., Glycoconj J, in press.

(63) **Structural Features of Galectins that Regulate Receptor Recognition and Intracellular Signaling in T Cell Death**

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The galectins have many functions, including regulation of cell proliferation, survival, adhesion, migration and death. Several galectins, including galectins-1, -2, -3, -7, and –9, have been reported to induce death of T cells and other types of cells. While the galectins have similar carbohydrate recognition domains (CRDs), distinct intracellular death pathways have been reported for

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galectins-1, -3, and –9. The mechanisms responsible for the utilization of different death pathways by various galectins are unknown. However, as galectins fall into three distinct structural sub-families, structural differences among galectins, in addition to differences among the CRDs, may contribute to the ability of galectins to trigger different intracellular signaling pathways. To elucidate the structural features responsible for triggering distinct cell death pathways, we made a series of galectin constructs, including bivalent galectin-1, two galectin-1 CRDs on a galectin-9 linker peptide, and two galectin-1 CRDs on a rigid spacer. We have compared these constructs with respect to potency in agglutination and cell death assays, glycoprotein receptor recognition, and intracellular signaling events. These data demonstrate that the presentation of the CRDs determines the cell death pathway triggered by galectin binding, and indicate that receptor recognition is regulated by CRD presentation as well as CRD specificity for glycan ligands. These findings contribute to our understanding of the distinct biologic roles played by different galectins in various tissues, and have implications for design of novel galectins targeted to specific cell types.

(64) **Structure and Biological Significance of *Trichomonas vaginalis* LPG**

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The parasitic protozoan *Trichomonas vaginalis* causes one of the most common non-viral sexually transmitted infections worldwide, and has been linked to increased incidence of HIV transmission, vaginitis, and several other complications related to women's reproductive health. The molecular pathways of inflammatory and immune responses to *T. vaginalis* are poorly understood. Previously, we reported the partial characterization of a major cell surface glycoconjugate, LPG, from *T. vaginalis*. Unlike other GPI molecules, TV-LPG contains no Man and has polylactosamine repeats. We extended our studies in further defining the biochemical nature and also the novel biological functions of LPG. Glycan fractions from LPG were released with enzymatic and mild acid treatments and were characterized by MALDI-TOF MS. Monosaccharide composition was analyzed by HPAE-PAD. The mild acid released PI- core portion of LPG contained GalN, Rha, Gal, GlcN, Glc, and Xyl (molar ratio 1:17.5:13.8:24:8.3:3.7). Mass spectra of released PI-core showed signals in the region m/z 8700 - 9300. Endo-β-galactosidase released saccharides predominantly correspond to lactosamine-based structures; further digestion with TFA led to diverse products, rich in Rhm. Intact TV-LPG (but not LPG from *T. foetus*, the causative agent of bovine trichomoniasis) induced IL-8 and MIP (macrophase inflammatory protein)-3α chemokines in human squamous epithelial cell types, representing the lower female genital tract mucosa. LPG fractions containing various domain(s) of the TV-LPG molecule initiated distinct dose-dependent inflammatory responses. We hypothesize that TV-LPG triggers the mucosal inflammatory reaction by signaling via receptors on the reproductive tract epithelial cells.

(65) **Dietary and Genetic Control of Pancreatic Beta-Cell Glucose Transporter Glycosylation Promotes Insulin Secretion in Suppressing the Pathogenesis of Type-2 Diabetes**

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We have previously reported that the *Mgat4a*-encoded Golgi-resident GlcNAcT-IVa (GnT-4a) glycosyltransferase is required for the production of an N-glycan structure that appears to act as a ligand for endogenous lectin receptors that maintain Glucose Transporter-2 (Glut-2) residency on the β cell surface. Administration of a high-fat diet, genetic disruption of *Mgat4a*, or competitive inhibition of lectin binding by glycan ligand mimetics all result in a severe reduction in Glut-2 cell surface expression, by inducing glycoprotein-selective and cell-type-specific endocytosis with redistribution of Glut-2 into β cell endosomes and lysosomes. GnT-4a deficiency thereby abolishes the first phase of glucose-stimulated insulin secretion resulting in hyperglycemia, increased circulating free fatty acids, elevated expression of liver gluconeogenic enzymes, hepatic steatosis, and insulin resistance. These phenotypic features encompass a metabolic disorder diagnostic of type 2 diabetes. Therefore GnT-4a glycosyltransferase expression and Glut-2 glycosylation are under dietary and genetic control, typically maintaining pancreatic β cell surface Glut-2 expression and insulin secretion in normal physiologic contexts and thereby suppressing the pathogenesis of type 2 diabetes. We have further investigated the cellular and physiologic processes regulating *Mgat4a* gene transcription in response to dietary intake and have analyzed human pancreatic β cells for the presence of a similar regulatory mechanism that controls glucose transporter glycosylation and endocytosis. Our current progress will be presented.

(66) **A Putative Role for the Involvement of β-N-Acetylglucoseaminyltransferase (OGT) in Membrane Associated Signaling**

Simon Amzalleg; Daniel Fishman; Jacob Gopas; Shira Elhyany-Amzalleg; Shraga Segal

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O-GlcNAc post translational modifications of cellular proteins have initially described in the early 80's (CR. Torres and G.W. Hart). Since then its high prevalence have been well documented, describing a wide range of O-GlcNAc modified nucleocytoplasmic proteins with different functions e.g. cytoskeleton proteins, transcription factors, chaperones, metabolic enzymes and major group of proteins involved in signal transduction pathways like kinases, phosphatases and adaptor proteins, establishing this form of post translational modification as inducible and dynamic.

The enzyme OGT which catalyzes the addition of O-GlcNAc moieties to the target proteins has been described to be predominatingly located to the cytoplasm and nucleus. Here we describe the localization of OGT to the cell surface membrane in three different cloned cell lines, employing confocal microscopy and immunoblotting. Distribution of OGT at the plasma membrane implies that the enzyme is localized to membrane associated micro-domains, thus contributing to the complexity of cellular signal transduction pathways.

(67) **The Research on the Antioxidant and Hypoglycemic Activity of Polysaccharide from Tea**

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Tea polysaccharide had high antioxidant activity and it could be used to cure diabetes. Antioxidant activity of tea polysaccharide (TPS) from three kinds of tea (green tea, oolong tea and black tea) were compared, the result indicated that oolong tea polysaccharide (OTPS)had the highest antioxidant activity. In order to explicate the mechanism of antioxidant and hypoglycemic activity, the streptozotocin(STZ)-induced diabetes mice model(DM) was established. The influence of OTPS on blood-glucose, content of MDA and NO, and activities of GSH-PX, SOD, NOS in serum, kidney and liver were investigated. The result showed that after four weeks injection of OTPS to DM mice, the blood-glucose of three treatment group reduced by 14.5%, 21.5% and 33.3%, respectively, comparing to the model control. The reduction effect of OTPS increased with the rise of dose. The activity of SOD and GSH-PX elevated significantly, while the activity of NOS decreased. The content of MDA and NO reduced significantly. The above results imply that antioxidant activity was enhanced. Comparing to XKW treatment, the effect of a dose of 300mg/(kg ·bw) OTPS was much better. The research showed that the OTPS had a significant effect on reducing blood glucose, and could enhance the antioxidant activity of DM mice.

(68) **Does the Cytoplasmic/Nuclear Tobacco Lectin Bind to Endogenous N-Glycans?**

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Treatment of tobacco leaves with the plant hormone jasmonic acid results in the expression of the Nicotiana tabacum agglutinin, also called Nictaba, in the nucleus and the cytoplasm. The possible in vivo interaction of Nictaba with endogenous glycoproteins was corroborated using a combination of biochemical analyses and confocal microscopy of an EGFP-Nictaba fusion protein expressed in tobacco BY-2 cells. In vitro binding studies demonstrated that the expressed EGFP-Nictaba possesses carbohydrate-binding activity. Microscopic analyses confirmed the previously reported cytoplasmic/nuclear localization of Nictaba and provided evidence that the lectin is not uniformly distributed over the nucleus and cytoplasm of BY-2 cells but specifically binds to certain cell structures or glycoproteins. Western blot analysis of extracts from whole BY-2 cells and purified nuclei revealed that Nictaba interacts in a glycan inhibitable way with numerous proteins including many nuclear proteins. Enzymatic deglycosylation with PNGaseF indicated that the observed interaction depends on the presence of N-glycans. Glycan array screening confirmed that Nictaba exhibits a strong affinity for high-mannose N-glycans. Though still preliminary the localization in the cytoplasmic/nuclear compartment of a plant lectin that has a high affinity for high-mannose N-glycans and specifically interacts with conspecific glycoproteins suggests that N-glycosylated proteins might be more important in the cytoplasm and nucleus than is currently believed.

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(This work was funded primarily by Ghent University and in part by NIGMS - The Consortium for Functional Glycomics GM62116.)

(69) **Tissue-specific Roles for GNE in Cell Growth**

zhiyun wang; Zhonghui Sun; Kevin J Yarema
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The UDP-GlcNAc 2-epimerase/ManNAc 6-kinase bifunctional enzyme, referred to as 'GNE,' is known to regulate metabolic flux into the sialic acid biosynthetic pathway. Consequently, single amino acid mutations in GNE found in the human congenital disease hereditary inclusion body myopathy (HIBM) were initially expected to reduce sialic acid biosynthesis in patients. Clinical evidence, however, has not borne out that a significant decrease in overall levels of sialic acid occurs in HIBM suggesting that GNE may have a second cellular role that contributes to disease abnormalities. Moreover, another unexplained aspect of HIBM is the mechanism that restricts symptoms to skeletal muscle. In this study, roles for GNE in different cell types were tested and the findings offer clues to explain mechanistic basis of HIBM. Over-expression of rGNE, or down-regulation by siRNA, in HEK293 (human embryonic kidney) and SJCRH30 (human skeletal muscle) cells led to opposite changes in mRNA levels for GM3 and GD3 synthases as well as for the biosynthetic products of these sialyltransferases, the GM3 and GD3 gangliosides. Similarly, exogenous GM3 and GD3 changed the expression of GNE in opposite directions in HEK293 and SJCRH30 cells and GNE over-expression or reduction led to opposite effects on cell growth in these lines. Finally, changes to BiP expression and ERK1/2 phosphorylation consistent with apoptosis and proliferation, respectively, were observed. These results establish roles for GNE outside of sialic acid production and lay out cell line-specific metabolic links between GNE and gangliosides that may explain the selective pathology of skeletal muscle in HIBM.

(70) **The Influence of Gestational Diabetes Mellitus on N-acetyl-β-D-hexosaminidase Activity in the Blood Serum**

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

Background: Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It is the most common medical condition of pregnancy. Diabetes in pregnancy poses numerous problems for both mother and fetus. Hyperglycaemia causes chronic tissue damage.

N-acetyl-β-D-hexosaminidase (HEX) catalyzes removal of N-acetylglucosamine or N-acetylgalactosamine residues from the non-reducing end of oligosaccharide chains of glycoconjugates. This enzyme activity is found in the biological fluid like blood serum as well as in tissues. Measurment of HEX activity in urine is going to be nephropathy, hepatopathy, abstinence monitoring in alcohol diseases, diabetes and porphyria damage indicator. A consistent increase of HEX activity in serum has been reported during pregnancy.

The main object of our investigation was the determination of the changes in HEX activity in gestational diabetes mellitus.

Material and Methods: Blood serum of seventeen pregnant women with diagnosed gestational diabetes mellitus (GDM) after a 75-g oral glucose tolerance test, mean age 32 years (range 24-39) and 19 non-pregnant healthy women mean age 29 years (range 22-37) as a control group.

N-acetyl-β-D-hexosaminidase activity (pKat/kg protein) was measured by the method of Chatteriee modified by Zwierz.

Results: The main activity of HEX was: patients with GDM - 85,476 pKat/kg protein, control group – 17,991 pKat/kg protein.

Conclusions: We observed statistically significant increase of HEX activity in GDM compared to the control group. We are going to compare HEX activity in pregnancy among women with and without GDM.

(71) **Modeling a CDG-Ib Intestine in vitro: Silencing Phosphomannose Isomerase Predisposes for Intestinal Epithelial Protein Leakage**

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Congenital Disorder of Glycosylation Ib (CDG-Ib) is caused by hypomorphic mutations in the gene encoding for phosphomannose isomerase (PMI), which result in 5-15% remaining PMI enzyme activity. Mannose supplements bypass the PMI bottleneck and correct the clinical symptoms, one of which is protein-losing enteropathy (PLE), the loss of plasma proteins through the intestine. PLE onset is often associated with infections and a pro-inflammatory state. Most intriguing is the specific loss of heparan sulfate (HS) proteoglycans (HSPG) from the basolateral surface of intestinal epithelial cells only during PLE episodes. We have shown in previous studies that loss of HS(PG) directly contributes to protein leakage and it also amplifies cytokine-induced protein leakage. However, mechanistic links between reduced PMI activity, loss of HS(PG), inflammatory cytokines, and PLE are unknown. We hypothesized that reduced PMI activity impairs HSPG N-glycosylation, affecting HSPG biosynthesis, intracellular trafficking, shedding or degradation. To verify this hypothesis, we used siRNAs to silence PMI activity (<10%) in human intestinal epithelial cells (HT29) and determined albumin flux in transwell assays. Reduced PMI activity amplifies TNFα-induced protein leakage, which can be completely reversed with mannose. However, effects of complete HS loss and reduced PMI activity are additive, suggesting that the effects of reduced PMI activity are not solely mediated through loss of HS(PG). We now aim to provide insights into alternative mechanisms that link reduced PMI activity with an increased incidence for PLE. (Supported by NIH R01 DK065091, R21 HL 078997, Children's Hearts Fund, and DFG BO 2488/1-1).

(72) **Ganglioside GM2/GM3 Inhibits HGF-Induced cMet Activation and Cell Motility in Bladder Epithelial Cells, through Functional Organization of Components in Glycosynapse**

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Glycosphingolipids (GSLs) at the cell surface membrane are associated or complexed with signal transducers (Src family kinases, small G-proteins), tetraspanins, growth factor receptors, and integrins. Such organizational framework, defining GSL-modulated or –dependent cell adhesion, motility, and growth, is termed "glycosynapse" (Hakomori S, Handa K, FEBS Lett 531(1): 88-92, 2002). We describe here differences in composition and functional organization of glycosynaptic microdomain in normal cells vs. highly-malignant tumor cells, taking as example transitional epithelial bladder cells and their transformants. Our results show that: (i) In normal bladder epithelial cells, ganglioside GM2 is complexed with tetraspanin CD82. This complex inhibited functional interaction ("cross-talk") of integrin □3 or □1 with cMet, whereby hepatocyte growth factor (HGF)-induced cMet tyrosine phosphorylation was strongly suppressed. Treating normal cells with P4, which depleted GM2 or abrogating CD82 expression by RNAi method, greatly enhanced HGF-induced cMet phosphorylation and cell motility. (ii) In contrast, highly invasive bladder cancer cells, YTS1, are characterized by HGF-independent cMet activation and cell motility. cMet activation and cell motility are inhibited by co-expression and mutual interaction of GM2 and GM3 with CD82, as observed in YTS1 cells transfected with CD82 gene; or by the exogenous addition of GM2 and/or GM3. Low ganglioside level in HCV29 cells caused focal adhesion kinase (FAK) translocation into glycosynapse and further activation of MAPK pathway.

(73) **Cell Signaling Mediated by Carbohydrate-to-Carbohydrate Interaction: GM3 Binds to EGFR via N-Linked Oligosaccharide and Regulates Autophosphorylation**

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EGFR is a transmembrane N-glycosylated glycoprotein with an intracellular kinase domain. EGFR undergoes dimerization by ligand binding and activated kinase domain, resulting in occurrence of autophosphorylation. Glycolipid GM3, having sialyllactose, binds to EGFR and thereby inhibits its kinase activity through receptor dimerization. We show that inhibition of GM3-dependent EGFR kinase is based on interaction between N-linked oligosaccharide of EGFR and oligosaccharide of GM3. GM3 oligosaccharide binds to N-glycan with terminal GlcNAc, but not to high mannose type or sialylated complex type N-glycans. Therefore, A431 cells were treated with two antisense oligomers which respectively knock down the β1,4galactosyltransferase (β4GalT) gene and mannosidase IB gene, thereby inducing enhanced expression of N-glycan with terminal GlcNAc and of high mannose type N-glycan associated with EGFR. This relationship was further supported by observed increase of WGA or ConA binding to EGFR bands, and increase of N-linked glycan with GlcNAc termini or with high mannose type structure. Subsequently, GM3-dependent effect on EGFR autophosphorylation was examined in A431 cells whose N-linked glycan was modified by knockdown of β4GalT and mannosidase IB as above. EGFR autophosphorylation was inhibited by GM3 more strongly in β4GalT knockdown cells than in control cells. On the other hand, EGFR autophosphorylation in A431 cells with mannosidase IB knockdown was not clearly inhibited by GM3. These results indicate that GM3 sugar chain

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recognizes the N-glycan of EGFR and that EGFR autophosphorylation is regulated by such interaction. Carbohydrate-to-carbohydrate interaction between glycolipid and glycoprotein appears to be a key factor for cell surface signaling.

(74) Core Fucosylation is Crucial for the Function of Growth Factor Receptor(s)

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alpha 1,6-Fucosyltransferase (Fut8) catalyzes the transfer of a fucose residue to N-linked oligosaccharides on glycoproteins via an alpha 1,6-linkage to form core fucosylation in mammals. Disruption of Fut8 induces severe growth retardation, early death during postnatal development and emphysema-like changes in mice. Absence of core fucosylation on EGF or PDGF receptor results in down-regulation of the receptors-mediated signaling which is plausible factor that may be responsible for the growth retardation. Reintroduction of the Fut8 gene to Fut8-null cells potentially rescued these receptors-mediated signaling impaired in null cells. We found that the core fucosylation of N-glycans is required for the binding of the EGF to its receptor, whereas no effect was observed for the expression levels of EGF receptor on the cell surface. Interestingly, the expression levels of VEGF receptor 2, an important factor for vascular development, were decreased in Fut8 null mice lungs. Now we focus on whether the alteration in VEGF receptor 2 is associated with apoptosis exclusively observed in Fut8 null mice lung. Collectively, these results suggest that core fucosylation regulates growth factor receptors-mediated biological functions with different manners.

(75) Structural and Functional Differences between Human and Non-Human Cell Expressed Human Cytokines and Growth Factors

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Glycosylation of recombinant proteins is dependent on the machinery of the cell line in which they are made. Hence recombinant human proteins made in a human cell line may differ significantly from the same protein made in NS0, CHO, *E. coli* or any other non-human cell line. In this study we looked at the structure and function of recombinant human cytokines and growth factors produced from human cells versus the same recombinant human proteins produced from non-human cell lines.

Our studies showed major differences in the glycosylation between recombinant human proteins produced from modified human 293 cells compared to CHO or NS0 cell line products. We have also found differences in in vitro and in vivo function, particularly immunogenicity, immunoreactivity and stability. For example, we have shown that glycosylated human cell expressed IL-4 is more stable in cell culture than non-glycosylated IL-4, and that recombinant human cell expressed erythropoietin (EPO) had a different immunoreactivity profile to rh EPO from CHO cells. Preliminary assays also showed a difference in the ability of the human versus non-human cell expressed growth factors to stimulate growth and differentiation of human cells.

These results support our hypothesis that production of human proteins from human cell lines, with unique human glycosylation patterns, results in proteins which function more effectively than recombinant human proteins expressed from non-human cell lines.

(76) Bacterial Symbionts Induce a *fut2*-dependent Fucosylated Niche on Colonic Epithelium Via a TLR-4 Sentinel that Activates ERK and JNK Signaling

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Mature mammalian gut contains a complex dynamic microbial ecosystem. This seems to be supported by the highly fucosylated glycans expressed on the epithelium of colonized adult gut. Initial intestinal colonization of mouse gut by adult microbiota coincides with an abrupt increase in fucosylated glycan expression. Uncolonized gut contains little fucosylated glycan, but contains a fucosylated form of TLR-4 not present in colonized gut. The fucosylated TLR-4 seems to act as a sentinel for bacterial species. Initial colonization, or recovery from antibiotic treatment, activates TLR-4-associated ERK and JNK pathways, elevating and activating transcription factors c-fos, ATF2, and c-jun; this results in transcription of *fut2*-mRNA, induction of fucosyltransferase activity, and expression of the highly fucosylated intestinal mucosa characteristic of adult mammalian gut. Blocking the ERK and JNK signaling cascade inhibits induction of *fut2*-mRNA levels and fucosyltransferase activity. We conclude that pioneer mutualist symbiont bacteria communicate their presence to the intestinal epithelium of the mammalian host by binding fucosylated TLR-4. This activates the ERK and JNK signaling cascades which induce transcription of *fut2*-mRNA, increase expression of fucosyltransferase activity, and expression of fucosylated glycans in the mammalian colon that promote succession of resident microbiota toward an adult microbial ecosystem.

(77) Disruption of O-GlcNAc Cycling Mimics Diabetes Mellitus in *C. elegans*

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A dynamic cycle of O-linked N-acetylglucosamine (O-GlcNAc) addition and removal, through the action of two highly conserved enzymes OGT (O-GlcNAc transferase) and OGA (O-GlcNAcase), alters the function of various protein substrates. In addition, these enzymes mediate the final step in the nutrient-driven 'hexosamine-signaling pathway', which may be deregulated in diabetes. Here, we describe the first viable and fertile animal knockouts of OGA and OGT. Further, we report that a loss of function of either enzyme, which effectively blocks O-GlcNAc cycling, caused changes in Ser- and Thr-phosphoprotein profiles as well as alterations in glycogen, trehalose and lipid stores. These striking metabolic changes prompted us to examine the insulin-like signaling pathway controlling nutrient storage, longevity and dauer formation. Dauer formation is a stress response in C.elegans that leads to a reversible growth arrest. To measure subtle effects on the ability to form dauer, we used a sensitized genetic background containing a temperature-sensitive mutant of the insulin receptor homologue, daf-2. In this background we found that the OGA null strain augmented dauer formation, which approximates an insulin-resistance model. Conversely, the OGT null strain diminished dauer formation, which approximates an insulin hyper-sensitivity model. Our findings suggest that the enzymes of O-GlcNAc cycling 'fine-tune' insulin-like signaling in response to nutrient flux. The knockout of OGA in C. elegans mimics human insulin resistance with respect to several metabolic and signaling changes. The C. elegans OGA mutant provides a viable and genetically amenable animal model for the study of non-insulin dependent diabetes.

(78) Heparan Sulfate Proteoglycan Modulation by Inflammation

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Heparan sulfate proteoglycans (HSPGs) mediate many cellular processes involving protein-protein interactions of the extracellular matrix or at the cell surface, e.g. cytokine or growth factor signalling. Local as well as systemic changes in HSPGs alter cellular and tissue functions. Heparinoids can compete for or replace HSPGs and are effective therapuetic agents. HSPG expression is decreased in inflammation and heparinoids are anti-inflammatory. Thus there appears to be an antagonistic relationship between HSPG synthesis and inflammation. We have explored this relationship by qPCR and RNAi. Expression of genes involved in inflammation, heparan sulfate synthesis, e.g. EXT1, EXT2, and the protein components of HSPGs, e.g. syndecans, glycpicans, perlecan, was measured by qPCR in rat chondrosarcoma cell cultures exposed to inflammatory agents. Similarly genes involved in expression of inflammation or HSPGs were knocked down by shRNAs and HSPG associated gene expression was measured.

(79) Non-Anticoagulant 2,3-de-O-Sulfated Heparin as a Potential Novel Therapeutic for Patients with Protein-Losing Enteropathy

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Subcutaneous injections of high-molecular weight, anticoagulant heparin (100-500 U/kg) reverse intestinal protein leakage in some patients with protein-losing enteropathy (PLE). However, the underlying mechanisms are unknown and long-term application has considerable side effects, e.g. bleeding, thrombocytopenia, and osteoporosis. Our aim was to establish cell-based assays and in vivo models to induce protein leakage, reverse it with heparin, and screen for non-anticoagulant heparin-like molecules that also prevent or reverse protein leakage, but without undesirable side effects. Screening of >30 different heparin-like compounds in our cell-based assay revealed that non-anticoagulant 2,3-de-O-sulfated heparin (2/3DOS) is as potent in reducing cytokine-induced protein leakage as heparin. Based on these in vitro results, we assessed its effects in the mouse. Mice were injected

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daily with either heparin (i.v., 100 U/kg; 714 μg/kg) or 2/3DOS (714 μg/kg). Intestinal protein leakage was induced with IFNγ/TNFα injections. Both heparin and 2/3DOS reduced cytokine-induced intestinal protein leakage by 50%. There was no significant difference between the effects of heparin and 2/3DOS. At a 5-fold higher concentration both heparin and 2/3DOS completely abolished IFNγ/TNFα-induced protein leakage. 2/3DOS has only 2% of the anticoagulant activity of unmodified heparin and therefore could be administered in even higher doses without this undesirable coagulation side effect. Since 2/3DOS has already passed phase-I clinical trials for other acute indications, it may be a safer and more efficient therapy for PLE patients in the future! (Supported by NIH R01 DK065091, R21 HL 078997, Children's Hearts Fund, and DFG BO 2488/1-1)

(80) **A Small Molecule Neutralizing Agent for Heparan Sulfate**

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Heparan sulfate plays an integral part in a variety of biological processes by binding and interacting with many growth factors and receptors, adhesion molecules and enzymes. To study the significance of these interactions, pharmacological agents to block binding could prove extremely useful. We have examined a number of different candidates and found a low molecular weight agent (MW = 372) that binds to heparin and neutralizes its anticoagulant activity. It works equally well on low molecular weight heparins, but not on the pentasaccharide, Arixtra®. Here we show that the compound also inhibits binding of FGF to cell surface heparan sulfate (IC_{50} ~3 μM), heparan sulfate dependent FGF signaling through its tyrosine kinase receptor in endothelial cells (IC_{50} ~10 μM), and FGF-stimulated branching morphogenesis of mammary epithelial cells (IC_{50} ~10 μM). It also blocks transfer of sulfate from PAPS to 2-O-desulfated heparin by recombinant heparan sulfate uronyl 2-O-sulfotransferase. The ability of the compound to bind and block heparan sulfate dependent interactions demonstrate the feasibility of finding agents to interfere with deleterious processes mediated by heparan sulfate, e.g., tumor angiogenesis and growth. Detailed structure-function studies are underway to determine the mechanism of binding and neutralization.

(81) **Modulation of the Secreted and Membrane Glycoproteome of Adipocytes via the Induction of Insulin Resistance**

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Insulin resistance precedes and is the hallmark of type II diabetes, a disease that affects more than 9% of the American adult population. We and others have demonstrated that elevated O-GlcNAc levels induce insulin resistance. Furthermore, we have recently demonstrated that elevated O-GlcNAc levels, similar to the induction of classical insulin resistance, modulate the secretion of a number of polypeptides, adipocytokines, from adipose tissue. Here we identify a number of novel secreted proteins from primary adipocytes that are regulated by the insulin sensitivity of the cell using shotgun proteomic techniques. Further, we characterize and quantify the glycans that are present on both the secreted as well as the membrane proteins of adipocytes under insulin responsive and insulin resistant conditions. Nanospray ionization (NSI)-linear ion trap mass spectrometry (MSn) was applied to characterize permethylated sodiated glycans. N-glycanase and beta elimination were used to release the N- and O- linked glycans, respectively. Relative glycan quantification was carried out via permethylation with methyl iodide-C12 or -C13 for insulin resistant and insulin responsive conditions, respectively. The results of these experiments highlight changes in glycoproteins secreted from adipocytes upon alterations in insulin sensitivity. This work is supported by the American Heart Association (LW) and NIGMS (MT).

(82) **Elevation of Intracellular Glycosylation, O-GlcNAc, Attenuates the Anti-Apoptotic Effect of Insulin in CHO-IR Cells**

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O-GlcNAc modification, a ubiquitous and dynamic intracellular glycosylation on serine and threonine residues of polypeptides, is implicated as a sensor for glucose flux through the hexosamine biosynthetic pathway and has been linked to the development of insulin resistance, a general clinical symptom for type II diabetes, in cell culture and animal models. Excess flux of hexosamine has been shown to attenuate the anti-apoptotic action of insulin, via the PI3K/Akt pathway, in retinal neurons. In this study, we show that insulin can protect CHO-IR cells from serum-withdrawal induced-apoptosis similar to that in retinal neurons. Moreover, elevation of global O-GlcNAc levels by either pharmaceutical or genetic approaches effectively diminishes the protective effect of insulin with a concomitant defect in Akt phosphorylation. However, elevated O-GlcNAc levels are unable to attenuate the anti-apoptotic effects of transiently transfecting cells with a constitutively active form of Akt, further supporting the findings that the functional target for O-GlcNAc modification is at or upstream of Akt in the signaling pathway. To further illustrate the impact of O-GlcNAc modification on the insulin signaling cascade through the PI3K/Akt pathway, signaling molecules participating in this pathway, including IRS, PI3K, PDK1, Akt, PTEN and SHIP2, are examined for the presence of the O-GlcNAc modification. These results, in conjunction with others, clearly demonstrate a role for O-GlcNAc levels in modulating insulin-mediated signal transduction pathways at or upstream of Akt. This work is supported by the Georgia Cancer Coalition (LW).

(83) **Down Regulation of EGFR-Trypsin-PAR2 Pathway in FUT8 Deficient Mice**

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Alpha1,6-fucosyltransferase (Fut8) plays important roles in physiological and pathological conditions. Fut8 deficient (Fut8-/-) mice exhibit growth retardation, earlier postnatal death and emphysema-like phenotype. To investigate the underlying molecular mechanism by which growth retardation occurs, we examined the mRNA expression levels of Fut8-/- embryos (18.5 dpc) using a cDNA microarray. The DNA microarray and real-time PCR analysis showed that a group of genes, including trypsinogens 4, 7, 8, 11, 16 and 20 were down-regulated in Fut8-/- embryos. Consistently, the expression of trypsinogen proteins was found to be lower in Fut8-/- mice in the duodenum, small intestine and pancreas. Trypsin, an active form of trypsinogen, regulates cell growth through a G-protein coupled receptor, the proteinase-activated receptor 2 (PAR-2). In a cell culture system, a Fut8 knockdown mouse pancreatic acinar cell carcinoma, TGP49-Fut8-KD, showed growth rate decreased, similar to that seen in Fut8-/- mice, and the decreased growth rate was rescued by the application of the PAR-2 activating peptide, SLIGRL-NH2. Moreover, EGF-induced receptor phosphorylation was attenuated in TGP49-Fut8-KD, which was highly associated with a reduction of trypsinogens mRNA levels. The addition of exogenous EGF recovered trypsinogen mRNA expression in TGP49-Fut8-KDs. Our findings clearly demonstrate that the EGFR-trypsin-PAR-2 pathway is suppressed in TGP49-Fut8-KDs as well as in Fut8-/- mice.

(84) **The Acute-Phase Protein α1-Acid Glycoprotein Induces Cytosolic Ca2+ Rises in Neutrophils via siglec-5 (CD170)**

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α1-acid glycoprotein (AGP) is a highly glycosylated acute-phase protein with unclear cell biological functions. AGP have been suggested to be able to modulate leukocyte functions. The aim of the present study was to elucidate possible signalling capacities of AGP on neutrophil granulocytes.

Flow cytometry studies showed that FITC labelled AGP bound specifically to the neutrophil surface. Furthermore, when passing a protein lysate from neutrophils over a column with immobilized AGP the neutrophil cell surface protein, siglec-5 (CD170) was bound to the column. Desialylation of AGP by neuraminidase treatment resulted in loss of binding to siglec-5.

Using fura-2-loaded neutrophils, we found that AGP induced a small rise in [Ca2+]i. However, incubating neutrophils with monoclonal antibodies against L-selectin markedly enhanced the following Ca2+ response induced by AGP. A src-tyrosine kinase inhibitor (PP2) reduced, and a phospholipase C inhibitor (U73122) abolished the effect of AGP

The effect of AGP was further characterised by pre-incubating neutrophils with antibodies directed towards siglec-5. We found that siglec-5 antibodies antagonised the AGP-induced rise in [Ca2+]i. The importance of sialic acid residues was confirmed by using a desialylated form of AGP. This form of AGP produced a significantly smaller rise in [Ca2+]i.

Based on the results, we suggest that AGP binds to siglec-5 on the surface of neutrophils and this interaction results in a rapid rise in [Ca2+]i. Thus, AGP may be considered as a signalling molecule that directly participates in the regulation of neutrophil functions.

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(85) **Specificity of Galectin-1 in Cell Composition**

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Galectins mediate cell-cell and cell-matrix adhesion, proliferation and apoptosis during inflammatory processes and malignant transformation. Although there have been many studies on specificity of galectins determined by solid phase assays, up to date there have been a few studies on the carbohydrate binding properties of cell-associated proteins. The aim of this work was to study galectin-1 specificity in cell composition and compare these data to that obtained by solid phase assays [1-3]. Galectin-1 was loaded on Raji cells (that do not express galectins) and probed with fluorescein labeled polyacrylamide glycoconjugates (Glyc-PAA-fluo) where Glyc is oligosaccharide; binding was determined by flow cytometry. Glycoconjugates containing terminal LacNAc bind to galectin-1-loaded cells, the degree of binding expanded as the number of LacNAc repeats in the glycans increased:

3´,6´-(LacNAc) $_2$-LacNAc ≈LacNAc1-3(GlcNAcβ1-6)LacNAc≈LN_3> LNnT> LN-3LN>

LN6-LN>>LacNAc. Linkage of terminal Gal residue in 3´,6´-(LacNAc) $_2$-LacNAc is highly important for binding because galectin-1 loaded cells lacked detectable binding to LacNAc1-6´(GlcNAcβ1-3´)LacNAc and 3´,6´(GlcNAcβ) $_2$LacNAc. 3´-O-Su-Le^c and LacNAc that displayed high affinity to galectin-1 in solid phase assays [1] did not significantly bind to galectin-1-loaded cells. (The work is supported by the grant of Russian Foundation for Basic Research N 04-04-49689).

1. (http://www.functionalglycomics.org)

2. Stowell et al., Glycobiology, 2004, 14, 157 – 167

3. Leppanen et al., J Biol Chem., 2005, 280, 5549 - 5562

(86) **Carbohydrate-Binding Properties of Galectins in Composition of Cellular Membrane**

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Galectins are expressed on various cell types and exhibit a variety of biological functions. Because of insufficient information regarding the carbohydrate specificity of cellular galectins an experimental model was developed where human galectins-1, -2, -3, -4, -7 were loaded on Raji cells (non galectin expressed) and probed with synthetic β-galactoside glycoconjugates PAA-fluorescein probes. A strong binding of LNnT to galectin-1, -2, -4 and -7 –loaded cells was observed. Galα1-3´LacNAc was most potent for galectin-3. $T_{\beta\beta}$, asialoGM1 and TF probes displayed high affinity to galectin-4-loaded cells. In order to elucidate how galectins are attached to the cell surface we performed number of inhibition experiments. Galectin-1 did not load on cells pre-incubated with PNA. Thus, galectin-1 dimer utilizes one of carbohydrate-recognition domain (CRD) for anchoring to surface glycoconjugates, whereas the second CRD remains free for external binding. On the contrary, galectin-3 preserved ability to bind cells in presence of PNA as well as to bind probes; cell loaded truncated galectin-3 (without collagen-like domain) did not bind Glyc-PAA-fluo. Thus, due to multivalent architecture galectin-3 potently competes with PNA for the binding to cell surface glycoconjugates. Masking of galectins by *cis*-ligands was studied. Degalactosylation of galectin-1- or galectin-3-loaded cells significantly improved binding to LacNAc, whereas binding to more affine LNnT and

Galα1-3´LacNAc remained unchanged. Thus, galectins can be selectively masked on cell surface by high-affinity *cis*-ligands. (The work is supported by the grant of Russian Foundation for Basic Research N 04-04-49689).

(87) **Site-Specific Glycosylation Analysis of hFSH Isoforms**

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Follicle-stimulating hormone (FSH) glycosylation is regulated by feedback from the gonads resulting in a bewildering array of glycans associated with FSH preparations. FSH glycosylation varies due to inhibition of FSHβ N-glycosylation and elaboration of 1-4 branches possessed by mature N-glycans. To characterize FSH glycosylation, FSH isoforms in pituitary gland extracts and a variety of physiological fluids are commonly separated by chromatofocusing. Variations in the ratios of immunological and biological activities in the resulting FSH isoform preparations are generally attributed to changes in glycosylation, which are defined in terms of sialic acid content. Human (h)FSHβ glycosylation inhibition assessed by Western blotting revealed 30 to 47% non-glycosylated hFSHβ associated with four of six hFSH isoform preparations derived by chromatofocusing. Glycopeptide mass spectrometry assessment of glycan branching in these isoforms extensively characterized two N-glycosylation sites, one at αAsn52, the critical glycan for FSH function, and the other at βAsn24. With 2-4 N-glycans/FSH molecule many combinations of charges distributed over these sites can provide the same isoelectric point. Indeed, several glycans were common to all isoform fractions analyzed. There was no trend showing predominantly monoantennary glycans associated with the high pI fractions and predominantly tri- and tetra-antennary glycans associated with low pI fractions. Thus, differences in receptor-binding activity could not be associated with any specific glycan type or location in the hormone. FSH aggregation was associated with reduced receptor-binding activity, but did not affect immunological activity. Supported by NIH RR16475 and RR017708, NSF under EPS-9874732, and matching support from the State of Kansas.

(88) **Polysialylation of the Neural Cell Adhesion Molecule in Mutant Mice with Variable Numbers of Functional Polysialyltransferase Alleles**

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Poly-α2,8-sialic acid (polySia) is an important post-translational modification of the neural cell adhesion molecule (NCAM) and plays an essential role during brain development. The biosynthesis of this unique carbohydrate polymer is catalyzed by two closely related polysialyltransferases, ST8SiaII and ST8SiaIV. To gain insight into the role that each enzyme plays in the polysialylation process, we inter-crossed ST8SiaII and ST8SiaIV knock-out mice and compared the NCAM polysialylation profiles in brain samples of nine generated genotypes with different combinations of polysialyltransferase allele numbers. Gene dose-dependent alterations in ST8SiaII and ST8SiaIV transcript levels resulted in distinct changes in the polySia level and the ratio of polysialylated to non-polysialylated NCAM. While in perinatal brains of wild-type and ST8SiaIV-null mice close to 100% of the expressed NCAM was in the polysialylated form, the fraction of polysialylated NCAM was reduced to 55% and 30% in ST8SiaII-/- ST8SiaIV+/+ and ST8SiaII-/- ST8SiaIV+/- mice, respectively. By contrast, a single functional allele of ST8SiaII (ST8SiaII+/- ST8SiaIV-/-) was sufficient to modify approximately 90% of the NCAM pool, demonstrating a remarkable imbalance in the ability of the two enzymes to modify the complete NCAM pool. Furthermore, using quantitative real-time RT-PCR, we determined that in perinatal wild-type brain the ST8SiaII transcript level is only two-fold higher than the ST8SiaIV level. In ST8SiaII and ST8SiaIV knock-out mice, loss of one enzyme was not compensated by increased transcription of the remaining polysialyltransferase gene, indicating independent gene regulation.

(89) **Translation Attenuation by PERK in Response to ER Stress Rectifies Impaired $Glc_3Man_9GlcNAc_2$-P-P-Dolichol Synthesis and N-linked Glycosylation**

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In the endoplasmic reticulum (ER), nascent polypeptides are glycosylated by the transfer of $glucose_3mannose_9GlcNAc_2$ ($G_3M_9Gn_2$) from the lipid-linked oligosaccharide (LLO) $Glc_3Man_9GlcNAc_2$-P-P-dolichol ($G_3M_9Gn_2$-P-P-Dol) to asparaginyl residues (N-linked glycosylation). Further digested N-linked $G_3M_9Gn_2$ (high-mannose processing intermediates) play important roles in protein folding, quality control, and degradation. Accumulation of LLO intermediates disturbs ER homeostasis and causes ER stress. ER stress activates a set of coordinated signals known collectively as the Unfolded Protein Response (UPR). In response to ER stress, autotransphosphorylation of resident ER membrane protein PERK (PKR-like kinase) results in phosphorylation of eukaryotic initiation factor 2α(eIF2α) and eIF2α-P interferes with translation initiation. As a result, translation attenuation by PERK diminishes the load of ER client protein, thus reduces stress. Several lines of evidence suggest that metabolic deficiencies affecting $G_3M_9Gn_2$-P-P-Dol synthesis or N-linked glycosylation might be compensated by ER stress responses.

Here we use brief glucose deprivation to simulate LLO biosynthesis disorders, and show that in response to ER stress, PERK reduces LLO consumption by

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attenuating synthesis of glycoprotein precursor polypeptides. This allows more time for extension of LLO intermediates to $G_3M_9Gn_2$-P-P-Dol, and corrects impaired N-linked glycosylation. This process requires both PERK kinase function and phosphorylation of $Ser^{51}$of eIF2α. Similar results can be achieved by activating cytoplasmic stress-dependent Ser^{51} kinases. In conclusion, by sensing ER stress from defective glycosylation, PERK rectifies impaired $Glc_3Man_9GlcNAc_2$-P-P-dolichol synthesis and N-linked glycosylation through translation attenuation.

(90) **Production of Recombinant Yeast Mannosyltransferase Complex M-Pol I.**

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N-glycans in *S. cerevisiae* consist of a core region to which large outer-chains consisting of an α1,6-linked mannose backbone substituted with mannose side chains can be attached. The M-Pol I complex consisting of Mnn9p and Van1p initiates polymerization of the α1,6-linked mannose residues [1].

To study its structure, sub-unit interactions and mechanism of action, M-Pol I was expressed in *P. pastoris* as a soluble secreted heterodimer. Constructs encoding Mnn9p and Van1p in which the N-terminal transmembrane domains were replaced with the α-factor signal sequence in pPICZαA and pPIC9 vectors, respectively, were used for sequential transformation of the *P. pastoris* KM 71 strain. Mg quantities of Mnn9p and Van1p were secreted in a 1:1 ratio by dual transformants. In contrast, neither of the single transformants secreted the proteins. Recombinant M-Pol I catalyzed the addition of at least two α1-6 mannose residues to α1-6 mannobiose from GDP-[^{3}H]mannose, as shown by digestion with linkage-specific α-mannosidases.

The M-Pol I complex and its sub-units will be purified for structure determination by X-ray crystallography to elucidate the catalytic mechanism of M-Pol I. This will be useful for design of novel antifungal agents since this yeast-specific enzyme activity contributes to virulence of *C. albicans* [2].

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(91) **Cloning and Expression of an α-KDOase from the Oyster, Crassostrea virginica**

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Despite the wide occurrence of KDO, very little is known about the degradation of this unique sugar. We have previously reported that the hepatopancreas of oyster (*Crassostrea virginica*) contains an α-KDOase capable of releasing KDO from lipopolysaccharides (Li, et al. *J. Biol. Chem.* 272: 26419, 1997). We have subsequently purified oyster α-KDOase and prepared tryptic peptides. Using the oligonucleotide primers designed from these peptides and the cDNA library of oyster hepatopancreas, we have cloned the gene (*kdo*) that encodes oyster α-KDOase. The open reading frame of the full-length gene (*kdo-full*) consists of 1,176-bp nucleotides encoding a protein with 392 amino acid residues (molecular mass 44,604; pI 9.20). The matured native protein contains only 237 amino acid residues (molecular mass 26,940; pI 9.56). The N-terminus of the mature protein starts at the S^{156} ($A^{466}GT$) of the full-length sequence. Two Asp boxes, S^{277}PDDGKTW^{284} and S^{386}EDSAEIW^{393}, commonly found in various sialidase sequences, are present in the KDOase sequence. It also contains a quasi Asp box, S^{156}EDSAEIW^{163}, at the N-terminus, but does not contain the FRIP-motif that is also conserved in sialidases. The *kdo-full* gene contains two initiation codons, M^1 and M^{18}. Employing plasmid pET12a and pET15b under a variety of conditions, we were unsuccessful in expressing an active α-KDOase in *E. coli* as detected by using 4-methylumbelliferyl-α-KDO (MU-α-KDO) as substrate. However, we were able to obtain a protein with MU-α-KDO cleaving activity expressed by plasmid pcDNA/*kdo-full* in CHO-S cells or by plasmid pYES/*kdo-full* in *Sccharomyces cerevisiae*.

(92) **The *C*-terminal Assembly Module of Endosialidases and Other Tail Spike Proteins: an Example for Divergent or Convergent Evolution?**

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Endosialidases are tail spike proteins of bacteriophages infecting *Escherichia coli* K1. The endosialidase-catalyzed degradation of the bacterial polysialic acid capsule represents the key step in the infection cycle of K1-specific phages. All endosialidases characterized so far are composed of three modules: a variable *N*-terminal capsid-binding domain, a central catalytic part, and a short *C*-terminal domain which is released by proteolytic cleavage. The intact *C*-terminal domain is essential for assembling the catalytic homo-trimer but is dispensable in the mature enzyme [1,2]. Using circular dichroism and size exclusion chromatography, we could show that the *C*-terminal domain of the endosialidase of bacteriophage K1F (endoNF) can be expressed separately with similar secondary and quaternary structures as the proteolytically processed *C*-terminal domain. We identified analogous *C*-terminal domains also in proteins lacking endosialidase activity, including the L-shaped tail fiber protein of Coliphage T5, the gp12 of Bacillusphage GA-1, and the K5-eliminase of an *E. coli* K5-prophage. Like in endosialidases, the *C*-terminal domain of these proteins is cleaved-off at a highly conserved serine residue, an exchange into alanine resulted in non-cleavable proteins. By generating an endoNF-gp12-chimera, we showed that the *C*-terminal domain of endoNF can be substituted by the analogous part of gp12. In summary, we identified a common *C*-terminal assembly module that is used for proper folding of otherwise unrelated phage proteins. Subsequent release by proteolytic cleavage might be essential to trap the *N*-terminal protein in a kinetically stable fold.

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(93) **Elevated Mannose-6-Phosphate In Mouse Embryonic Fibroblast Cells Is Associated With Release Of Free Glycan from Lipid-linked Oligosaccharide**

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Lipid-linked oligosaccharides (LLOs) are the precursors of asparagine (N)-linked glycans. Completed LLOs have the structure $Glc_3Man_9GlcNAc_2$-P-P-dolichol. LLOs can also be hydrolyzed. Mannose-6-Phosphate (M6P) was previously found to increase the hydrolysis of $Glc_3Man_9GlcNAc_2$-P-P-dolichol in vitro, forming the free glycan $Glc_3Man_9GlcNAc_2$ and dolichol phosphate (Dol-P). Here, Fluorophore-assisted Carbohydrate Electrophoresis (FACE) was used to analyze monophosphate sugars and free glycans in cultured mouse embryonic fibroblasts (MEFs). ER stress increased M6P levels, and was accompanied by elevated luminal free glycans and Dol-P, and diminished $Glc_3Man_9GlcNAc_2$-P-P-dolichol. When MEFs were cultured with D-mannose, which increased M6P levels without causing ER-stress, free luminal glycans also increased. Methods that blocked M6P increases during ER stress also blocked the increase of free glycans. These results suggest that ER stress-induced M6P causes hydrolysis of $Glc_3Man_9GlcNAc_2$-P-P-dolichol in MEFs. Current experiments are investigating the potential role of these free glycans in the ER stress response, and the biochemical target of M6P.

Supported by NIH grant GM38545

(94) **Glycoengineered Plants for the Production of Recombinant Glycoproteins with Humanized N-Glycosylation**

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Plants are attractive hosts for the production of recombinant proteins of pharmaceutical interest as they are versatile systems, amenable to rapid and economical scale-up. Plant derived recombinant glycoproteins contain N-glycans with β1,2-linked xylose and core α1,3-linked fucose residues, which are not present in humans and thus can cause allergenic and/or immunogenic reactions. Therefore, approaches have to be developed to eliminate these non-human structures. Here we report on two approaches for the modulation of the N-glycosylation pathway:

First, triple knockout *Arabidopsis thaliana* plants were generated, which lack active forms of β1,2-xylosyltransferase (XylT) and core α1,3-fucosyltransferase (FucT). These plants are completely devoid of N-glycans with immunogenic β1,2-xylose and core α1,3-fucose residues, but grow normal under standard growth conditions. Analysis of the N-glycans of a recombinantly expressed human IgG by Western blot and MS revealed the complete absence of β1,2-xylose and core α1,3-fucose residues and the presence of predominantly GnGn ($GlcNAc_2Man_3GlcNAc_2$) structures.

Second, posttranscriptional gene silencing was used to "knockdown" the expression of XylT and FucT in *Nicotiana benthamiana* plants. Analysis of endogenous N-glycans revealed that β1,2-xylose and core α1,3-fucose, respectively, were dramatically reduced in transgenic lines expressing the silencing constructs. An antibody expressed in plants harboring gene silencing constructs for both enzymes displayed a N-glycan profile with high levels of

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terminal GlcNAc residues and undetectable amounts of β1,2-xylose and core α1,3-fucose.

Our glycoengineered plants provide a new platform for the production of recombinant glycoproteins with humanized N-glycosylation.

(95) **Investigating the Physiology of Mammalian N-Glycan Branching Contributed by the *Mgat4b*-encoded GlcNAcT-IVb Glycosyltransferase**

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Studies of animal models rendered genetically deficient in specific glycosyltransferases and glycosidases have provided a wealth of physiologic information on the structure-function relationships associated with mammalian glycan biosynthesis. Recently, we have found that the *Mgat4a*-encoded GlcNAcT-IVa glycosyltransferase plays a pivotal role in pancreatic beta cell function by supporting glucose transporter residence at the cell surface, thereby promoting insulin secretion and suppressing the pathogenesis of type 2 diabetes. The mammalian genome also contains an apparent isozyme of GlcNAcT-IVa, which is expressed by the *Mgat4b* gene and encodes the GlcNAcT-IVb glycosyltransferase. These glycosyltransferases operate in vitro on the same acceptor substrate, although the specific activity of GlcNAcT-IVb is quantitatively lower than that of GlcNAcT-IVa. Furthermore, human RNA expression analyses indicate that *Mgat4b* gene expression occurs among mostly different cell and tissue types as compared with *Mgat4a* expression. In order to understand why this isozyme exists and the role of GlcNAcT-IVb in N-glycan branching in vivo, we have produced mice that lack the *Mgat4b* gene using gene-targeting and Cre-loxP recombination in embryonic stem cells. *Mgat4b*-deficient animals are viable and appear grossly normal in early post-natal development, similar to mice lacking the *Mgat4a* gene. Analyses of N-glycan branching and phenotype formation have been initiated on mice bearing the *Mgat4b* deficient genotype and which have been back-crossed 7-9 generations into the C57BL/6 background. Our progress on this project will be presented.

(96) **Man2C1, an α-Mannosidase Involved in the Trimming of Free Oligosaccharides in the Cytosol**

Tadashi Suzuki; Izumi Hara; Miyako Nakano; Masaki Shigeta; Takatoshi Nakagawa; Akihiro Kondo; Yoko Funakoshi; Naoyuki Taniguchi
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The endoplasmic reticulum-associated degradation (ERAD) of misfolded (glyco)proteins ensures that only functional, correctly folded proteins exit from the ER and that misfolded ones are degraded by the ubiquitin-proteasome system. During the degradation of misfolded glycoproteins, they are deglycosylated by the peptide: *N*-glycanase (PNGase) (1). The free oligosaccharides released by PNGase are known to be further catabolized by a cytosolic α-mannosidase, although the gene encoding this enzyme has not been identified unequivocally. The human Man2C1 orthologue, previously known as ER/cytosol mannosidase, was found to be localized in the cytosol, and its enszymatic properties were found to be similar to cytosolic α-mannosidases so far characterized from animal sources. The downregulation of Man2C1 by siRNA drastically changed the amount and structure of oligosaccharides accumulating in the cytosol, demonstrating that Man2C1 indeed is involved in free oligosaccharide processing in the cytosol (2). The oligosaccharide processing in the cytosol by PNGase, endo-β- *N*-acetylglucosaminidase (3) and α-mannosidase may represent the common "non-lysosomal" catabolic pathway for *N*-glycans in animal cells (4).

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(97) **Neofunctionalization in Legumes: the Example of a Novel Family of Plant Lectins Evolutionary Related to Class V Chitinases**

Els J.M. Van Damme[1]; Raphael Culerrier[2]; Annick Barre[2]; Richard Alvarez[3]; Pierre Rougé[2]; Willy J Peumans[1]
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A lectin has been identified in the bark of black locust (Robinia pseudoacacia L.) bark that shares approximately 50% sequence identity with plant class V chitinases but is essentially devoid of chitinase activity. Specificity studies indicated that the Robinia pseudoacacia chitinase-related agglutinin or RobpsCRA preferentially binds to high mannose N-glycans comprising the proximal pentasaccharide core structure. Closely related orthologs of RobpsCRA have been identified in the legumes Glycine max, Medicago truncatula and Lotus japonicus but in no other plant species, suggesting that this novel lectin family most probably evolved in an ancient legume species or possibly an earlier ancestor. The present identification of RobpsCRA not only illustrates neofunctionalization in plants but also provides the first firm evidence that plants are capable of developing a sugar-binding domain from an existing structural scaffold with a different activity and accordingly sheds a new light on the molecular evolution of plant lectins.

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(98) **In vitro Synthesis of Corneal Keratan Sulfate**

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Corneal keratan sulfate(KS) plays important roles in maintaining corneal convex curvature and its transparency by keeping uniform structures and arrangement of collagen fibers in stromal extracellular matrix. Nevertheless, biosynthetic pathway of KS hasn't been well established yet. We previously proposed that sulfation on 6-O position of non reducing GlcNAc end by corneal GlcNAc 6-O-sulfotransferase(hCGn6ST/GlcNAc6ST-5/GST4β) is a critical step for production of highly sulfated KS in human cells. β1,3-*N*-acetylglucosaminyltransferase7(GnT7) and β1,4-galactosyltransferase4(GalT4) are suggested to be responsible enzymes among eight β1,3-*N*-acetylglucosaminyltransferases and seven β1,4-galactosyltransferases reported to date.

In this study, we examined ability to produce KS carbohytrate in vitro by using GnT7, GalT4 and hCGn6ST. For this aim, we constructed expression vectors for soluble form protein of each enzyme, and produced proteins in cell culture medium by transfecting the vectors in a CHO line. Using the concentrated medium as a source of each enzyme and a sulfated oligosaccharide SO3-6-O-GlcNAcβ1,6-Manβ1-2Manα-octyl, as an acceptor substrate, we analyzed production of KS in vitro. With GnT7 and GalT4, we detected reaction products, which are elongated forms of the substrate, up to hexa-saccharide structure, whereas we observed much longer products up to deca-saccharide in the presence of three enzymes, GnT7, GalT4 and hCGn6ST. By HPLC analysis, we found that the longer products were preferentially sulfated by hCGn6ST (in deca-saccharide product, 1S:2S:3S:4S=0%:0%:35%:65%). These results suggest that sulfation on GlcNAc by hCGn6ST is a crucial step for elongation of KS chain by GnT7 and GalT4.

(99) **The Degradation Complex of Misfolded Glycoproteins**

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Following their translation and glycosylation in the ER, N-linked glycoproteins that incorrectly fold are degraded by a complex proteasome-related system in the cytoplasm. Mouse peptide N-glycanase (mPNGase) cleaves the N-glycan chain from misfolded glycoproteins and glycopeptides. Previously, mPNGase was found to mediate the formation of a ternary complex containing mHR23B, mPNGase and mp97. We have now found that the cytoplasmic protein mp97 participates in the formation a complex containing five proteins, mAMFR, mY33K, mp97, mPNGase, and mHR23B. This assemblage recruits the cytosolic mPNGase close to the endoplasmic reticulum (ER) membrane, where the retrotranslocation of misfolded glycoproteins is thought to occur. This complex may serve to couple the activities of retrotranslocation, ubiquitination, and deglycosylation and thereby route misfolded glycoproteins to the proteasome more efficiently. Furthermore, PUB domain of mPNGase is found to interact with p97. Currentlywe are developing methods to isolate this pentameric complex and hope to study of the roles of its components in the degradation of glycoproteins.

(100) **Evidence that Cellulose is a Heteropolymer**

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Cellulose, the most abundant polysaccharide in the biosphere, is conventionally described as a substance composed of β-1,4-glucan chains

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aggregated by hydrogen bonds within and between the chains. Whereas the scientific literature presumes that cellulose is comprised solely of glucose, acid hydrolysis of native

and regenerated cellulose from several sources releases not only glucose, but several oligosaccharides, mannose, galactose, s-inositol, iditol, and sorbitol. Cellulose isolated from cotton linters, cotton fiber from two Acala (Upland) cultivars, two Pima cultivars, bamboo fibers, white pine fibers, regenerated bamboo fibers, regenerated cellulose sponge, coconut fibers, ivory nut shavings, cotton fabric, and the commonly used cellulose standard, Avicel® were investigated. In all cases, similar products were released by sequential acid hydrolysis. Samples were subjected to repeated hydrolysis in 6N HCl until completely dissolved. The hydrolysis products were released in relatively constant proportions throughout the sequential hydrolyses. Previous reports of the presence of monosaccharides, other than glucose, in acid hydrolyzates of cellulose often have been attributed to sample contamination. We believe this is the first report that shows that constituents other than glucose are present in sequential, exhaustive degradations of the polymer. The consistent presence of these minor constituents throughout the entire course of the progress stages of hydrolysis is considered an indication that the constituents are present within the cellulose itself. The description of cellulose as a substance composed solely of glucose originates from 1921. This early work used techniques now recognized as not capable of identifying minor constituent monosaccharides in an acid hydrolyzate.

(101) **PNGase F Treatment of Glycoproteins: Evidence for Selective Release of Glycans, Part II**

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In order to address the many concerns surrounding the previously presented data regarding glycan selectivity of PNGase F, a new and more complex glycoprotein has been examined. In these experiments, the subject glycoprotein is much larger and contains 4 glycosylation sites. It has been subjected to denaturation with heat and SDS to linearize the protein; thereby, alleviating concerns involving protein structure related steric hinderances. Further, the glycoprotein has been subjected to proteolysis with Endo Lys-C in order to generate smaller (and therefore more accessible) glycopeptides.

The data presented here supports the presence of glycan selectivity in PNGase F.

(102) **Role of Conformational Dynamics in the C-Terminal Region Of Alpha-1,3 Galactosyltransferase in Catalysis**

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α-1,3 Galactosyltransferase (α3GT) catalyzes galactose transfer from UDP-gal to β-linked galactosides, and in the absence of an acceptor substrate, to water at a lower rate. α3GT is a retaining glycosyltransferase, which might be expected to proceed through a double displacement mechanism involving a β-galactosyl-enzyme covalent intermediate; however current evidence does not support this. Crystallographic studies of complexes of a recombinant catalytic domain with substrates and products indicate that UDP binding produces the restructuring of the C-terminal 11 residues (358-368), a region that is largely disordered in the apo-enzyme. This transconformation is linked to the formation of the binding site for acceptor substrates. C-terminal truncations show that the three C-terminal residues (366-368) can be removed with little effect on catalytic properties but the additional truncation of Arg365 lowers kcat by more than two orders of magnitude. In crystallographic structures of enzyme complexes with UDP-gal, the C-terminal region is highly ordered but the hydrolysis products, UDP and galactose are observed in the active site. However the structure of the equivalent complex of an active site mutant contains intact UDP-gal and has a partial ordered C-terminus. A combination of mutational and structural data suggests that the binding of Mn2+ cofactor and donor substrate induces a partial structural change in the C-terminal region associated with Michaelis complex formation, the additional binding of acceptor substrate completes the structural transition to the state observed in the enzyme-UDP (product) complex. This step may be a key to UDP-galactose bond cleavage and galactose transfer.

(103) **Normal Secondary Branch Formation in the Outer Chain of *Candida albicans* *N*-Glycans Requires Tertiary Branch Mannosylphosphorylation**

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The pathogenic yeast *Candida albicans* produces large protein *N*-glycans containing only mannose residues. The glycan outer region comprises a primary branch with multiple secondary branches and tertiary branches. Tertiary branches are linked to secondary branches by phosphodiester bridges. In the current model of outer chain elongation, synthesis of the branches occurs sequentially, primary to tertiary. Thus, disruption of mannosylphosphorylation, the initial step in tertiary branch formation, should not affect primary or secondary branch production. However, compared to wild-type, a mutant defective in outer region mannosylphosphorylation (*mnn4Δ/mnn4Δ*) made outer regions with reduced susceptibility to low acid acetolysis treatment. We have begun to determine how the outer region has changed in the mutant. Higher acid acetolysis conditions were required to release the secondary branches from the primary branches. Fluorophore Assisted Carbohydrate Electrophoresis analysis revealed that the mutant produces only a subset of the wild-type secondary branches and synthesizes at least one unique oligosaccharide. Alkaline phosphatase treatment of released secondary branches suggests that some of the branches missing in the mutant are due to loss of phosphate. Reintegration of the *MNN4* gene into the mutant restored normal outer region formation, demonstrating that abnormal *N*-mannan synthesis in the mutant was directly due to loss of *MNN4* expression. We conclude from these results that the initial step in tertiary branch formation is required for normal secondary branch production and suggest that secondary and tertiary branch formation in *C. albicans* are interdependent events, and occur concurrently, rather than sequentially.

(104) **Phosphoglucomutase (PGM) is Located in the Glycosomes of *Trypanosoma cruzi* Different Forms**

Luciana L. Penha; Celso B. Sant'Anna; Narcisa Cunha-e-Silva; Lucia Mendonça-Previato; Norton Heise; José Osvaldo Previato; Ana Paula C. A. Lima
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The surface of *T. cruzi* is covered by a coat of *O*-glycosylated sialoglycoproteins and glycoinositolphosphoglycans, which are highly galactosylated and thought to play a role in host cell adhesion and invasion. Since *T. cruzi* is incapable of metabolizing galactose, the formation of UDP-galactopyranose by the parasite is dependent on the epimerization of UDP-Glu. Phosphoglucomutase (PGM) is the pivotal enzyme that catalyses the interconversion of Glu-6-P into Glu-1-P, an intermediate required for the synthesis of UDP-Gal*p*. In *S. cerevisiae* and in higher eukaryotes PGM is exclusively found in the cytoplasm. However, several enzymes that participate in sugar metabolism in trypanosomatids are confined to peroxisome-like organelles called glycosomes. *In silico* predictions of putative glycosomal enzymes showed that pmm-like genes of *T. cruzi* and *L. major* present a peroxisomal targeting signal sequence which is absent from PGM. In order to investigate the sub-cellular localization of PGM in these parasites, we used His-tagged recombinant PGM of *T. cruzi* to produce polyclonal antibodies. Immunofluorescence microscopy of different forms of *T. cruzi* and of promastigotes of *L. major* showed co-localization of PGM with GAPDH, a *T. brucei* glycosomal marker. Partition of membrane and soluble fractions of epimastigote extracts using Triton X-114 revealed that PGM was found in the aqueous phase. Sub-cellular fractionation of *T. cruzi* organelles by centrifugation in a sucrose gradient followed by Western blotting revealed that PGM is present in the glycosomal enriched fraction. Taken together, our results suggest that in *T. cruzi*, PGM is a soluble protein and associated with the glycosomal fraction.

(105) **A Challenge to Describe the Functional Networks of Glycoconjugates: "Glyconet" in a Database "Glycoconjugate Data Bank"**

Nobuaki Miura[1]; Kazuko Hirose[2]; Tomonori Ito[2]; Ryo Hashimoto[2]; Nobuhiro Fukushima[2]; Kenji Monde[1]; Shin-Ichiro Nishimura[1]
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Glycoconjugates play an essential role in biological systems. Understanding the significances of post-translational modifications and functions of glycoconjugates at molecular level enables us to develop new strategy for the tailor-made therapy and design of the novel therapeutic reagents.

There are a lot of databases related to carbohydrates in the world. Almost of them are based on the earlier developed databases called CCSD and CARBBANK with respect to structure of carobhydrates and are uniquely expanded by themselves. Although some of these databases are very informative and helpful, all these databases lack the information of the functional networks of the glycoconjugates except for biosynthetic pathway. Thus we have developed a database focused on the functional networks which is a part of our "Glycoconjugate Data Bank (http://www.glycoconjugate.jp/)".

Our data model is very simple. We obtained data from research papers. In one research paper, some experimental facts are described via a certain assay

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method. We have stacked the information by using binary relations with respect to biosynthetic pathway, inhibition of enzymes, phenotypes, and diseases, which correlate to the functions of glycoconjugates. The first attempt to represent molecular functions in binary relations was KEGG BRITE database by Prof. Kanehisa, Kyoto University (http://www.genome.jp/kegg/brite.html). In this model, we can concentrate to a paper without relation to results of other paper. We consider that it makes updates of the database easier.

(106) **Unusual *N*-Glycans from α-Mannosidase II/IIx Double Knockout Mice Identified by a Systematic Glycomic Approach using MDSF Method in MALDI-TOF/TOF-MS**

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Alpha-mannosidase IIx (MX) is closely related to the Golgi *N*-glycan processing enzyme, α-mannosidase II (MII). Recently, we generated MII/MX double knockout mice and found that both enzymes catalyze the conversion of hybrid to complex-type *N*-glycans in mutually compensatory manner and that most double knockouts die shortly after birth due to respiratory failure (1). In the present study, we analyzed extensively the structures of *N*-glycans from wild type, MII null, MX null and MII/MX double null mice, at embryonic day 15.5. Quantitative profiling of all *N*-glycans identified totally 37 *N*-glycan species, and structures of 27 of them were determined by 2D-LC mapping technique. However, the structures of 10 species were not readily determined.

We then employed a systematic glycomic approach with two-dimensional LC mapping database and matrix dependent selective fragmentation (MDSF) technique in MALDI-TOF/TOFMS (2). This advanced technique enabled us to identify 6 unusual *N*-glycan structures from MII/MX double knockouts, which were among the 10 species described above. A series of structural characterization using MDSF revealed that 6 species were unusual pseudo-complex-type *N*-glycans and 2 of them were completely new glycoforms. Our study demonstrated that use of versatile MDSF method in MALDI-TOF/TOFMS greatly accelerates the detailed analysis of uncommon and minute N-glycans.

References

(1) Akama, T. O. et al. *Proc. Natl. Acad. Sci.* (2006) **103**. 8983-8988

(2) Kurogochi, M. et al. *Anal. Chem.* (2004) **76**. 6097-6101

(107) **Comprehensive Analysis of the Polysialyltransferase from *Neisseria meningitidis* and Identification of Functional Motifs in Bacterial Sialyltransferases**

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Neisseria menigitidis (*Nm*) is a leading cause of bacterial meningitis and severe sepsis that in addition to sporadic outbreaks periodically spreads in epidemic waves. The meningococci produce extracellular polysaccaride capsules known to be major virulence factors. Serogroup B strains (*Nm*B), the primary disease causing isolates in Europe and America, are encapsulated in α-2,8 polysialic acid (polySia).

In *Nm*B capsule biosynthesis, polySia is synthesized from activated sialic acid (CMP-Neu5Ac) by the polysialyltransferase (*Nm*B-polyST). However, only little biochemical data of this important enzyme are reported, mainly due to the lack of expression and assay systems. Here we present a comprehensive characterization of *Nm*B-polyST. The enzyme was expressed as soluble fusion protein, purified and kinetically analyzed by establishing a continuous spectrophotometric assay for polysialyltransferases. Our data show, that the optimal *Nm*B-polyST acceptor structure contains at least three α-2,8-linked sialic acid residues (dp3) and that building of the polySia-chain proceeds in a non-processive manner.

Sequence alignments of *Nm*B-polyST with bacterial sialyltransferases of different CAZY[1] families revealed two conserved motifs. Single point mutations introduced into these motifs generated mutant *Nm*B-polyST enzymes with low or no activity, highlighting the critical role of the identified conserved residues for active sialyltransferases. This is to our knowledge the first description of functional motifs relating bacterial sialyltransferases of diverse CAZY families.

[1] Coutinho et al. (2003), J. Mol. Biol. 328:307-317

(108) **Mammalian O-Mannosylation is Sequon-Independent and cis-Controlled by an Upstream Trigger Sequence**

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Protein O-mannosylation is a very rare modification in mammals, ocurring only on a limited number of glycoproteins in brain, nerve, and skeletal muscle. The most investigated O-mannosylated protein is alpha-dystroglycan. Its O-mannose glycan chains play an important role in protein-protein interactions by mediating binding to the laminin G domains. Initiation of O-mannosylation is catalyzed by a functional O-mannosyltransferase complex (POMT1/POMT2). Enzymatic activity of these enzymes was demonstrated in vitro, but little is known about a potential sequence dependency of protein O-mannosylation. We have expressed a series of recombinant glycosylation probes in human cell lines corresponding to sections of human alpha-dystroglycan that covered the mucin domain and adjacent stretches of the protein. The fusion proteins were isolated from the supernatants by affinity and reversed-phase chromatography and subjected to structural analyses of their O-glycosylation. These analyses comprized monosaccharide composition analysis of glycan alditols for identification of the core sugar, oligosaccharide characterization by ESI-MS/MS of methylated glycans and ESI-ion trap (electron-transfer-dissociation/collision-induced dissociation) MS/MS for localization of O-mannosylated sites in tryptic glycopeptides. We could demonstrate that O-mannosylation occurs within the mucin domain of alpha-dystroglycan, but is independent of specific sequons within this domain. The same sites were mucin-type O-glycosylated in vivo, if amino acid sequences upstream of the mucin domain were absent in the glycosylation probe. Mammalian O-mannosylation is apparently not controlled by the amino acids surrounding the putative target sites of O-mannosylation, but by more distant sequences, which may serve for binding and activation of the functional POMT1/POMT2 complex in the ER.

(109) **Biosynthesis of Sialylated Lewis Antigens in Human Gastric Carcinoma Cells: Combined Role of alpha2,3sialyltransferases and alpha3/4fucosyltransferases**

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Sialyl Lewis A (SLeA) and sialyl Lewis X (SLeX) are cancer-associated carbohydrates, and their biosynthesis involves sialylation of type 1 and type 2 precursor structures by alpha2-3sialyltransferases and consecutive fucosylation by alpha1-3/4fucosyltransferases. We studied the role of alpha2-3sialyltransferases and alpha1-3/4 fucosyltransferases in the biosynthesis of sialylated Lewis antigens in human gastric carcinoma cells. Expression and enzyme activity of alpha1-3/4fucosyltransferases were characterized in MKN45 and IPA220 before and after stable transfection with ST3GalIV and ST3GalVI. Stable transfection of MKN45 with alpha2-3sialyltransferases induced limited expression of sialyl Lewis antigens. Expression of FUT3 was low in MKN45 and the effect of FUT3 methylation on SLeA and SLeX expression was studied using 5-aza-2'deoxycytidine. In MKN45 transfected with ST3Gal IV and VI, expression of SLeA increased slightly after demethylation while expression of SLeX suffered no alteration. Expression of LeX, in the absence of LeA and SLeA, together with the observation that MKN45 extracts have preferential activity on type 2 acceptors, indicates this cell line has predominant alpha1-3fucosyltransferase activity, possibly FUT4 or FUT9. Comparatively, the alpha1-3/4fucosyltransferase activity of MKN45 was very low/absent for sialylated type 1/type 2 structures. Another cell line, IPA220, expressing FUT3, has alpha1-4fucosyltransferase activity towards type 1 acceptors and synthesizes LeA; still no SLeA or SLeX are expressed. Taken together, the results for both cell lines indicate that FUT3 has preferentially alpha1-4fucosyltransferase activity towards type 1 precursor structures in vivo, whereas other alpha1-3fucosyltransferases to be identified are involved in the synthesis of SLeX in gastric carcinoma cells. Support: FCT (POCI/SAU-OBS5668/2004); AICR (Grant 05-088).

(110) **Motifs Analysis in the Sialyltransferase Protein Family**

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Sialic acids are increasingly recognized as the key determinants of a diverse oligosaccharide structures involved in a large variety of biological events. The transfer of sialic acid to such diverse carbohydrate structures is mediated by sialyltransferases (ST), a group of enzymes that transfers sialic acid from its common activated nucleotide sugar donor, CMP-NeuAc. So far, total 20 members of this enzyme family with distinct carbohydrate linkage specificity have now been cloned. These entries account for Neu5Acα2,6Gal (ST6Gal I & II), Neu5Acα2,3Gal (ST3Gal I – VI), Neu5Acα2,6GalNAc (ST6GalNAc I – VI), and Neu5Acα2,8Neu5Ac (ST8Sia I – VI). Comparative peptide sequence analysis of these cloned mammalian type II transmembrane glycoproteins showed the presence of four conserve sialylmotifs in the catalytic domain, namely L-, S-, -III and -VS, which are common to all of this protein family. Experiments by site-directed mutagenesis showed the evidence that these motifs contribute to the binding of either donor or the acceptor or both. Experimental evidence also showed the presence of a disulfide linkage between the L-sialylmotif and the S-sialylmotif. Apparently this disulfide linkage brings all of these motifs closer together facilitating interaction of these motifs with the substrates. Although there is no structural evidence of any of these mutagenesis studies, studies by fold recognition and comparative modeling techniques supports these findings. In addition, although there is no experimental evidence, comparative sequence analysis also suggests a strong correlation of linkage specificity of these enzymes with the peptide sequence closer to these sialylmotifs.

(111) **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 released and permethylated N-linked oligosaccharides from the DSiaT have been analyzed by both MALDI-MS and ESI-MS/MS. More than ten different N-glycans were detected in the DSiaT glycoprotein with GlcNAc2Man3Fuc and GlcNAc2Man3Fuc2 as the main released glycans. ESI-MS/MS data of individual glycans will be presented to unambiguously characterize the structure of the additional N-linked glyacns in DsiaT.

The sites of N-linked glycosylation will also be determined using online ESI-MS/MS analysis of the glycopeptides from the DSiaT glycoprotein.

(112) **Futile Cleavage of UDP-GlcNAc by Recombinant Soluble Human GlcNAc-Phosphotransferase**

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GlcNAc-phosphotransferase catalyzes the initial step in the synthesis of mannose 6-phosphate, the determinant for intracellular targeting of lysosomal hydrolases to the lysosome. GlcNAc-phosphotransferase is a multisubunit, membrane-bound enzyme with an $\alpha_2\beta_2\gamma_2$ arrangement. The α− and β-subunits contain the catalytic domain, whereas the γ-subunit functions in lysosomal hydrolase recognition. A recombinant soluble GlcNAc-phosphotransferase (mixture of $\alpha'_2\beta'_2\gamma_2$ and $\alpha'_2\beta'_2$) containing transmembrane-deleted α− and β-subunits was produced and used for *in vitro* phosphorylation. Approximately half of the purified recombinant enzyme lacks the γ-subunit based on quantitative amino-terminus sequencing. GlcNAc-phosphotransferase transfers GlcNAc 1-phosphate from UDP-GlcNAc to mannoses on the high-mannose type N-glycans, generating UMP as a byproduct (Man-R + UDP-GlcNAc → GlcNAc-P-Man-R + UMP). Although the soluble enzyme has a Km for lysosomal hydrolases similar to the native bovine GlcNAc-phosphotransferase, the Vmax for the reaction was lower than the native enzyme. We found that a major side reaction was catalyzed by the soluble enzyme, namely the transfer of GlcNAc-phosphate to water, generating GlcNAc 1-phosphate and UMP (H2O + UDP-GlcNAc → GlcNAc 1-P + UMP). Since the native enzyme does not have this activity, this reaction might be catalyzed by the enzyme lacking the γ-subunit or as a result of the absent transmembrane domains. This side reaction might contribute to the lower Vmax. GlcNAc-phosphotransferase in MLIIIC patients who lack the functional γ-subunit might have the same activity. This reaction has not been reported for the other enzymes that transfer GlcNAc 1-phosphate from UDP-GlcNAc, such as dolichyl-phosphate GlcNAc 1-P transferase or bacterial capsular polymerases.

(113) **Evidence of Exo-Sulfatase Activity in Quail Egg White**

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Sulfatases specific for sulfate (SO_4^{2-}) at the non-reducing ends of glycosaminoglycans (GAG) have been identified previously. We tested quail egg white for its ability to remove sulfate moieties at the non-reducing ends of exogenous dermatan sulfate substrates (DS_{18} and DS_{28}). Quail egg white depleted of its albumin by adsorption with rabbit anti-chicken egg albumin was adjusted to 0.066 M NaOAc, pH 5.60. Inhibitors of exoglycosidase enzymes known to degrade DS were added at the final concentrations indicated: Maleic acid (3 mM), an α-L-iduronidase inhibitor; D-GlcNAc (1 mM) and D-GalNAc (1 mM), inhibitors of β-N-acetylhexosaminidase; and D-GlcA (5 mM), a β-glucuronidase inhibitor. Egg white-inhibitor mixture (275 ml) was combined with substrate (108 mg), placed in a dialysis membrane and dialyzed against 1 L of 0.066 M NaOAc, pH 5.60, under nitrogen at 37° C for 13 hr. Quail egg white-inhibitor mixture without albumin (275 ml) was used as control. Sulfate in the reaction dialysates was quantified. The yields were 22.29 μmol from DS_{18}, 29.58 μmol from DS_{28} and zero from the control. The substrates (DS_{18} and DS_{28}) were re-isolated from the respective non-dilysable materials and digested with β-N-acetylhexosaminidase. The yields of D-GalNAc were 1.0987 μmol from DS_{18}, 0.307 μmol from DS_{28}, and zero from the control. The data show that exogenous substrates, DS_{18} and DS_{28}, contain, respectively, 4.00 % and 1.04 % sulfated D-GalNAc at their nonreducing ends, and identify quail egg white is an alternative source GAG exosulfatase(s).

(114) **Prediction of Mucin-Type O-Glycosylation using Variation Profiling**

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O-Glycosylation is a key post-translational modification of proteins that is considerably altered in certain pathologies (e.g., cancer). Therefore, owing its potential therapeutic relevance, some algorithms for the prediction of OG sites were developed. Nonetheless, these algorithms exhibit rather low specificity in predicting true OG sites. Based on experimentally mapped mucin-type OG residues, we developed an algorithm, namely O-Glycosylation Prediction Electronic Tool (OGPET), which shows high sensitivity and specificity. OGPET makes amino acid (aa) prediction motifs considering 5 relevant positions (-3, -1, +1, +3, and +4) around the possible Thr/Ser residue (position 0) that are known to influence the interaction of the polypeptide GalNAc-transferase (ppGalNacT) with the target protein. Furthermore, analysis of the physical and chemical properties of aa made possible to indistinctively switch aa at any of the 5 relevant positions without increasing the rate of false-positive predictions. Our results showed a sensitivity of 0.97 and a specificity of 0.98 for standard performance tests. OGPET predicted true-positive sites despite mutations on the primary sequence using the aa variation approach (variation profiling). Finally, a set of prediction constraints was able to find novel sites that were not included on the training sets. OGPET is currently available through the WWW (http://129.108.112.23/OGPET/).

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(115) **Identification and Characterization of the cis-Regulatory Elements of Human Mucin Core 2 β1,6 N-Acetylglucosaminyltransferase-M Gene**

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Mucin glycan constitutes 60-90 % of mucin molecules by weight and is the primary determinant of mucin functions. The three mucin glycan branch structures, including core 2, core 4, and blood group I, expand the repertoire of peripheral carbohydrate structures and thus the functional potential of mucins. All three branch structures can be synthesized by mucin core 2 β1,6 N-acetylglucosaminyltransferase-M (C2GnT-M). Therefore, alteration of C2GnT-M gene expression is expected to have a profound effect on mucin functions. Real-time PCR analysis showed that this gene was expressed primarily in mucus-secretory tissues. 5'Rapid amplification of cDNA ends (RACE) analysis coupled with sequence alignment with human genome database showed that this gene contained three exons and two introns. Northern blotting using exon 1 probe detected this exon in all three different size transcripts identified with exon 3 probe, suggesting the presence of cis-

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regulatory elements in the proximal region upstream of the longest E1. Analysis of this region of DNA plus 187 bp of E1 by the promoter-reporter transient transfection assay and linker scanning mutagenesis revealed two positive regulatory regions, including –291 to –282 and –62 to –43. Correlated with C2GnT-M enzyme activity, the promoter activity was enhanced by retinoic acid, suggesting that retinoic acid treatment activated promoter-specific transcription factors. Thus, the cis-regulatory elements identified are specific for hC2GnT-M gene and may be useful for construction of a mucus cell-specific vector for therapy of mucus hypersecretory diseases. (Supported by NIH RO1 HL48282 and Cystic Fibrosis Foundation)

(116) **Large-Scale Biosynthesis of (iso)Globotrihexose with a New Three-Enzyme System**

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The resistance to current complexity of chemical syntheses of isoglobotrihexose and globotrihexose, which are the trisaccharide donors for the biomedically important glycolipids isoglobotrihexosylceramide (iGb3) and globotrihexosylceramide (Gb3), inspired us to investigate an efficient approach to synthesize those trisaccharides in large-scale. Taking advantage of the high efficiency of enzymatic synthesis and commercially available UTP at low price, isoglobotrisaccharide and globotrisaccharide could be produced in gram scale by a new three-enzyme system with the yield of 78% and 82%, respectively. Compared with current chemical syntheses of iGb3 and Gb3 trisaccharide donors, the present methodology dramatically shortens the synthetic route from 17 steps to 3 steps. Furthermore, the substrate specificity of the enzymes in the new three-enzyme system can be tuned so that unnatural substrate analogs can be readily incorporated into the synthesis and ended up in the products, thus offering an attractive way to obtain structurally modified oligosaccharides.

(117) **Production of Mucin-Type Glycoprotein in Yeast**

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Mucin-type sugar chain is one of the typical O-linked sugar chains commonly observed in mammals. In cancer, mucins are aberrantly O-glycosylated, and consequently, they express tumor-associated antigens such as the Tn determinant (-GalNAc-O-Ser/Thr). As compared with normal tissues, they also exhibit a different expression pattern. It has been evaluated extensively as a potential diagnostic marker and several Tn-based vaccines are in clinical trials. To develop therapeutic glycoproteins such as erythropoietin and anticancer vaccines based on the Tn antigen, we attempt to produce mucin-type sugar chains in yeast.

Three genes, encoding Bacillus UDP-GalNAc 4-epimerase (GalE), human UDP-Gal/GalNAc transporter (UGT2) and human ppGalNAc-T1, were used for transformation of Saccharomyces cerevisiae W303-1A cells. Next, MUC1a peptide was expressed in the transformant. The secreted MUC1a peptide was purified by reversed-phase HPLC and analyzed by MALDI-TOF MS. The protonated molecular ion of purified MUC1a was observed at m/z 2136, which was corresponding to the mass of HexNAc containing MUC1a peptide. The peptide was also analyzed on lectin micro array, and it showed signals specific for GalNAc specific lectins. The GalNAc-peptide was secreted over 1 mg/L to the medium. By introducing Drosophila beta-1,3-GalT gene into the GalNAc-peptide expressing yeast strain, we succeeded in production of a core1-type (Gal-GalNAc-O-Ser/Thr) MUC1a peptide in yeast. An artificial fusion protein (FGF with O-GalNAc) was also produced in the GalNAc-peptide expressing yeast strain as a host. This technology will be useful to make a large amount of recombinant mucin-type glycoproteins with low cost.

(118) **Production of Recombinant β-Hexosaminidase A that is Applicable to Enzyme Replacement Therapy for GM2 Gangliosidosis, in Methylotrophic Yeast**

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Human β-hexosaminidase A (HexA) is a heterodimeric glycoprotein composed of α- and β-subunits, which degrades GM2 gangliosides in lysosome. Although the homodimers HexS (αα) and HexB (ββ) exist as isozymes, only HexA (αβ) is capable of hydrolyzing GM2 gangliosides. The inherited deficiency of HexA causes accumulation of GM2 gangliosides and leads to a type of lysosomal storage diseases (LSD) known as GM2 gangliosidosis. We aim to establish enzyme replacement therapy (ERT) for GM2 gangliosidosis, as it has already been applied clinically for several other LSDs. In ERT, administrated recombinant enzymes are incorporated into the cell via mannose-6-phosphate (M6P) receptor localized at the cell surface. As a large amount of M6P-containing enzyme is required, recombinant HexA was produced from the methylotrophic yeast *Ogataea minuta*, instead of mammalian cells commonly used for production of recombinants for ERT. The problem of antigenicity due to the difference in *N*-glycan structure between yeast and mammalian glycoproteins were resolved by using *och1* disruptant yeast as host. Of the total *N*-glycans of purified recombinant HexA, 6.9% were phosphorylated. The mannosidase-treated HexA was prepared to expose M6P residues at the non-reducing end of *N*-glycans (M6PHexA). The enzyme incorporation into the cultured fibroblast derived from a patient and the intracellular GM2 degradation were detected for M6PHexA but not for HexA, suggesting that M6PHexA is properly recognized by M6P receptors on the cell surface and correctly functions in the lysosome. These results demonstrated that yeast recombinant M6PHexA is suitable for ERT of GM2 gangliosidosis.

(119) **Molecular Cloning and Characterization of a Novel 3'-Phosphoadenosine 5'-Phosphosulfate Transporter, PAPST2**

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Sulfation is an important posttranslational modification associated with a variety of molecules. It requires the involvement of the high energy form of the universal sulfate donor, 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Recently, we identified a PAPS transporter gene in both humans and *Drosophila*. Although human colonic epithelial tissues express many sulfated glycoconjugates, *PAPST1* expression in the colon is trace.

In the present study, we identified a novel human PAPS transporter gene that is closely related to human *PAPST1*. This gene, called *PAPST2*, is predominantly expressed in the human colon tissues. The PAPST2 protein is localized on the Golgi apparatus in a manner similar to the PAPST1 protein. By using yeast expression studies, PAPST2 protein was shown to have PAPS transport activity with an apparent K_m value of 2.2 μM, which is comparable with that of PAPST1 (0.8 μM). Overexpression of either the *PAPST1* or *PAPST2* gene increased PAPS transport activity in human colon cancer HCT116 cells. The RNA interference of the *PAPST2* gene in the HCT116 cells significantly reduced the reactivity of G72 antibody directed against the sialyl 6-sulfo *N*-acetyllactosamine epitope and total sulfate incorporation into cellular proteins. These findings indicate that *PAPST2* is a PAPS transporter gene involved in the synthesis of sulfated glycoconjugates in the colon.

(120) **Structural and Functional Studies of Glycosyltransferases Involved in Biofilms Development in Pseudomonas aeruginaosa**

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Pseudomonas aeruginosa (PA) represents a versatile opportunistic pathogen that is capable of thriving in diverse environments ranging from water and soil to plant and animal tissues. This bacteria, which has an extensive arsenal of virulence factors, causes chronic infections upon biofilm formation and is able to colonize human lungs during cystic fibrosis pathogenesis. Bacteria within biofilms are adherent aggregates of bacterial cells that form on biotic and abiotic surfaces, including human tissues. They are attached to either a substratum or each other and are embedded in a matrix of extracellular polymeric substance (EPS), which may consist of proteins, polysaccharides and nucleic acids. Biofilms resist antibiotic treatment and contribute to bacterial persistence in chronic infections. Hence, the elucidation of the mechanisms by which biofilms are built may assist in the treatment of chronic infections, such as P. aeruginosa in the airways of patients with cystic fibrosis.

Recently, a transcriptome analysis has revealed that the psl and pel operons are involved in production of carbohydrate-rich components of the biofilm matrix which are therefore critical for the autoaggregative properties of PA.

Several glycosyltranferases (GTs) encoded by these loci are mostly responsible for the expolysaccharidic matrix during biofilm formation1-3. Our aim is to characterise these GTs by solving their 3D structures using X-ray

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crystallography combined with functional studies. These results will identify the structural determinants necessary for their substrate specificity and unveil the catalytic reaction mechanism. This will bring essential information on their mode of action and help the development of new antibiotics.

(121) **Function and Structure Correlation between Family 20 and 21 Carbohydrate-Binding Modules in Glucoamylase**

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Glucoamylase (GA) is a commonly used glycoside hydrolase in industry. Fungal GAs present a common primary structure constituted by two parts: the catalytic domain and the starch binding domain (SBD). SBDs are found in carbohydrate-binding module (CBM) families 20, 21, 25, 26, 34, 41 and 43 and the most generalized and studied is the CBM20. In the present study, we constructed the CBM21 from Rhizopus oryzae GA (RoGACBM21) and studied its function and structure relationship. The molecular model of RoGACBM21 was constructed according to the result of structure-based sequence alignment using Aspergillus niger GA (AnGACBM20) as the template. The result shows that RoGACBM21 has an all-β-sheet structure, similar to that found in AnGACBM20. Through the use of molecular modeling, we identified a number of key residues involved in ligand binding and validated experimentally by site-directed mutagenesis, chemical modification, and quantitative binding assay. In the present study we have for the first time demonstrated that the key ligand-binding residues of RoGACBM21 can be identified and characterized by a combination of novel bioinformatics methodologies.

(122) **During N-Glycosylation the Dolichyl Carrier Lipid is Recycled to the Cytoplasmic Monolayer of the ER as Dolichyl Monophosphate**

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During protein N-glycosylation, the dolichyl carrier lipid is discharged as the pyrophosphate (Dol-P-P), and then rapidly converted to Dol-P by the CWH8 phosphatase at the lumenal face of the endoplasmic reticulum (ER) membrane. However, it is not known if lumenal Dol-P returns to the cytoplasmic leaflet as the monophosphate, or is first dephosphorylated to dolichol and then rephosphorylated by cytoplasmic CTP-dependent dolichol kinase (DK) activity. To answer this question, sealed ER vesicles from calf brain were incubated with acetyl-Asn-Tyr-Thr-NH_2, to generate lumenal Dol-P-P, and then assayed for the appearance of endogenously-generated Dol-P (using Man-P-Dol synthase (MPDS)) or free dolichol (as a substrate for DK) on the cytoplasmic surface. Incubation with 0.1 mM acetyl-Asn-Tyr-Thr-NH_2 increased MPDS activity, but not DK activity, under conditions that the initial enzymatic rates were dependent on endogenous acceptor substrate levels. When CHO cells were gently permeabilized with streptolysin-O (and therefore not subject to physical perturbation of microsomes), N-glycopeptide synthesis requiring multiple cycles of the dolichol pathway occurred in the absence of CTP and in the presence of apyrase. These results indicate that recycled Dol-P returns directly to the cytoplasmic monolayer without dephosphorylation/rephosphorylation involving DK. Since spontaneous transmembrane diffusion of Dol-P is energetically unfavorable, it is possible that the flip-flopping of Dol-P is mediated by a Dol-P flippase, providing a mechanism for the recycling of Dol-P generated from Dol-P-P, Man-P-Dol-mediated reactions in N-, O- and C-mannosylation of proteins, GPI anchor assembly, and the Glc-P-Dol-mediated reactions in LLO biosynthesis.

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(123) **Different Protein forms of UDP-Xylose Synthase**

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Xylose is the first sugar residue within the core of all proteoglycans but is also found in O-glycans of other proteins. UDP-Xylose, substrate for all xylosyltransferases, is produced from UDP-glucuronic acid within the Golgi lumen by UDP-xylose synthase (UXS), formerly also named UDP-glucuronic acid decarboxylase. The sequence predicts UXS to be a type-II transmembrane protein and experiments indeed confirmed this. Human UXS is made in two splice variants differing in only 5 amino acids just after the predicted transmembrane domain. Expression of a tagged recombinant form of one of the splice forms resulted in the formation of two different proteins. The largest polypeptide ran on an SDS-PAGE as predicted for the full size protein, whereas the smaller form was shortened at the N-terminus and is, as judged from the size, lacking the transmembrane domain. This form can theoretically be formed by two mechanisms; translation from an alternative start codon present just after the transmembrane domain or by proteolytic cleavage of the full size form. Although previous experiments had shown that a recombinant construct of UXS lacking the transmembrane domain could actively be expressed in the cytoplasm, different experiments showed that the observed N-terminally truncated form is probably a result of proteolytic cleavage. Mutation of the potential alternative start codon did not result in the disappearance of the short form of the protein and the short form was secreted in the medium of cells expressing recombinant UXS. UXS therefore exists as a membrane bound Golgi protein and a secreted soluble form.

(124) **Structural and Functional Characterisation of an Epimerase Involved in the Sialic Acid Metabolism of Clostridium Perfringens**

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Clostridium perfringens (C.perf) is an ubiquitous pathogenic bacterium known to cause gas gangrene, acute food poisoning and antibiotic-associated diarrhea in humans. Bacterial growth, and therefore disease progress, are highly dependent on the availability of nutrients, including sialic acid found in abundance in mucines of the intestinal tract. C.perf has developed a pathway for degrading sialoglycoconjugates to N-acetylglucosamine-6-phosphate (GlnNac6P), which is essential for the bacterial cell wall synthesis.

We are focusing our efforts on NanE a N-acetylmannosamine-6-phosphate epimerase, which catalyzes the last step of the pathway: conversion of N-acetylmannosamine-6-phosphate (ManNac6P) into GlnNac6P. The aim of the project is to study the structure of the enzyme and establish its catalytic mechanism.

Here we present the structure of NanE solved at 1.7Å resolution. NanE is a dimer showing a pseudo-domain swapping. It folds into two (alpha/beta)8 barrels which mutually exchange their 8th helix in C-terminal position through the dimeric interface. The structure of NanE in complex with GlnNac6P has been solved at 1.9Å resolution; no conformational changes have been observed upon the binding of the

ManNac6P and its epimerisation into GlnNac6P. The orientation of the ligand into the active site have permit us to identify Lys66 as a putative catalytic residue. Recently we have solved the structure of the catalytic mutant of NanE (NanEK66A) in complex with unprocessed ManNac6P, at 1.4 Å resolution. These results consolidate the hypothesis that Lys66 is involved in the catalytic mechanism.

(125) **Isolation and Characterization of a Putative *Trichoplusia ni* Core alpha 1,3 Fucosyltransferase Gene**

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As part of a broader research program in insect glycobiology, we have isolated a core alpha 1,3 fucosyltransferase gene from the lepidopteran insect, *Trichoplusia ni*. Initially, we used a degenerate PCR approach to isolate an internal fragment of this gene. This fragment was cloned and sequenced and the results were used to isolate a larger fragment of this gene, which was designated TnFT3. Ultimately, a full-length TnFT3 sequence was assembled using these data and additional data obtained from 5'- and 3'-RACE experiments. Due to the presence of three regions of nucleotide sequence with over 80% G+C content, the determination of this sequence required the development of new RACE methods, which have been reported (X. Shi and D.L. Jarvis, Analyt. Biochem., in press) and will be presented in this poster. The final TnFT3 cDNA sequence determined in this study is 3263 bp in length and encodes a theoretical translation product of 449 amino acids, which is 50% identical and 65% similar to the functionally characterized *Drosophila melanogaster* core alpha 1,3 fucosyltransferase, FUT3A (G. Fabini et al., 2001. J. Biol. Chem. 276:28058). Expression, purification, and biochemical and cell biological characterization of the TnFT3 gene product are currently underway. Interestingly, preliminary experiments have indicated that a GFP-tagged form of TnFT3 co-localizes with neither the Golgi stain, BODIPY TR C5-ceramide, nor the lysosomal stain, LysoTracker Red DND-99.

(126) **Expression and Isotope Labeling of ST6Gal1—Enabling NMR Characterization of Glycosylated Proteins**

Lu Meng; John Glushka; Leslie Stanton; Tian Fang; Robert Collins; Greg Carey; Greg Wiley; Zhongwei Gao; James Prestegard; Kelley W. Moremen
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Sialic acid residues are present at the non-reducing termini of many glycans structures associated with glycoproteins and glycolipids where they play roles

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in critical biological functions including cell-cell communication, cell-substrate interaction, adhesion and protein targeting. In mammalian cells the synthesis of sialic acid linkages is catalyzed by sialyltransferases in CAZy family GT29. Despite the key roles that sialic acid linkages play in glycoprotein/glycolipid maturation, immune function and development, no structures of any GT29 family members have yet been determined and little is understood about their mechanisms of action.

We have expressed recombinant rat α-2,6-sialyltransferase (ST6Gal1) in mammalian HEK293 cells in >10mg batches and we have also adapted the expression protocol for selective 15N amide-labeling of amino acids. We successfully labeled the 16 Phe residues of ST6Gal1 as indicated by mass spectrometry and HSQC-NMR and additional amino acid labeling has also been accomplished. We have performed detailed kinetic analyses and surface plasmon resonance studies to examine the interactions between ST6Gal1 and substrates or substrate analogs. Ongoing studies are attempting to crystallize the enzyme as a model for structural and functional studies on this key family of glycosyltransferases. (Supported by NIH grant RR005351)

(127) **Comparing Glycan Presentation and Dynamics in a Bacterial and Eukaryotic N-Glycosylated Protein**

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A crystal structure of the N-glycosylated protein PEB3, identified as a major antigenic protein in *Campylobacter jejuni* [1], has been solved [2]. However, the glycan itself is not present in the crystal structure. We have employed molecular modeling techniques to add the bacillosamine-linked glycan [3] to the protein. In addition, since bacterial and eukaryotic N-glycosylation share the requirement for an Asn-X-Ser/Thr sequon, we wished to examine the differences in glycan presentation between these two systems. To do this, we generated a hypothetical glycoprotein employing the PEB3 protein, in which a high-mannose type N-linked glycan was attached in place of the native glycan.

Both the bacterial glycoprotein PEB3 and its hypothetical eukaryotic analog were subjected to computational simulations to examine the differences in glycan presentation and dynamics. These results may be particularly valuable in light of the proposal that bacterial glycosylation may be employed in "glycoengineering" [4].

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(128) **Heterologous Expression of Rat ST6Gal1 in *Pichia pastoris* for Structural and Functional Studies**

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ST6Gal1 plays an important role in immune regulation mediated via the lectin CD22. The enzyme catalyses transfer of sialic acid to form a Siaα2-6Galβ1-4GlcNAc (Sia6LacNAc) tri-saccharide product, which is the ligand for CD22. 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 p*PIC*-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. Purified uniformly labeled or unlabeled recombinant ST6Gal1 were characterized by enzyme assays and SDS-PAGE. A preliminary characterization revealed a K_m of 180μM for the sugar donor CMP-Sia. 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. We have subsequently eliminated the glycan heterogeneity by treating recombinant ST6Gal1 with Endo H followed by chromatography over ConA-Sepharose. Further characterization of the purified deglycosylated enzyme by NMR and crystallization are presently underway. (Supported by NIH grant RR005351)

(129) **N-Linked Glycans are Required to Improve Catalytic Activity of BjussuSP-I, a New Thrombin-Like Glycoprotein Isolated from Bothrops jararacussu Snake Venom**

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Snake venoms contain several glycoproteins, but there are few studies on the role exerted by glycans on their biological activities. A new serine-protease, BjussuSP-I, was isolated from Bothrops jararacussu snake venom, and its nucleotide sequencing and conceptual translation product were determined. Two potential sites of N-glycosylation and one of O-glycosylation were predicted in BjussuSP-I by using the NetNGlyc 1.0 and NetOGlyc 3.1 softwares. The SDS-PAGE analysis of BjussuSP-I appears as a single 61 kDa band. After treatment with 0.1M H2SO4 or PNGase-F, the apparent MM of BjussuSP-I has decreased to 54 and 37 kDa, respectively, strongly suggesting that the molecule contains sialic acid and N-glycans. In order to determine the influence of N-glycans on the BjussuSP-I thrombine-like activity, purified samples, treated or not with PNGase-F, were assayed for interference on coagulation kinetics. The formation of fibrin clot was reduced in 50% by deglycosylation of BjussuSP-I. Then, the three-dimensional structure of the BjussuSP-I was built by comparative protein modeling using SWISS-PDB-VIEWER. The structure of the enzyme was modeled on the basis of structural similarity with the AAV-SP-I and AAV-SP-II, which are glycosylated serine-proteases from Agkistrodon acutus venom. The BjussuSP-I homology model demonstrated that the catalytic triad (residues His40, Asp85 and Ser178) is located in a groove between the two N-glycosylated sites. Our results indicate that the N-glycans of BjussuSP-I facilitate its serine-protease activity presumably through stabilizing the catalytic site interactions with substrates. Thus, glycosylation can be relevant for the optimization of the biological activities of snake venom toxins.

(130) **Mechanism of Substrate Binding and Catalysis for Class I (GH 47) α1,2-Mannosidases: the Effect Ca^{2+} Coordination on Catalysis**

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Mammalian Class I (GH 47) α1,2-mannosidases play critical roles in the maturation of Asn-linked glycoproteins as well as influencing the timing and recognition for disposal of terminally misfolded proteins during ERAD. Prior structural studies on human ER mannosidase I (ERManI) indicated an inverting catalytic mechanism involving the conformational distortion of the glycone to a novel $^3H_4$ transition state influenced by an association of two substrate hydroxyls with an enzyme bound Ca^{2+} ion. The enzyme directly bound the divalent cation through only two points of coordination to Thr^{688}, with the other four points of the unusual 8-fold pentagonal bipyramidal coordination to water molecules that indirectly bridged to enzyme side chains. Previous mutagenesis studies demonstrated that T688A mutation did not reduce Ca^{2+} affinity for the enzyme, but reduced the k_{cat} 61-fold and increased glycan binding affinity by 50-fold concluding that Ca^{2+} tethering through Thr^{688} directly facilitated catalysis. Here we present the co-crystal structure of the T688A mutant with an α1,2 mannobiose thiodisaccharide and compared the structure to a co-complex of the thiodisaccharide with the wild type enzyme. The disaccharide and Ca^{2+} ion were bound in identical positions in the T688A mutant. However, the 8-fold Ca^{2+} coordination of the wild type enzyme was altered to a 7-fold coordination in the mutant enzyme. The conformation of the glycone residue was also altered to an intermediate between a $^1C_4$ and $^3H_4$ conformation. Implications of the altered Ca^{2+} coordination and substrate conformation on the catalytic mechanism will be discussed. (Supported by NIH grant GM047533)

(131) **Glycosphingolipidomic Analysis of *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), characteristic glycosphingolipids of fungi whose biosynthesis is essential for normal growth and life cycle. Interestingly, structural features are shared between the GIPCs and capsular polysaccharides of *C. neoformans*, especially the GalXM. We speculate that one or more Xylβ1,2-transferases (XTs) involved in capsule synthesis might also add xylose to the GIPCs. We have already disrupted several genes responsible for xylose metabolism to study *C. neoformans* polysaccharide biosynthesis. We plan to use these strains to test our hypothesis with respect to GIPC biosynthesis as well. So far, we have compared GIPCs in wild type JEC21 (serotype D) and a strain from which xylose has been eliminated by disruption of the UDP-GlcA decarboxylase gene, *UXS1*. As expected, the characteristic Xylβ1,2 residue was missing from GIPCs of the knockout. Furthermore, a Manα6 residue, which elongated the Manα3Manα4Galβ core in the JEC21 wild type, was absent in the mutant. Comparative analysis of GIPC structural profiles in these strains and a series of XT knockouts will be presented.

(132) **Isolation, Genotyping, and Phenotypic Analysis of Mouse Embryo Fibroblasts from Mgat-V Knock-out Mice**

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The purpose of this experiment is to establish cell cultures of primary mouse embryonic fibroblasts (MEFs) from Mgat-V mice for experimentation on the effects of N-acetylglucosaminyl transferase V (Gnt-V) on cellular adhesion and motility. The Mgat-V knock-out mouse is a genetic murine knockout model for the gene encoding Gnt-V. Heterozygotes were bred and the females harvested on day 13.5 of pregnancy. Fetuses were collected and then prepared for cell culture. MEFs were then amplified and harvested. The harvested cells were then lysed and DNA was extracted. The resulting DNA samples were then quantitated and 25ng/µl solutions were prepared. Different polymerase chain reaction (PCR) protocols were used to determine the genotypes of the MEF cultures. The results from the PCRs confirmed the genotypes of each culture, homozygous knockouts (-/-), heterozygotes (+/-) and wild types (+/+) for the Gnt-V gene. Phenotypic changes were noted in the cell cultures. The Gnt-V knock-out cells had increased cell-to-cell adhesion and decreased cell motility as compared to the wild type MEF cells. On cell culture plates, the confluent Gnt-V (+/+) cells formed a fibrillar pattern, whereas the Gnt-V (-/-) cells formed a tight and relatively irregular monolayer. The null mice also displayed increased alpha 5, beta 1 subunits in lysates and on the cell surface which increased the cell matrix adhesion and decreased the cellular migration of fibronectin. Further experiments are being performed to obtain a more indepth analysis of this cell line's intracellular phenotypic changes.

(133) **Mucin-Type O-Glycosylation and O-GlcNAc Found in Rice Seed Storage Protein Prolamin Fraction**

Michelle Kilcoyne[1]; Miti Shah[1]; Jared Gerlach[1]; Amy Smith[1]; Kazuhito Fujiyama[3]; Veer Bhavanandan[1]; Ulf Summers[2]; Catherine Costello[2]; Lokesh Joshi[1]
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Mucin-type O-glycosylation has been well characterized in mammalian systems. We investigated the purified prolamin fraction of Shirikiku rice for O-linked oligosaccharides. SDS-PAGE and MS analysis of the purified prolamin fraction showed the presence of the 14-kDa and 16-kDa prolamin families while lectin blotting with PNA and VVA indicated the presence of Gal-beta-(1→3)-GalNAc. Endo-alpha-N-acetylgalactosaminidase digestion, subsequent 2-aminobenzamide (2-AB) fluorescent labeling and normal phase HPLC analysis revealed a peak consistent with Gal-beta-(1→3)-GalNAc-2AB. The mass spectrum was consistent with this structure and the MSMS fragmentation was similar to that of the commercial standard. Monosaccharide analyses by hydrolysis with HPAEC-PAD revealed the presence of galactose, galactosamine, glucose and, unexpectedly, glucosamine. Western blotting of the purified prolamin fraction with mouse anti-O-GlcNAc antibodies confirmed the presence of O-GlcNAc in both molecular weight families. The function of O-glycans and O-GlcNAc in seeds is currently unknown. We speculate that these structures may have roles in protein stability and signaling.

(134) **Monoclonal Antibody Glycosylation – A Study of Culture Media and Expression System Effects**

Ken Lawson; Yu-Heng Ma; Bernice Yeung; Jennifer Liu
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Monoclonal antibodies (mAbs) have relatively simple glycoforms comprised primarily of core-fucosylated, complex type bi-antennary N-glycans situated within the Fc region of the molecule. Though often shielded and not exposed directly, characterization of these glycans is necessary to ensure both product comparability and safety through the identification of potential immunogenic structures.

In this poster we will present the characterization of a recombinant mAb and the impact of both cell culture media composition and expression system on its glycoform distributions. While initial CHO production cultures showed very high protein expression levels, the resulting product contained significant levels of high mannose glycan structures due to incomplete glycan processing. In an effort to improve the glycan synthesis efficiency, the cell culture conditions and media were optimized, resulting in a significant decrease in the level of high mannose structures while maintaining relatively high protein titers. Parallel evaluations of a hybridoma expression system showed a nearly complete absence of these high mannose structures; however, unique glycoforms were generated, including several α-galactose and sialic acid variant species. Analysis of the N-glycan structures was performed using high pH anion exchange chromatography of fluorescently labeled N-glycans with characterization by MALDI-TOF mass spectrometry and exoglycosidase specific digestion.

(135) **Characterization of Glycan Moieties in Vitellogenin of the Freshwater Prawn Macrobrachium Rosenbergii, and Bioinformatics Comparison with other Decapod Crustaceans**

Ziv Roth; Shmuel Parnes; Simy Weil; Amir Sagi; Isam Khalaila
Ben-Gurion University, Beer-Sheva, Israel

Vitellin is the primary storage protein composing the egg yolk in oviparous species including decapod crustaceans. Vitellogenin (Vg) is the precursor of vitellin and in most decapod crustaceans it is synthesized in the hepatopancreas, secreted to the hemolymph and transported to the ovary. Vg undergoes vast modifications; and has been characterized as lipoglycocarotenoprotein. Glycosylation is an important post translational modification. The glycan moieties might influence the structure and activity of the protein and in some cases they were shown to have an essential role in the folding, processing and transporting of the protein. Until now Vg's of ten crustacean species where sequenced. In this study a bioinformatics approach has been used to retrieve the glycosylation characteristics of the known sequences of crustacean Vg, in addition to biochemical and structural characterization of the glycan moiety of the prawn M. rosenbergii. The Vgs were found to be conserved between the ten species. However, with respect to putative glycosylation sites there is a significant difference between different crustacean suborders. On the deduced Vg of M. rosenbergii, three putative N-glycosylation sites were found, that seem to be conserved in other crustaceans, however, no O-glycosylation sites were predicted by the web-based algorithm. The biochemical results showed that two of the three Vg subunits purified from M. rosenbergii (the 89 and 170 kDa) are indeed N-glycosylated with high mannose structures. Lectin study showed that the 89 kDa subunit also possess O-glycosylation modification although such modification was not predicted by the web based algorithm.

(136) **Expression of Human N-Acetylneuraminic Acid Phosphate Synthase and Bacterial N-Acetylneuraminic Acid Synthase in Tobacco Plants**

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The inability of plants to routinely generate terminally sialylated N-glycans poses a serious problem in their use as bioreactors for production of recombinant mammalian glycoproteins. In silico analyses of plant genomes have shown that although plant cells are probably capable of transporting and transferring sialic acid to potential acceptors, their ability for sialic acid synthesis is yet to be established. In contrast, in higher eukaryotes sialic acid biosynthesis is an active pathway involving conversion of UDP-GlcNAc to ManNAc-6-P, its condensation with PEP to NeuNAc-9-P, which after removal of the phosphate group is activated to CMP-NeuNAc - a substrate for sialyltransferases. In microorganisms the pathway is shorter as PEP condenses with ManNAc to form directly NeuNAc. The present work reports for the first time that human and bacterial enzymes involved in sialic acid biosynthesis can be functionally active in plant cell environment. Both, human N-acetylneuraminic acid phosphate synthase and Campylobacter jejuni N-acetylneuraminic acid synthase isoenzyme I genes were independently

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transferred to N. bentamiana L. plants by Agrobacterium-mediated transformation. The production of stable transgenic lines was verified by molecular analyses and the activity of the proteins was confirmed by in vitro enzyme assays. The results from the ectopic expression of human N-acetylneuraminic acid phosphate synthase and bacterial N-acetylneuraminic acid synthase suggest the feasibility of a transgenic approach for generating plant bioreactor capable of sialylating protein therapeutics.

(137) **A Model for the Biosynthesis of Xylans in Plant Secondary Cell Walls**

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The secondary cell walls of plants are the dominant component of biomass, and understanding their biosynthesis is key to renewable energy and biorefinery technologies. Xylans typically constitute 30% of the secondary cell wall and are the second most abundant biopolymer made by plants. Although enzymes responsible for the biosynthesis of cellulose and lignin, the other major components of secondary cell walls, have been identified, the biosynthesis of xylans is poorly understood. Expression of a large number of putative glycosyl transferases is correlated xylan biosynthesis, a result that is hard to reconcile with the relatively simple structures of xylans.

We identified several putative glycosyl transferase mutants in Arabidopsis thaliana that fail to generate normal secondary cell walls. Spectroscopic analysis was used to show that these mutants produce reduced amounts of xylan with atypical chemical structures. Specifically, the amount of an oligosaccharide that appears to act as a primer for xylan synthesis varies significantly in the mutant plants. Some mutants (class 1) produce a low amount of this oligosaccharide and reduced amounts of high-molecular weight xylan. Another mutant (class 2) produces normal amounts of this oligosaccharide, but produces moderately reduced amounts of xylan with a very low molecular weight. These results suggest a testable model in which class 1 glycosyl transferases are involved in initiation of xylan biosynthesis and the class 2 glycosyl transferase is involved in elongation of the xylan chain.

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(138) **Conformational Aspects of Polypeptide GalNAc Transferase Substrate Triplet Mucin Motifs**

Andrew Borgert; Mian Liu; George Barany; David Live
University of Minnesota, Minneapolis, MN

Structural knowldege of mucin proteins is important both in understanding interactions with the glycans they present, and in the level of specificity displayed by glycotransfeases in protein glycosyolation. Studies on smaller mucin glycopeptide constructs support the contention that these display conformational properties reflective of such segments in the native protein, making it possible to use synthetic, well-defined and tractable mucin glycopeptides to gain insights into the organization of mucin glycoproteins. Using NMR spectroscopy, we have examined structures of a series of glycopeptides based on the MUC 2 related sequence PTTTPLK, which has also been examined as a substrate for polypeptide GalNAc transferases. All permutations with GalNAc attached to one, two or three of the T residues have been made. These allow us to examine structural features as a function of the density and distribution of glycosylated sites. The comparison of four NMR derived structures lead to several interesting conclusions. First, comparing the orientation of the glycans on the fully glycosylated variant with previous work on the glycopeptide S*T*T*AV (* = GalNAc) shows that glycan disposition appears to be independent of whether the glycosylated residue is S or T in the triplet. Second, the structure seems largely invariant to the larger sequence context in which the glycosylated triplet appears. Results on the three doubly glycosylated constructs indicate a similar disposition of the respective glycans to the fully glycosylated construct even in the absence of one GalNAc. This offers insights into the degree of glycosylation needed to initiate the structural motif.

(139) **D-Configuration Peptides that Bind with High Affinities to Carbohydrate Binding Proteins**

Byron E. Anderson[1]; Joseph Firca[1]; Carrie Cook[1]; Eric Johnson[2]; William Tepp[2]
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Several publications suggest that aromatic compounds, including aromatic peptides, can bind to lectins, and to or near the carbohydrate binding sites. In this study we used a library of peptides of the D-configuration aromatic amino acids, phenylalanine, tyrosine and tryptophan, together with D-alanine and glycine, to synthesize penta-peptides attached to Tenta-Gel beads. The synthesis method results in each bead having multiple copies of a single D-configuration aromatic peptide (DAP) sequence. The library was screened for binding of a number of lectins at nM concentrations using various detection methods. The numbers of positive beads binding a particular lectin indicated a high degree of selectivity for most of the lectins as well as high binding affinities. Sequences were obtained from positive beads and DAPs were identified as binding with: ConA, PSA and GS1-B4 lectins; the botulinum toxin serotypes A, B and E, as well as the B complex; ricin and cholera toxins; antibodies to alpha-Gal and to Ley epitopes; as well as the proteins TNFalpha, TGFbeta1, and the protective antigen of the anthrax toxin. Synthesized DAPs conjugated to microtiter wells were used to estimate the Kd values of binding for ricin (20 nM) and the bot toxins A and B (0.5 - 2 nM). Particular DAPs were inhibitory of the binding of alpha-1-acid glycoprotein to bot B and E, and of ConA to alpha-methyl-mannoside.These results suggest that aromatic peptides can bind with high affinities to many lectins; such binding may potentially have many analytical, diagnostic and therapeutic uses.

(140) **Dolichol Kinase Deficiency Causes a New Inherited Disorder with Death in Early Infancy**

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The present study describes the discovery of a new inherited metabolic disorder, dolichol kinase (DK1) deficiency. DK1 is responsible for the last step of the de novo biosynthesis of dolichol phosphate, which is involved in several glycosylation reactions like N- glycosylation, GPI-anchor biosynthesis and C- and O-mannosylation.

The four patients described here were found to be homozygous for one of two mutations (c.295 T>A (99 Cys>Ser); 1322 A>C (441 Tyr>Ser)) in the corresponding hDK1 gene. The residual dolichol kinase activity was 2-4 % of control cells. The human wildtype allele complemented the temperature sensitive growth phenotype of dolichol kinase deficient yeast cells, whereas the mutated alleles failed to restore this growth phenotype.

Affected patients showed a very severe clinical phenotype with death in early infancy.

(141) **Epitope Characterization of Tamarind Xyloglucan Reactive Monoclonal Antibodies**

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The microstructure and dynamics of plant cell walls during development and in response to stress remain largely obscure, albeit general wall composition is well known. Owing to the specificity of antibody-antigen interactions, monoclonal antibodies can be utilized as probes for changes in cell wall fine structure. These antibody probes are most useful when their binding sites (epitopes) on the polysaccharide are known in detail. We have produced tamarind (*Tamarindus indica*) xyloglucan-reactive monoclonal antibodies that show differential recognition patterns against various purified plant cell wall carbohydrates in ELISAs. The antibodies can be grouped into at least four subgroups with distinct specificity patterns against polysaccharides from tamarind, tomato and sycamore. Immunofluorescence labeling of tamarind seed sections shows diverse labeling patterns for epidermal cell walls, further corroborating the four distinguishable specificities observed in vitro. All of the antibodies label the thick storage cell walls in the seed. Detailed epitope characterization using various methods, such as isothermal titration calorimetry, is under way for these monoclonal antibodies in order to understand the precise structures recognized by each of them. Knowledge of the epitope structures will permit more accurate interpretation of the observed differences in labeling patterns.

This work was supported by National Science Foundation Grant DBI-0421683.

(142) **Heparan Sulfate GlcNAc N-deacetylase/N-sulfotransferase Isoforms Differentially Generate Ligand Binding Sites**

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Heparan sulfate is a linear copolymer assembled from N-acetylglucosamine and glucuronic acid units extensively modified by a relatively ordered series of reactions involving an epimerase and four families of sulfotransferases

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which differentially place N- and O-sulfate groups. These modifications result in the generation of a very high degree of heterogeneity, which is thought to be largely responsible for differential binding of various ligands. The first step in heparan sulfate remodeling involves the action of N-deacetylase/N-sulfotransferase (NDST) isoforms that catalyze the removal of acetyl groups from N-acetyl glucosamine residues and then the transfer of sulfate to the newly deacetylated amino groups. The NDST gene family is composed of four distinct isoforms that exhibit differential spatial and temporal expression and show marked differences in the relative strength of their two catalytic activities. To date little is known about the specific functions of these isoforms and what effects they have on final heparan sulfate structure. We have developed a scheme to test the effects of all four NDST isozymes expressed in a mutant cell line which is null for the entire NDST family. With the use of specific biological assays that monitor the interactions between heparan sulfate and heparan sulfate-binding molecules along with chemical analysis of heparan sulfate structure by liquid chromatography tandem mass spectrometry we have found that distinct NDST isoforms have differing effects on heparan sulfate generation of binding sequences for apoE, FGF-2, FGF-18, VEGF165, FGFR1, HSV-1 glycoprotein gD, and antithrombin.

(143) **Proteoglycan-Driven Lipoprotein Metabolism in the Liver: Clearance of Triglyceride-Rich Particles Independent of Low-density Lipoprotein Receptors**

Jennifer M. MacArthur[1]; Kristin I. Stanford[1]; Joseph R. Bishop[1]; Lianchun Wang[1]; André Bensadoun[2]; Joseph L. Witztum[1]; Jeffrey D. Esko[1]
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Clearance of triglyceride-rich lipoproteins (TRLs) by the liver occurs when particles pass through the fenestrated endothelium, become sequestrated in the space of Disse and bind to receptors expressed by hepatocytes. The primary lipoprotein receptors include the LDL receptor (LDLR), LDL-receptor related protein (LRP) and one or more heparan sulfate proteoglycans (HSPGs). HSPGs may play multiple roles in clearance by sequestering lipoproteins in the space of Disse, by serving as a co-receptor for LRP, or as independent endocytic receptors. The identity of the active proteoglycans in this process (membrane-bound and secreted) and the relationship of heparan sulfate structure to lipoprotein binding and uptake in vivo remain unknown. We recently found that hepatocyte-specific inactivation of the heparan sulfate biosynthetic enzyme, GlcNAc N-deacetylase/N-sulfotransferase (Ndst1), led to a marked accumulation of triglyceride-rich/apoE bearing lipoproteins, with characteristics similar to VLDL and remnant particles derived from chylomicrons. Accumulation of these particles results from defective clearance, as demonstrated in vivo and in isolated hepatocytes. Heparan sulfate isolated from mutant hepatocytes bound poorly to apoE, providing a mechanism to explain altered clearance of the particles. Current studies focus on the identification of the proteoglycans that mediate lipoprotein clearance. These mice provide a potential model for studying certain forms of hypertriglyceridemia in humans.

(144) **Enzymatic Activity of Mutant Sulfotransferases Found in Macular Corneal Dystrophy Type II**

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In human, CHST6 encodes corneal GlcNAc 6-O sulfotransferase (hCGn6ST, also known as GlcNAc6ST-5 and GST4β), which is an essential enzyme for production of sulfated keratan sulfate (KS), and mutation on the gene leads to a hereditary eye disease, macular corneal dystrophy (MCD), which the patients develop clouding cornea. MCD is categorized into 3 subtypes by immunological examinations; no sulfated KS in cornea and serum (type I), presence of KS in cornea and serum, even at much reduced level in some case (type II), and no KS in serum and corneal matrix but present in stromal keratocytes (type IA). In the previous studies, we found loss-of-function mutations of CHST6 on genomes of MCD type I patients, and DNA rearrangements at putative promoter/enhancer region of CHST6 on genomes of MCD type II patients. From the finding, we hypothesized that complete lack of hCGn6ST activity leads to MCD type I phenotype and cornea-specific loss of CHST6 expression results in MCD type II phenotype. To date, several mutations on CHST6 have been reported to be responsible for MCD including type II. In this study, I examined sulfotransferase activity of mutant hCGn6STs, which have been found in patients of MCD type II, whether the mutants have ability to produce sulfated KS. By immunological and in vitro enzymatic analyses, I found 2 missense mutants out of 6 MCD type II mutants actually possess sulfotransferase activity and concluded that sulfotransferase activity is still present in all case of MCD type II mutation reported, supporting our original hypothesis.

(145) **Site-Mapping and Glycan Characterization of Functional Alpha-Dystroglycan**

Sana Hashmi[1]; Stephanie Hammond[1]; Jae-Min Lim[1]; Kazuhiro Aoki[1]; Mindy Perlman[1]; Gerardo Gutierrez-Sanchez[1]; James Wheeler[1]; James M. Ervasti[2]; Carl Bergmann[1]; Michael Tiemeyer[1]; Lance Wells[1]
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Alpha-Dystroglycan (aDG) is a highly O-mannosylated glycoprotein that in a multiprotein complex serves as a bridge between the intracellular cytoskeleton and the extracellular matrix. In several forms of congenital muscular dystrophy, mutations exist not in aDG but in the glycosyltransferases necessary for the O-mannose addition and extension of the glycan structure necessary for proper aDG function. Hypoglycosylated aDG has also been associated with oncogenesis and metastasis. Given the importance of glycosylation of aDG in disease, the work presented here is our current progress in fully site mapping and characterizing the glycans on aDG isolated, initially, from rabbit skeletal muscle. The sites of glycosylation are being mapped using mass spectrometry techniques for O-glycosylation including neutral-loss MSn directly on the glycopeptides and beta-elimination/Michael addition approaches. Released permethylated glycans are also being characterized by MSn approaches. Our glycan analysis and site-mapping data to date include several O-Man and O-GalNAc initiated structures on multiple residues of aDG. Furthermore, we have recently developed a laminin-1 binding assay using surface plasmon resonance and shown binding of the aDG preparation. We are currently using a host of glycosidases to elucidate the glycans necessary for aDG interaction with laminin-1. We will be using all this information to determine the key functional sites of modification and glycan structures on aDG for laminin-1 binding . Following mapping and characterization of the glycans on aDG from rabbit muscle, aDG glycosylation will be studied in other tissues, tumors, and mouse models of congenital muscular dystrophy. This work is supported by the MDA.

(146) **Glycoprotein Labeling and Detection: Novel Click Chemistry-Based Applications for Gel Electrophoresis, Flow Cytometry, and Fluorescence Microscopy**

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We demonstrate highly-selective and sensitive labeling methods for the detection of specific glycoprotein subclasses, including cell surface N- and O-linked glycoproteins and intracellular O-GlcNAc modified proteins, utilizing the copper-catalyzed cycloaddition reaction between azides and alkynes, or click chemistry. The two-step labeling technique involves the incorporation of unnatural azide-modified sugars into protein glycan structures and subsequent ligation with fluorescent or UV-excitable azide-reactive detection probes. Both metabolic and enzymatic labeling techniques were utilized for the incorporation of azide residues depending upon the cell or tissue source. Enzymatic labeling of O-GlcNAc-modified proteins, isolated from cell or tissue extracts, was accomplished using a permissive beta-Gal-TI transferase that accepts unnatural azido-modified UDP substrates. Metabolic labeling of cell surface O-linked or sialic acid containing glycoproteins, or intracellular O-GlcNAc modified proteins, was accomplished by feeding various cell types the unnatural tetraacetylated azide-modified sugars GalNAz, ManNAz, or GlcNAz, respectively. For detection, azido-modified glycoproteins were reacted with fluorescent or biotinylated azide-reactive probes. Assay readout formats include detection of azido-labeled glycoproteins by 1-D and 2-D electrophoresis, Western blot, FLOW cytometry, and fluorescence microscopy. Demonstrated detection sensitivities of the glycoprotein labeling approach were in the low femtomole range as determined by 1-D electrophoresis. This novel click-based glycoprotein detection strategy provides selectivity and sensitivity that is currently unachievable with presently available lectin-based and antibody-based methods

(147) **Profiling of Polysaccharide–Receptor Interaction with Recombinant Innate Immunity Receptor-Fc Fusion Proteins**

Shih-Chin Cheng[1]; Wen-Bin Yang[2]; See-Wen Chin[1]; Chun-Cheng Lin[2]; Chun-Hung Lin[2]; Yu-Ju Chen[2]; Po-Chiao Lin[2]; Ming-Fung Wu[1]; Chi-Huey Wong[2]; Shie-Liang Hsieh[1]
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Host immune cells are equipped with many surface receptors, such as Toll-like receptors (TLRs), lectins and immunoglobulin-like (Ig-like) receptors, to recognize the polysaccharides on the cell surface of pathogens. On the other hand, the immune modulation properties of many herb drugs, such as the medical fungus Reichi (Ganoderma lucidum), are attributed to the

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polysaccharides contained in the extracts that interact with these surface receptors. Due to the complexity of polysaccharides, it is difficult to perform quantitative and qualitative analysis of this class of molecules in the samples for the study of their functions. Lectins are sugar-binding proteins that bind sugar through the carbohydrate recognition domain (CRD). Recent studies also indicate that the Ig-like proteins, such as TREM and TREM-like transcripts (TLTs) are capable of interacting with polysaccharides. In this study, we cloned the extracellular domains of lectins, TREMs and TLTs, and fused with the Fc-portion of human IgG1 to generate receptor-Fc fusion proteins as probes to profile their interaction with different polysaccharides by enzyme-linked immunosorbent assay (EIA). It was found that polysaccharides from various sources display different profiles as fingerprints. The polysaccharides isolated from Ganoderma lucidum, for example, were found to bind not only Dectin-1, but also DC-SIGNR, Kupffer cell receptor (KCR), and TLT-2. This high-throughput profiling provides a new effective method not only for the functional study of polysaccharide but also for the characterization of polysaccharides in herbal medicines and natural products.

(148) **The Studies on the Extracting Technologies and Purification of Fucoidan from Laminaria japonica**

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Abstract The extracting technologies of three sets of enzymatic hydrolyzing for fucoidan from Laminaria japonica were studied in this paper. Through the orthogonal tests, the optimum factors were determined by the yield rates of fucoidan extract, the contents of SO42- and polysaccharides. The optimum factors for Novozymes Viscozyme L were: the Novozymes Viscozyme L added 0.06%, temperature 40℃, pH 3.5 and time 30 minutes. The optimum factors for Celluclast BG were: the Celluclast BG added 0.208%, temperature 50℃, pH 4.5 and time 50 minutes. The optimum factors for Cellulase and pectase were: the Cellulase added 0.221%, the pectase added 0.074%, temperature 50℃, pH 4.5 and time 50 minutes. The yield rate obtained by Cellulase and pectase hydrolyzing and the purity of fucoidan product were higher than by the traditional hot water extracting technology. The natural unique structure and the SO42- content of fucoidan were the key factor of functional activities. Therefore the Cellulase and pectase hydrolyzing were used for further fucoidan extractions.

Through anion-exchange chromatography DEAE－52, FuⅡ, FuⅣ and FuⅤ were isolated from fucoidan extracted by Cellulase and pectase hydrolyzing. Then FuⅡ, FuⅣ and FuⅤ were further purified by chromatography Sephadex G-200 and ten fractions were obtained. Five fractions were with large molecular weights and they are possibly the aggregates of fucoidan. The other five fractions of them were arranged with the molecular weights of about 57.8KDa, 115KDa, 125KDa, 154KDa and 206KDa.

(149) **A Zebrafish Model for Mucolipidosis II**

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The lysosomal storage disorder, mucolipidosis II (MLII), is caused by highly deficient or absent activity of UDP-GlcNAc:lysosomal enzyme GlcNAc-1-phosphotransferase, the enzyme that catalyzes the first step in the biosynthesis of mannose-6-phosphate residues. Patients with MLII exhibit multiple clinical features shortly after birth including skeletal and cardiac defects, coarse facial features and psychomotor retardation. Due to the lack of an appropriate animal model, there has been little insight to date regarding the mechanisms by which loss of mannose-6-phosphate residues on proteins results in the developmental abnormalities of the disease. Using a morpholino-based knockdown strategy, we have generated a vertebrate model for MLII in zebrafish (Danio rerio). Injection of one-cell stage zebrafish eggs with a translation-blocking morpholino resulted in a dose-dependent reduction of phosphotransferase activity in embryos yielding several developmental defects consistent with the human disease. Morpholino-injected zebrafish embryos exhibited enlarged hearts, craniofacial defects and abnormal pectoral fin development. In addition, phosphotransferase deficient embryos have a pronounced motility defect, possibly attributed to abnormal neuromuscular junction organization and/or impaired motoneuron migration. Appearance of specific phenotypes directly correlated with increasing loss of phosphotransferase activity. In preliminary experiments, injection of translation-blocking morpholinos towards the cation-independent but not cation-dependent mannose-6-phosphate receptor resulted in similar but not identical phenotypes. This work establishes the first known vertebrate model for MLII. Such a model may facilitate further insight into the molecular pathogenesis of MLII.

(150) **A New Mutation that Alters Tissue-Specific Expression of N-Linked Glycans in the *Drosophila* Embryo**

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Glycosylation influences multiple developmental processes that control differentiation and morphogenesis. Mechanisms that regulate the full diversity and dynamic flux of glycan expression in the embryo remain largely unknown. 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 random screen for mutations that affect HRP-epitope expression, we generated a new mutation that abolishes almost all HRP-epitope expression without affecting early steps in N-linked glycosylation. We named this mutation *sugar-free frosting* (*sff*) because the nerve cord still retains a hint of HRP-epitope, which has the appearance of lightly frosting the axon scaffold. Our single mutant allele of *sff* (sff^{B22}) is viable and temperature sensitive with respect to embryonic HRP-epitope expression and homozygous adults display a behavioral phenotype. Wild type adults climb rapidly up the side of their vials (negative geotaxis), but sff^{B22} mutants are significantly slowed. The geotaxis phenotype is rescued pharmacologically by acute administration of a tricyclic antidepressant, indicating an underlying neuropathology. The mutation maps to 61E1-F7, a chromosome interval lacking fucosyltransferase genes. Characterization of the N-linked glycan profile of sff^{B22} embryos verifies the loss of α3-linked Fuc and detects the presence of major α6-monofucosylated glycans. Therefore *sff* is a regulator of tissue-specific glycosylation, not a component of the biosynthetic machinery that assembles the HRP-epitope. Supported by funding from NIH/NIGMS. The first two authors contributed equally to this work.

(151) **Identification and Characterization of a Novel *Drosophila* 3' -Phosphoadenosine 5' -Phosphosulfate Transporter**

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Sulfation of macromolecules requires the translocation of a high energy form of nucleotide sulfate, *i.e.* 3' -phosphoadenosine 5' -phosphosulfate (PAPS), from the cytosol into the Golgi apparatus. In this study, we identified a novel *Drosophila* PAPS transporter gene *dPAPST2* by conducting data base searches and screening the PAPS transport activity among the putative nucleotide sugar transporter genes in *Drosophila*. The amino acid sequence of dPAPST2 showed 50.5 and 21.5% homology to the human ortholog PAPST2 and SLALOM, respectively. The heterologous expression of dPAPST2 in yeast revealed that the dPAPST2 protein is a PAPS transporter with an apparent K_m value of 2.3 μM. The RNA interference of *dPAPST2* in cell line and flies showed that the *dPAPST2* gene is essential for the sulfation of cellular proteins and the viability of the fly. In RNA interference flies, an analysis of the genetic interaction between *dPAPST2* and genes that contribute to glycosaminoglycan synthesis suggested that *dPAPST2* is involved in the glycosaminoglycan synthesis and the subsequent signaling. The *dPAPST2* and *sll* genes showed a similar ubiquitous distribution. These results indicate that dPAPST2 may be involved in Hedgehog and Decapentaplegic signaling by controlling the sulfation of heparan sulfate.

(152) **Role of O-Glycosylation in Quality Control of Notch Folding**

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Notch is a cell surface receptor that is vital to the development of many organisms. The extracellular domain of Notch consists of up to 36 tandem epidermal growth factor-like (EGF) repeats, each of which contains 6 cysteines forming three disulfide bonds. Formation of the proper disulfide-bonding pattern for each EGF repeat in an extracellular domain with over 200 cysteines presents a formidable challenge to cellular folding machinery. Many of the EGF repeats of Notch contain consensus sequences for O-fucosylation or O-glucosylation. Protein O-fucosyltransferase 1 (Pofut1, responsible for addition of O-fucose to EGF repeats) is a well-studied enzyme that is essential for Notch signaling, is a soluble enzyme in the ER, and is able to distinguish between folded and unfolded EGF repeats. Pofut1 is believed to be essential for proper folding of Notch. Although protein O-

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glucosyltransferase (Pogut) has not yet been identified, the activity shares a number of similarities with Pofut1. Pogut also appears to be a soluble enzyme localized in the ER with the ability to distinguish between folded and unfolded EGF repeats. Mutation of specific O-fucose or O-glucose modification sites causes a decrease in cell surface expression of mouse Notch1, adding support to a possible role for these modifications in quality control. To further address whether O-fucose or O-glucose play important roles in quality control of Notch receptor folding, we are examining processing and cell-surface expression of Notch in cells deficient in either GDP-fucose or UDP-glucose biosynthesis. This work was supported by NIH grant GM61126.

(153) **Dissecting the Biological Role of Mucin Type O-Glycosylation using RNA Interference in Drosophila Cell Culture**

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Mucin type O-glycosylation is initiated by the family of evolutionarily conserved polypeptide N-acetyl-α-galactosaminyltransferases (ppGaNTases or pGalNAcTs in mammals and pgants in Drosophila). In order to dissect the biological function of the family members, we set up an RNA interference (RNAi) system in Drosophila cells to monitor changes in cellular viability, morphology and sub-cellular architecture upon the loss of particular isoforms. We performed RNAi screens with a set of double-stranded RNAs (dsRNAs) targeting the 12 potential pgant members in Drosophila cell culture. Additionally, dsRNA to proteins known to be expressed in the Golgi apparatus as well as the yellow fluorescent protein were used as positive and negative controls, respectively. We quantitated the expression levels of each isoform after RNAi treatment using real-time PCR. Fluorescence microscopy was used to visualize morphological alterations in treated cells. Using this approach, we obtained specific knockdown of individual isoforms and associated changes in cell viability and morphology. Therefore, this cell culture-based assay should allow us to begin to understand the cellular role of each pgant, thereby providing the basis for a more complete view of the mechanism of mucin-type O-linked glycosylation in biology.

(154) **In Vivo Functional Studies of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases In Drosophila melanogaster**

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O-Linked protein glycosylation is initiated by the action of a 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 in an O-glycosylic linkage to either Thr or Ser has a profound structural impact on a polypeptide backbone and thus highlights the unique physicochemical properties of O-glycosylated proteins such as mucin glycoproteins. 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. Previous work in our group demonstrated enzymatic conservation between mammalian and Drosophila orthologues within this gene family. One member, pgant35A, was found to be essential for viability and development in Drosophila. Our current work continues to focus on the biological function of additional members of this enzyme family. 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 each gene at specific times and in specific organs systems will aid us in deciphering the role of O-glycoproteins in vivo.

(155) **Glycotranscriptome Analysis during Differentiation of Murine Embryonic Stem Cells Assayed by High-Throughput Real-Time RT-PCR**

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Elucidating the regulatory mechanisms for mammalian cellular differentiation is perhaps one of the most critical areas of contemporary research in developmental biology because of its potential applications for embryonic stem (ES) cell-derived therapeutics in the treatment of human disease. The molecular mechanisms that regulate differentiation of stem cells into specific cell lineages are just beginning to be revealed. In an effort to define the changes in cell surface glycan structures as markers for differentiated phenotypes, we have performed focused transcriptome analysis of undifferentiated ES cells, retinoic acid (RA) induced neuroectoderm lineages, and differentiated mixed cell populations generated by growth of aggregates in suspension (embryoid bodies, EBs). A comprehensive list of >740 glycan-related genes was assembled from multiple sources to cover all the glycosyltransferases, glycosidases, glycan modifying enzymes, enzymes involved in sugar-nucleotide biosynthesis and interconversions, transporters, lectins, and other glycan-associated proteins from the mouse genome. A quantitative high-throughput SYBR Green qRT-PCR method was used to examine transcript abundance of glycan-related genes revealing numerous differences in relative transcript abundance between the three cell populations. Surprisingly, numerous transcripts that were not detected in ES cells, were induced by >1000 fold upon RA-induced differentiation or differentiation into EB, while a smaller number of transcripts were down-regulated upon differentiation. Correlations of transcript levels and glycan structures are presently underway and the poster will highlight examples where changes in transcript levels led to identification of altered cell surface glycan structures as potential markers for differentiated cell types. (Supported by NIH grant RR018502)

(156) **Glycomic Analysis of Oligosaccharides that Bind Sperm**

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Interactions between glycans and lectins on the cell surface and extracellular matrix mediate many cell-cell interactions, including those between sperm and the oviduct and sperm and the egg. Immature (uncapacitated) sperm bind to the oviduct, which maintains viability and is believed to promote capacitation. Capacitated sperm bind to the egg extracellular matrix (zona pellucida) and then undergo acrosomal exocytosis before penetrating the zona. Studies using competitive inhibitors have identified candidate mediators of sperm binding but the specific glycans in the oviduct or zona pellucida that bind sperm remain controversial. We took an alternate approach and identified glycans capable of binding sperm using a glycan array. An array containing 264 glycans and glycoproteins was used in an adhesion assay to identify glycans possibly involved in sperm-oviduct and sperm-egg interactions. Live porcine sperm stained with DiC18 were incubated with the array and fluorescence of bound sperm was assessed. Uncapacitated, capacitated, and acrosome-reacted sperm bound to neutral and charged glycans. When ranked by the number of sperm bound, no glycans were among the top 5% in binding sperm of all three maturational stages but several bound two stages. Most glycans bound only a specific maturational stage of sperm. Sperm bound to glycans in a charge- and conformation-specific manner and bound several types of glycans, suggesting the presence of multiple lectins on sperm. The glycans that bind sperm may be useful to block fertility or develop laboratory fertility tests. (Glycan arrays and detection were provided by the Consortium for Functional Glycomics Grant number GM62116).

(157) **Immunological Studies of Plant Cell Wall Glycome Dynamics**

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The cell walls of plants play a prominent role in determining the structure and shape of individual cells, and ultimately the morphology of the plant as a whole. Chemical studies have provided an overall picture of the structure and organization of the major wall polymers. However, these analyses do not provide complete information about wall structure and dynamics at the cellular and sub-cellular levels. Antibodies provide highly specific and sensitive tools to monitor the composition of cell walls at the cellular level. We are currently generating and characterizing an expanded set of monoclonal antibodies that bind to diverse carbohydrate epitopes residing on hemicelluloses, pectins, and glycoproteins. We have employed these antibodies (CCRC series), and those generated by others (MAC, JIM and LM series), for immunofluorescent and immunogold electron microscopic studies in *Arabidopsis thaliana*. Tissue sections examined were taken from leaves and flowers, and from different points along roots and stems. The labeling patterns observed show a diversity of cell- and tissue-specific localizations of carbohydrate structures in cell walls and organelles. In addition, both appearance and disappearance of carbohydrate structures in the walls of specific cell types were observed along developmental gradients in some of the tissues examined. These results demonstrate the utility of monoclonal antibodies to provide new insights into the diversity and plasticity of the glycome of plant cells. *[Supported by grants from DOE (DE-FG02-96ER20220 and DE-FG02-93ER20097) and NSF (DBI-0421683 and RCN-0090281).]*

(158) **Isolation of Glycoproteins from *Caenorhabditis elegans* by Lectin Affinity Chromatography**

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Caenorhabditis elegans has been used as a model system for distinct studies to building an understanding of animal genetics, development and behavior. We have used this organism to find glycoproteins related to animal development. Previous investigations of the N-glycans in *C.elegans*, including our own, have frequently employed global N-glycan release, but not addressed to entities that glycans are coupled, the glycoproteins. Then, we have been stained *C.elegans* with FITC-labeled lectins and showed that jacalin, KM+ and lentil lectin stained differentially parts of *C.elegans* body. For isolation of glycoproteins we have used lectin affinity chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis from glycoproteins retained in jacalin and lentil lectin affinity chromatography has exhibited very similar profiles. These glycoproteins present a range of 14 to 97 kDa. Subsequently, the gel was stained by the standard periodic acid-Schiff base method in the Fuchsia Glycoprotein Gel Stain Kit to confirm the presence of oligosaccharides in these proteins. It has been observed that glycoproteins of 14 to 35 kDa were strongly stained. It has been showed that jacalin recognize N-Acetyllactosamine oligosaccharides with terminal α-galactose residues as well as glycopeptides containing O-linked oligosaccharides. Lentil lectin recognize oligosaccharides with chitobiosyl core and the α-(1-6)-linked fucose residue attached to the reducing terminal GlcNAc. According these results, we suggest these oligosaccharides can be found in the same glycoproteins. To step to the next level, we have identified these glycoproteins by mass spectrometry. FAPESP, CAPES and FAEPA.

(159) **Role of Skp1 Prolyl Hydroxylation and Glycosylation in Oxygen-Dependent Development in Dictyostelium**

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A biochemical screen for glycosylation of cytoplasmic proteins previously netted a novel glycosylation pathway acting on Skp1 in the social amoebazoan Dictyostelium. The glycan chain is assembled on 4-hydroxyproline, a modification that depends on P4H1, the Dictyostelium ortholog of cytoplasmic animal prolyl 4-hydroxylases hypothesized to be physiological oxygen sensors regulating the half-life of HIFalpha. New biochemical and informatics evidence indicates that a related hydroxylation/glycosylation pathway exists in the intracellular human pathogen Toxoplasma gondii. Skp1 is best known as a subunit of E3(SCF) ubiquitin ligases responsible for regulating the lifetime of cell regulatory proteins. In Dictyostelium, a terminal developmental transition (culmination) is inhibited by mild hypoxia. Genetic inactivation of P4H1 also inhibits culmination, and inhibition is rescued by overexpression of normal but not catalytically-inactive P4H1. P4H1 overexpression also bypasses the hypoxic blockade suggesting that the hypoxic signal is mediated by P4H1 oxygen-substrate starvation. Overexpression of protein kinase A, which regulates culmination, bypasses inhibition mediated by either hypoxia or inactivation of P4H1, indicating that P4H1 and oxygen act upstream. Genetic inactivation of the second glycosyltransferase does not inhibit culmination, whereas the first (alphaGlcNAcT1) glycosyltransferase gene resists inactivation suggesting an essential function. Biochemical complementation suggests that Skp1 is the only substrate of the P4H1/alphaGlcNAcT1 enzyme pair, apparently encoded by a single gene in other protists, that accumulates in P4H1-null cells. These results indicate that P4H1 is a critical mediator of oxygen-dependent development, but further evidence is required to establish that this enzyme functions via the predicted target substrate Skp1. (Supported by NIH GM-03759)

(160) **Knock-Down of Galectin-1-like Proteins in Zebrafish (*Danio rerio*) Reveals a Muscle and Heart Developmental Phenotype**

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We have previously identified and characterized four galectin-1-like proteins in zebrafish, (Drgal1-L1, Drgal1-L2, Drgal1-L3, and a spliced variant of Drgal1-L2) with distinct spatial and temporal expression patterns. Drgal1-L1 is maternal, Drgal1-L2 is zygotic and strongly expressed in the notochord, while Drgal1-L3 is both maternal and zygotic. We have recently identified and characterized an additional galectin-1-like protein (named Drgal1-L4), which shows ontogenic expression similar to Drgal1-L2. Knock-down experiments in zebrafish embryos using morpholino-modified antisense oligo targeted to the Drgal1-L2 5'-UTR sequence resulted in a phenotype with a short and bent tail, and disorganized muscle fibers. A stronger phenotypic effect (bent tail, disorganized muscle, under-developed heart, disrupted blood circulation (lower cell numbers and flow rates) was observed when Drgal1-L4 was knocked-down along with Drgal1-L2. This effect was dose-dependent as follows: 68-78% at 17 ng, 35-40% at 8.5 ng, 16-20% at 4.2 ng, and 10-12% at 2.1 ng. However, these morphological defects were not observed in the Drgal1-L1 knock-downs, indicating that the phenotype is sequence-specific, and not due to the toxicity of the morpholino-modified oligos. As the notochord serves as the primary source of signaling molecules required for proper patterning of adjacent tissues such as neural tube, somites, and heart, galectins produced by the notochord may also play a key role in somitic cell differentiation and heart development. The mechanism by which galectins may participate in this process will be discussed. (Supported by NIGMH Grant R01 GM070589-01 to GRV)

(161) **The N-Glycome of Human Embryonic Stem Cells**

Tero Satomaa[1]; Annamari Heiskanen[1]; Milla Mikkola[2]; Cia Olsson[2]; Maria Blomqvist[1]; Taina Jaatinen[3]; Jari Helin[1]; Jari Natunen[1]; Timo Tuuri[4]; Timo Otonkoski[5]; Juhani Saarinen[1]; Jarmo Laine[3]
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Complex carbohydrate structures, glycans, are crucial components of glycoproteins, glycolipids, and proteoglycans. While individual glycan structures such as the SSEA and Tra antigens are already used to define undifferentiated human embryonic stem cells (hESC), the whole spectrum of stem cell glycans has remained unknown. We undertook a global study of the asparagine-linked glycoprotein glycans (N-glycans) of hESC and their differentiated progeny using MALDI-TOF mass spectrometric profiling and proton NMR spectroscopy of unmodified N-glycosidase F liberated glycans. The data demonstrated that stem cells have a unique N-glycome which consists of a constant part and a variable part that changes during hESC differentiation (circa 75%/25%, respectively). Significantly, certain hESC-associated N-glycans were lost and novel glycans emerged in the differentiated cells. By use of novel quantitative data analysis methods for the mass spectrometric glycan profiles, we were able to deduce N-glycan structural features typical to each cell type and also evaluate the extent of the changes in the N-glycome. The applicability of the analysis methods were verified by use of nano-scale proton NMR N-glycan profiling as well as specific exoglycosidase digestions. We found that both N-glycan core structures and their decorations were changed during hESC differentiation, while complex fucosylation was the most characteristic glycosylation feature of undifferentiated hESC. These results provide an overview of the glycobiology of hESC and form the basis for strategies to target stem cell glycans.

(162) **Mice with a Human-like Deficiency in N-glycolylneuraminic Acid Biosynthesis Mimic Aspects of the Human Condition**

Maria Hedlund[1]; Pam Tangvoranuntakul[1]; Hiromu Takematsu[2]; Jeffrey Long[1]; Gary D. Housley[1]; Yasunori Kozutsumi[2]; Akemi Suzuki[3]; Anthony Wynshaw-Boris[1]; Allen F. Ryan[1]; Richard Gallo[1]; Jerrold Olefsky[1]; Nissi Varki[1]; Ajit Varki[1]
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Humans and chimpanzees share >99% identity in protein sequences. One rare biochemical difference determined by protein sequence changes results from an Alu-mediated human-specific inactivating deletion in the CMAH gene, which determines biosynthesis of the sialic acid N-glycolylneuraminic Acid (Neu5Gc). However, Neu5Gc expression has been found in human cancers and fetuses, and trace amounts reported in normal human tissues. We used two approaches to inactivate the CMAH gene in mice, and studied the consequences in vivo. We found no evidence for an alternative biosynthetic pathway for Neu5Gc in normal, fetal or malignant tissue. Rather, null fetuses accumulated Neu5Gc from heterozygous mothers and dietary Neu5Gc was incorporated into malignant tumors induced in adult mice. As with humans, all tissues also showed an accumulation of the precursor N-acetylneuraminic Acid (Neu5Ac) and small increases in sialic acid O-acetylation. While viable and fertile under vivarium conditions, the null mice showed several abnormalities, some reminiscent of the human condition. Adult animals showed delayed skin wound healing and a tendency to become obese on a high carbohydrate (but not on a high fat) diet. The mice also showed a diminished acoustic startle response and variable prepulse inhibition of this response. In keeping with this, marked histological abnormalities of the inner ear developed in older mice, which also had impaired hearing. Thus, the loss of Neu5Gc production that occurred in the human ancestral lineage ~2-3 million years ago would have had both immediate and long-term consequences for the human condition, some of which appear to remain extant today.

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(163) **Polysaccharide Microarray Technology for the Serodiagnosis of *Burkholderia mallei* Infection in Horses**

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Burkholderia mallei is an obligate mammalian pathogen that causes the zoonotic disease glanders. *B. mallei* is a biothreat agent and classified as category B pathogen by the Centers for Disease Control and Prevention. The complement fixation (CF) test is the traditional procedure for the serodiagnosis of glanders. CF test interpretation is often subjective. Furthermore, the CF test is based on crude whole-cell preparation or extracts of the bacteria, and therefore, the potential for false-positive serodiagnosis cannot be ruled out. In this study, we developed a polysaccharide microarray platform, using well-characterized antigens (capsular polysaccharides and *O*-antigen saccharides). These polysaccharides were isolated from irradiated bacteria (*B. mallei* and *B. pseudomallei*) and then immobilized onto glass slides. This polysaccharide array was tested successfully for detecting *B. mallei* antibodies in the sera of glanders- infected horses.

(164) **Detection of Different Glycosylation by a New Proteome Platform**

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Introduction: A new proteome platform 2DICAL (2-Dimensional Image Converted Analysis of Liquid chromatography and mass spectrometry) we introduced last year (Ono et al., MCP, 25:1338-1347, 2006) has become enable to compare a large number of samples equipping a new 2-dimensional analysis system. The new 2-dimensional image consists of the axes with retention time (RT) and sample using the advantage of the mass to charge ratio (m/z) precision of mass spectrometer. By this system, different glycosylation modification is easily compared on the same peptide of different samples. In this conference we report the new system to analyze N-linked glysosylation modification of CEA (carcinoembryonic antigen) and introduce new software to recognize glycosylation from MS/MS data.

Materials and Methods: Four commercial CEAs were prepared to the concentration of 0fmol, 500fmol and 5pmol with 1pmol albumin. They were totally digested by trypsin and resuspended in 0.1% formic acid solution. Performing LCMS measurement, the data was analyzed by 2DICAL.

Results: The peptide fragments without modification were visualized in the same pattern among different CEAs by 2DICAL. But the peptide fragment with LQLSNGN*R which was modified with N-glycosylation at N* were differently visualized at m/z of 1059 among the CEAs. Two of them had strong peaks, one had weak peak and the rest one was not visible.

Conclusion: 2DICAL can detect the difference of differently glycosylated peptide fragments. 2DICAL has a great possibility to analyze the glycosylation difference of glycoproteins.

(165) **Advances in Purification Methods of Serum Glycoproteins for MALDI-MS Analysis of N- Glycome in Patients with Glycosylation Disorders**

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Genetic defects of the N-glycosylation pathway, named Congenital Disorders of Glycosylation (CDGs), result in abnormalities of N-glycome with aberrant glycan structures and changes in the relative levels of normal glycan moiety. Understanding N-glycan profile may be useful for characterization of known CDG types and to identify glycosylation processing defects in unsolved patients.

CDG are heterogeneous disorders with variable clinical findings and multisystem involvement. As glycosylation defects are usually associated with abnormal glycoprotein folding and activity, it is plausible that the variety of clinical signs in CDG underlies abnormalities in a plethora of glycosylated molecules. Serum Transferrin was widely used so far to characterize N-glycan profile in patients with CDG; an alternative approach was based on the analysis of N-linked glycan released from total plasma. Our present work on N-glycome analyses in patients with CDG and related disorders is based on the systematic characterization, in addition to Transferrin, of multiple abundant serum glycoproteins, including acute-phase proteins. On this regard, we are working for creation of N-glycan profiling panel of each patient by the following steps: 1) purification of target glycoproteins by using sequentially, selective immunoaffinity columns on a few amount of unique serum sample. 2) characterization of the intact glycoprotein by MALDI mass spectrometry: this fundamental step allows us to analyze the rate and extent of deglycosylation (N-glycosylation site underoccupancy). 3) MALDI analyses of N-glycan structures. The observed occurrence of underglycosylation and abnormal glycan structure of AAT in CDG-Ia may link to possible unbalance of protease/antiprotease system in these patients.

(166) **All-in-One Processing of Oligosaccharides on Solid-Support (A1-POS); A General Protocol with *Glycoblotting* for Functional Glycomics (Part 1)**

Yoshiaki Miura[1]; Jun-ichi Furukawa[1]; Yasuro Shinohara[1]; Hiromitsu Kuramoto[2]; Masaki Kurogochi[1]; Hideyuki Shimaoka[2]; Shin-Ichiro Nishimura[1]
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Here we present a so-called all-in-one sample prep solution for the analysis of functional glycome, that combines oligosaccharide-capturing onto beads, methyl esterification, and fluorescent-labeling in a single workflow on a multiwell filter plate, allowing robotic application. For the development of the all-in-one method, we designed a fluorescent probe consisting of a fluorophore, hydrazide, and thiol functionalities. The hydrazide and thiol group owe the chemoselective enrichment of oligosaccharides and introduction of the probe on the surface of solid supports, respectively. Since stabilization of sialic acids are essential for the quantitative mass analysis (MS) of sialyloligosaccharides, a novel methyl esterification of sialic acid carboxylates was incorporated into the protocol (Y. Miura et al., in preparation). Enzymatically-released *N*-glycans prepared from human serum according to our recent report[1] were subjected to the *A1-POS* protocol. Upon capturing the released *N*-glycans via hydrazone linkage (Glycoblotting[2]), the facile on-bead methyl esterification was followed in order to analyze simultaneously both the neutral and acidic glycomes by MS. For the HPLC analysis with fluorescent monitoring in amine-adsorption or WAX mode, the esterification step can be omitted for the charge-based separation. The effectiveness of this procedure was demonstrated through negligible sample loss and quantitative detection of serum and cellular glycomes. The results provide a generalization of sample preparation that will facilitate functional glycome analysis across a wide variety of biological samples.

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(167) **MALDI-TOF/TOF-MS for the Analysis of Pyrenebutyric Hydrazide-Derivatized Keratan Sulfate Oligosaccharides**

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In recent years, MALDI-TOF/TOF tandem mass spectrometry has proven to be a very powerful tool for oligosaccharide structure elucidation due to its simplicity, speed of analysis, and comparatively increased sensitivity. Compared to peptides, the relatively low signal intensity still limits the utility of MALDI-MS for analysis of oligosaccharides, especially for acidic oligosaccharides, such as polysulfated oligosaccharides. Keratan sulfate (KS), is a glycosaminoglycan having a linear backbone consisting of repeating disaccharide units composed of alternating residues of D-galactose (Gal) and N-acetyl-D-glucosamine (GlcNAc) linked β-(1-4) and β-(1-3), respectively. In some domains of KS from most tissues, the hydroxyl groups at the C-6 positions of both Gal and GlcNAc residues are sulfated. In the present work, analysis of pyrenebutyric hydrazide-derivatized keratan sulfate oligosaccharides was achieved in combination with 2,5-dihydroxybenzoic acid (DHB) matrix using MALDI-TOF/TOF-MS under negative ionization mode. The results show that the pyrene-derivatization can extremely increase the intensity of KS oligosaccharides. The MS1 spectra of mono-sulfated disaccharides exhibit a peak at m/z 746.304 corresponding to the molecular ion [M – H] –, The MS1 spectra of multiply sulfated KS oligosaccharides reveal a singly charged ion corresponding to the molecular ion [M + nNa – (n+1)H] –, and the number of sulfates equals n+1. The MS2 spectra of pyrene-derivatized keratan sulfate oligosaccharides give linkage and sequence information. Moreover, two sialyated KS disaccharide isomers were distinguished through MALDI-TOF/TOF-MS.

(168) **The Monitoring and Characterization of Endoglycosidase H Released N-Glycans on Monoclonal Immunoglobulin G**

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The recombinant monoclonal immunoglobulin G (IgG) contains mainly biantennary complex-type asparagine-linked oligosaccharides and small amounts of high-mannose type and hybrid type N-glycans. The type of N-glycans on IgG may influence antibody biological function. The quantitative monitoring of IgG N-glycan species with traditional oligosaccharide mapping by HPLC is a laborious process. A simple and robust method to quantitate high-mannose and hybrid type glycans on IgG by CE-SDS, following treatment with endoglycosidase H, has replaced the HPLC method. However, with the CE-SDS method, the % high-mannose and hybrid type N-glycans are indirectly quantitated by measuring the % increase in the unglycosylated IgG heavy chain peak and does not offer the qualitative identification of the Endo H sensitive glycans. The Endo H sensitive glycans have been identified by sequential exoglycosidase treatment and followed by Normal-phase HPLC. The peak identifications were then verified by MALDI-TOF analysis. With all the glycans characterized, it was then possible to determine the % contribution of the high mannose type glycans on IgG.

(169) **Neuraminidase Assay Based on Fluorescent Oligosaccharide Substrates**

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Fluorescent neuraminidase (NA) assay has been developed. Ion-exchange 96-well plates were used for separation of BODIPY-labeled neutral product from non-reacted negatively charged substrate followed by fluorescent detection of both components. Twenty probes in four replicates could be analyzed at the same time, giving us a possibility to study kinetics of enzyme-substrate interaction. So, comparing the slopes of the starting region of the concentration kinetic curves for different substrates, we got a pattern of NA substrate specificity, while evaluating V_{max} and K_M, we could elucidate the reasons determining this profile. Specificities of six influenza H1N1 virus NAs towards six BODIPY-labeled sialooligosaccharides, 3`SiaLa$^c$, 3`SiaLacNAc, SiaLe^c, SiaLe^a, 6`SiaLac and 6`SiaLacNAc, have been evaluated. The obtained results evidence that influenza virus NA can discriminate not only the type of bond between Neu5Ac and Gal residues but also distinguish the structure of substrate at tri- and tetrasaccharide level.

(170) **RINGS: Resource for INformatics of Glycans at Soka**

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In recent years, many glycome informatics methods have been published, theoretically providing insights into glycan structure and function either for specific diseases or on a comprehensive scale. However, these methods are not available at any particular site for ease-of-use, to our knowledge. Furthermore, the application of these methods to a particular research project would require the knowledge of an informatician to implement it. Knowing that this is infeasible for many, we present a new resource that centralizes these methods for free use by the glycobiology community, called RINGS (Resource for Informatics of Glycomes at Soka). Currently based on data from KEGG, RINGS provides links between glycans and protein sequence data such that relevant glycans to a specific protein may be queried by BLAST easily. The 3D protein structure and reaction information can be easily viewed as well. 2D queries are also possible simply by using the mouse to input structures through a Java applet. In the near future, RINGS will provide tools for analyzing microarray expression data of glycosyltransferases, analyzing mass spectroscopy data, finding profiles of glycans based on probabilistic models, mining patterns in sets of glycans, and classifying glycans based on kernel methods, to name a few. By putting these tools into practical use, and in turn receiving feedback, these tools can be further enhanced to make informatics techniques useful for meaningful research. We are dedicated to providing the community with pertinent and valuable tools to advance glycobiology. RINGS is currently available at http://rings.t.soka.ac.jp.

(171) **Determination of the Protein Concentration and Product Quality in Conditioned Media by Two-Dimensional Chromatography on-Line with Mass Spectrometry (2D-LC/MS)**

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BiogenIdec, Cambridge, MA

The physicochemical characterization of recombinant protein biopharmaceuticals plays a critical role not only for product release but also during the biopharmaceutical process development. The integration of different analytical methodologies enables identification and characterization of complex biologics in order to meet requirements of Food and Drug Administration (FDA) for a consistent drug production process and product quality. However, the ability to identify different sources of a recombinant protein heterogeneity in complex sample matrices in a timely and quantitative manner remains a significant challenge.

In this work, we describe a 2-dimensional liquid chromatography approach with on line UV and mass spectrometric (MS) detection to assess protein titer and quality of a recombinant glycoprotein in conditioned media samples. This rapid and automated analytical approach provides important information to enable timely and efficient control and optimization of protein production process. The method is based on the use of a home built 2D-LC/MS system that employs ion exchange chromatography followed by reverse-phase chromatography with UV and MS detection. The set-up includes a switching valve to redirect the column flow from ion-exchange column onto a reverse-phase column for further separation and protein titer quantitation by UV absorbance at 280 nm. An additional switching valve has been used to couple 2D-LC system with an on-line electrospray ionization quadrupole time of flight mass spectrometer (ESI-qToF-MS) for analysis of protein glycoforms. Details of the assay development and performance will be discussed.

(172) **Structural Analysis of *O*-Glycopeptides Employing Negative- and Positive-Ion MS^n Spectra Acquired by nanoHPLC/ESI-Linear Ion Trap Time-of-Flight Mass Spectrometer**

Hiroki Ito[1]; Kisaburo Deguchi[1]; Kuriko Yamada[1]; Shinji Nagai[2]; Masataka Fumoto[3]; Hiroshi Hinou[1]; Hiroaki Nakagawa[1]; Yasuro Shinohara[1]; Shin-Ichiro Nishimura[1]

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Recently, we reported that MS^n spectral matching simply based on correlation coefficient calculations between positive/negative-ion MS^n spectra is useful and applicable to the structural assignment of PA *N*-glycans and *N*-glycans binding to peptides without releasing them, enzymatically or chemically. In this presentation, this approach is extended to the structural assignment of *O*-glycan of glycopeptides. This direct assignment method of *O*-glycans is particularly suitable for *O*-glycopeptides which is still missing a versatile enzyme like PNGase F for *N*-glycopeptides.

Experiments were performed by using a Hitachi NanoFrontierL system consisting of a capillary HPLC and an ESI-Linear IT-TOF MS. The samples used were synthetic *O*-glycopeptides binding a sialyl *N*-acetyllactosamine (3'-SLN) and a sialyl Lewis x (sLe^x). The samples were dissolved in water/acetonitrile. Flow rate was 200 nL/min. MS^n spectra were acquired varying CID gain (energy) in 0.6-2.6.

Positive-ion MS^2 spectra caused neutral losses of a fucose and a sialic acid, and therefore, they were useless for the structural assignment of *O*-glycans. In the negative-ion mode, the CID MS^2 spectra of *O*-glycopeptides showed a relatively abundant glycoside-bond cleavage between the core *N*-acetylglucosamine (GlcNAc) and serine that yields deprotonated C_3-type fragment ions of *O*-glycan. The structure of sLe^x oligosaccharide was simply assigned by comparing the CID MS^3 spectrum derived from the C_3-type fragment ion with the CID MS^2 spectra of the sLe^x and sLe^a standards (i.e., negative-ion MS^n spectral matching). The amino acid sequence of the peptide including the glycosylation site was determined from the MS^2 spectrum in the positive-ion mode.

(173) **Novel Chiroptical Analysis of Glycoconjugates by Vibrational Circular Dichroism (VCD)**

Kenji Monde; Tohru Taniguchi; Masami Fukuzawa; Mai Hashimoto; Atsufumi Nakahashi; Nobuaki Miura; Shin-Ichiro Nishimura

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Vibrational Circular Dichroism (VCD) is differences of vibrational spectra with respect to left vs. right circularly polarized radiation. Due to recent commercial available instruments, various fields of research have been investigating for bio-macromolecules such as proteins, DNA as well as determinations of absolute configurations for small chiral molecules with the aide of the theoretical calculation. The VCD is considered as a simple CD measurement in the IR region, having some advantages compared to well-known UV-VIS CD such as wide range of application. Glycoconjugates as represented by carbohydrates have many chiral carbon centers, which accordingly create their structural information. However, representative analytical techniques such as MS, NMR can not extract there chiral information, but diastereomeric one. All carbohydrates show IR absorption and thus we are focusing on their chiroptical analysis to extract the chiral structural information of glycoconjugates by VCD.[1]

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Systematic VCD measurements of typical mono- and di- saccharides revealed that VCD in the Mid-IR region could distinguish their glycosidic linkage[2] and also VCD pattern in the C-H IR region was significantly sensitive against their glycosidic linkage sites. To extend the VCD study of glycoconjugates, we also applied this new technique to glyceroglycolipids and artificial glycolipids.[3]

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(174) **An Efficient, Rapid, Differential N-Glycan Profiling from Mouse Tissues**

Masaki Kurogochi[1]; Yasuro Shinohara[1]; Yoshiaki Miura[1]; Jun-Ichi Furukawa[1]; Hideyuki Shimaoka[3]; Hiromitsu Kuramoto[3]; Yoko Kita[4]; Mika Nakano[4]; Hiroki Ito[5]; Hiroaki Nakagawa[2]; Kisaburo Deguchi[2]; Shin-Ichiro Nishimura[1]

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The importance of oligosaccharides in nature has been well recognized. Recently, we established a high-efficient, rapid, automatable analytical method for N-glycan from serum. This method is composed of optimized digest condition containing the release of N-glycans, and efficient oligosaccharides purification ("Glycoblotting"), derivatization of oligosaccharides using chemo-selective reactions, we demonstrated that N-glycome profiling via this method can provide high-quality and quantitative information. As a result of comparative glycomic analysis between normal and db/db model mouse serum, we found that mouse serum N-glycome changed dramatically. In order to elucidate whether the disease-associated serum N-glycans correlate with several tissues N-glycome, we challenged the analysis of N-glycan from several tissues according to the above mentioned methods, and compared with each N-glycan profile.

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(175) **Glycomics Analyses: Automatic Annotation of Glycopeptide Spectra**

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Recent advances in mass spectrometric techniques have enhanced the sensitivity and possibilities of specific cleavage/mass mapping strategies in glycopeptide analysis. Online nanoLC-MS and MS/MS experiments using QTOF instrumentation have become popular techniques and have been used successfully in a number of challenging analyses. With these advances, however, the volume of data generated has become vast and the amount of time required to perform a complete analysis becomes greater as the sample complexity increases, to the point where it is entirely possible to spend months analysing a single sample.

To this end, we are developing prototype software for the automated analysis of glycopeptide spectra, as a logical continuation of our work on Cartoonist, a system capable of automatically annotating N-glycan spectra. The software examines each spectrum in an LC/MS series, hunting for isotope envelopes of ions that have sufficient resolution to determine the charge, and that have the appropriate shape for a glycopeptide. It then builds a table containing the masses of all tryptic glycopeptides from the target molecules and for each mass computed from the LC/MS spectra checks for a matching target mass in the table. Once a peak has been identified as having the mass of a tryptic glycopeptide, a plausible cartoon is assigned to the glycan, using an improved version of the Cartoonist algorithm.

This software, whilst not yet fully developed, promises to either supersede current manual interpretation of complex glycopeptide data sets or more likely to augment it, vastly reducing the time required for a complete analysis.

(176) **Webapplication "Glyco-Peakfinder" – Automated Annotation for MS Peaks of Glycoconjugates combined with Database Searches**

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Mass spectrometry is the key technology for the identification of glycan structures. The web-application "Glyco-Peakfinder" was developed to assist people in structure identification in MS spectra. In contrast to other known tools, such as "GlycoMod", which are basically focused on complete structures, MSn spectra with different ion series (A-, B-, C-, X-, Y-, Z- ions) can be calculated in parallel. "Glyco-Peakfinder" calculates all possible theoretical compositions for a peak from a given mass list. The calculation of multiply-charged ions increases the range of applications to techniques other than MALDI-MS. As additional features, modifications of an residue (such as sulphation) and modifications of the complete structure (such as permethylation) can be included. It is also possible to handle glycan structures which are attached to an aglycon (such as amino acid sequence) or which are modified at the reducing end (such as 2-aminopyridin). In addition, to assist in structure identification, the results from "Glyco-Peakfinder" can be used for a database composition search of reported oligosaccharides in GLYCOSCIENCES.de (http://www.glycosciences.de). "Glyco-Peakfinder" was developed for the EUROCarbDB project (http://www.eurocarbdb.eu) to allow for fast annotation of most types of MS spectra. The option of detecting differently- and multiply-charged ions in one calculation cycle provides a complete annotation of the whole spectrum. The first version of "Glyco-Peakfinder", as well as annotation results from different sample spectra and a perspective for integration of the software tool into the EUROCarbDB project will be presented.

(177) **Tools for Glycomics: Isotopic labeling of Glycans with ^{13}C for Relative Quantitation**

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Analysis of permethylated oligosaccharides by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has enabled the investigation of the glycan repertoire of tissues and organisms with high resolution and sensitivity. It is difficult, however, to correlate the expression of glycosyltransferase transcripts with the glycan structures present in a particular cell because the use of MALDI-TOF MS for quantitative purposes has significant limitations. In order to develop a technique that would allow glycan quantification by MS analysis, a procedure was developed for the isotopic labeling of oligosaccharides with ^{13}C labeled methyl iodide using standard permethylation conditions. Separate aliquots of oligosaccharides from human milk were labeled with ^{12}C or ^{13}C methyl iodide, the labeled and non-labeled glycans were mixed in known proportions, and the mixtures analyzed by MALDI-TOF-MS. Results indicated that the isotopic labeling described here was capable of providing relative quantitative data with a dynamic range of at least two orders of magnitude, adequate linearity and reproducibility. This procedure was successfully used to analyze N-linked oligosaccharides released from mixtures of α-1 acid glycoprotein, bovine fetuin and human orosomucoid; however it can also be used to analyze oligosaccharides from O-linked sites and glycolipds, thereby representing an effective procedure for quantitative glycan analysis by MALDI-TOF-MS.

(178) **Methodology for High-Sensitivity Analysis of the Glycomes of Glycolipids and Glycoproteins from a Single Tissue**

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The availability of genome sequences, and the development of transcriptomic and proteomics technologies has led to expression profiling of many genes and proteins within biological systems, but little attempt has been made to tie in the glycomic component of the systems. Already, glycosylation has been associated with a range of diseases, so it is essential to develop methodologies for analysing as much of the glycome as possible from a single sample. Previously, we have optimised methods for analysing the glycoproteins of tissues and cells. Here, we report a rapid, sensitive mass spectrometric screening strategy for sequentially profiling the glycolipid and glycoprotein glycomes from a single tissue or cell preparation. After cellular disruption, (glyco)proteins were precipitated and separated from glycolipids by

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centrifugation. Glycans were subsequently released from glycolipids by ceramide glycanase digestion, permethylated and analysed by MALDI-MS. Glycoproteins were reduced, alkylated, digested with trypsin and treated with PNGase F to release N-glycans. O-glycans were also efficiently obtained from the tryptic glycopeptides by reductive elimination. N- and O-glycans were subsequently permethylated and analysed by MALDI-MS. Sequence information was also obtained by MALDI-TOF/TOF and ESI-MS/MS. This method will be useful as a component of systems biology studies, and for routine screening of knock-out mice where it is possible to assess the role of glycosyltransferases and glycosidases at the cellular and whole organism level.

(179) **Structural Characterisation of Permethylated Glycans using MALDI-TOF/TOF**

Jihye Jang Lee; Bérangère Tissot; Maria Panico; Howard R. Morris; Anne Dell; Stuart M. Haslam
Imperial College, London, United Kingdom

Glycans have been implicated in numerous biological roles. However, in order to rationally address the function of glycans in biological systems, knowledge of molecular structures is essential. Because only minute quantities of material are often available and there are increased demands to obtain as much structural information as possible from a single sample, the development and optimisation of analytical techniques is the focus of considerable attention. MALDI-TOF/TOF is a recently introduced technology capable of addressing such problems. Here, we present data obtained using this recent technology to sequence permethylated glycans derived from various biological sources. Results show that MALDI-TOF/TOF experiments yield structurally informative fragment ions which are useful for unambiguous sequencing of glycan structures that are present in very low abundance. Furthermore, the examination of permethylated glycans with this technology increases the sensitivity and induces predictable fragmentation patterns assisting sequence determination. Therefore, our results show that MALDI-TOF-MS/MS is a powerful tool for high-sensitivity and high-throughput glycomic analyses. Our methodologies are currently being employed by the Analytical Core of the Consortium for Functional Glycomics (www.functionalglycomics.org).

(180) **Pathogen Antigens Probed by On-Cell Solution NMR**

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Many polysaccharide (PS) vaccines have been produced using the purified PS from bacterial capsules. Unfortunately, efforts to obtain a reliable vaccine against *Neisseria Meningitidis* B by this technique have been unsuccessful. Among the reasons considered for this failure is that the pure PS may differ structurally respect the PS on cells, specifically by the presence of lactones.

In vitro Nuclear Magnetic Resonance (NMR) has long been appreciated for its capacity to study biomolecules in solution, usually at physiologically relevant temperature and pH. Nevertheless, the cellular environment is arguably different in other respects, important for functioning and recognition of molecules. There have been recent antecedents of in-cell NMR studies, all related to proteins, showing the influence of the environment over them. Here we report an on-cell multidimensional solution NMR study on a modified *E. coli* K1 strain expressing the same capsular PS as *N. Meningitidis* B. To facilitate NMR studies the pathogenic bacteria were genetically engineered to selectively label the capsular PS with ^{13}C and ^{15}N.

We obtained 1- and 2-dimensional NMR spectra, including triple resonance experiments, of the on-cell PS and compared it to the spectra of the pure PS (with and without lactonization) and monomeric sialic acid. Data analysis shows no evidence for lactonization or other structural differences between free and on-cell PS, under the experimental conditions used. In addition, we demonstrate the method to be suitable for a wide range of *in-vivo* NMR experiments.

(181) **Comparative Glycomics of Connective Tissue Glycosaminoglycans using Mass Spectrometry**

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Chondroitin Sulfate is a glycosaminoglycan (GAG) present in connective tissues. Structural analysis of GAG oligosaccharides from connective tissues is challenging because of the tissue complexity, and to date, mass spectrometry has been applied to analysis of CS oligosaccharide glycoforms from cartilage. This work describes the glycoform analysis of GAGs extracted from other joint connective tissues, including tendon, muscle, ligament and synovium. Stable isotopic labeling and mass spectrometric analysis of extracted GAG oligosaccharides allows quantification of different glycoforms. This work also describes a greater depth of structural characterization of GAGs extracted from cartilage tissue. The goal is to generate an unparalleled level of detail in profiling structural changes to connective tissue-derived GAGs during developmental and disease processes.

GAGs extracted from tendon, muscle, ligament and synovium connective tissues were stable isotope labeled and analyzed by LC-tandem mass spectrometry. The percent total ion abundances of light and heavy predictive ions containing the reducing end (Y_1^{1-}, Y_3^{2-}, and $[M\text{-}H\text{-}SO_3]^{2-}$) were calculated. Predictive ion contributions from three replicate tissue samples were put into a set of three equations and solved for three unknowns representing the percentage of CSA, CSB, and CSC in each sample. The results demonstrate that tandem mass spectrometry can be used for the isotopic quantification of glycoforms of stable isotopically labeled GAGs from a variety of intact connective tissues.

GAGs extracted from bovine and human cartilage tissue were analyzed by nano-ESI/MS for the analysis of lower abundance compositions, including over- and under-sulfated sequences, and saturated oligosaccharides containing the non-reducing chain termini.

(182) **Development of New Fluorocarbon-Based HPLC-MS methods for glycosylinositol phosphorylceramide analysis**

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Glycosylinositol phosphorylceramides (GIPCs) play an important role in the life cycle of fungi. Improved methods of structural elucidation and quantitation of constituent GIPCs will aid functional studies. This presentation will highlight new developments in our laboratory that have resulted in the generation of derivatives that make use of Fluorous™affinity tags (F-tags; Fluorous Technologies Inc., Pittsburgh, PA). The method utilizes a combination of sensitive HPLC detection and mass spectrometry analyses for quantitation and structural profiling of GIPCs. For this study the generation of primary amine groups is accomplished by the enzymatic deacylation of the ceramide moiety of GIPCs using the enzyme sphingolipid ceramide N-deacylase (SCDase). To facilitate detection the resulting free amine groups of the lyso-GIPCs are then derivatized by the addition of selected chromophoric and/or fluorophoric N-reactive F-tags, N-[4-(1H,1H,2H,2H-perfluoroalkyl)benzyloxycarbonyloxy]succinimide (F-Cbz-OSu) and 1-({[2,7-bis(1H,1H,2H,2H-perfluorooctyl)-9H-fluoren-9-ylmethoxy]carbonyl}oxy)-2,5-pyrrolidinedione (F-Fmoc). The derivatives are separated and quantified using Fluorous™ HPLC with UV and/or fluorescent detection, and the structural profiling carried out by MALDI-TOF and ESI mass spectrometry. The long term goal of this work is to incorporate automation and directly interfaced ESI-MS^nanalysis.

(183) **A Novel Glycoproteomic Approach for the Complete Characterization of Glycopeptides from Complex Biological Mixtures**

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The field of glycomics is currently focused on the structural characterization of released glycans. Typically, glycans are released from cells or tissues then analyzed by mass spectrometry either in their native state or following chemical modification. However, this procedure results in a complete loss of information regarding the glycoproteins from which the glycans originated. This is unfortunate because relevant biological information can be obtained by understanding both glycoprotein and glycan expression. To analyze glycoprotein expression, lectin affinity chromatography (LAC) has previously been employed. The advantage of LAC is that the glycoproteins or glycopeptides are separated from the non glycosylated species, thus simplifying further analyses by MS/MS. The specificity of the lectin however, does not facilitate the global isolation of glycoproteins or glycopeptides with a diverse population of glycan structures. In this study, we describe the development of a highly selective and sensitive method for the unambiguous isolation and characterization of glycopeptides from complex mixtures. Human blood serum glycoproteins were enzymatically digested with trypsin. The peptide/glycopeptide mixture was desalted on a Sep-Pak and the glycopeptides were isolated by normal phase chromatography. Characterization of the peptide sequences and glycan structures of intact glycopeptides were performed by collision induced dissociation, electron capture dissociation, and infrared multiphoton dissociation - tandem mass spectrometry. This approach is noteworthy because it was capable of characterizing the glycan populations on over 100 individual N-linked sites

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from serum glycoproteins, and appears to be a robust procedure for the high throughput characterization of glycopeptides from complex mixtures.

(184) **Sialoside Analog Arrays for Identifying High Affinity Analogs of Siglec Ligands**

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The siglec family of glycan binding proteins recognizes sialic acid containing glycans of glycoproteins and glycolipids as ligands. Most are differentially expressed on various white blood cells that mediate immune function, and one of them, myelin associated glycoprotein (MAG), is expressed on glial cells and functions in myelin-axon interactions. Most siglecs recognize sialic acid containing ligands on the same cell (*cis* ligands) and on adjacent cells (*trans* ligands). To investigate the roles of the ligand binding in the functions of Siglecs we are attempting to develop high affinity ligand analogs as functional probes that compete with *cis* ligands and bind to siglecs on native cells. To this end we employ chemo-enzymatic synthesis using glycosyltransferases to synthesize the preferred ligand of a siglec of interest. Taking advantage of the tolerance of most sialyltransferases for substituents at the 9- and 5-positions, we have worked out flexible synthesis schemes for production of siglec ligands containing sialic acid analogs. Recent efforts have been focused on the creation of glycan-microarrays containing a panel of sialoside analogs that can be used for rapid screening of siglecs for 9- and 5-substituents that increase binding affinity. Results suggest that this technology is suitable for rapid identification of substituents that increase affinity of sigelcs and can provide the basis for creating high affinity probes for members of the siglec family. (Supported by NIGMS grant GM60938)

(185) **Combined Use of Hydrazide Functionalized Polymer and Sequential Tag Exchange; A General Protocol with *Glycoblotting* for Functional Glycomics (Part 2)**

Jun-ichi Furukawa[1]; Yoshiaki Miura[1]; Hiromitsu Kuramoto[2]; Hideyuki Shimaoka[2]; Masaki Kurogochi[1]; Mika Nakano[3]; Yasuro Shinohara[1]; Shin-Ichiro Nishimura[1]
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Glycoconjugates play important roles in many fundamental biological processes. Although these glycan entities are specifically responsible for their function, the major difficulty in glycomic analysis is a consequence of the fact that the purification of trace amounts of oligosaccharides often requires tedious multistep processes. Recently, we communicated that rapid purification of carbohydrates can be achieved by employing glycan-specific chemical catch onto aminooxy-functionalized polymers, which we termed "Glycoblotting." Aiming to further expand this technique widely applicable for structural and functional glycomic study, we evaluated the usefulness of various imine exchanges to achieve simultaneous recovery and probing of blotted oligosaccharides. The efficiencies in imine exchange were first evaluated in solution. We found that the conversion from hydrazone to oxime proceeded most efficiently, and the reaction conditions were optimized to maximize the conversion efficiency. Next, we prepared a bead bearing hydrazide groups in high-density as a platform for high-throughput, automatable and quantitative glycomics. In the system, the glycans captured on the polymer could be transferred to a small tag molecule possessing a desired function such as high sensitivity in MS analysis, fluorescence, and stable isotopes via the imine exchange. These approaches may have a great impact on a variety of glycomic study since it enables rapid, highly efficient tag conversion, and is applicable to oligosaccharides derived from biological samples in a practical manner.

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(186) **Glycomic Sequencing of Complex Glycans from Glycosphingolipids by High Energy CID MS/MS and Validation of Linkage Specific Fragmentation Characteristics**

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Mass spectrometry (MS) based glycomic analysis entails not only high sensitivity profiling but also de novo sequencing that would allow structural definition. Among the more daunting tasks is a need to discriminate between co-existing isomeric mixtures of type 1 and 2 chains, and with it, the various blood group antigens, particularly those differing only in linkages. Characteristic features of high energy CID MS/MS as implemented on a MALDI-TOF/TOF have recently been established against permethylated N-glycans and other smaller glycans, and proved to be highly effective in delivering linkage information. In particular, specific cleavage ions were identified which allow systematic assignment of substituents around the ring. In conjunction with chemical and/or enzymatic manipulation, we have unambiguously shown that glycan chains carried on the lactosylceramides of a human colonic adenocarcinoma cell line, Colo205, comprise a complex mixture of type 1 and 2 hybrids, as well as those exclusively of linear and branched extended type 1 chains. To delineate the precise epitopes recognized by therapeutic monoclonal antibodies raised against the glycolipids of Colo205, a panel of cancer cell lines were screened for their respective glycolipids by means of MS and peaks of interest were directly sequenced by MS/MS. The derived glycomic maps include those that expressed rare blood group A and B in tandem with Lewis antigens, on linear or branched glycans. Together with MS/MS data obtained on synthetic standards, the most critical series of linkage specific ions afforded by high energy CID MS/MS can now be reliably compiled and applied.

(187) **Further Improvement of the System for Evanescent-Field Fluorescence-Assisted Lectin Microarray**

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Structural glycomics requires a key technology, which enables high-throughput analysis of complex features of glycans. Of emerging techniques, lectin microarray developed recently in our laboratory is of excellent performance: it dose not require washing procedures before scanning, while other related techniques do. Since lectin-glycan interactions are relatively weak compared with antigen-antibody interactions, a method enabling in situ observation of their binding under the equilibrium conditions is required. To realize this, we adopted an evanescent-field fluorescence-detection principle[1]. The developed method also proved to be useful for differential profiling of cultured cells, of which glycosylation pathways are different[2].

However, generally available bio-samples, e.g., cells, tissues and body fluids, are limited in their amounts. Moreover, natural abundance of glycans, which can be useful bio-markers, are supposed to be very low. To overcome this difficulty, we made various attempts to improve sensitivity even for crude clinic samples. As a result, >10-fold higher S/N ratio was attained with increased sensitivity. The obtained performance makes it much easier to analyze low-abundance samples for investigation of useful bio-markers. Thus, our new platform of lectin microarray is expected to have much wider applications.

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(188) **Molecular Cloning of Two Distinct Sialyltransferases, α-2,3- and α-2,6-sialyltransferases, from a Marine Bacterium**

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We have previously cloned α-2,3- and α-2,6- sialyltransferases from marine bacteria such as Photobacterium damselae. We herein report cloning and characterization of two distinct sialyltransferases, α-2,3- and α-2,6-sialyltransferases, from marine bacterium Photobacterium sp. JT-ISH-224. The α-2,3-sialyltransferase activity was first identified in JT-ISH-224 in a screening program for bacteria with sialyltransferase activity. Because DNA fragments that hybridized to the α-2,3-sialyltransferase from P. phosphoreum JT-ISH-467 was detected by Southern analysis in JT-ISH-224, the gene for the sialyltransferase was cloned from a genomic library constructed from JT-ISH-224 using a probe from JT-ISH-467. Homology in amino acid sequence between the α-2,3-sialyltransferase from JT-ISH-224 and the one from JT-ISH-467 was 92.2% whereas that between the α-2,3-sialyltransferase from JT-ISH-224 and sialyltransferase form other bacteria was less than 65%. The activity of α-2,6-sialyltransferase was also found in JT-ISH-224, and the corresponding gene was isolated from the genomic library by using the α-2,6-sialyltransferase gene from P. damselae JT160 as a probe. Homology in amino acid sequence between the α-2,6-sialyltransferase from JT-ISH-224 and the one from JT160 was 54.5% whereas that between the α-2,6- and α2,3-

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sialyltransferase from JT-ISH-224 was only 33.7%. This is the first report of the isolation of two distinct sialyltransferases from one prokaryotic organism. The recombinant, truncated forms of both of the sialyltransferases exhibited a number of useful features.

(189) **Molecular Cloning and Characterization of α-2,6-Sialyltransfease from Vibrionaceae Photobacterium sp. JT-ISH-224**

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A novel α-2,6- sialyltransferase was cloned from Vibrionaceae Photobacterium sp. JT-ISH-224 and expressed in Escherichia coli in this study. The gene encoding α-2,6- sialyltransferase from Photobacterium sp. JT-ISH224 contained an open reading frame of 1545 base pairs encoding for a protein of 514 amino acid residues. We previously reported cloning of several sialyltransferases from various kinds of bacteria and demonstrated that productivity in E. coli of the recombinant sialyltransferases that lacked putative signal peptides was higher than that of full-length sialyltransferases. In this study, we constructed a series of proteins truncated from α-2,6-sialyltransferase from Photobacterium sp. JT-ISH224 to improve the productivity of this enzyme. The DNA fragments that encoded for the full length protein (N0C0) and truncated proteins, which lacked the signal peptide (N1C0: NΔ17), additional peptides of various lengths (N2C0: NΔ62, N3C0: NΔ110, N3.1C0: NΔ127) or C-terminal peptides (N0C1: CΔ82), were amplified by PCR and cloned into expression vector pTrc99A. All of the genes were expressed in E. coli. N1C0 showed the highest activity, being followed by N3C0, N0C0 and N2C0 in order of decreasing. N3.1C0 and N0C1 completely lost the sialyltransferase activity. The level of production of N1C0 was over 10,000U/L culture or more, and this was the highest among the sialyltransferases that have been expressed to date. These results indicate that the residues 110-514 might be essential for the sialyltransferase activity.

(190) **Neoglycolipids Prepared via Oxime-ligation for Microarray Analysis of Carbohydrate-Protein Interactions**

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Affinities of most carbohydrate-protein interactions are so low that multivalent forms of ligand and protein are required for detecting interactions. The neoglycolipid (NGL) technology was designed to address the need for microscale presentation of oligosaccharides in a multivalent form for studying carbohydrate-protein interactions [1] and is now the basis of a state-of-the-art carbohydrate microarray system [2]. Conventional NGLs are prepared by conjugating oligosaccharides to 1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine, by reductive amination. The NGLs derived from tri- or larger oligosaccharides have performed well for the majority of carbohydrate-recognition systems. However, ring-opening of reducing-end monosaccharides often limits applicability to very short oligosaccharides and may limit applicability to long oligosaccharides if an intact reducing-end monosaccharide is a part of the recognition motif. In this communication, we describe a simple method for preparing NGLs by chemoselective oxime-ligation, by which diverse reducing mono- and oligosaccharides are conjugated with high efficiency to an aminooxy (AO)-functionalized lipid (submitted for publication). Comparative binding studies with conventional NGLs in microarrays show advantages of AO-NGLs for presenting (a) short oligosaccharides, e.g. Lewisx (Lex) trisaccharide to anti-Lex antibodies and sialyllactose analogues to siglecs, and (b) N-glycans to Pisum sativum agglutinin, which requires both core and backbone regions to be intact for strong binding. Thus, AO-NGLs have broadened the applicabilities of NGLs as probes in studies of carbohydrate-protein interactions. (Supported by MRC and UK 'Glycochips' consortium.)

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(191) **Electrospray Ion Mobility Spectrometry of Isomeric Carbohydrates**

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Glycoconjugate glycans consist of mixtures of variants, known as glycoforms, on a common core structure. These variants arise as a result of biosynthetic events under complex regulation. One of the challenges in mass spectral analysis of glycoconjugate glycans is that the ion signals corresponding to a given oligosaccharide composition may be produced by a mixture of structural isomers. Ion mobility spectrometry (IMS) entails passing ions through a mobility cell operated at elevated pressure, relative to vacuum. For a given charge state, the mobility time increases with the collisional cross section of the ions. The goal of this work is to determine the extent to which carbohydrate isomers may be resolved using ion mobility.

Electrospray ion mobility spectra were acquired using a modified Waters QTOF Premier mass spectrometer equipped with a traveling wave ion guide operated at 1 mbar. The following compound classes were studied: native glycosaminoglycan disaccharides, native and permethylated milk oligosaccharides, and native and permethylated high mannose N-linked oligosaccharides. This series of compounds were selected to evaluate the extent to which carbohydrate positional isomers present collisional cross sections that may be resolved using ion mobility spectrometry. The results showed that lactosamine isomers and some sulfation isomers may be resolved using the experimental system. The results were complex, in that different trends were observed, depending on the charge of the ion (negative or positive). In addition, contour mobility plots were useful for visualizing complex heparin oligosaccharide mixtures.

(192) **A Survey of Siglec Binding Preferences using Carbohydrate Microarrays**

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Carbohydrate microarrays are a powerful means of surveying oligosaccharide repertoires that can be bound by receptors of the immune system. Having established a microarray system of ~250 sequence-defined oligosaccharide probes linked to lipid [1, 2] we are using this to examine fine specificities of siglecs. We have reported previously results of binding studies using microarrays of Lewis^x (Le^x)- and sialyl-Le^x-related probes with different sulphation patterns [3]. We observed that unlike L-selectin [4] and Langerin [5], siglecs (human Siglec- 7, 8, 9 and murine Siglec F and CD22) do not give detectable binding signals with sulphated analogues of sialy-Le^x that are lacking sialic acid. However, the sulphate groups modulate positively or negatively the siglec binding intensities to the sialyl-Le^x sequence. Such differences in recognition of sulpho motifs may have functional significance and translate into co-operative or competing effects on leukocyte targeting and signalling, e.g. at different sites on lymphoid microvascular surfaces where various sulphation patterns of sialyl-Le^x occur. In this communication, we will describe observations on multi-sialylation and multi-sulphation, with or without fucosylation, on siglec binding using screening microarrays as well as tailor-made dose-response microarrays of selected probes. (Supported by UK Basic Technology Initiative, "Glycochips")

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(193) **Comparative Glycoproteomics of the Trypanosoma Cruzi Lifecycle**

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Trypanosoma cruzi (T. cruzi) is a protozoan parasite endemic to Latin America and the causative agent of Chagas' disease. The life cycle of T. cruzi is complex, with multiple developmental stages persisting between a variety of mammalian host (including humans) and insect vectors. Although the cell surface glycoproteins of T. cruzi have been implied in numerous critical functions, such as host cell recognition, host cell invasion, and immune evasion, little is actually known on this topic.

We have used a lectin/isotopic labeling strategy for identifying the classes of N-linked glycans present on specific sites of individual glycoproteins -- information typically missed by both proteomic and glycomic methodology. We have also applied this strategy to compare the stage specific glycoproteome of T. cruzi. These studies were able to identify the N-linked glycosylation sites occupied by high mannose glycans on 17 glycoproteins, all of which were membrane associated. This study also provides the first evidence for the expression of 7 putative trypomastigote cell surface glycoproteins including a member of the dispersed gene family 1 and a novel senescence-specific cysteine protease. All of these proteins had escaped

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identification during an exhaustive whole cell proteome, suggesting that the lectin chromatography step allows for further enrichment of peptides from membrane proteins. These studies are expected to reveal the glycoprotein changes observed by lectin blot analysis, which in turn, may provide information on the pathogenicity of T. cruzi.

(194) **Glycan Array and Structural Analysis of AAV Capsid – Receptor Binding Specificity**

Mavis Agbandje-McKenna[1]; Edward B Miller[1]; Michael DiMattia[1]; Brittney Gurda-Whitaker[1]; Lakshmanan Govindasamy[1]; Robert McKenna[1]; Nicholas Muzyczka[1]; Sergei Zolotukhin[1]; Richard A Alvarez[2]; Ola Blixt[3]; James C Paulson[3]

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Receptor-mediated attachment and entry are essential first steps in the parvoviral life cycle. Initial viral attachment frequently determines tissue tropism and subsequent interactions determine transduction efficiency for the Adeno-associated viruses (AAVs). Studies have shown that the AAVs recognize and bind cell surface carbohydrates for infectious entry. However, other than AAV2, which binds heparan sulfate proteoglycan, little is known about the receptor interaction characteristics of the other AAV serotypes. We have used the resources of core H of the Consortium for Functional Glycomics (CFG), to screen the carbohydrate binding properties of three AAVs, AAV1, AAV2, and AAV5, with distinct cellular tropisms. The screen utilized a printed glycan array (PA ver 2) containing 264 different natural and synthetic glycans, including sialylated sugars with different linkages and modifications, e.g. sulfation, but not heparin sulfate. AAV1 and AAV5 bound to specific glycans with terminal sialic acids consistent with cell binding and transduction assays. AAV2 recognized glycans with sulfated groups but did not show particular specificity, consistent with the absence of heparin sulfate from the array. We have obtained the glycans recognized by AAV1 from core D of CFG for soaking into crystals of AAV1 to enable the mapping of the carbohydrate binding site. For AAV5, the sulfated sugars identified in the array are not yet available from core D, but the structure of the AAV5-sialic acid complex has been determined. The glycan array data plus the structures of the AAV capsid-receptor complexes will be presented.

(195) **Mass Spectrometry of Fluorocarbon-Modified Glycosphingolipids. Potential for Comprehensive High-Throughput Structural Profiling**

Yunsen Li; Emma Arigi; Steven B. Levery

University of New Hampshire, Durham, NH

Because glycosphingolipids (GSLs) are extracted with a variety of other lipids from the cell membrane, and because they are highly varied in their structures, their resulting physico-chemical properties, and their distribution patterns, development of "universal" high throughput protocols for their extraction and analysis has been an elusive goal. Our goal is to develop multi-purpose methodologies which are well-suited for sensitive, accurate, rapid, and comprehensive profiling of GSL expression, applicable to tissues or cells from any species, which can be adapted for quantitative comparisons of GSL expression, but which also allow for identification and detailed characterization of novel and/or isobaric structures. The analytical method which will be described involves the use of the enzyme sphingolipid ceramide N-deacylase (SCDase) to remove the fatty acid of the sphingoid, followed by the incorporation of fluorocarbon tags (F-Tags^{TM}; Fluorous Technologies, Pittsburgh, PA) at the free amine site. This will be used to separate GSLs from crude lipid mixtures for analysis by mass spectrometry (MALDI-CFR, MALDI-QIT and LTQ). In preliminary trials, SCDase-treated purified gangliosides GM1, GD1a, and GT1b were derivatized by the prototype F-Tag in essentially quantitative yield and recovered by Fluorous^{TM} solid phase extraction (F-SPE). ESI-MS and MS^n spectra consistent with the expected products were acquired. Further trials have been carried out on SCDase-treated crude ganglioside mixture extracted from bovine brain. Further development of practical protocols requires experiments carried out with crude cell lipid extracts, which are ongoing.

(196) **Microwave Assisted Glycoprotein Labeling and Detection**

Kapil Kumar; Tamara Nyberg; Courtenay Hart; Nancy Ahnert; Brian Agnew

Molecular Probes - Invitrogen, Eugene, Oregon

Protein glycosylation is an important focus of investigation in proteomics. We have recently developed a novel platform for the detection, quantification, and proteomic analysis of glycoproteins using metabolic or enzymatic labeling with detection based on the copper (I) catalyzed [3+2] cycloaddition (click chemistry). In this report, we present data to demonstrate the advantage of using a CEM microwave system to enhance both enzymatic and non-enzymatic reactions used in our platform. We investigated the effect of microwave irradiation on the activity of a mutant β-1,4-galactosyltransferase-1 enzyme (Gal-T1 (Y289L)) in (1) a pure O-GlcNAc-modified glycopeptide labeling reaction, (2) a pure O-GlcNAc-modified glycoprotein labeling reaction, and (3) labeling of endogenous O-GlcNAc-modified glycoproteins in a cell extract. The effect of microwave irradiation on the deglycosylation activities of PNGase F, Endo Hf and Endo M were also compared. We also investigated the ability of the microwave to achieve glycoprotein labeling in the click reaction in presence of lower-than-normal copper (I) catalyst. Preliminary results show a significant enhancement in the rate of Gal-T1 (Y289L) mediated glycopeptide labeling. Significant differences were found between the activities of PNGase F, Endo Hf, and Endo M upon microwave irradiation suggesting that the effect of microwave irradiation on enzyme kinetics can vary depending upon the nature of enzymes used. Finally, under defined microwave-assisted conditions, the click-mediated labeling of model glycoproteins can be carried out in the presence of at least 4 fold less copper (I) concentration. We conclude that the CEM microwave can be used to enhance glycoprotein analyses.

(197) **Novel Isotope Coded Affinity Tag for Quantitative Mass Spectrometry of Glycans**

Baoyun xia[2]; Christa Feasley[1]; Goverdhan P. Sachdev[1]; Richard D Cummings[2]

[1]The University of Oklahoma health Sciences Center, Oklahoma City, OK; [2]Emory University School of Medicine, Atlanta, GA

Many diseases and disorders are related to expression of abnormal glycan structures in qualitative and/or quantitative terms. There is a need for novel stable isotope-coded affinity tag (ICAT) methods for glycomic analyses to allow microchemical characterization of glycan expression. ICAT approaches have been successfully used in proteomic studies, but no successful ICAT approaches have been developed for glycans. Here we report the development of a novel approach for direct covalent conjugation of glycans using commercially available stable isotopes (light and heavy), which we have termed glycan-ICAT. The glycan derivatives can be directly quantified and compared by mass spectrometry, such as by matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS). Conjugation is very efficient with free glycans, including commonly studies N-glycans and O-glycans derived from Asn- and Ser/Thr-linked glycans, respectively. Conjugated glycans can be easily separated from reactants. An additional advantage of the glycan-ICAT method described here is that the conjugates absorb ultraviolet light, allowing their direct quantification, and both isotope derivatives have similar absorbance spectra. This method can be applied to characterize glycosylation-related diseases where difference in glycosylation between individuals or samples from individuals are of interest, including CDG (Congenital disorders of glycosylation), Cystic Fibrosis, cancer, and autoimmune-related diseases, such as IgA nephropathy. This presentation will demonstrate the use of this novel approach to characterize glycan compositions from several biological sources.

(198) **Characterization of Unknown Oligosaccharides in Glycoprotein and Milk**

Michael A. Madson; Srinivasa Rao; Chris Pohl

Dionex Corporation, Sunnyvale, CA

The need for methods of analysis of oligosaccharides is growing due to the increased demand for analytical methods to study carbohydrate post-translational modifications. Although there are methods for the analysis of N-linked and O-linked oligosaccharides but none, to our knowledge, that simply separate the two sets of glycoforms from the same sample. Separation of the N- and O-linked glycoforms aids in identification and structural analysis of glycoprotein structure. We have used this approach to analyze less than mg quantities of glycoprotein.

Although some reports of successful handling, isolation, analysis and structural identification are known, difficulties in the isolation and analysis of sulfated and sialylated carbohydrates from biological sources are evident in the literature. We have developed methods for the isolation and partial structural identification of an unknown oligosaccharide alditol from bovine thyroglobulin. We have also discovered an unknown disaccharide in bovine and goat milk. We report the partial structural identification of oligosaccharide from milk and the O-linked oligosaccharide from bovine thyroglobulin, the chromatography of these molecules and the ESI MS and MS2 of these molecules.

We have proposed a structure. We used mass spectral analysis and ion and HPAEC-PAD chromatography to refine the structure. We have used ion chromatography with conductivity detection to show the presence of sulfate

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and phosphate. We used anion exchange AS11HC (Dionex, Corp.) guard and analytical columns for the separation and identification of phosphate and sulfate. We will report on these findings.

(199) **Identification and Analysis of Genes Involved in Glycan Synthesis in *Aspergillus fumigatus***

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Aspergillus fumigatus is one of the most ubiquitous of the airborne saprophytic fungi, which acts as an opportunistic pathogen causing pneumonia and other fatal invasive infections in immuno-compromised hosts, particularly among patients undergoing cytotoxin chemotherapy or bone-marrow transplantation. During the last decade, there has been a dramatic increase in severe and usually fatal invasive aspergillosis caused by *A. fumigatus*. Therefore, the investigation of virulent factors and potential chemo-therapeutical targets of *A. fumigatus* are of clinical interests. Although *A. fumigatus* produces various glycoconjugates, little is known about their syntheses and functions. We have analyzed the N- and O-glycan from *A. fumigatus*. It turns out that the mature N-glycan is close to the core structure of mammnian N-glycan, while the O-glycan is identified as O-linked mannose. To study the biosynthetical pathway and function of glycans, several genes involved in the biosynthesis of glycans in *A. fumigatus* have been identified. The genes responsible for activation of Man, O-mannosylation, and GPI assmbly are knoked out or disrupted. The analyses of phenotype and virulence of these mutants reveal that the activation of Man is vital to viability. The mutants that defect in GPI-anchor and O-mannosylation of protein result in deficent cell wall integrity and show attenuated virulence in mouse model.

(200) **Biorecognition of E. coli K88 Adhesin for Glycated Porcine Albumin**

Andrei Sarabia-Sainz; Luz Vázquez-Moreno; Gabriela Ramos-Clamont
Centro de Investi. en Alimentación y Desarrollo, Hermosillo Sonora, México

Escherichia coli (E. coli) that expresses galactose-reactive lectins, like K88 adhesin, causes high morbidity and mortality among piglets. Carbohydrates that compete for adhesion attachment could serve as an alternative for disease prevention. Porcine serum albumin (PSA) obtained by hydrophobic interaction chromatography followed by pseudo-affinity chromatography was modified by non-enzymatic glycation with lactose in a solid state. Lactose was non-enzymatically attached to the protein free amino groups. Disaccharide and PSA were lyophilized together, kept under 43 % of relative humidity for 7 days, and heated at 60 °C for 4, 6 and 8 h. PSA lactosylation was confirmed by Ricinus comunis lectin binding to PSA-lactose conjugates. Conformational changes of PSA-lactose in the course of glycation were evaluated by fluorescent spectroscopy. Conjugates from 8 h treatment showed less intrinsic fluorescence that either 6 h or 4 h treatment. Biotin labeled E. coli K88 recognized PSA-lactose from 6 and 8 h treatments. These results suggest that neoglycoconjugates obtained by non-enzymatic glycation of proteins may serve in the prophylaxis of diarrhea in piglets.

(201) **Functional and Structural Analysis of N-Linked Glycans of Trichomonas vaginali**

Kuo-Yuan Hwa[1]; Hsingshen Hung[1]; Kay-Hooi Khoo[2]
[1]National Taipei University of Technology, Taipei, Taiwan, ROC; [2]Academia Sinica, Taipei, Taiwan, ROC

Trichomonas vaginalis, a protozoan parasite is the causative agent of trichomoniasis, a sexually-transmitted human disease. Surface glycoconjugates, such as lipophosphoglycan and glycoproteins can mediate the interaction between parasites and the host cells. Our goal is to examine if N-lined glycans of glycoproteins have functional roles in cell interaction. Attachment of the parasites to host cells was significantly inhibited in vitro by pretreatment of tunicamycin and PNGase F. Results from fluorescence flow cytometry indicated that the parasites interacted with concanavalin A and wheat germ agglutinin. Consistently, compositional analysis of N-linked glycans revealed that the principal components were mannose and N-acetyl-glucosamine. Surprisingly, we also detected xylose as a minor component. Further detail analyses with mass spectrometry of N-linked glycans revealed the high mannose type structures and a novel core structure. We had also used bioinformatics approaches to identify sequences similar to known xylosyltransferases by constructing a theoretical hidden Markov model and by in silico screening of EST database of T. vaginalis. Eight sequences with high similarity to beta-1, 2 xylosyltransferase were found. We are currently in the process to verify the results by cloning the genes and by assaying the enzymatic activity.

(202) **Alteration of Expression of Syndecan-4 in Gastric Cell Line Induced by Helicobacter pylori**

Ana Magalhães[1]; Nuno T. Marcos[1]; Ana Sofia L. Carvalho[1]; Maria Oliveira[1]; Nuno Mendes[1]; Céu Figueiredo[1]; Tim Gilmartin[2]; Steven R. Head[2]; Celso A. Reis[1]
[1]Institute of Molecular Pathol. Immunol. IPATIMUP, University of Porto, Portugal; [2]The Scripps Research Institute, La Jolla, CA, USA

Helicobacter pylori (Hp) is a bacteria that causes gastritis, duodenal ulcer and is involved in gastric carcinogenesis. Bacterial virulence factors have been associated with the development of chronic inflammation and with the pathogenicity mechanism. The syndecan family is constituted by 4 members of transmembrane heparan sulfate proteoglycans implicated in inflammation. Syndecan-4 has been shown to be induced by Hp through NF-kB pathway.

This study evaluates whether the induction of expression of syndecan-4 is associated with known virulence factors of Hp strains (cag PAI).

Human gastric cell line MKN45 was infected with each of the 5 cag PAI (+) and the 5 cag PAI (-) strains, and gene expression alterations were evaluated in by semi-quantitative PCR and microarray analysis using the GLYCOv2 array from the Consortium for Functional Glycomics. All the cag PAI (+) Hp strains induced increased expression of syndecan-4, whereas no alteration of expression was observed by cag PAI (-) Hp strains. Further evidence was obtained by microarray analysis that showed that the highly pathogenic cag PAI (+) Hp strain 26695, but not the cag PAI (-) Tx30a strain, induced significant alterations in syndecan-4 expression. Neither other members of the syndecan family nor the other 23 proteoglycans analysed showed alterations of expression. These results strongly suggest that the induction of syndecan-4 expression in gastric cell lines is associated with the Hp cag PAI. Supported by FCT (POCI/SAU-OBS/56686/2004), AICR (Grant 05-088). Gene Microarray Core resources and collaborative efforts provided by Consortium for Functional Glycomics funded by NIGMS - GM62116.

(203) **Trehalose Synthase Converts Glycogen to Trehalose**

Alan D. Elbein
University of Arkansas for Medical Sciences, Little Rock, AR

Trehalose Synthase Converts Glycogen to Trehalose

Y.T. Pan, J. David Carroll, Naoki Asano and Alan D. Elbein, Departments of Biochemistry and Microbiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Trehalose (α,α1,1,glucosyl-glucose) is essential for growth of mycobacteria, and these organisms have 3 pathways that can produce trehalose. One pathway involves transfer of glucose from UDP-glucose to glucose-6-P to form trehalose-6-P, then removal of phosphate to give trehalose. Another pathway involves rearrangement of the reducing end of glycogen to transform the α1,4-bond to the α,α1,1-bond of trehalose, then cleavage to give free trehalose. The third potential pathway involves the enzyme described here, i.e., trehalose synthase (TreS) which catalyzes the interconversion of maltose and trehalose. In this report, we show that TreS from Mycobacterium smegmatis has amylase activity, as well as TreS activity. Thus, when TreS is incubated with glycogen, both maltose and trehalose are produced. Interestingly, the TreS activity, but not the amylase, is strongly inhibited by validoxylamine, whereas the amylase, but not the TreS, is inhibited by acarbose. An M. smegmatis mutant lacking all three pathways cannot grow in synthetic media unless it contains exogenous trehalose. However, transfection of this mutant with the TreS gene allows it to grow without added trehalose. Interestingly, the mutant that only contains TreS and grows without added trehalose will not grow in the presence of validoxylamine, but will grow with validoxylamine when exogenous trehalose is also added to the growth media.

(204) **Glycomimetic Compound GMI-1051 inhibits Pathogenic Functions of the Virulence Factor Lectins, PA-IL and PA-IIL, from Pseudomonas aeruginosa**

Theodore Smith; Arun Sarkar; John Patton; John L. Magnani
GlycoMimetics Inc., Gaithersburg, MD

Upon productive infection, Pseudomonas aeruginosa undergoes quorum sensing control in which virulence factors are expressed in concert and aid the progression of disease in the host. Two such virulence factors are the lectins, PA-IL and PA-IIL. Both soluble lectins exist as tetramers, but differ in their carbohydrate binding specificity. PA-IL binds galactose, whereas PA-IIL binds fucose. Based on detailed epitope analysis of lectin binding, one glycomimetic compound, GMI-1051, was rationally designed to inhibit both lectins with higher affinity than either fucose or galactose for their respective lectin. GMI-1051 is able to inhibit the pathogenic functions of both lectins in vitro. Explants of ciliated tracheal epithelial cells maintain a rhythmic beating frequency while in culture. PA-IIL binds these cells and at 4uM will inhibit

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ciliary beat frequency within 2 to 3 hours. Addition of fucose can partially rescue these cells from inhibition; however GMI-1051 completely blocks the inhibition of ciliary beat frequency caused by PA-IIL over a 24 hour period. PA-IL and PA-IIL lectins also bind to human neutrophils, which are an important component of a chronic infection. GMI-1051 inhibits binding of both lectins to neutrophils. PA-IL lectin is also a potent stimulus of apoptosis of neutrophils and GMI-1051 completely blocks this pathogenic function.

(205) **Carbohydrate Epitopes are Immunodominant at the Surface of Infectious *Neoparamoeba spp***

Margarita Villavedra[1]; Joyce To[1]; Susan Lemke[1]; Kevin Broady[1]; James Melrose[2]; Debra Birch[3]; Michael Wallach[1]; Robert L. Raison[1]
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Amoebic gill disease, caused by the parasite *Neoparamoeba spp*, can be induced by exposure of salmon to parasites freshly isolated from infected fish. Cultured amoebae are non-infective. To characterise the surface of infective parasites we produced monoclonal antibodies (MAb) using a subtractive immunisation method. Mice inoculated with non-infective parasites were treated with cyclophosphamide to deplete reactive lymphocytes and then immunised with antigen preparations from infective parasites. The high percentage (97%) of MAbs recognising carbohydrate epitopes on the infective parasites suggests that the dominant epitopes unique to infective parasites are carbohydrate in nature. MAb 44C12 is one of a group that recognise carbohydrate epitopes on a cluster of high molecular weight antigens (HMWA) present in the infective parasites. These HMWA represent 19% of the total protein in the soluble fraction of the parasite and exhibit a range of pIs between 4 and 9. Treatment of this HMWA with trifluoromethanesulfonic acid indicated that the carbohydrate portion constitutes more than 70% of the total molecular weight. While a similar HMWA complex is present in non-infective parasites, these glycoproteins are not recognised by MAb 44C12. HMWA are resistant to degradation by PNGase F, O-glycosidase + neuraminidase. Three MAbs specific for the parasite HMWA did not react with any of 260 glycans tested by the Consortium for Functional Glycomics. Our results suggest that the key difference between infective and non-infective parasites are novel glycans expressed in the glycocalyx of *Neoparamoeba spp*.

(206) **Gene Expression Alterations Mediated by *Helicobacter pylori* Strains of Different Pathogenicity – a Focus on Glycosylation**

Nuno T Marcos[1]; Bibiana Ferreira[1]; Ana Magalhães[1]; Maria J Oliveira[1]; Tim Gilmartin[2]; Steven R Head[2]; Céu Figueiredo[3]; Ana S Carvalho[1]; Leonor David[3]; Filipe Santos-Silva[1]; Celso A Reis[1]
[1]*IPATIMUP, Porto, Portugal;* [2]*The Scripps Research Institute, La Jolla, CA;* [3]*Medical Faculty of the University of Porto, Porto, Portugal*

Gastric carcinoma is the second cause of cancer death worldwide. *Helicobacter pylori* (Hp) is a bacteria that causes gastritis and is involved in gastric carcinogenesis. Hp binding/interaction with host cells is known to alter the host's gene expression profile. Expression of inflammation-associated sialyl-Le^X antigen is induced during persistent Hp infection, suggesting that Hp triggers the host tissue to retailor its gastric mucosal glycosylation patterns to a more favorable environment.

The biosynthesis of complex carbohydrate structures that may be altered by Hp and may function as ligands for bacterial adhesins remains largely unknown. Therefore, we performed a large scale gene expression analysis and searched for alterations induced by two Hp strains differing markedly in virulence.

We observed that Hp induced significant expression alterations in 168 of the 1031 genes tested. The most virulent Hp strain led to increased expression of glycosyltransferases participating in the biosynthesis of the lactoseries and neo-lactoseries on glycolipids, which can explain the synthesis of Sialyl-Le^X antigen, the ligand of Hp SabA adhesin. In addition, our results showed that genes involved in the regulation of the inflammatory response displayed the most remarkable increases, and are related with the NF-κB.

This study shows that Hp is capable of altering several glycosylation-related genes and that the strains' factors of virulence are profoundly related with gene expression alterations induced in host cells. Supported by FCT (POCI/SAU-OBS/56686/2004) and AICR (Grant 05-088). The Gene Microarray Core resources and collaborative efforts provided by The Consortium for Functional Glycomics were funded by NIGMS - GM62116.

(207) **The Role of Cell Surface Glycoconjugates in the Pathogenesis of *Trichomonas vaginalis***

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University of California, Los Angeles, Los Angeles, CA

Trichomonas vaginalis is a protozoan parasite that is responsible for trichomoniasis, the most common non-viral sexually transmitted infection. Attachment to host cells plays a significant role in the establishment of infection, yet *T. vaginalis* receptors on host cells have not been identified. A candidate molecule for host-pathogen interactions on the surface of *T. vaginalis* is its lipophosphoglycan (LPG)-like molecule. In order to study the importance of LPG in infection, mutants with altered LPG were generated by chemical mutagenesis followed by selection by lectin agglutination. Selected mutants have clear differences in the monosaccharide composition of their LPG molecules. Differences in adhesion and cytotoxicity to ectocervical cells suggest that LPG is involved in parasite-mediated cytotoxicity. These mutants underscore the importance of *T. vaginalis* LPG in establishing infection. Because of the abundance of LPG molecules on the parasite surface, it is possible that *T. vaginalis* utilizes host cell lectins as receptors. An S-type lectin found on host cells called galectin-1 has been recently implicated in binding a variety of pathogens. We now show that *T. vaginalis* binds to galectin-1 in a lectin-specific manner. *T. vaginalis* LPG may be the specific molecule that binds to this lectin, as the LPG mutants do not bind galectin-1. Studies are currently in progress to determine whether *T. vaginalis* is capable of utilizing this host cell lectin as a receptor to initiate binding of the parasite to host cells.

(208) **Expression Cloning of Cholesterol α-Glucosyltransferase, that can be Inhibited by Gastric Mucin *O*-Glycans with Antibiotic Activity, from *Helicobacter pylori***

Heeseob Lee[1]; Motohiro Kobayashi[1]; Ping Wang[1]; Jun Nakayama[2]; Peter H. Seeberger[1]; Minoru Fukuda[1]
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Helicobacter pylori infects over half the world's population but only 3% of those infected develop peptic ulcer, gastric carcinoma, and malignant lymphoma. *H. pylori* preferentially colonizes in the surface mucosa in those asymptomatic individuals. Deeper portion of the mucosa is characterized by the presence of MUC6 that express α1,4-GlcNAc-capping structure on core 2 branched *O*-glycans. Previous our studies showed that mucin-type glycoproteins containing α1,4-GlcNAc capping structure inhibit *H. pylori* growth by inhibiting the synthesis of α-glucosyl cholesterol, the major constituent of *H. pylori* cell wall. This finding is the first example for natural antibiotic function of human mucins (Science, 305, 1003-1006, 2004). Here, we identified cholesterol α-glucosyltransferase (CHLαGcT) using an expression cloning strategy and the His-tagged enzyme was expressed in *Escherichia coli*. This enzyme is distinct in being inhibited by mucin-type *O*-glycans and among mucin-type *O*-glycans tested, GlcNAcα1-4Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα1-octyl, which represents *O*-glycans present in MUC6, had the highest inhibitory activity. Moreover, inactivation of CHLαGcT by homologous recombination led to retarded *H. pylori* growth or almost lethal effect to *H. pylori*, while control homologous inactivation of vacuole A did not impair *H. pylori* growth. These results indicate that *H. pylori* CHLαGcT is a unique enzyme targeted by a natural antibiotic mucin and constitutes an excellent therapeutic target for developing drugs to prevent and possibly treat *H. pylori*-induced peptic ulcer, gastric carcinoma, and malignant lymphoma. This work was supported in part by NIH grants CA33000, CA71932, and grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

(209) ***L. major* UDP-Glucose Pyrophosphorylase: Characterisation of Ligand Binding Properties and Substrate Specificity using NMR Spectroscopy**

Anne-Christin Lamerz[1]; Thomas Haselhorst[2]; Anne Bergfeld[1]; Sebastian Damerow[1]; Mark von Itzstein[2]; Rita Gerardy-Schahn[1]
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The protozoan parasite *Leishmania* causes a group of diseases collectively known as *Leishmaniasis*. *Leishmania* express various glycoconjugates on their cell surface allowing the survival and proliferation in the sand fly vector and mammalian host. The biosynthesis of glycoconjugates essentially depends on the availability of activated nucleotide sugars. The UDP-glucose pyrophosphorylase (UGP) represents a key position in the activation of glucose and galactose, both major components of *Leishmania* glycoconjugates. UGP catalyses the synthesis of UDP-glucose from glucose-1-phosphate and UTP. Formation of UDP-glucose is a prerequisite for the synthesis of UDP-galactose. Using a gene deletion approach we identified UGP to exert an important function for *L. major* virulence.

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To gain a first insight into the catalytic mechanism and thus provide a starting point for the design of specific inhibitors, we characterised the protein-ligand interactions of *L. major* UGP by saturation transfer difference (STD-) NMR. Using this technique, we could directly prove that the enzyme follows an ordered bi-bi reaction mechanism with UTP preceding binding of glucose-1-phosphate. Interestingly, UDP and UMP were not recognized by the enzyme demonstrating the relevance of the gamma-phosphate group of UTP.

Though the nucleotide part is important for binding, the substrate specificity is influenced by the sugar moiety. Epimerisation of position 4 of the hexose is sufficient to prevent binding, as demonstrated by the absence of binding of the C4-epimer UDP-galactose. Thus the correct orientation of the C4-hydroxyl group mediates substrate specificity of *L. major* UGP.

(210) **Biosynthetic Pathway of GDP-D-*glycero*-α-L-gluco-Heptose from Campylobacter jejuni**

Christopher W. Reid[1]; David J. McNally[1]; Joseph Hui[2]; Andrea Graziani[3]; Frank St. Michael[1]; Paul Kosma[3]; J.R. Brisson[1]; Evelyn Soo[2]; Christine Szymanski[1]

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Capsular polysaccharides (CPS) comprise the outer most structure of the bacterial cell and play an important role in the interaction between the pathogen, host, and the environment. Recently, we demonstrated that the CPS of Campylobacter jejuni NCTC 11168 was a tetrasaccharide repeat composed of β-D-Rib, β-D-GalfNAc, α-D-GlcpA6(NGro) - a uronic acid with 2-amino-2-deoxyglycerol at C-6, and the unique 6-O-methyl-D-glycero-α-L-glucoHep as a side-branch. This is the first report of the L-gluco configuration in nature. In our ongoing effort to exploit the glycome of C. jejuni for glycoengineering, we have undertaken a study of the heptose biosynthetic pathway to identify novel enzymes that can be utilized for the construction of glycoconjugates. Through CPS mutagenesis, mass spectrometry and high-resolution magic angle spinning NMR studies, we identified several genes encoding enzymes involved in the biosynthesis of this complex heptose branch. A selective method for the detection of nucleotide-linked sugars using CE-ESMS and precursor ion scanning was utilized to identify mutants that caused the accumulation of GDP-Hep. Three enzymes were identified using this metabolomics approach, Cj1427 (putative NAD dependent 4-epimerase), Cj1428 (putative dehydrogenase/reductase), and Cj1430 (putative 3,5-epimerase/reductase).These enzymes were cloned and over-expressed as N-terminal His-fusion proteins. CE-UV has been used to assess the ability of each enzyme to utilize GDP-D-glycero-α-D-manno-heptose. Initial results indicate that all three enzymes are capable of acting on the GDP-D,D-Hep precursor. A proposed biosynthetic pathway for C. jejuni D-glycero-α-L-gluco-heptose will be presented.

(211) **A New Perspective on Mycobacterial Cell Wall Biosynthesis and the Identification of Potential Drug Targets**

Luke J Alderwick[1]; Mathias Seidel[2]; Lothar Eggeling[2]; Gurdyal S Besra[1]

[1]University of Birmingham, Birmingham, United Kingdom; [2]Institute for Biotechnology, Research Centre Juelich, Germany

Tuberculosis is a global catastrophe caused by the highly infective pathogen Mycobacterium tuberculosis which causes around 8 million new cases and 3 million deaths annually around the world. A major feature that contributes to its survival and persistence is its unusual cell wall, which consists of a mycolyl-peptidoglycan-arabinogalactan (mAGP) complex. Much interest has focused on the biosynthetic machinery involved in the production of the highly impermeable mAGP, which is the target for numerous anti-tuberculosis agents. Here, we report on a novel arabinofuranosyl transferase (AftA) involved in the initial steps of AG biosynthesis. This "priming" enzyme was cloned, expressed and confirmed as a bona fide glycosyltransferase which transfers the initial arabinose units to the galactan backbone. Interestingly AftA was unaffected by Ethambutol, the frontline drug which inhibits down-stream arabinosyltransferases. Further to this, the crucial enzyme (UbiA) involved in the production of the arabinan precursor decaprenylmonophosphoarabinose (DPA) was deleted in the close relative Corynebacterium glutamicum as a means to study otherwise essential genes in M. tuberculosis. Subsequent glycosyl and biochemical analyses revealed a cell wall completely devoid of arabinose and bound mycolates. These enzymes represent ideal candidates for chemotherapeutic exploitation, especially with the advent of multi-drug-resistant tuberculosis (MDR-TB). However, many important glycosyltransferases involved in the biosynthesis of the crucial arabinogalactan complex remain elusive. Here we present a contemporary perspective of the enzymes involved in arabinogalactan biosynthesis, the mechanisms of polymerisation and recent advances in the physiology of cell wall production.

(212) **Role of the Lipopolysaccharide Structure in the Resistance of *Yersinia pestis* to the Bactericidal Action of Polymyxin B and Serum**

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Yersinia pestis, the cause of plague, circulates in natural foci, which involve a rodent reservoir and an insect vector. The rough-type ipopolysaccharide (LPS) of *Y. pestis* is one of the bacterial features that counteract mammalian and insect antimicrobial factors, assuring maintenance of the pathogen in these hosts during the transmission cycle. Cultivation at various temperatures [mammalian (37°C), flea (25°C) or winter-hibernation (6°C)] was shown to affect the LPS composition and structure [Knirel et al. *Biochemistry*, **44**, 1731-1743 (2005)] as well as the susceptibility of various *Y. pestis* subspecies grown at these different temperatures to antimicrobial cationic peptide polymyxin B and complement-mediated serum killing [Anisimov et al. *Infect. Immun.*, **73**, 7324-7331 (2005)]. To elucidate the significance of these LPS phase variations we studied the relationship of the LPS structure to the biological properties of *Y. pestis*. Mutation tests, using a non-polar single mutation in each of the LPS biosynthesis genes, showed that the impaired inner heptose region of the LPS core, but not the terminal outer-core monosaccharides, is crucial for the resistance of *Y. pestis* to both bactericidal factors. The polymyxin B resistance requires also a high content of the cationic sugar, 4-amino-4-deoxy-L-arabinose, in lipid A. At flea temperature the LPS structure synthesized confers resistance to polymyxin B whereas resistance to normal serum killing was manifest at both mammalian and flea temperatures. At winter-hibernation temperature the bacterium synthesizes an LPS conferring sensitivity to both anti-microbial factors, which are not expressed in hibernating rodents.

(213) **Structural Characterization of PEB3, a Putative Adhesin of Campylobacter jejuni and a Natural Substrate for Its N-Glycosylation System**

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Campylobacter jejuni is unusual among bacteria in possessing a eukaryotic-like system for N-linked protein glycosylation at Asn residues in sequons of the type Asp/Glu-Xaa-Asn-Xaa-Ser/Thr (Kowarik, M. et al., 2006). However, little is known about the structural context of the glycosylated sequons, limiting the system's usefulness for producing novel recombinant glycoproteins. PEB3 (Cj0289c) is a major antigenic glycoprotein from C. jejuni whose sequence similarities to other proteins suggest it has a role in adhesion and/or small molecule transport. We have determined its crystal structure as a complex with bound citrate, at 1.6Å resolution. PEB3 is a dimeric protein, both in the crystal structure and in solution. It has the class II periplasmic binding protein fold, with each monomer having two domains with a ligand-binding site located between them. The structure of PEB3 is most similar to bacterial molybdate- and sulfate-binding proteins. The residues that form the major structural features and the citrate binding site are conserved in the adhesin Paa from enteropathogenic E. coli, strengthening the case for PEB3 being an adhesin. The sequon around Asn90 is located within a surface-exposed loop that could be accessible to the PglB oligosaccharyltransferase. Hence N-glycosylation may be able to occur after the protein has folded in the periplasm.

[1] Kowarik, M., Young, N.M., Numao, S., Schulz, B.L., Hug, I., Callewaert, N., Mills, D.C., Watson, D.C., Hernandez, M., Kelly, J.F., Wacker, M., and Aebi, M. (2006) Definition of the bacterial N-glycosylation site consensus sequence. EMBO J. 25, 1957-1966.

(214) **The glycome of *Campylobacter jejuni* – Dissection of the Bacterial *N*-Linked Glycosylation Pathway**

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Campylobacter jejuni possesses a functional *N*-linked glycosylation system that attaches a unique heptasaccharide to greater than 30 proteins. We

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therefore developed a universal method to isolate *N*-linked glycans from glycoproteins in complex bacterial and eukaryotic protein mixtures based on a combination of non-specific proteolytic digestion and permethylation. In addition to detecting the *N*-linked heptasaccharide in whole cell extracts of *C. jejuni* we also observed an unexpected free heptasaccharide that required a functional protein glycosylation (*pgl*) pathway. Currently, experiments are underway to elucidate the role of this novel intermediate in the *N*-glycosylation pathway of *C. jejuni*.

We are also interested in *pgl* gene expression and the influence of the pathway on other cellular functions. RT-PCR analyses identified several transcripts within the 13 gene *pgl* locus. This finding was corroborated by promoter mapping studies using 5' RACE that revealed the presence of at least three transcriptional start sites within the *pgl* locus. Interestingly, the consensus sequence for the *pgl* transcripts differs from the three well characterized *C. jejuni* sigma factor binding sites (σ^{70}, σ^{54}, and σ^{28}). To study the potential impact of *N*-glycosylation on other pathways, the transcriptome of the *C. jejuni* wild-type was compared to several *pgl* mutants using *C. jejuni* amplicon-based DNA microarrays. Expression of several genes is altered in the *pgl* mutants and the profiles also differ among the *pgl* mutants indicating that the *N*-glycan pathway influences multiple cellular functions in *C. jejuni* consistent with the observation that mutation of this pathway results in pleiotropic biological effects.

(215) ***Caenorhabditis elegans* Functional Glycomics - Elucidating Bt Toxin Resistance**

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Glycoconjugates are important contributors to a diverse range of cellular biological events. As we attempt to better comprehend these events a knowledge of the structures of the participants is vital. In recent years our lab has been involved in defining the glycome of *Caenorhabditis elegans*. We are utilizing this knowledge to investigate Bt toxin resistance in *C. elegans*. We have previously demonstrated that (i) the major mechanism for Bt toxin resistance entails a loss of glycolipid carbohydrates; (ii) Bt toxin directly and specifically binds glycolipids; and that this binding is carbohydrate-dependent. Here we describe the structural characterization of glycolipid derived and glycoprotein derived glycans from *bre*-1 mutant animals which have a low resistance to Bt toxin. We demonstrate that *bre*-1 mutant animals are defective in production of fucosylated glycolipids. Remarkably, we also show that *bre*-1 mutant animals, although viable, show a lack of fucosylated N- and O-glycans. Thus, *C. elegans* can survive with little fucose and can develop resistance to crystal toxin by loss of a monosaccharide biosynthetic pathway.

(216) **Structural Characterization of Glycosphingolipids and Toxin Receptor Gangliosides by IRMPD with TLC/VC-MALDI-FTMS**

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Biological roles of glycosphingolipids and gangliosides are dependent on the structures of both the oligosaccharide and the ceramide. We initially assumed that GD1a ganglioside of human intestinal T84 cells, the presumed receptor for the LTIIb toxin, is not functional in this cell type due to ceramide structural variation. To test this idea, we analyzed toxin receptor ganglioside structures, using our previously developed method of directly coupling TLC plates with vibrationally cooled (VC)-MALDI-FTMS. The desorbed ions were fragmented by SORI-CAD and IRMPD.

IRMPD of the glycolipids resulted in fragmentation of both the ceramide and oligosaccharide moieties, whereas SORI-CAD required multi-step fragmentation with individual optimization of the SORI pulse for each fragment. For glycolipids having larger glycans, fragmentation within the lipid portion could only be obtained by IRMPD. More fucosylation was observed in the T-84 gangliosides than in the Vero-cell line; both had extended glycan moieties as compared to common gangliosides. The high separation efficiency of the HP- and 2D-TLC methods resolved numerous ceramide homologs, and each of these was desorbed directly off the TLC plates. GD1 gangliosides were not found migrating in the expected region; the glycolipid glycans identified, however, contained both NeuAc and Fuc residues. Vero-cells had poly-sialylated gangliosides in the expected patterns. Vibrational cooling resulted in stabilization of the labile sialic acid and fucose glycosidic linkages.

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(217) **Search for Aryl *N*-Acetyl-A-D-Glucosaminides which Suppress the Growth of *Helicobacter pylori***

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Helicobacter pylori (*H. pylori*) is Gram-negative bacteria, which causes chronic gastritis, peptic ulcer, and gastric cancer. *H. pylori* colonizes surface mucous cell-type mucin, while this microbe is rarely found in gland mucous cell-type mucin which specifically contains α1,4-GlcNAc-capped *O*-glycans. Recently we have demonstrated that α1,4-GlcNAc-capped *O*-glycans function as natural antibiotics against *H. pylori* by inhibiting the biosynthesis of cholesteryl-α-D-glucopyranoside, a major cell wall component (Science **305:** 1003-1006, 2004). In the present study, we have examined the antimicrobial activity of various synthetic aryl *N*-acetyl-α-D-glucosaminides *in vitro*. *H. pylori* was cultured in the presence of these compounds, and we found that these aryl glycoside having phenyl, p-nitrophenyl, p-methoxyphenyl, and o-nitrophenyl suppressed the bacterial growth in a dose-dependent manner. By contrast, such growth suppression was not found when *H. pylori* was cultured with aryl glycosides of p-aminophenyl, m-hydroxyphenyl, and phenyl having lactone. We then examined quantitative structure-activity relationship of these glycosides to determine the dominant structural parameters for antimicrobial activity by using the genetic algorithm-based partial least squares method. In this analysis, we found that the predicted antimicrobial activity could be given by three parameters of these aryl glycoside; i.e, hydrophobic effect, molar refractivity, and Connolly solvent-excluded volume. These data will provide the basis for development of new drugs for *H. pylori* infection. Supported by Grants-in-Aid for Priority Area 14082201 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Scientific Research C-185501600002 from the Japan Society for the Promotion of Science, and NIH grant CA33000.

(218) **Bioinformatic Analysis and Characterization of Sialidases and Trans-sialidase-Related Genes Using Phylogenetic Approach**

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Sialidases belong to a class of glycosyl hydrolases that release terminal N-acyl-neuraminate residues from glycoproteins, glycolipids, and polysaccharides. The functions of sialidases are poorly understood and until recently, their biochemical and evolutionary relationships were unclear. From a systematic search of databank, using a bacterial sialidase as a query sequence, we have identified 155 putative sialidase sequences in 59 organisms belong to four kingdoms except plant. Approximate 75% putative sialidases were enzymes derived from pathogenic bacteria and protozoa. Multiple amino acid sequence alignments of sialidases showed these enzymes have aspartate-box sequence domains, Ser-x-Asp-x-Gly-x-Thr-Trp, at their C-terminal region. Phylogenetic tree analysis indicated that sialidase would be split into two distinct groups; Bacterial sialidase and Protozoal sialidase. Interestingly, most eukaryotic enzymes, which are ganglioside sialidases integrated to plasma membrane, were clustered with bacterial sialidases. It was implied that eukaryotic sialidase genes would be transferred from bacterial origins by horizontal gene transfer. Indeed, most bacterial enzymes were detected from parasitic microorganisms in the human body, suggesting that bacterial sialidases could play an important role in establishing the host-parasite relationship. Topology analysis of eukaryotic and bacterial sialidases indicates that one protozoan and three bacteria might have a trans-sialidase. We also identified that a bacterial trans-sialidase can transfer α2,3- and α2,6-linked sialic acid to Gal-acceptor by characterizing bacterial recombinant enzymes. This is the first report on biochemical and genetic characterization of bacterial trans-sialidase. Database mining and subsequent phylogenetic analysis of sialidase will provide the information of evolutionary relationship through parasite invasion of host.

(219) **Glycophorin A-Knockout Mice are Resistant to Rodent *Babesia* Infections**

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Babesiosis is a major infection of domestic animals tropical and subtropical areas worldwide and also gaining increasing interest as an emerging zoonosis in humans. The intraerythrocytic development of *Babesia* parasites causes clinical manifestations, such as fever, anemia and hypotensive shock syndrome; however, the precise molecular mechanisms of the development within the red blood cells (RBC) are largely unknown. Glycophorins are major surface sialoglycoproteins on the RBC. In the present study, we characterized RBC of Glycophorin A (GPA)-knockout mice and their susceptibility to the rodent *Babesia* infections. Flow cytometric analysis showed an apparent reduction of *Maackia amurensis* lectin II (MAL II) binding to GPA homozygous (-/-) RBC compared with wild-type and GPA heterozygous (+/-) RBC, indicating the remarkable loss of α2-3 linked sialic acids on the surface of GPA-/- RBC. In the western blot analysis with MAL II and a monoclonal antibody TER-119, which has been previously reported to react with murine GPA, approximately 97, 66, 42 and 33 kDa bands were abrogated from the RBC membrane of GPA-/- mice. The GPA-/- mice were resistant significantly to the infections of *Babesia microti* as compared with wild-type and GPA+/- mice. *B. rodhaini* caused a lethal infection in wild-type and GPA+/- mice, the infected GPA-/- mice showed clearly low parasitemia and survived. These results indicate that sialoglycoproteins that were not present in GPA-/- RBC might be involved in growth of rodent *Babesia* parasites as invasive receptors for these parasites.

(220) Unique Structural Requirements for Chemically Modified Reduced-Charge Heparin Derivatives to Selectively Bind HS-Binding Proteins

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Heparan sulfate (HS) is a cell-surface glycosaminoglycan that plays a profound role in numerous physiological processes. Over twenty therapeutic applications have been proposed for molecules to bind specific HS-binding proteins and block or modulate HS-mediated biological activities. The discovery of such molecules, including efforts to chemically modify natural polysaccharides such as the HS-like glycosaminoglycan heparin, has primarily focused on optimizing the degree and spatial orientation of anionic substituents on saccharide based or non-carbohydrate based core structures. Due to the cationic nature of HS-binding sites in HS-binding proteins, the discovery of polyanionic molecules that bind to HS-binding proteins is typically not a problem. The problem is identifying molecules that selectively, if not specifically bind the HS-binding site of individual HS-binding proteins. Negatively charged molecules that bind to a target HS-binding protein often have affinity for many other HS-binding proteins. We previously reported the diversity-oriented chemical modification of heparin through a N-desulfonation/N-acylation strategy affords charge-reduced heparin derivatives having increased binding affinity and increased binding selectivity for certain HS-binding proteins. In the work presented here, the unique structural requirements for select N-desulfonated/N-acylated heparin derivatives and heparin amides to bind certain HS-binding growth factors, coagulation factors and pathogen surface proteins will be discussed. Results of studies using these structure-affinity relationships to further create minimally-charged heparin-derived oligosaccharides that selectively bind to individual HS-binding proteins involved in host-pathogen interactions will also be presented.

(221) Characterization of D-Arabinopyranose-containing Glycosylinositolphospholipids from Leishmania major

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Arabinose-containing glycoconjugates are plentiful in microbes, plants, and protozoan parasites, but are absent in mammalian cells. Unlike other monosaccharides, arabinose can occur naturally in glycoconjugates in pyranose or furanose conformations and as D- and L- stereoisomers. Not much is known of the metabolism of D-arabinopyranose and its importance as a substituent of glycoconjugates. In the infectious part of their life cycle, Leishmania major use D-arabinopyranose to cap lipophosphoglycan (LPG, a dominant adhesion molecule) enabling the parasite to detach from the midgut epithelium of their sand fly vector. Besides LPG, it has been suggested that Leishmania express other D-arabinose-containing glycolipids. To begin investigating these other Ara-containing glycolipids, L. major promastigotes were metabolically labeled for 2 h with 3H-arabinose and extracted. The 3H-Ara-lipids exhibited the following notable characteristics: 1) comprised ~33% of the total 3H-arabinose label incorporated into glycoconjugates; 2) was susceptible to nitrous acid deamination, indicating a GPI-anchor; and 3) was susceptible to mild acid hydrolysis (0.02 N HCl; 5 min; 60°C), suggesting the presence of phosphodiester linkages. Furthermore, 3H-arabinose radiolabeling of the glycolipids was precluded in L. major gene knock-out mutants defective in either GDP-sugar transport or UDP-Gal transport in the Golgi apparatus as well as in a gene knock-out of ether lipid synthesis. These characteristics are consistent with arabinose-containing glycosylinositolphospholipids (GIPL) assembly in the parasite's Golgi apparatus. The precise structure of the Ara-GIPL as well as details regarding it subcellular localization and possible functions are underway.

(222) Elongating Mannosylphosphoryltransferase from Leishmania donovani: Solubilization and Partial Purification

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Lipophosphoglycan (LPG) is the predominant glycoconjugate of Leishmania parasites and plays important roles. Structurally, LPG consists of a polymer of Gal(beta1,4)Man-phosphate repeat units attached to a GPI anchor. The number of repeat units varies depending on the stage of the life cycle: n~15 in procyclic and n~30 in metacyclic promastigotes, and n=0 in the intracellular amastigote form of the parasite. At least two mannosylphosphoryltransferases (MPTs) have been implicated in repeat unit assembly: an "initiation" specific MPT (iMPT) which uses the LPG core as acceptor and an "elongation" specific MPT (eMPT) which uses repeat units as acceptor. We previously identified the LPG4A gene by functional complementation of an lpg- mutant of L. donovani which synthesized only a single repeat unit. LPG4A gene encodes a predicted type II transmembrane protein of 1,375 amino acids and has significant homology to mammalian UDP-GlcNAc phosphotransferase. Our findings suggest that that the protein encoded by the gene is the eMPT or a component of an eMPT complex. Dodecylmaltoside was the best detergent for both solubilizing LPG4A from microsomes obtained from lpg- cells transfected with epitope-tagged LPG4A and retaining eMPT activity. However, solubilized eMPT was extremely unstable. While glycerol and Mn and Mg cations were helpful for stabilizing enzymatic activity, only ~20% activity remained after 14 h of incubation at 4°C. Improvements in stabilization, purification, and characterization of eMPT are ongoing. Characterization of eMPT will be critical for understanding how the number of repeat units in LPG is developmentally regulated in the parasite's infectious cycle.

(223) Comparasion of Protein Profiling of Central Nervous System from Wild Type and Galectin-3 Knockout Mice Infected with *Toxoplasma gondii*

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Galectin 3 is a multifunctional protein expressed in a variety of cell types in the immune system, constitutively or in response to microbial invasion. These studies indicate an implication of galectin-3 in both innate and adaptive immune responses, where it participates in the activation or differentiation of immune cells. Bernardes et al. (Am. J. Pathol, 168:p.1910, 2006) have previously described that galectin-3 knockout mice (gal3–/–) infected with Toxoplasma gondii mount a higher Th1-polarized response, which was characterized by a higher ratio of T. gondii-specific IgG2a/IgG1 and increased levels of IL-12. In addition, they identified CD11c+ dendritic cells as responsible for the increase in IL-12 production, and consequently for the higher Th1 response in these mice.

Here we are investigated protein profiling expression of central nervous system (CNS) of gal3–/– mouse infected with T. gondii (KO-gal3-TOXO), and wild type (WT-TOXO) compared to uninfected gal3–/– (KO-gal3) and wild type mouse (WT). CNS protein extracts were prepared for 2D gel electrophoresis. Two dimensional maps showed an average of 457 spots stained by coomassie blue in WT, WT-TOXO, KO-gal3 and KO-gal3-TOXO, and only on the basis of spots with variation of more than two fold (100%) spots were selected for protein identification by MALDI-TOF-MS after trypsin digestion. Twenty four proteins were differentially expressed and classified as being part of metabolism like enolase and aldolase, and cytoesqueleton as non muscular cofilin. These preliminary results indicate that protein profiling maybe contribute to understanding the role of galectin-3 during infection by *T. gondii*.

(224) The Immune Response to Linear and Clustered β-Mannan Epitopes of *Candida albicans*

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A β1,2-linked trisaccharide of mannopyranosyl residues is the epitope recognized by monoclonal antibodies that confer passive protection against Candida albicans infection [1]. Unpublished work in our group showed the same trisaccharide conjugated to tetanus toxoid induced antibodies and conferred a significant level of protection against live *Candida* challenge in a rabbit model of disseminated candidiasis.

The use of synthetic oligosaccharides for vaccine formulation offers many advantages over conjugate vaccines composed of polysaccharides derived from microbial sources, however, several challenges must be addressed prior to practical applications. Amongst these is the introduction of modifications for improved antigen presentation or *invivo* persistence. Oligosaccharide immunoconjugates for eventual use as vaccine candidates may also benefit from novel conjugation strategies that maximize B-cell epitopes while preserving T-cell epitopes, and perhaps from introduction of isosteric functionality that maintain the fidelity of immunological recognition, yet prolong bioavailability of the antigen.

We report here a comparison of the immune response to several synthetic β-mannan based vaccine constructs that employ clustering of B-cell epitopes, and substitution of the inter-glycosidic oxygen atom by sulphur to create antigens with enhanced *invivo* persistence. The immune response to these antigens is compared with the response to a simple trisaccharide tetanus toxoid antigen.

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(225) **Sialic Acid Binding and Release in Myxoviruses**

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Influenza virus gains entry into the host cell via binding of hemagglutinin (HA) to sialic acid receptors. Human viruses recognize predominantly α2,6 linked sialic acids while avian viruses show preference for the α2,3 linkage., A Fujian-like H3N2 human influenza virus, A/OK/323/03, agglutinates human red blood cells but does not elute from them, indicating that NA activity does not cleave the receptor bound by the HA (U. Gulati et al., Virology 339, 12-20, 2005). When OK/03 was passaged in MDCK cells we found a decrease in NA activity. RT-PCR results confirmed that after passaging at non limiting dilution, NA was deleted from its normal length of 1467 nucleotides to a fragment of 300-800 nucleotides with conservation of both the 5' and 3' ends. The virus grows efficiently in MDCK cells even after the deletion of NA activity, indicating that the virus is not dependent on receptor destroying activity, at least when grown in MDCK cells.

To further characterize the receptors bound by recent human influenza viruses, we used Alexa Fluor ®488 labeling and glycoarray analysis (Core H of the Consortium for Functional Glycomics) to determine the specificity of binding by A/Oklahoma/323/03 (Fujian-like), A/Wyoming/03 (PR8 reassortant, egg adapted vaccine strain), A/California-like H3N2 isolates from Oklahoma City, 2005 and Human Parainfluenza viruses hPIV1 and hPIV3. The influenza viruses bound preferentially to long glycans containing α2-6 linkages while PIV1 and PIV3 show significant differences in binding to glycans containing α2,3-linked sialic acid.

(226) **A Galectin from Hemocytes of the Oyster (*Crassostrea virginica*) is a Potential Receptor for the Parasite *Perkinsus marinus***

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Although the Eastern oyster (*Crassostrea virginica*) is endowed of efficient innate immune recognition and effector mechanisms that are successful in fighting most potentially pathogenic microbes, they become readily infected when exposed to *Perkinsus marinus*, a protozoan parasite responsible for mass mortalities in native and farmed oyster populations in the Atlantic and Gulf coasts of the USA. We have cloned and characterized the cDNA and the gene organization of a galectin of unique domain organization present on the surface of the oyster hemocytes that may function as a receptor for the protistan parasite *P. marinus*. The 1668 nucleotides-long transcript, encoding 555 amino acid residues (CvGal), revealed the presence of four galectin-like carbohydrate recognition domains (CRDs). The CvGal gene is composed of 12 exons divided by 11 introns, none of which are present within the regions encoding each CRD. CvGal is mostly expressed in hemocytes, and its binding activity is strongly inhibited by lactose, N-acetyllactosamine and thiodigalactose, and several glycoproteins, including lactoferrin, laminin, thyroglobulin, and asialofetuin. Comparative binding studies that included bacteria, algae and the Perkinsus spp revealed that CvGal binds very efficiently to the surface of *Perkinsus* spp trophozoites, and that the binding is carbohydrate-mediated, This evidence, together with the observation that *P. marinus* efficiently abrogates the respiratory burst elicited upon phagocytosis, suggests that this recognition system may have been possibly subverted as an infectivity mechanism by the parasite *P. marinus*. (Supported by NIH Grant R01 GM070589-01 and NSF grant MCB-00-77928 to GRV)

(227) **Mechanisms and Consequences of Sialic Acid de-O-Acetylation in Group B Streptococcu**

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Group B Streptococcus (GBS) is a common cause of neonatal sepsis and meningitis. A major virulence factor of GBS is its sialic acid-containing capsular polysaccharide. Recently, we discovered the presence and genetic basis of sialic acid O-acetylation in GBS, a modification missed during three decades of previous studies. We now characterize a sialic acid O-acetyl esterase, which may regulate the degree of O-acetylation on the GBS surface. The enzyme domain responsible for hydrolyzing Sia O-acetyl esters is fused to the GBS CMP-Sia synthase, which are together encoded by the neuA gene. A genetic approach in multiple GBS strains revealed accumulation of intracellular O-acetylation upon deletion of neuA and reduction of surface O-acetylation upon over-expression of neuA. In a defined biochemical system, purified GBS NeuA de-O-acetylated free 9-O-acetyl-N-acetylneuraminic acid in a CTP-dependent manner. Moreover, CMP-9-O-acetyl-N-acetylneuraminic acid was able to compete with the unactivated substrate. Taken together, our data are consistent with a processive model in which the fused domains of GBS NeuA act in a cooperative manner to CMP-activate, then de-O-acetylate intracellular sialic acid. During infection, capsular sialic acids serve an anti-phagocytic role by interfering with host complement deposition. Preliminary in vivo studies suggest that high level O-acetylation is detrimental for bacterial survival in an acute mouse infection model, compared to an isogenic strain with little O-acetylation. Thus, the de-O-acetylation capacity of the NeuA esterase may serve an important role in bacterial pathogenesis by preventing excessive O-acetylation, which is deleterious for bacterial survival in the host.

(228) **Intravenous Immune Globulin Treatment for Hereditary Inclusion Body Myopathy: A Pilot Study**

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Hereditary Inclusion Body Myopathy (HIBM) is an autosomal recessive, adult onset neuromuscular disorder with no effective treatment. The causative gene, GNE, catalyzes the first two reactions in the synthesis of sialic acid (SA). Reduced sialylation of muscle glycoproteins, such as α-dystroglycan and neural cell adhesion molecule (NCAM), is observed in HIBM. In this pilot study we treated 4 HIBM patients with intravenous immune globulin (IVIG), which contains 8μmol of SA/g IgG, as a means of providing high quantities of SA. IVIG was infused at a dose of 1g/kg on two consecutive days followed by 3 doses of 400mg/kg at weekly intervals. The primary outcome was muscle strength assessed by Quantitative Muscle Testing. Mild improvements in strength were recorded. Function of the right and left quadriceps improved by 13-154% and 8-48%, respectively, in 3 patients. Similarly, shoulder abduction improved by 24-79% on the right and 13-184% on the left in 3 patients. Esophageal motility and lingual strength improved in the 2 patients with abnormal barium swallows. Minimal to modest qualitative improvements in daily activities were experienced in 3 patients. No unexpected side effects occurred. Muscle immunohistochemistry and western blot analysis for α-dystroglycan did not demonstrate any appreciable changes, while NCAM expression on western blotting decreased post-IVIG in 2 patients, suggesting decreased denervation/regeneration. The absence of inflammation in HIBM muscle suggests that the noted mild benefits were not related to the anti-inflammatory effects of IVIG. The uses of IVIG and other sources of SA are being explored as treatment options for HIBM.

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(229) **Molecular Basis for Equilibrium Between Non-Covalent Dimer and Monomer of Myelin P0 Glycoprotein in *Xenopus laevis* Peripheral Nerve**

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Myelin protein zero (P0), an integral membrane glycoprotein, has a major role in the formation and maintenance of myelin. Mutations and deletions in the P0 gene correlate with hereditary peripheral neuropathies. P0 glycans are proposed to play an important role in cell-cell adhesion.

Studies on bovine, murine, and human P0 suggest that P0 exists as tetramers in the myelin membranes of these species. However, for Xenopus, dimeric P0 predominates. Based on the results from SDS-PAGE and Mini-Prep-Cell™, we propose that the Xenopus PNS P0 dimer is stabilized by non-covalent interaction(s). MALDI and ESI mass spectrometry and tandem MS strategies, including CID, Q2CAD on qQq-FTMS, were utilized to explore the factors affecting the aggregation of Xenopus P0 and the post-translational-modification (PTMs) profiles of the monomers and multimers. Xenopus P0 contained a series of high mannose, hybrid, and complex glycans whose structures we determined. Asn92 was confirmed as the single fully occupied *N*-glycosylation site. Cys152 was found to be acylated with stearic acid. The PTMs in Xenopus P0 identified here differ from those reported for other species such as bovine. We propose that its unique acylation and glycosylation could underlie the unusual aggregation forms of P0 from *Xenopus laevis*.

The studies on the aggregation behavior of P0, the major adhesive protein in peripheral myelin, will likely contribute to an understanding of the phylogenetic development of P0's adhesion role in myelin.

This work was supported by NIH-NCRR P41-RR10888 and S10-RR15942 (to CEC). Research at Boston College was supported by institutional-research-funds (to DAK).

(230) **Characterization of N-Linked Glycans on the Prion Glycoprotein (PrPc) by Mass Spectrometry**

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Prion diseases are a class of neurodegenerative diseases in which conversion of a normal cellular glycoprotein, the prion protein (PrPc), to a misfolded form (PrPSc) generates a protein, which is believed to be the sole component of the transmissible agent of the diseases. It is the accumulation of PrPSc in the CNS that causes neurodegeneration, and gliosis, which are characteristic of the diseases. Conformational changes in PrPc that lead to PrPSc cause the protein to become proteinase resistant and to form fibrils.

PrPc is a glycoprotein expressed by many cells including neurones and muscle cells. However, it is predominantly expressed at synapses suggesting that, functionally, PrPc is important for neuronal activity. The fusion glycoprotein was expressed in CHO cells and purified with protein G beads.

The aim of this study has been to characterize the N-linked oligosaccharides on the prion protein. The released and permethylated N-linked oligosaccharides were analyzed by both MALDI-MS and ESI-MS. The structure of N-linked glycans was confirmed by MS/MS analysis of permethylated glycans.

The N-linked profiling experiment produced a complicated set of data with more than twenty different oligosaccharides that are present on this glycoprotein. The main structures were the fucosylated biantennary structure with one galactose and the fucosylated biantennary structure with two galactose residues. Larger triantennary and tetraantennary structures were also detected.

(231) **Neural Differentiation of Human Stem Cells *via* Metabolic Sialic Acid Engineering**

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Stem cells hold enormous promise for the treatment of disease and for regenerative medicine. The clinical development of stem cell therapies hinges upon reliable methods to differentiate pluripotent or multipotent cells into specific and homogeneous lineages. Up to now numerous efforts to develop cocktails of growth factors and signaling molecules, to mimic the complex signal transduction cascades that guide differentiation in the developing embryo, have been reported. By contrast, our strategy of exploiting small molecules to modulate glycosylation represents a novel approach to regulating stem cell fate. Specifically, we show that metabolic substrate-based sialic acid engineering methods can be applied to stem cells by designing a small molecule, *N*-thioglycolyl-D-mannosamine peracetate (Ac_5ManNTGc, **1**), that induced neuron-like differentiation of human embryoid body-derived (hEBD) stem cells. hEBD cells process **1** through the sialic acid biosynthetic pathway resulting in the expression of thiols on cell surface sialoglycans, in the form of *N*-thioacetyl-neuraminic acid (Neu5TGc). Upon incubation with **1** for two weeks, hEBD cells experienced morphological changes suggestive of differentiation to neurons; neural differentiation was confirmed by staining for neuronal markers and the accumulation and nuclear localization of β-catenin, a central protein involved in Wnt-signaling pathways. Culturing the cells on gold-coated cover slips in the presence of **1** enhanced the differentiation process indicating the importance of complementary interactions between cell surface thiols and the substrate. Finally, these effects were not observed in cells treated with *N*-glycolyl-D-mannosamine peracetate (Ac_5ManNGc, **2**), indicating that neuron differentiation was thiol-specific and not merely a consequence of disturbing sialic acid metabolism.

(232) **Polysialic Acid Determines Cell Fate of Neural Precursor Cells in Mouse Brain Development**

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Polysialic acid, a homopolymer of α2,8-linked sialic acid, is a unique and essential carbohydrate highly expressed in developing central nervous system. This post-translational modification of the neural cell adhesion molecule (NCAM) by two polysialyltransferases, ST8SiaII (STX) and ST8SiaIV (PST), is required for normal brain development. To determine the role of polysialic acid, distinguished from the role of NCAM, in neural development, we analyzed mutant mice lacking ST8SiaII and ST8SiaIV. In contrast to NCAM knockout mice and ST8SiaII or ST8SiaIV single knockout mice, double mutant mice display severe defects in brain development such as thin cerebral cortex, thin corpus callosum and enlarged lateral ventricle. Immunostaining for neural markers demonstrated that distribution of glial cells, GABAergic neurons and pyramidal neurons is impaired in the double mutant mice. BrdU-labeling experiments showed that migration of cortical neurons generated in ventricular zone of polysialic acid-deficient mice was slower than that of wild type mice. Thus, polysialic acid deficiency with NCAM expression *in vivo* resulted in deficient cell migration of neurons and glial cells. Secondly, neuronal projections without polysialic acid were immature *in vivo* and *in vitro*. Furthermore, the loss of polysialic acid enhances PDGF-directed differentiation of glial cells in neurosphere assays. Thirdly, many neural cells in double mutant mice underwent apoptosis, which was not found in NCAM null mice. These studies collectively show that polysialic acid plays critical roles in regulating cell migration, affecting neural cell differentiation and development. Supported by NIH grant CA33895.

(233) **Dietary Ganglioside and Neurochemistry in the Developing Rat**

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Background- Gangliosides are biologically important glycosphingolipids that are concentrated in the central nervous system. Gangliosides are involved in the growth and development of neuronal function, signaling, and the recovery of injured neuronal tissues. Recent experiments show that ganglioside content in neurological tissues is sensitive to dietary alterations. Human breast milk is a dietary source of ganglioside for the newborn infant. Infant formulas fed as acceptable alternatives to mother's milk to do not include significant amounts of ganglioside. The study objective is to determine if changing dietary ganglioside alters the lipid profiles of synaptosomal membrane and myelin fractions of developing rat brain.

Methods-Weanling rats were fed diets differing in ganglioside content, or a control diet with no ganglioside. After two weeks of feeding, synaptosomal membrane and myelin fractions were extracted from the whole brain tissue. The ganglioside, sphingolipid, phospholipid and cholesterol contents of these fractions were analyzed. Na-K ATPase activity was measured in fresh synaptosomal membrane as an indication of fraction purity, as well as neuronal function.

Results-Brain fractions contained the following gangliosides: GQ1b, GT1b, GT1a-GD1b, GD1a, GD3, GM1, GM2, GM3, GM4. Dietary treatment did not alter lipid profiles, with the exception of GQ1b in myelin and GM4 in synaptosomal membrane. There was a non-significant trend in the values for

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Na-K ATPase, showing increased activity when the diet contained long-chain polyunsaturated fatty acid in addition to ganglioside.

Conclusion-This study provides insight into the previously unstudied relationship between dietary ganglioside and the lipids of important fractions in the developing rat brain.

(234) **Neural Expression of β4GalNAcTA is Required for Normal Crawling Behavior in *Drosophila***

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A genetic approach to study Drosophila glycosyltransferases previously generated mutations in *Drosophila* homologs of the vertebrate β1,4-galactosyltransferase family. These *Drosophila* enzymes are β1,4-N-acetylgalactosaminyltransferases, transferring GalNAc to terminal GlcNAc in a β4 linkage and thus generating LacDiNAc. *Drosophila* mutant for β4GalNAcTA display a behavior/locomotion phenotype in adults, indicating a role for this enzyme in the neuromuscular system. Here we report that larvae mutant for β4GalNAcTA also display a locomotion phenotype demonstrating that the functional role for this enzyme is not restricted to adults. Mutant larvae are sluggish and display spontaneous backward crawling. In addition to this locomotion phenotype loss of β4GalNAcTA is associated with morphological and functional changes at the larval neuromuscular junction. These defects do not easily account for the crawling phenotype associated with loss of the enzyme. We go on to selectively express β4GalNAcTA using the Gal4-UAS system. Neural expression of the enzyme rescues the mutant crawling phenotypes but not the neuromuscular junction defects. This suggests that β4GalNAcTA and one or more of the LacDiNAc contain glycoconjugates it generates is required in the larval nervous system for normal crawling behavior and that the enzyme plays a separate role in fine-tuning the morphology and function of the neuromuscular junction.

(235) **Global Expression Analysis of Glycoconjugates in Rat Central Nervous System using Lectin Histochemistry**

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The interactions between glycans and their receptors (lectins) have proven to be vital for normal cellular functioning. The disruption of these interactions plays an important role in a range of disease processes, including neurological disorders. Global profiling of glycans and their corresponding lectins is therefore critical in understanding molecular mechanisms of neurological diseases.

Lectin histochemistry has proven to be an excellent tool to study expression of glycoconjugates in a variety of organs from healthy to diseased states. This technique has also been applied to study central nervous systems (CNS) of invertebrate and vertebrate animals.

In the present study we have used a variety of lectins and carbohydrate specific antibodies to characterize glycoconjugates expression and cell types in the CNS of adult rats. Preliminary results on the use of labeled glycans to map the location of the corresponding lectins will also be reported. This global expression analysis of glycoconjugates and lectins in CNS may lead to subsequent characterization of different regions and cell types of the brain and spinal cord.

(236) **The Expression and Function of *Drosophila* Sialyltransferase in the Central Nervous System**

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We study *Drosophila DSiaT* gene that encodes a functional α-2,6 sialyltransferase. DSiaT is so far the only characterized sialyltransferase in protostomes. DSiaT protein shows significant homology to the ST6Gal family of mammalian sialyltransferases. In our previous studies, we found that developmentally regulated expression of DSiaT is restricted to the central nervous system (CNS). Using several molecular markers, we characterized DSiaT-expressing cells. DSiaT expression was detected in many interneurons and motor neurons during larval stages, as well as in the optic lobe and central brain region of imago. The DSiaT-expressing cells in the central brain of adult flies were identified as the projection neurons that are known to convey information from olfactory neurons to the mushroom body and have been recently also implicated in memory formation. We also found that DSiaT mutants have significantly reduced life span and fertility. The analysis of DSiaT expression and the phenotype of *DSiaT* mutants suggest the involvement of DSiaT in neural transmission and development. To further corroborate this hypothesis and to reveal the genetic pathway in which *DSiaT* is involved, we are currently using genetic approach to assay interactions between *DSiaT* alleles and mutations in genes involved in different aspects of neural activity and development. We will discuss potential molecular mechanisms underlying the function of DSiaT in the nervous system of *Drosophila.* This work was supported in part by the NIH grant GM069952 to V.P.

(237) **A Structural Role for O-GalNAc Protein Glycosylation in alpha-Dystroglycan**

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The glycoprotein alpha-dystroglycan is an important component of the extracellular matrix in muscle and other tissues. There has been particular interest in the mannose O-linked glycosylation because of pathologies related to errors in assembly of the associated tetrasaccharide. These glycans are found in the central region of the glycoprotein where there is a mucin-like amino acid sequence. Electron microscopy has indicated that this region is extended and not globular. Analysis indicates that the majority of glycosylated S or T residues in this region bear the mannose linked oligosaccharides, but there are a significant number with glycans originating in GalNAc as well. Earlier studies showed that conventional extended mucin structures arise as a consequence of the stereochemistry of the glycosidic linkage facilitating interactions of functional groups on GalNAc with the peptide backbone. With mannose, the relevant N-acetyl group is not present, suggesting that this sugar could not induce the extended structure. NMR studies on several synthesized glycopeptides from the alpha-dystroglycan central region incorporating mannosylated S and T residues indicated that introduction of mannose had only a modest conformational effect. In contrast, when GalNAc was substituted for mannose, a significant conformational effect was seen. We hypothesize that it is the presence of GalNAc based glycans which induce the extended conformation that aid in displaying the mannose tetrasaccharides. To better understand the post-translational modification of this protein, the ability of polypeptide GalNAc transferase isofirms to incorporate GalNAc residues in proximity to O-mannosylated residues in glycopeptides mannosylated at selected sites is being studied.

(238) **Lewis X glycan Decreases Neural Progenitor Proliferation by Preventing FGF-2 Binding**

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It is well established that neurogenesis (generation of functionally integrated neurons from undifferentiated, multipotent cells) continues in discrete regions of the adult brain. Reliable markers are needed to identify, isolate and study these cells and to develop novel treatments for neural diseases. The brain is a highly specialized tissue where complexities of cellular architecture and connectivity suggest important roles for glycans, which cover all cellular surfaces. Here, we report generation of a new antibody (3A8), which recognizes progenitor/stem cells of the hippocampus. This antibody demonstrates high specificity for the alpha-1-3-linked fucose moiety of Lewis X (LeX) on an N-glycan-bearing protein. Interestingly, LeX is expressed by a subset of progenitors, which have the potential to become neurons, and expression is abolished upon differentiation. Using the 3A8 antibody to sort adult rat hippocampal progenitors (AHP), we show that the LeX+ population initially proliferates more rapidly than the LeX- cells. Likewise, incubating cells with the 3A8 antibody increases AHP proliferation. Further analyses revealed that 3A8 masks an endogenous inhibitory effect of LeX on proliferation. Indeed, multiple approaches show that removal of this masking effect actually decreased AHP proliferation. Finally, we show a direct interaction between LeX and FGF-2 and propose that the LeX bearing N-glycans on progenitor cells prevent FGF-2 binding to its receptor, possibly by occupying the heparin sulphate proteoglycan binding site of FGF-2. Further studies are needed to evaluate if LeX mediated loss of FGF-2 signaling in AHPs causes differentiation into neurons in vivo, thus enhancing hippocampal neurogenesis-dependent processes.

(239) **Glycosidase Activity and Lysotracker Staining Pattern in Breast Cancer Cell Lines Compared with a Normal Breast Cell Line**

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Dramatic alterations in glycosylation have been reported in breast and other solid tumours. Despite some understanding of the glycosylation changes in cancer the role of glycosidase enzymes remains an under-researched area of glycobiology.

We assayed ten glycosidases in five breast cell lines using PNP-sugars. In our model, four glycosidase enzymes: alpha fucosidase, beta mannosidase, beta-N-acetylgalactosaminidase and beta-N-acetylglucosaminidase showed increased activity in the breast cancer cells: BT 474, MCF 7, MDA MB 435, ZR 75-1 as compared to the normal breast cell lysate, HB4a ($p<0.05$).

To investigate whether the increased activity of these enzymes correlated with the intracellular location and size of lysosomes we used RND 99 lysotracker dye (Molecular Probes) and confocal microscopy to study the lysosomal size and distribution in HB4a and MCF 7 cells. The results showed an increase in the number of lysosomes in MCF 7 compared to HB4a and this correlated with the glycosidase activity. After 48 hours of serum starvation, a further increase in lysosome number was observed in the HB4a cells compared with the MCF 7 cell line. A study of the media indicated secretion of glycosidases into the media but there was no correlation between the enzyme activity in the media and number or size of the lysosomes. These data suggests that the breast cells may use an alternative route to secrete their glycosidase into the media.

(240) **Carboxylated N-Glycans in Inflammation-Mediated Colon Cancer**

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Patients with inflammatory bowel diseases (IBD) are at increased risk for developing colorectal cancer (CRC) and several lines of evidence point to chronic inflammation of the colon as an important predisposing factor to CRC in IBD. However, the molecular basis of the association between the two disease entities remains poorly understood. We previously showed that carboxylated N-glycans mediate colitis by activation of NF-kB. We therefore hypothesized that carboxylated N-glycans expressed on cell surface receptors on macrophages and tumor epithelial cells exert tumor-promoting functions in the setting of inflammation through sustained activation of NF-kB in one or both cell types. To test this, we examined the effects of the anti-glycan antibody mAbGB3.1 in a mouse model of colon carcinogenesis. We induced colitis associated cancer in CD-1 mice using a single low dose of azoxymethane (AOM) followed by a single week of administration with 2% DSS in drinking water. A control group of mice did not receive AOM or DSS. We treated separate groups of AOM/DSS mice with iv injections of mAbGB3.1 or isotype control antibody at 5mg/gm body weight. Antibodies were administered at the beginning and every week until the end of the experiment. We found that administration of mAbGB3.1 reduced DSS induced inflammation and the incidence of dysplasia by about 50% at 6 weeks and incidence of dysplasia by about 75% at 12 weeks after AOM/DSS. These findings strongly suggest that carboxylated glycans play an important role in chronic colonic inflammation and inflammation-mediated progression to cancer.

(241) **Enzymatic Large-Scale Synthesis of MUC6-Tn Glycoproteins for Anti-Tumor Vaccination**

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Mucins are aberrantly O-glycosylated in cancer, and consequently, they express tumor-associated antigens such as the Tn determinant (alpha-GalNAc-O-Ser/Thr). Some of them exhibit a different pattern of expression as compared to normal tissues. In particular, MUC6, which is normally expressed only in gastric tissues, has been detected in intestinal, pulmonary, colorectal and breast carcinomas. Recently, we have shown that the MCF7 breast cancer cell line expresses MUC6-Tn glycoproteins in vivo. Cancer-associated mucins show antigenic differences from normal mucins and, as such, they may be used as potential targets for immunotherapy.

In order to develop anti-cancer vaccines based on both MUC6 and the Tn antigen, we prepared several MUC6-Tn glycoconjugates. To this end, we performed the GalNAc enzymatic transfer to recombinant MUC6 proteins by using UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltransferases (ppGalNAc-Ts), which catalyze in vivo the Tn antigen synthesis. We used either a mixture of ppGalNAc-Ts from MCF7 breast cancer cell extracts or recombinant ppGalNAc-Ts.

These glycoproteins displayed a high level of Tn antigen, although the overall density depends on both enzyme source and protein acceptor. These MUC6-Tn glycoconjugates were recognized by anti-Tn monoclonal antibodies, specific for human cancer cells.

However, not all the obtained glycoproteins were capable of eliciting antibodies that recognize human tumor cells, suggesting that particular Tn density and/or position is essential to mimic Tn expressing mucins produced by cancer cells.

In conclusion, the production in large amounts of MUC6 with tumor-relevant glycoforms holds considerable promise for developing effective anti-cancer vaccines.

(242) **A Glycomic Approach to Drug Resistance: Direct Interaction between Cisplatin and *N*-Glycans**

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Cisplatin, *cis*-diamineplatinum-(II) dichloride (CDDP), is a critical chemotherapeutic agent against cancer. In many cases, however, tumors gain acquired or intrinsic resistance to treatment. Although widely investigated, mechanisms underlying CDDP resistance are unclear. A CDDP-resistant line (KCP-4) was isolated from KB-3-1 human carcinoma cells, and a revertant line (KCP-4R) was made from KCP-4 cells. Here, we compared neutral *N*-glycans from parental, resistant and revertant cells using pyridylamination and high performance liquid chromatography. We analyzed detailed structures and ratios of 16 *N*-glycans and show that high-mannose type oligosaccharides are increased and lactosamine type oligosaccharides are decreased in resistant cells and that revertant cells show the same profile as parental cells. CDDP interaction with oligosaccharides was analyzed using cold spray ionization-mass spectrometry. That analysis showed that high-mannose type oligosaccharides bind CDDP with greater affinity than do lactosamine type oligosaccharides. These results suggest that CDDP tolerance is correlated with specific *N*-glycan profiles and that CDDP interaction with high-mannose oligosaccharides may underlie that tolerance.

This work was supported by the National Project on Functional Glycoconjugates Research for New Industry from the Ministry of Education, Science, Sports, and Culture of Japan.

(243) **Mgat5 Specific ShRNA Suppress the Growth of Mammary Adenocarcinoma Cells in vivo and Stimulating Th1 Cells Activation**

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Golgi β1, 6N-acetylglucosaminyltransferase V (Mgat5) is required in the biosynthesis of β1, 6GlcNAc-branched N-linked glycans attached to cell surface and secreted glycoproteins. Amounts of Mgat5 glycan products are commonly increased in malignancies, and correlate with disease progression. In this study, a Mgat5 specific-shRNA eukaryotic expression vector which can efficiently downregulate the level of mouse Mgat5 was constructed and selected by RT-PCR and FITC-L-PHA labeling flow cytermetry analysis. The mgat5 specific-shRNA and control shRNA were transfeted into mammary adenocarcinoma cells MA 782 and then planted into 8-weeks BalB/C mice. We found that mgat5-specific shRNA could suppress mammary adenocarcinoma tumor cells growth in vivo. And Th1 cells and macrophages were activated in Mgat5-shRNA knockdown mice. The levels of TNF-b were significantly increased in Mgat5-shRNA knockdown mice, and the level of IFN-g were also enhanced in CD4T cells, but the level of IL-4 was not changed significantly. RT-PCR showed that the expression of transcription factor T-bet of Th1 cytokine was increased as well. We propose that Mgat5 modified N-glycans on tumor surface may regulate Th1 cell activation.

(244) **Antitumor Activity of a Novel Lectin from the Alga Dasa villosa**

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A novel lectin was purified from alga Dasa villosa (DVL) by affinity chromatography on bovine thyroglobulin-Sepharose 4B followed by gel fitration on Sephadex G-200. The lectin exhibited a native molecular mass of 571 kDa by gel fitration on Sepharose CL-4B. Hemagglutination of rabbit erythrocytes by the purified lectin was best inhibited by bovine thyroglobulin. The hemagglutinating activity of DVL was independent of the divalent cations Ca2+ and Mg2+. The lectin isn't sensitive to temperature. Heating of the lectin solution at 100 oC for 30 min, their activity remains 50 %. Antitumor effect of

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DVL on locally implanted ascitic-type hepatic carcinoma H22 cells in BALB/c nude mice in vivo. DVL 65, 130, 260 mg/kg inhibited xenograft with inhibitory rate of 21 %, 27 % and 40 %, respectively. At the same time, average weights of the spleens (0.1 mg per gram body weight) were significantly greater than those of positive control groups, indicating that the lectin could increase the weight of immune organ.

(245) ***N*-Glycans Correlate 5-FU Resistance in Colon 26 and Derived Cell Lines**

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N-Linked oligosaccharides play diverse roles in living organisms. In cancer cells, glycans function in metastasis and cancer progression, but little is known about the relationship between chemo-resistance and glycoform transformation. We investigated the role of *N*-linked oligosaccharides in chemo-resistance. To do so, we used the well-known anti-cancer drug 5-FU to make a chemo-resistant cell line from colon 26 cells, a murine colorectal cancer cell line. First, we analyzed various glycoforms seen in 5-FU-resistant cell lines by high performance liquid chromatography using an ODS column and precisely compared those forms with those seen in parental cells. This analysis suggested a correlation between specific glycoforms and chemo-sensitivity. Next, we used swainsonine, an inhibitor of *N*-linked oligosaccharide processing, to alter glycans in resistant cell lines to investigate their potential roles in chemo-sensitivity. Swainsonine treatment of cultured cells dramatically altered cellular glycoforms. The same treatment also significantly reduced 5-FU-tolerance of resistant cells, although 5-FU sensitivity of the parental cell line was not affected. These observations strongly indicate that alterations in *N*-linked oligosaccharides affect 5-FU resistance of cancer cells.

(246) **A Novel Drug Delivery System. Carbohydrate Recognition-Based and Controlled Release System using Intraperitoneal Macrophages as a Cellular Vehicle**

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Gastric cancer killed 49,958 people in Japan in 2001. Almost all the patients died due to the progression in the peritoneal cavity. The lymphoid tissue in the omentum, at the so-called milky spots, is known as an initial place for disseminated cancer cells to develop into solid tumours. In the present study, we developed a new drug delivery method so as to control tumour development at milky spots. Intraperitoneal macrophages significantly took up oligomannose-coated-liposomes (OML) that were injected into the peritoneal cavity, and then gradually accumulated in the omentum and the other lymphoid tissues within 24 h of intraperitoneal injection of OMLs. When 5-FU was encapsulated in the OMLs, more than 60% of administered 5-FU accumulated in the omentum. Treatment of macrophages at 39°C for 30 min led to the release of 5-FU from the macrophages, suggesting that controlled release from macrophages could be achieved by mild hyperthermia. We encased magnetic nanoparticles, which are known to convert electromagnetic energy to heat, in the OMLs to achieve in vivo hyperthermia at the site. Using this system in a mouse intraperitoneal metastasis model, we successfully controlled tumour development by co-administration of OML-encased 5-FU and OML-encased magnetic nanoparticles, followed by treatment with an alternating magnetic field. No apparent reduction was seen in tumour growth with the administration of OML-encased magnetic nanoparticles or OML-encased 5-FU alone. Thus, we have established the use of intraperitoneal macrophages as a novel drug-delivery system for the control of cancer metastatic to milky spots. (NEDO:04A01548a)

(247) **Alterations in *N*-Glycans Seen in Drug-Resistant Human Hepatocellular Carcinoma**

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Correlations of disease phenotypes with alterations in glycosylation have been intensively evaluated in the field of tumor biology. Glycoforms associated with carcinogenesis, tumor progression and metastasis have been identified. Although multiple mechanisms mediate resistance of cancer cells to anticancer drugs, including overexpression of transporters, the relationship between anticancer drug resistance and glycosylation requires further analysis. We established epirubicin (EPI)- and mitoxantrone (MIT)-resistant cell lines (HLE-EPI and HLE-MIT, respectively) from a human hepatocellular carcinoma cell line (HLE). HLE-EPI cells overexpressed the multidrug resistance protein 1 (MDR1)/ABCB1, and HLE-MIT overexpressed the breast cancer resistance protein (BCRP)/ABCG2. We then compared the glycomics of these resistant cells to the parental cell line and observed that the core-fucosylated triantennary oligosaccharide (310.8) Galβ4GlcNAcβ2(Galβ4GlcNAcβ4)Manα3(Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAc was increased in resistant cells. Expression of glycosyltransferases catalyzing synthesis of this oligosaccharide was examined by RT-PCR in HLE, HLE-EPI and HLE-MIT cell lines. In HLE-MIT cells, expression of *N*-acetylglucosaminyltransferases GnT-IVa and GnT-IVb was decreased compared with HLE and HLE-EPI cells. In HLE-EPI cells, GnT-IVa expression was decreased and GnT-IVb expression was increased compared to that seen in the other two cell lines. By contrast, α1,6-fucosyltransferase was highly expressed in HLE-MIT cells compared to the other two lines.We revealed that glycosyltransferase expression and *N*-glycan profiles in cancer cells are altered by giving chemotherapic agents for long term and such phenomena are suggested to correlate with the acquisition of drug resistance for hepatocellular carcinoma.

(248) **Identification of Novel Carbohydrate Binding Receptor on the Lung Endothelial Cell Surface Responsible for Carbohydrate Dependent Cancer Metastasis**

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Many studies have shown that carbohydrates on cancer cell surface are related to poor clinical outcome including metastasis, suggesting a mechanism for carbohydrate-dependent cancer metastasis. Previously, we found that intravenously injected sialyl Lewis X (sLeX)-expressing B16 cells colonize the lung in mutant mice deficient in both E- and P-selectins (Zhang, J. et al., Cancer Research 62: 4194-4198, 2002). This suggested the existence of novel carbohydrate binding endothelial receptor(s) distinct from selectins. Since a selectin ligand mimicry I-peptide inhibited this colonization, a putative receptor was designated as I-peptide receptor (IPR). In this study, we identified by proteomics the major and minor IPRs as pre-mRNA splicing factor and annexin 1, respectively. In vitro assays including Glycoarray demonstrated these proteins produced in bacteria bind to N-acetyl-lactosamines with or without fucose or sialic acid. When a mouse was intravenously injected with liposomes composed of I-peptide and an apoptosis inducing ganglioside GD3, some lung endothelial cells underwent apoptosis, and the lung colonization of sLeX-B16 cells did not occur in I-peptide/GD3 treated mice. Since annexin 1 has been identified as specific endothelial marker of tumor vasculature, we tested if I-peptide can be used as a targeting vehicle against the tumor vasculatures. We found that intravenously injected I-peptide/GD3 liposomes containing anticancer drug, doxorubicin, suppress tumor growth in the mouse. These results suggest significant potential of I-peptide in therapies against cancer.

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.

(249) **GnT-V Expression Correlates with Patient Survival in Bladder Cancer**

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Purpose: *N*-Acetylglucosaminyltransferase V (GnT-V) is an enzyme that catalyzes beta1-6 branching of *N*-acetylglucosamine on asparagine (*N*) -linked oligosaccharides (*N*-glycans) of proteins. We examined the relationship between GnT-V expression and the clinicopathologic features of patients with bladder cancer.

Material and Methods: We examined GnT-V expression by immunohistochemistry of paraffin-embedded bladder cancer specimens using

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an anti-GnT-V monoclonal antibody. We compared GnT-V expression with cause-specific survival as indicated by Kaplan-Meier survival curves of bladder cancer patients treated by radical cystectomy. Univariate and multivariate analyses were carried out to compare GnT-V expression with other clinical and pathologic variables. We also evaluated GnT-V mRNA expression and N-linked oligosaccharide structures in bladder cancer specimens.

RESULTS: Immunohistochemistry revealed that GnT-V expression is inversely correlated with tumor grade and stage. The incidence of positive GnT-V expression in bladder cancer was significantly higher in low-grade/superficial cancer than in high-grade/invasive cancer. Patients whose tumors were GnT-V positive survived significantly longer than those whose tumors were GnT-V negative. Univariate and multivariate analyses revealed that GnT-V expression was an independent predictor of a patient's prognosis. Expression of GnT-V mRNA determined by reverse transcription-PCR was consistent with immunohistochemistry results of tumor samples. Carbohydrate structural analysis revealed that superficial bladder cancer is rich in branched N-linked oligosaccharides, whose biosynthesis requires GnT-V.

CONCLUSIONS: Expression of GnT-V and resultant beta1-6 branching *N* -linked oligosaccharides is significantly with a low malignant potential and a favorable prognosis for bladder cancer patients.

(250) **Carbohydrate Structure of Prostate-Specific Antigen and Its Distinct Affinity to *Maackia amurensis* Lectin between Cancer and Non-Cancer Source**

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Purpose: Prostate-specific antigen (PSA) is a glycoprotein which has one N-glycosylation site. To elucidate cancer-associated carbohydrate alterations of PSA, we first analyzed the carbohydrate structure of *N*-glycans expressed on PSA from human seminal fluid. We then tried to identify a lectin to demonstrate cancer-associated carbohydrate alteration on PSA.

Materials and Methods: *N*-glycans from human seminal fluid PSA was pyridylaminated and analyzed by high performance liquid chromatography (HPLC). *Lens culinaris* (LcH), *Aleuria aurantia* (AAL), *Sambucus nigra* (SNA), and *Maackia amurensis* (MAA) lectins were tested for their binding affinity to the carbohydrates on PSA. To detect cancer-associated carbohydrate alterations on PSA, lectin affinity column chromatography was carried out. Seminal fluid and benign prostate hypertrophy (BPH) tissue were the source for non-cancer PSA, LNCaP culture supernatant with serum free medium was for cancer-associated PSA.

Results: The predominant core structure of *N*-glycan of seminal fluid PSA was a complex type biantennary oligosaccharide and was consistent with the structure reported previously. However, we found sialic acid alpha2-3 galactose linkage as a terminal carbohydrate structure on seminal fluid PSA. Among the lectins examined, MAA-bound fraction of free PSA showed the most significant difference between non-cancer source and LNCaP supernatant. Plasmon resonance (SPR) analysis also supported the distinct binding affinity of PSA to MAA lectin.

Conclusions: We demonstrated the distinct binding affinity of PSA to MAA lectin between cancer and non-cancer source.

(251) **Increased α1,6-Fucosylation of *N*-Glycan in Serum Glycoprotein of *db/db* Mice**

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The *N*-glycan profile of serum glycoproteins is known to change in various pathological states. However, *N*-glycans have been little investigated in diabetes mellitus. To examine potential alteration of serum *N*-glycans in diabetes, we compared the *db/db* mouse diabetic model with *db*/+ controls. For a comprehensive analysis, serum *N*-glycans were fluorescence-labeled and analyzed by high-performance liquid chromatography. Reproducible differences in *N*-glycan profiles were seen between *db/db* and *db*/+ mice. Oligosaccharide structures, whose levels differed in both mice, were analyzed further using a two-dimensional mapping method and matrix-assisted laser desorption ionization-time of flight mass spectrometry, combined with exoglycosidase digestion. Those analyses revealed an increase in *N*-glycans possessing core α1,6-fucose in the serum of *db/db* mice compared to *db*/+ controls. In *db/db* mice, levels of α1,6-fucosyltransferase (FUT8) mRNA were also increased in liver but not in epididymal adipose tissues or kidney. The observed marked change in serum *N*-glycans seen in *db/db* mice may be due in part to increases in liver FUT8 mRNA levels. These changes in glycosylation may affect protein activities and be associated with the pathophysiology of type 2 diabetes accompanied by obesity.

(252) **Construction of MUC1 Related Compound Library**

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Dynamic structural alteration of O-glycan chains of MUC1 has been implicated in a variety of cancer. In breast, ovary, and other carcinomas, it has been known that the MUC1 mucin is aberrantly glycosylated in comparison with mucin from corresponding normal tissues. Although the structures of oligosaccharide moiety as well as peptide chains at the MUC1 is interesting in terms of cancer related epitopes, the functional role of the oligosaccharide structure in the MUC1 has not been revealed yet due to the extremely complicated structures and myriad glycosylation patterns. Taking this into consideration, synthetic compound library of the MUC1-related glycopeptides having various mucin core structures will become key in the fulfillment of their essential biological roles. In addition, it seems likely that the synthetic MUC1 glycopeptides will greatly accelerate the systematic analysis of epitopes recognized by various antibodies as well as discovery research of diagnostic tools and cancer vaccines. We communicate herein the rapid and combinatorial synthesis of MUC1 glycopeptide derivatives by the tandem synthetic process of microwave-assisted solid-phase chemical syntheses and solution phase enzymatic syntheses using a molecular shuttle which acts as a suspension bridge between two different polymer platforms.1,2 Compound library constructed in this study allowed for investigating essential structures required for MUC1 functions as tumor-associated glycoproteins.

1) Fumoto et al. J. Am. Chem. Soc. 127, 11804-11818 (2005).

2) Matsushita et al. J. Org. Chem. 71, 3051-3063 (2006).

(253) **Glycomic Mapping and Identification of Sialyl Le^x and Sialyl Le^a on Mucins from Human Ovarian Cyst Fluid**

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Expression of sialyl Lewis x (NeuAcalpha2→3Galβ1→4[Fucalpha1→3]GlcNAc, sLe^x) and sialyl Lewis a (NeuAcalpha2→3Galβ1→3[Fucalpha1→4]GlcNAc, sLe^a) on cell-surface glycoproteins endows cells with the ability to adhere to E-, P- and L-selectins present on endothelia, platelets or leukocytes. In this report, O-glycans of secreted neoplasm-associated sialoglycoproteins with sLe^x and sLe^a epitopes from human ovarian cyst fluid (HOC 350) were characterized by tandem mass spectrometry (MS) analyses and immuno-/lectin-chemical assays. The results showed that HOC 350 carries a large number of bioactive epitopes for sLe^x, sLe^a and Le^a active antibodies, whereas the desialylated product bound well to many Galβ1→3/4GlcNAc (I/II), Galβ1→3GalNAc (T) and GalNAcalpha1→Ser/Thr (Tn) specific lectins. Advanced MS/MS sequencing data revealed that the O-glycans from HOC350 are mostly of type 2 core structures, extended on both arms and branched with both type I and type II LacNAc chains, with variable degrees of terminal sialylation and/or fucosylation to give the sLe^x or sLe^a epitopes. Other distinguishing structural features include i) a further branching on the 3-arm chain, at -3,6Galβ1-3GalNAc, which is itself mostly extended with a type 1 unit, as noted before; ii) exposure of incompletely synthesized core structures, Tn and sialyl Tn; and iii) a significant degree of sulfation not previously appreciated. This study provides evidence that secreted and water soluble glycoproteins in neoplasm formation may express, among others, the selectin carbohydrate ligand, sialyl Lewis x, besides the well-known sLe^a as a tumor marker, in high valency.

(254) **Identification of *N*-Glycans Related with Cartilage Deterioration**

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Osteoarthritis (OA) is the most common of all joint diseases, but the molecular basis of its onset and progression is controversial. Several studies have shown that modifications of *N*-glycans on proteins contribute to the pathogenesis. However, little attention has been given to those of articular cartilage. In this study, we identified disease specific *N*-glycan expression profiles seen in degenerated cartilage induced by anterior cruciate ligament transection (ACLT) in a rabbit OA model.

Methods. Cartilage was harvested at 7, 10, 14 and 28 days after ACLT and assessed for cartilage degeneration and alteration in *N*-glycans isolated from glycoproteins. In sham control rabbits, arthrotomy without ACLT was performed. *N*-Glycans from cartilage were analyzed using high performance liquid chromatography and mass spectrometry to detect potential differences.

Results. Histological analysis showed significant changes in cartilage in all cases from 10 to 28 days after ACLT. We observed that in normal cartilage *N*-glycans existed in almost 30 different forms and there were apparent alterations in peak patterns from 7 to 28 days after ACLT. Such changes were apparent prior to changes in tissue morphology.

Conclusion. These observations indicate that alterations in *N*-glycans accompany the pathogenesis of cartilage degeneration. Understanding mechanisms underlying changes in glycans seen in patients with OA may be of therapeutic value in treating cartilage injury.

(255) **A Combined Proteomic and Metabolomic Investigation of Glioblastoma Multiforme Cell Lines Treated with Wild-Type p53 and Cytotoxic Chemotherapy**

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Profiling was performed of proteins and sphingolipids in glioma cell lines with mutant p53 (apoptotic phenotype), compared to cells with wild-type p53 (apoptosis-resistant phenotype) and wild-type p53 cells treated with cytotoxic chemotherapy (apoptotic). In each investigation, approximately one million cells were analyzed. The proteomic investigation was performed by two-dimensional electrophoresis, image analysis and mass spectrometry of tryptic digests of the separated proteins, followed by database searches. Through the proteomic approach, we identified several proteins that were up- or down-regulated in different cellular states. Of these proteins, the level of galectin-1 expression was determined to mirror accurately the degree of apoptosis observed in the cell lines. Galectin-1 is a carbohydrate-binding protein with affinity for beta-galactoside, a carbohydrate epitope that may be found on glycoproteins or glycolipids. We studied changes in the membrane lipid profiles of the cell lines by liquid chromatography and tandem high resolution mass spectrometry. The resulting sphingolipid profiles, taken together with proteomic data, provide a more complete understanding of the underlying biochemical mechanisms than can be gained by analysis of either the proteomic or metabolomic data alone.

This work was supported by NSF DMR 0084173, NHMFL, and Florida State University.

(256) **The Molecular Basis for Recognition of Metastatic Colorectal Cancer by the Lectin HPA**

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The lectin HPA from the Roman snail Helix pomatia binds to solid tumours and is closely associated with lymphatic metastases, poor patient prognosis and shortened disease free interval. Although there has been considerable interest in identifying the HPA binding partners in cancer, the glycoproteins recognised by HPA have remained elusive.

We used two human colorectal cancer cell lines SW480 (faint binding, non-metastatic) and HT29 (intense binding, metastatic) as a model to identify HPA binding glycoproteins.

The cell membrane-associated HPA binding glycoproteins identified in this study included integrin alpha 6 and alpha v subunits, annexins 2 and 4. These proteins were found complexed with actin, tubulins, cytokeratins and heat shock proteins 70/90. The interaction between the HPA-binding proteins was inhibited using GlcNAc, GalNAc and / or sialic acid (SA) at a concentration of 50mM – 250mM.

Serendipitously we found that commercial preparations of HPA contain two GalNAc-binding lectins and a further protein with sequence similarity to the SA binding lectin of the Garden snail Cepea hortensis. Sequence analysis of this protein suggests it adopts a C1q fold. Purified recombinant SA-like lectin showed similar binding characteristics on Western blots of HT29 cell membrane proteins as native HPA.

We conclude that the utility of HPA binding to metastatic cancer is via at least a dozen proteins involved in cell migration and signalling. A hitherto unknown protein in the commercial HPA preparations, designated SA-like lectin, was found to contribute to HPA binding in the metastatic cancer cell line HT29.

(257) **Monitoring Differential Expression of Sialyted Glycoproteins in HeLa Cells using the Staudinger Ligation**

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Sialic acid presented on cell surface glycoproteins is known to mediate a variety of cellular interactions during cell development, differentiation and tumor progression. Studies have indicated that enhanced sialyltransferase (ST) activity resulting in hypersialylation is implicated in up-regulation of metastatic potential. Understanding the details of sialoside-mediated biological events is imperative to any drug development pertaining to ST activity. Therefore, a tool to screen sialic acid expression on the cell surface would be extremely useful to monitor these metabolic functions.

Numerous labs have used cell-surface engineering employing bioorthogonal functional groups to study various cellular phenomena. In this study azide-modified mannose, a precursor to sialic acid, was incorporated into the sialic acid biosynthesis pathway. Once incorporated, the differential expression of sialic acid on cell surface glycoproteins was monitored using a Staudinger ligation. The Staudinger ligation is a reaction between azides and a specific phosphine derivative to yield an amide bond. In this regard, a FLAG-phosphine was employed because the FLAG epitope provides a versatile module for various immunochemical techniques. Thus the FLAG-phosphine acts as a probe for capture of the azide-modified glycoproteins. Utilizing this technique, two different cell populations of HeLa were studied, one in which the sialic acid population was down regulated and another cell population where the sialic acid pathway was not perturbed. The differential presentation of sialic acid was analyzed using immunoprecipitation and subsequent western blotting experiments.

(258) **Increased Frequency of Incomplete L-selectin Ligands, Non-sulfated Sialyl Lewis X, on HEV-like Vessels in Gastric Mucosa-associated Lymphoid Tissue Lymphoma**

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The majority of primary gastric lymphomas is mucosa-associated lymphoid tissue (MALT) lymphoma. 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 regulated 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 indicate that HEV-like vessels in gastric MALT lymphoma express incomplete (non-sulfated) L-selectin ligands probably due to impaired expression of sulfotransferases, namely GlcNAc-6ST-2.

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(259) **Phyllodes Tumors of the Breast: A Heparan Sulfate Perspective**

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Heparan sulfate, a polyanionic glycosaminoglycan made up of repeating disaccharide subunits of glucuronic/iduronic acid and glucosamine, is a biomarker of infiltrating ductal carcinoma of the breast. To determine if it plays a role in other breast neoplasms, we examined its expression in phyllodes tumors using immunohistochemistry. Phyllodes tumors of the breast, also known as cystosarcoma phyllodes, are characterized by stromal hypercellularity and elongated mammary ducts with leaf-like patterns, and have a higher incidence in women of Asian origin. The expression pattern of heparan sulfate in these tumors was examined by immunohistochemical staining of archival specimens on tissue microarray slides using HepSS-1, a monoclonal anti-heparan sulfate antibody. The staining intensities of both ductal and stromal components were analyzed and compared against clinicopathological parameters. Strong stromal expression of heparan sulfate was found to be significantly associated with malignant tumors. In addition, HEPSS-1 expression correlated with the presence of hypercellularity and cytologic atypia in the stroma. Taken together, these results suggest that heparan sulfate is a useful biomarker of phyllodes tumors, and may be involved in regulating growth of malignant tumors.

(260) **Investigation of the E-cadherin Glycoprotein by Mass Spectrometry**

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Introduction

E-cadherin is a 120-kDa membrane glycoprotein expressed in epithelial cells; it is the main player in establishing adherens junctions between cells. Alterations in the assembly or disassembly of adherens junctions occur in association with major changes in the state of the cells, including differentiation and proliferation, as well as in cancer progression. Recent studies have indicated that the *N*-glycosylation pattern of E-cadherin has a role in the molecular organization of adherens junctions. Specifically, the presence of complex *N*-glycans is associated with destabilized adherens junctions.

Method

E-cadherin from dense cultures of non-malignant and malignant cells is purified using ion exchange chromatography with Q Sepharose and affinity capture with antibodies specific to E-cadherin. Protein

and oligosaccharide analysis is accomplished utilizing a Bruker Reflex IV mass spectrometer for MALDI-TOF MS and an Applied Biosystems Sciex Pulsar *i* QoTOF mass spectrometer (QStar) coupled to a Waters CapLC for LCMS-MS/MS.

Results

The forms of E-cadherin in malignant cells are more highly glycosylated than are those in normal cells. MALDI-TOF MS analysis of in-gel enzymatically released *N*-glycans suggests E-cadherin contains predominantly complex *N*-glycans. Definition of the *N*-glycoform structures is a part of continued work. This research highlights that cell context, the recruitment of protein binding partners, and specific structural modifications to E-cadherin may define the overall stability of E-cadherin mediated adhesion.

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(261) **MUC2 Mucin *O*-glycosylation Patterns in Sigmoid Colon of Patients with Ulcerative Colitis**

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Novel proteomic and glycoproteomic methods were used to study sigmoid colon biopsies from ulcerative colitis (active and inactive) and control patients, a total of 50 patients. The guanidium chloride insoluble mucins from two biopsies were extracted, the disulfide bonds reduced/alkylated and the mucins separated by SDS-polyacrylamide/agarose composite gel electrophoresis. Several mucin bands were visualized by staining for protein or negatively charged oligosaccharides. The three major bands were trypsin digested and identified as MUC2 by nanoHPLC-FT-ICR MS^n representing different oligomeric forms of MUC2. The monomeric form of MUC2 was semiquantified in 46 patients as Sypro Ruby stained protein bands. The individual differences in MUC2 amounts were large (5-10-fold differences) in the three patient groups.

The *O*-glycosylation of colonic MUC2 was studied after transfer of protein to PVDF membranes and release of oligosaccharides by reductive β-elimination from the monomeric MUC2 band, followed by nanoLC/MS^n in negative ion mode, using graphitized carbon (Hypercarb) as separating medium. A very high sensitivity has been reached when using columns with 100 μm i.d. and an in-house developed nanoLC/MS interface. More than 50 *O*-linked oligosaccharides were identified, many with a high degree of sialylation. Some of the elucidated glycan structures, are to our knowledge, not described previously. The glycans present in more than 10 patients (26 glycans) were further semiquantified. A subpopulation of patients showed an accumulation of the precursor glycans with a decrease of more complex glycans.This glycan pattern was more common among the active ulcerative colitis patients.

(262) **Carbohydrate-short Chain Fatty Acid (SCFA) Hybrids as Anti-cancer Prodrugs: The Sugar Matters**

Srinivasa-Gopalan Sampathkumar[1]; Christopher T. Campbell[1]; M. Adam Meledeo[1]; Sean S. Choi[1]; Mark B. Jones[1]; Tony Sheh[1]; Mathew David[1]; Kiren Khanduja[1]; Jie Fu[1]; Tim Gilmartin[2]; Steven R. Head[2]; Justin Hanes[1]; Kevin J. Yarema[1]

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Butyrate, a natural SCFA, remodels chromatin by acting as a histone deacetylase inhibitor (HDACi), increases $p21^{WAF1/Cip1}$ expression, and thereby reactivates defective cell cycle checkpoints found in cancer. To overcome its poor pharmacological properties, a necessity for clinical translation, butyrate has been ester-linked to various scaffolds including carbohydrates. So far, these prodrugs have utilized innocuous carbohydrate carriers, missing an opportunity to co-deliver sugars actively involved in key glycosylation pathways and thus address a second hallmark of cancer, the display of aberrant glyco-epitopes. We have developed a novel compound, But_4ManNAc (**1**) that delivers both butyrate and ManNAc, the metabolic precursor for sialic acid biosynthesis. Western analysis, reporter-gene assays, and cell cycle studies show that **1** exhibits characteristic SCFA activity consistent with butyrate and periodate-resorcinol assays demonstrate a dramatic increase in sialic acid consistent with ManNAc. In *in vitro* tests, **1** ensured 'arrest & execution' of cancer cells and reduced their metastatic potential. By contrast, But_4GlcNAc (**2**), But_5Man (**3**) and tributyrin (**4**) – compounds that use other sugars as delivery vehicles for butyrate – provided transient cell cycle arrest but not toxicity or inhibition of metastasis. Microarray analysis of breast cancer cells treated with **1** and **3** using GLYCOv3 revealed sugar-dependent effects on gene expression. For example, a key metastatic tumor marker MUC1 was down regulated by **1**, but was up regulated by **3**, a finding consistent with effects on cell invasivity. In continuing work, the efficiency of **1** is being optimized for in vivo delivery by microparticle encapsulation and enclosure in polymer-wafers.

(263) **Roles of Carbohydrates and Pro-inflammatory Cytokine in Determining the Metastatic Potential of Human Prostate Cancer (LNCaP) Cells**

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The goal of current study was to identify carbohydrate epitopes and their synthetic and degradative enzymes, and/or binding proteins that determine the metastatic potential of cancer cells. Human lymph node-derived metastatic prostate cancer (LNCaP) cells, including androgen-sensitive low passage (C-33) and androgen-resistant high passage (C-81) cells, which contained similar levels of functional androgen receptor and mimicked the clinical progression of prostate cancer, were used. Western blotting showed higher levels of epitopes recognized by MECA 79 and P selectin in C-81 clone. The MECA 79 epitopes were associated with mucin glycans assessed by susceptibility to O-sialoglycoprotein endopeptidase. DNA arrays and RT-PCR analysis showed increased expression of galectin-1, heparanase, IL-8, □1,6N-acetylglucosaminyltransferase (C2GnT-1), fucosyltransferase-VII, and sialyltransferases (ST3Gal-IV&VI) in C-81 clone. The results predicted in C-81 clone higher levels of selectin ligands, such as sLex with or without 6-sulfation on GlcNAc, consistent with increased in vitro invasion property of C-81 clone on Matrigel and polyethylene terapthalate membranes. In addition, expression of those genes except C2GnT-1 for both clones was further elevated by treatment with a pro-inflammatory cytokine, TNFα. These results suggested that the metastatic potential of both LNCaP cell clones could be potentiated under inflamed conditions, which supports the well-accepted hypothesis that inflammation contributes to cancer metastasis. The roles of above-mentioned genes, carbohydrate epitopes, and pro-inflammatory

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cytokines in metastasis of these LNCaP cells will be examined in animals in the future. (Supported in part by an Eppley Cancer Center pilot project, the Nebraska Research Initiative-Cancer Glycobiology Program, and Gene Microarray Core of GM62116)

(264) **A Chemical Reporter Strategy to Probe Glycoprotein Fucosylation**

David Rabuka[1]; Sarah C. Hubbard[1]; Scott T. Laughlin[1]; Sulabha P. Argade[2]; Carolyn R. Bertozzi[1]

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Of the nine monosaccharide building blocks employed by vertebrates, fucose has drawn considerable attention for its roles in a number of cellular processes. Fucosylated glycoproteins are involved in many cell-cell recognition events and are markers of embryonic and malignant tissues. Increased levels and novel expression of fucosylated antigens in cancer suggests that these glycans represent potential biomarkers. We have previously shown that various azide-labeled precursor sugars can be incorporated into cell surface and intracellular glycoproteins and subsequently detected in a secondary labeling step. Incorporation of modified sugars into cell-surface glycans is detected in live cells using a phosphine probe via the Staudinger Ligation. Additionally, "click" chemistry can be employed for examining low-abundant proteins modified with azidosugars in total cell lysates. Here we describe the synthesis of azidofucose derivatives as well as a method for the rapid profiling of fucosylated glycoproteins from human cancer cells, using 6-azidofucose as a chemical reporter. Metabolic labeling with this unnatural sugar provides a means for subsequent identification of fucosylated glycoconjugates and for profiling changes in fucosylation as a function of normal cellular development or malignant transformation.

(265) **Hetero-bifunctional CD22 Ligands Drive IgM Binding and Complement Killing of B Cells**

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Nature utilizes multivalency to achieve stable binding with low affinity protein-carbohydrate interactions. However, except for highly multivalent polymers and neoglycoproteins, design of high affinity multivalent glycan ligands have met with limited success. CD22 is a B cell molecule that recognizes sialosides containing the sequence NeuAcα2-6Galβ1-4GlcNAc with a Kd of ~0.2 mM. The high concentration of NeuAcα2-6Gal on the surface of B cells causes CD22 to be masked, preventing the binding of multivalent probes. We recently reported a synthetic ligand with 9-biphenylcarbonylamido (BPC)-NeuAc (9-BPC-NeuAcα2-6Galβ1-4GlcNAc or 9-BPC-Sialoside) that bound CD22 with 100 fold higher avidity than the natural ligand, and when incorporated into high molecular weight polymers (n > 500) could bind to native B cells. To design a more chemically defined probe, we considered a hetero-bifunctional ligand approach to link the high affinity ligand of CD22 to an antigen, nitrophenol (NP), recognized by a decavalent IgM. Synthesis was achieved by coupling 9-BPC-Sialoside-β-O-ethylamine to N-hydroxysuccinimide ester activated 4-hydroxy-3-nitrophenylacetic acid (NP) to 9-BPC-Sialoside-NP. Surprisingly, 9-BPC-Sialoside-NP is able to efficiently assemble IgM complexes with CD22 on both asialo- and native B cells. The low valency of the IgM/BPC-Sialoside-NP/CD22 complex (n=10), demonstrates the importance of orientation and spacing of the glycans. The IgM bound to CD22 was also found to initiate complement mediated killing of B lymphoma cells expressing CD22, suggesting a therapeutic utility for treatment of B cell related diseases. (Supported by NIH grants GM60938, AI50143 and GM62116).

Reference: Collins, B. E. et al., Journal of Immunology (2006) in press

(266) **Nanoparticle-Based Sensing of Glycan-Lectin Interactions**

Joseph Wang; Yun Xiang; Zong Dai; Jared Gerlach; Jeffrey La Belle; Lokesh Joshi

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Glycosylation is recognized as one of the most crucial post-translational modifications in eukaryotic organisms as every cell is covered with glycan moieties. The glycosylation profile (glycosignature) on glycoconjugates and the cell surface is dynamic and its alterations are indicative of changes in cellular environment and physiology. Most chronic and immunological diseases are accompanied by corresponding glycosignature changes of affected cells and circulatory proteins. We have recently demonstrated the feasibility of a nanoparticle-based biosensing of sugars based on their interaction with surface-functionalized lectins. Our novel bioassay involves an immobilized lectin onto a gold surface, a competitive nanocrystal tagged sugar and the target sugar, and electrochemical monitoring of the competitive assay. The lectin acts as the carbohydrate recognition element for the competition between a nanocrystal-labeled sugar and the target sugar for the carbohydrate binding sites on lectins. The extent of competition is performed through highly sensitive electrochemical voltametric stripping detection of the captured nanocrystals.

The assay has been demonstrated using surface-bound pure Arachis hypogaea (peanut agglutinin - PNA) lectin and various analytes including cancer-associated T antigen beta-D-Gal-[1-3]-D-GalNAc disaccharide moiety. This protocol exhibits excellent discrimination between target and non-target sugars. With feasibility shown, next, multiplexing the system to detect more than one target marker should be possible. An array of sensors, each with a multiplexed four nanocrystal assemblies that can be used to detect: T, Tn, ST, and STn antigen for example. This type of multiplexing would be most beneficial in high throughput screening for point-of-care applications.

(267) **The Role of Differential Carbohydrate Related Gene Expression in Metastasis**

Michelle Lum[1]; Stephen T. Koury[1]; Tim Gilmartin[2]; Steven R. Head[2]; Anne Dell[3]; Stuart M. Haslam[3]; Simon J. North[3]; Jamie Heimburg[1]; Sue Morey[1]; James C. Paulson[2]; Kate Rittenhouse-Olson[1]

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Carbohydrate adhesion interactions may be involved in metastasis. Four closely related breast carcinoma lines (4T1 and derivatives) with different metastatic potentials were analyzed to study metastasis and carbohydrates. These lines were analyzed by Glyco-gene chip, confirmatory real-time RT-PCR, flow cytometry, and glycan profiling. RNA from each cell line was analyzed by the CFG using Glyco-gene chip analysis, a microarray capable of detecting over 1,800 genes related to cell surface carbohydrate expression. The metastatic cells were found to be more closely related to each other than the non-metastatic cell lines. VCAM-1 showed down-regulation in the non-metastatic 67NR cell line, while CD34 showed increased expression in this cell line. Several sialyltransferases showed higher expression levels in the non-metastatic cell lines, whereas several galactosyltransferases and GalNAc transferases showed higher levels in the metastatic cells. Real-time quantitative RT-PCR for 13 genes was used for validation. CD34 and VCAM-1 gene data were confirmed by flow cytometry analysis of cell surface expression patterns. ST6Gal I expression data was also confirmed by flow cytometry, using SNA, a lectin specific for the ST6Gal I product. Elevated sialyltransferase activity in non-metastatic lines was confirmed through increased binding of the lectin MAL II to the non-metastatic 67NR cell line. Preliminary MALDI analysis of the surface glycans showed increased sialylation on the N-linked glycans of the non-metastatic cells, consistent with the gene data. This analysis can be utilized to further understand, and to develop therapy for the prevention of metastasis.

(268) **Sensitive and Rapid Electrochemical Bioassay of Glycosidase Activity**

Jared Q. Gerlach; Tanin Tangkuaram; Veer P. Bhavanandan; Jeffrey T. La Belle; Joseph Wang; Lokesh Joshi

Biodesign Institute at Arizona State University, Tempe, AZ

Glycosidases trim sugars from oligosaccharides attached to glycoconjugates. The action of glycosidases is a regular part of signaling networks in a variety of biological processes. However, many diseases, including various forms of cancer and autoimmune disorders, display irregular glycosidase activity as a symptom of disease progression. Current methods for reliable measurement of glycosidases are tedious and time consuming. In order to utilize glycosidases as disease markers, there is a need to develop sensitive, specific, and simple methodologies. We have developed a novel electrochemical assay technique adaptable to both enzyme and substrate screening. This assay utilizes the electrooxidation and subsequent detection of para-nitrophenol released from sugar substrates by glycosidases in solution. The amperometric responses seen in the enzymatic screening demonstrate a detectable range of 0.2 to 1.0 mU of endo-a-N-acetylgalactosaminidase in less than 60 seconds (Tangkuaram et al, 2006, Analyst, 131, 889). This method overcomes the high background associated with conventional optical assays. In addition, this electrooxidation method is adaptable to electrode systems where the reaction occurs directly at substrates immobilized on solid-supports. Our continuing research centers on expansion of electrochemical biosensor technologies to fit a variety of specific applications including early disease detection.

(269) **High Throughput Technology for the Identification and Characterization of Glycan Binding Peptides**

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The Biodesign Institute at ASU, Tempe, Arizona

Modification of cell surface glycosylation is a common property of cells undergoing growth, development or disease. Unique glycan motifs associated with the state of a cell provide an opportunity for use as a biomarker to predict the cell's health status. Traditional methods for analyzing cell surface glycans include the use of lectins that bind to and recognize specific glycan structures. Recently, limited success has been reported in discovering peptides capable of binding to glycans using phage display of random peptide libraries. We are currently developing a novel high throughput technology for the discovery of peptides that bind to glycans, therefore mimic carbohydrate binding domains. We have discovered peptides that can bind to and distinguish between closely related glycan structures. We have now extended this technology to identify GlycoSignatures of cells. We have applied this technology to discover peptides that bind to cell surface glycans of cultured cells from pancreatic, lung, colon and breast cancers. These peptides and glycans are being validated and characterized. Our high throughput approach for the identification and characterization of cell specific biomarkers will provide a valuable tool to not only detect the GlycoSignatures of cells, body fluids and glycoconjugates but also for imaging, diagnostic and therapeutic applications

(270) **Label-free Realtime Detection of Sugars on Lectin-modified High-Resolution Differential (HRD) Surface Plasmon Resonance (SPR) Sensors**

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Arizona State University, Tempe, AZ

Surface plasmon resonance (SPR) sensing is a label-free sensing method with a low detection level used in applications such as protein discovery, food safety, medical diagnostics, and environmental analysis. In our particular case, a HRD-SPR system is capable of detecting low molecular weight sugars based on a differential SPR signal from a sensing area with respect to a reference area modified with an inert layer. In the present work, *Arachis hypogaea* lectin (Peanut agglutinin, PNA) is bound to the sensing area via anti-PNA antibody attached to the gold surface. The cancer associated T antigen (Galβ1-3GalNAc) binding with the lectin is detected. The reference area modified with an inert layer of IgG-antiIgG is used to subtract non-specific interaction and to eliminate error due to background noise. The label-free target sugar is passed over the sensor surface and the SPR response is monitored in real time. Lectin-Sugar interactions are measured using SPR differential signals down to the nM concentration range. The corresponding affinity constants are assessed for chemical binding characterization. SPR is a promising technology capable of monitoring lectin-sugar interactions and quantifying low concentrations.

(271) **Disaccharide Analogs Inhibit Selectin-Mediated Tumor Metastasis**

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The cell surface carbohydrate antigen, sialyl Lewis X (sLe^x), is expressed on many carcinomas and facilitates tumor metastasis by binding to selectin cell adhesion receptors present on platelets and endothelia. Patient survival studies after surgical resection of tumors indicate higher mortality for those patients whose tumors express sLe^x. Thus, the development of a pharmacological approach to inhibit sLe^x on tumor cells could improve patient survival. We showed previously that different tumor cells will take up and deblock acetylated GlcNAcβ1-3Galβ-O-naphthalenemethanol and use the disaccharide as a primer of oligosaccharide synthesis, diverting the assembly of the chains from endogenous glycoproteins and inhibiting sLe^x expression on cell surface mucins (Brown et al. 2003 J. Biol. Chem. 278:23352; Fuster et al. 2003 Cancer Res. 63: 2775). GlcNAcβ1-3Galβ-O-naphthalenemethanol also blocks spontaneous metastasis when administered to mice bearing subcutaneous tumors (Brown et al. 2006 Clin. Cancer Res. 12:2894). Here we report on disaccharide analogs in which the 3-OH and 4-OH of the terminal GlcNAc residue have been substituted with –H, -F, $-OCH_3$, and $-NH_2$. These compounds no longer act as primers, but retain sLe^x inhibitory activity when fed to U937 lymphoma cells. Kinetic analysis revealed that the 4-deoxy analog acts as a competitive inhibitor of bovine β4galactosyltransferase, using either GlcNAcβ1-3Galβ-O-naphthalenemethanol or ovalbumin as substrates. Treatment of murine Lewis lung carcinoma cells inhibited experimental metastasis to the lungs of syngeneic mice by blocking tumor cells from forming P-selectin ligands. These new compounds represent more specific second-generation disaccharide-based inhibitors for blocking tumor cell dissemination and metastasis.

(272) **Glycoproteins Carrying the Characteristic MBP-Ligand Oligosaccharides on Human Colon Cancer Cells**

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The serum mannan-binding protein (MBP) is a Man/GlcNAc/Fuc specific C-type lectin, which has growth inhibitory activity to human colon cancer cells. We previously isolated MBP-ligand oligosaccharides from a human colon cancer cell line, SW1116, and characterized them as highly fucosylated polylactosamine type structures (1). In this study, we isolated and characterized a glycoprotein, which carried the characteristic MBP-ligand oligosaccharides (MBP-ligand glycoprotein). The cell surface MBP-ligand glycoproteins were isolated from the biotin-labeled SW1116 cells by an avidin-column, followed by an MBP column. A 120-kDa protein was detected as a major cell surface MBP-ligand protein. PNGase F digestion reduced the molecular size of the protein with almost complete loss of the binding activity to MBP, indicating that the cell surface MBP-ligand glycoproteins consisted mainly of the N-glycans. Then, the MBP-ligand glycoproteins were isolated from the cell lysates by an AAL- and an MBP-column. The major components were identified as CD26 and CD98 heavy chain by MS analysis. The digestion of CD26 with PNGase F resulted in the complete loss of the MBP-binding on the lectin blotting, whereas the digestion with endo H resulted in a partial loss of the binding, suggesting that the characteristic MBP-ligands are expressed on CD26. The CD26 was purified with an anti-human CD26 mAb column. MALDI-MS analysis of the N-glycans, released from the CD26 with PNGase F indicated the presence of a series of tandem repeats of 2 fucosylated LacNAc.

1) Terada, M. et al.: J. Biol. Chem., 280, 10897-10913, 2005

(273) **GalNAc Glycoproteins in Breast Cancer**

Anthony J Leathem; Anthony J Leathem
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GalNAc glycoproteins, as detected in cancer tissue paraffin sections by the lectin from the snail Helix pomatia, appear to predict aggressive behaviour of cancers. In particular, detection of GalNAc glycoproteins is associated with breast cancer metastasis to lymph nodes. To understand these glycoforms, we have used affinity chromatography to isolated HPA binding glycoproteins from fresh breast cancer tissue and from serum of patients. Breast cancer is an extremely heterogeneous disease, with variable behaviour so we need to compare the glycans and glycoproteins from the different types of breast cancer. This is assisted by the breast cancer tissue and sera library developed here at University College London. In addition to these 'cancer related glycoproteins', most of our patients with invasive breast cancer show antibodies in their serum against GalNAc glycans on these glycoproteins. At present we are trying to identify the glycans, so we may develop a more specific antibody assay. The clinical significance of these antibodies is not yet known but may provide a tool for earlier diagnosis or even treatment.

(274) **Analysis of the Protein-Linked Cancer Glycome in Discovery of New Cancer Associated Antigens**

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Human cells are covered by a layer of glycans that change in cancer and contribute to cancer progression and metastasis. We have studied the protein-linked glycomes of tumors and normal tissues from cancer patient archival tissue samples by MALDI-TOF mass spectrometry combined with specific enzymatic and chemical glycan modifications. The protein-linked glycome profiles of malignant tumor and control tissues were shown to be significantly different in a panel of over 100 tumor and control tissue samples from human patients. A number of distinct glycan signal groups were identified as the dominating tumor-associated protein-linked glycans in major human cancer types. In lung carcinomas, the analysis led to identification of a tumor-associated glycan group characterized by abnormal terminal β-N-acetyl-D-glucosamine (GlcNAc) residues. Similar glycan antigens were also expressed in glycolipids extracted from the same tumors, suggesting a common biosynthetic background. The results suggest that global analysis of the cancer glycome is an effective tool for discovery of new therapeutic targets in cancer. Furthermore, the glycomics method was able to discriminate between benign

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and malignant carcinoma samples. As an example, data from ovary and colon carcinomas and corresponding benign tumors are shown.

(275) **Profiling Glycosyltransferase Activities in Cancer Cells using Chemically-synthesized, Well-defined Acceptors**

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Changes in glycan profiles associated with cancer progression may be driven by altered activities of the glycosyltransferases involved in their biosynthesis. We systematically evaluated changes in the glycosylation capacity of cells using enzymatic assays coupled with quantitative RT-PCR analysis of specific glycosyltransferases. Such studies focus on three classes of enzymes: i) Those decorating the non-reducing terminus of glycoproteins, including α1,2 L–fucosyltransferase (FT), sialyltransferases, Gal3:O-sulfotransfrases and α(1,4) N-acetylglucosaminyltransferase (GlcNAcT). ii) Those modifying residues other than the terminal sugars e.g. α(1,3/4)FTs and GlcNAc6:O-sulfotransferases, and iii) Those initiating specific branching patterns during glycan assembly, e.g. GlcNAcTs in O-glycans and N glycans. Chemical synthesis approaches were applied to produce a series of well-defined oligosaccharide acceptors for enzymes and some of these acceptors were modified with O-methyl or fluoro groups to obtain specific acceptors for unique glycosyltransferases. Experiments with human prostate cancer cell line LNCaP expressing prostate-specific antigen (PSA) shows elevated levels of α1,2-FT but not α1,3/4-FT. LNCaP also expresses elevated sialyltransferases ST3Gal-I/II, but have low activities of both ST3Gal-IV and ST6Gal-I. Thus LNCaP is likely to express N glycans having Galβ/GalNAc1→4GlcNAc and Fuc1α→2Galβ1→4GlcNAcβ.These predictions are supported by mRNA level studies and published data on glycans of PSA from LNCaP. Similar studies were performed with breast cancer and colon cancer cell lines. Data from other prostate cell lines and prostate tumor tissues will also be presented. Together, the data suggest that studies of glycosyltransferases can aid the identification of cancer-specific unique glycan biomarkers.

(276) **In vivo and Genetic Analyses of Mammalian Core 2 O-Glycan Function**

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Core 2 O-glycans are among the most abundant forms of O-glycosylation that exist in mammals. Three genes within the mammalian genome encode separate glycosyltransferases with Core 2 GlcNAcT activity. These three glycosyltransferases are termed Core 2 GlcNAcT-1, Core 2 GlcNAcT-2 and Core 2 GlcNAcT-3. The presence of three isozymes implicates Core 2 O-glycans in multiple biologic functions. Previously, Core 2 GlcNAcT-1 deficient mice were generated by this laboratory and found to exhibit phenotypic abnormalities that were unexpectedly segregated to specific hematopoietic cell types and involved deficits in selectin-mediated leukocyte trafficking during inflammation. The in vivo biologic functions of Core 2 GlcNAcT-2 and Core 2 GlcNAcT-3 have been unexplored but remain of significant interest in order to understand the evolutionary purpose for the maintenance of these three separate isozymes. We have therefore developed mice that lack Core 2 GlcNAcT-2 and Core 2 GlcNAcT-3 in order to study the consequences of their ablation on Core 2 O-glycan biosynthesis and mammalian physiology. Mice lacking either Core 2 GlcNAcT-2 or Core 2 GlcNAcT-3 are viable and appear normal during early post-natal development. These mutant alleles have been crossed for more than 5 generations into the C57BL/6 genomic background during which homozygotes emerge at normal Mendelian frequencies. Current studies are focused on detecting changes in O-glycan structure and the presence of phenotypic consequences due to the loss of these highly conserved glycosyltransferases. Our progress on this project will be presented.

(277) **Mucin-type *O*-linked Oligosaccharides are Dispensable for Lymphocyte Homing: Novel Roles of *N*-glycan-based L-selectin Ligands**

Junya Mitoma[1]; Xingfeng Bao[1]; Bronislawa Petryanik[2]; Jean-Marc Garguet[3]; Patrick Schaevli[3]; Shin Yi[4]; Hiroto Kawashima[1]; Hideo Saito[1]; Kazuaki Ohtsubo[5]; Kay-Hooi Khoo[4]; Jamey D. Marth[5]; Ulrich von Andrian[3]; John B. Lowe[2]; Minoru Fukuda[1]
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Lymphocyte homing is mediated by specific interactions between L-selectin on lymphocytes and sulfated carbohydrate-addressin expressed in the high endothelial venules (HEV) of lymph nodes. These sulfated carbohydrates are attached to mucin-like scaffold glycoproteins. Here we demonstrated by inactivating core 1 extension and core 2 branch enzymes that loss of 6-sulfo sialyl Lewis X on *O*-glycans including MECA-79-positive addressin has minimum effect on lymphocyte number, although the number of T lymphocytes increased and compensated for the loss of B lymphocytes. The results suggest that T and B lymphocytes compete for the same counter-receptor. Further, mice deficient in both core 1 extension and core 2 branch enzymes exhibit only marginally compromised lymphocyte homing and inflammatory response, despite the fact that no 6-sulfo sialyl Lewis X exists on *O*-linked oligosaccharides. The remaining L-selectin ligands were removed by *N*-glycanase treatment of HEV and CD34 blotted on the membrane. The residual lymphocyte homing was abrogated by preinjection of *N*-glycan-specific E-PHA. Furthermore, 6-sulfo sialyl Lewis X was demonstrated by mass spectrometric analysis in *N*-glycans of lymph nodes. Lymphocyte rolling was observed on CHO cells expressing 6-sulfo sialyl Lewis X only in *N*-glycans. These results show that mucin-type *O*-linked oligosaccharides are dispensable for lymphocyte homing and novel roles of *N*-glycan-based L-selectin ligands for lymphocyte homing and trafficking. 6-sulfo sialyl Lewis X on *N*-glycans and *O*-glycans, however, apparently cooperate in lymphocyte recruitment during inflammatory response. The work is supported by NIH grants CA71932, CA48737, and in part by CA33000.

(278) **Chemokine-glycosaminoglycan Interactions Participate to the Endothelium Organospecificity and Cellular Addressing**

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Supporting chemotaxis and transendothelial migration of leukocytes, chemokines and their receptors play a crucial role in the organo-specific recognition between endothelial cells (ECs) and leukocytes. Apart from their receptors, chemokines interact with glycosaminoglycans (GAGs) on cell surfaces. This binding increases their local concentration creating a chemoattractant gradient and favors their presentation to the cell surface receptors.

From various human endothelial cell lines established in our laboratory, we demonstrated specific activities of given chemokines towards given EC lines. Lymphocyte adhesion increased on 6CKine-stimulated peripheral lymph nodes ECs or mesenteric lymph nodes ECs but it decreased on GAGs treated ECs.

Our aim is to understand the molecular mechanisms by which chemokine-GAG interaction participate to the endothelium organospecificity and lymphocyte recruitment.

Binding of chemokines to other structures than their receptors, namely to GAGs, on ECs, was clearly shown by flow cytometry and fluorescence microscopy analysis.

A detailed understanding of chemokine-GAG interactions may be a potentially useful therapeutic approach, especially in inflammation.

To better characterize the specificity of the chemokine-GAG interactions, surface plasmon resonance experiments were performed. According to the chemokine and to the GAG, the binding kinetics were very different. RANTES formed a very stable complex with chondroïtin sulfate E compared with heparan sulfate. Sulfation level of GAGs is an important feature for the interaction.

Determination of specific GAGs fragments which would allow to inhibit the interaction of a specific chemokine with GAGs and further inhibit lymphocyte recruitment represent a new strategy for modulating chemokine function in various diseases treatment.

(279) **Quantitative Analysis of High Endothelial Venule-like Vessels in Association with Clinical Activity in Ulcerative Colitis**

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Ulcerative colitis (UC) is an inflammatory disease involving the colonic mucosa with unknown etiology, and over 80,000 individuals in Japan are suffering from this disease in 2004. UC is histopathologically defined as diffuse lymphoplasmacytic infiltration in the lamina propria in addition to crypt abscess/cryptitis formation, and this lymphocytic infiltration is thought to be facilitated by the interaction between L-selectin on lymphocytes and its ligands 6-sulfo sialyl Lewis X on high endothelial venule (HEV)-like vessels. However, precise analysis of the interaction between L-selectin and its ligands

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in association with clinical manifestation of UC has not yet been done. Based on our hypothesis that the frequency of HEV-like vessels attributes to the severity or activity of UC, we performed immunohistochemical analysis of 44 (34 and 10 samples in active and remission phases, respectively) biopsied specimens with UC. We counted the number of MECA-79- and CD34-positive vasculatures, and calculated the percentage of MECA-79-positive vessels. We found that the percentage of MECA-79-posivie HEV-like vessels in active phase was larger than that in remission phase of UC. Moreover, triple immunostaining for MECA-79, CD3, and CD20/CD79α revealed that lymphocytes attached to luminal surface of MECA-79-positive HEV-like vessels were composed mainly of T cells, suggesting that T cell recruitment via HEV-like vessels might play an important role for the pathogenesis of UC, especially in active phase. This work was supported by Grant-in-Aid for Young Scientist B-1879240 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and NIH Grants CA71932.

(280) **E-selectin-specific Antagonist GMI-1077 Totally Abrogates Rolling and Adhesion of Neutrophils on Human Endothelium Stimulated by Glycated Serum Proteins of Diabetes**

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Many recent studies have demonstrated a direct correlation between circulating levels of soluble E-selectin and the presence of type 2 diabetes and its accompanying conditions. Endothelial cell activation is present in patients with diabetes and the resulting abnormalities in microvascular flow explain a wide variety of disease-related complications such as retinopathy, nephropathy and poor wound healing in extremities. High levels of glucose in the blood glycates serum proteins through the Amadori rearrangement and are markers for the disease. Glycated serum proteins have been shown to stimulate expression of E-selectin on endothelial cells. Here, we demonstrate that treatment of human endothelial cell monolayers with either glycated serum albumin or glycated hemoglobin will cause increased rolling and adhesion of neutrophils in a cell adhesion flow chamber as determined by digital image analysis. GMI-1077 is a rationally designed potent small molecule glycomimetic antagonist with high specificity for E-selectin. When tested in the flow chamber, GMI-1077 inhibits the increased rolling resulting from glycated serum albumin. At 2uM, GMI-1077 inhibited about 50%, whereas higher doses essentially eliminated (90 -95%) glycated albumin-induced rolling and adhesion of neutrophils. Abnormalities in microvascular flow associated with diabetes have been shown in both humans and mice by intravital microscopy and the effects of GMI-1077 are being tested in mouse models.

(281) **The Anti-Rheumatic Gold Salt Aurothiomalate Curbs Il-1beta Induced Hyaluronan Release by Suppressing has 1 Transcription**

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Gold compounds are among the oldest disease modifying drugs and are still widely used today for treating rheumatoid arthritis. Despite decades of use, little is known about the mode of action of this class of drugs. Here we demonstrate that aurothiomalate (AuTM) suppresses hyaluronan accumulation by blocking IL-1beta induced hyaluronan synthase-1 transcription. We further demonstrate that in fibroblast-like-synoviocytes (FLS) AuTM acts as a specific COX-2 inhibitor in that IL-1beta induced COX-2 transcription is blocked while COX-1 transcription and translation is unaffected. As a consequence, PGE2 levels released by FLS are dose dependently reduced in cells exposed to AuTM. Of similar importance is the demonstration that AuTM does block NF-kappaB-DNA interaction. In addition, two other transcription factors implicated in inflammatory events, namely AP-1 and STAT3, are blocked as well. The effect on NF-kappaB likely explains the inhibition of COX-2 as well as that of HAS1, since both are genes that depend on the activation of NF-kappaB. Interestingly, AuTM does not interfere with IL-1beta induced Inhibitor-kappa-B-alpha degradation, in most cases a prerequisite for subsequent NF-kappaB activation. Furthermore, evidence is presented that in FLS, AuTM blocks NF-kappaB-DNA interaction neither by binding to NF-kappaB binding sites nor by interacting with activated NF-kappaB proteins. Taken together, AuTM treatment of FLS blocks two of the most important proinflammatory events that are associated with RA. AuTM blocks the release of PGE2 and prevents the activation of NF-kappaB, therefore blocking IL-1beta induced HA accumulation and likely a series of other proinflammatory NF-kappaB dependent genes.

(282) **L-selectin Preferentially Recognizes Glycosulfopeptides Containing Sulfated Tyrosine Modeled after Endoglycan and PSGL-1**

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Endoglycan is a mucin expressed by vascular endothelial cells and some leukocytes and has been shown to bind to L-selectin, a C-type lectin important in lymphocyte homing to secondary lymphoid organs and leukocyte extravasation to inflammatory sites. Here, we show that recombinant L-selectin and human T lymphocytes that normally express L-selectin bind to synthetic glycosulfopeptides of thirty seven residues modeled after the N-terminus of human endoglycan containing one or two tyrosine sulfates along with a nearby core-2 based Thr-linked O-glycan with sLex (C2-sLex). Tyrosine sulfate at position Tyr118 was more critical for binding than at Tyr97. The presence of C2-sLex at Thr124 was highly important for L-selectin recognition because glycosulfopeptides lacking sLex did not bind to L-selectin. We also showed that L-selectin and T lymphocytes bound to glycosulfopeptides modeled after PSGL-1, a physiological ligand for P- and L-selectin that is expressed on leukocytes, but did not bind appreciably to sulfated carbohydrate epitope, 6-sulfo-sLex under similar conditions. These results demonstrate that sulfated tyrosine residues in association with a core-2 based sLex moiety within endoglycan and PSGL-1 preferentially contribute to L-selectin recognition over that toward 6-sulfo-sLex alone.

(283) **Gangliosides Improve Bowel Survival in Necrotizing Enterocolitis by Suppressing Inflammatory Signals During Infection and Hypoxia**

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Background - Necrotizing enterocolitis is an inflammatory bowel disease of neonates and remains the leading gastrointestinal emergency in premature infants. The underlying pathogenic mechanisms remain elusive but prematurity, infection, ischemia-hypoxia and enteral feeding are established risk factors while breastfeeding is protective. The role of local vasoactive and inflammatory mediators in the pathogenesis of necrotizing enterocolitis is unclear due to limitations in current models. The objective of this study is to develop a human neonatal bowel model of necrotizing enterocolitis and to determine whether gangliosides, human milk glycolipids with microbial and anti-inflammatory properties, reduce necrosis and pro-inflammatory signals in human neonatal bowel exposed to E. coli lipopolysaccharide and hypoxia.

Methods - Immunohistochemistry was used to access viability of full thickness bowel specimens from 0-3 month-old infants undergoing intestinal surgery. In culture, neonatal bowel was treated with E. coli lipopolysaccharide and hypoxia in the presence and absence of a ganglioside pre-exposure. Bowel necrosis and production of nitric oxide, eicosanoids and pro-inflammatory cytokines was measured.

Results - Analysis of hematoxylin/eosin stained neonatal bowel revealed viable tissue with intact villi and no infiltration of immune cells. Gangliosides reduced bowel necrosis triggered by E. coli lipopolysaccharide. We demonstrate that gangliosides reduce lipopolysaccharide- and hypoxia-induced bowel production of nitric oxide, LTB4, IL-1 beta, IL-6 and IL-8.

Conclusion – A human neonatal bowel model that incorporates multiple risk factors for necrotizing enterocolitis was developed. The findings show that gangliosides improve neonatal bowel survival by down-regulating pro-inflammatory signals released by neonatal bowel during infection and hypoxia.

(284) **Characterization of PEGylated Glycosulfopeptides as Inhibitors of P-selectin**

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Glycosulfopeptides (GSPs) modeled after the N-terminal region of P-selectin glycoprotein ligand-1 (PSGL-1) effectively inhibit the binding of P-selectin to its ligand both in vitro and in vivo. However, such GSPs have only a limited potential as anti-inflammatory therapeutic agents because of their rapid clearance from the circulation following intravenous injection (half-life of less than two minutes), as measured previously in mice. In an attempt to increase the circulatory half-life of GSPs, we covalently modified GSPs with polyethylene glycol (PEG) moieties and assessed the efficacy of the GSP-PEG derivatives to act as antagonists of P-selectin/PSGL-1 interactions during neutrophil rolling on human P-selectin in parallel plate flow chamber experiments. The results demonstrate that GSP-PEG acts as a potent P-

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selectin antagonist. We also determined the circulatory half-life of the PEGylated GSPs after intravenous injection into mice. The results indicate that PEG-derivatives of GSPs may be highly useful as antagonists in blocking inflammatory responses involving P-selectin.

(285) **Sialidases Neu1 and Neu3 on the Surface of Human Monocyte-derived Dendritic Cells may Influence Cell Activity by Desialylating GM3 Ganglioside**

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Sialidases influence cellular activity by removing terminal sialic acid from glycoproteins and glycolipids. Four genetically-distinct sialidases (Neu1-4) have been identified in mammalian cells. We have previously shown that both lysosomal Neu1 and plasma membrane-associated Neu3 are expressed in human lymphocytes and monocytes and that their expression is up-regulated during cell activation and differentiation. The enhanced sialidase activity in activated lymphocytes and in monocyte-derived dendritic cells influences cytokine production by these cells, likely by modulating intracellular signaling. We show here that both Neu1 and Neu3 are present on the outer cell surface of monocytes and monocyte-derived dendritic cells, as well as in intracellular locations. Cell surface Neu1 was found predominantly associated with protective protein cathepsin A, an enzyme that stabilizes and activates Neu1 in lysosomes. Gangliosides are major regulatory molecules that are present in the cell membrane and are potential substrates for both Neu1 and Neu3. To determine the effect of sialidase activity on ganglioside composition during monocyte differentiation, monocytes were differentiated in vitro into dendritic cells and gangliosides were pulse-labeled with [3H]-sphingosine. GM3 was the predominant ganglioside present in monocyte-derived dendritic cells. GM3 has been shown elsewhere to influence the differentiation and immunological responses of monocyte-derived cells. Differentiation of monocytes in vitro in the presence of sialidase inhibitors led to a decrease in cytokine production and to an increase in the amount of the sialylated GM3. Thus, Neu1 and/or Neu3 sialidases may modulate the production of cytokines in human dendritic cells by altering the composition of cell surface gangliosides.

(286) **Antibody Blockade of the L-Selectin Ligand Sulfoadhesin Blocks Disease in Mouse Collagen Arthritis**

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Leukocyte recruitment across blood vessels is fundamental to immune surveillance and inflammation. Lymphocyte homing to peripheral lymph nodes is mediated by the adhesion molecule, L-selectin, which binds to sulfated carbohydrate ligands on high endothelial venules (HEV). These glycoprotein ligands are collectively known as peripheral node addressin (PNAd) or sulfoadhesin, and are defined by the function-blocking monoclonal antibody known as MECA-79. The sulfation of these ligands depends on the action of two HEV-expressed N-acetylglucosamine 6-O-sulfotransferases: GlcNAc6ST-2 and to a lesser degree GlcNAc6ST-1. Induction of PNAd has also been shown to occur in a number of human inflammatory diseases including rheumatoid arthritis (RA). In murine collagen-induced arthritis (CIA) we have shown that PNAd is expressed in the vasculature of arthritic synovium in B10RIII mice immunized with collagen but not in the normal synovium of control animals. This de novo expression of PNAd correlated strongly with induction of transcripts for both GlcNAc6ST-1 and GlcNAc6ST-2, as well as the expression of GlcNAc6ST-2 protein. In order to probe whether PNAd and GlcNAc6ST induction are relevant to the disease process in this model, we treated immunized mice with MECA-79 IgM or a control rat monoclonal IgM. Despite poor pharmacokinetic properties of MECA-79 IgM in mouse plasma, animals treated with the MECA-79 antibody appeared to enjoy statistically significant protection from disease in the early stage of the model. Our results demonstrate that antibody blockade of PNAd or, by inference, inhibitors of the sulfotransferases GlcNAc6ST-1 and 2 may have therapeutic benefit in this widely-used mouse model of RA.

(287) **The Role of Sialic Acid Residue in Tumor Immunogenicity**

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The role of sialic acid (SA) in determining the immunogenicity of tumor cells and their interactions with immune surveillance mechanisms has not yet been thoroughly studied.

We have used a unique model of immunogenic and non-immunogenic tumor cell lines. 3-methylcholantrane-induced fibrosarcoma cell lines originated in IL-1alpha knockout mice are immunogenic and fail to produce tumors in mice, whereas similarly induced fibrosarcoma originated in control BALB/c mice are non-immunogenic and develops tumors in mice.

In this study, we have examined the relation between SA expression by malignant cells and their patterns of interaction with immune cells. Removal of SA residues, by sialidase, was used as an experimental tool. After such treatment, SA residues reappeared after 50 hours. Addition of sialidase-treated tumor cells to normal spleen cells, resulted in an increase IFN-gamma secretion, compared to non-treated cells. Injection of treated cells into mice resulted in retardation, of about 2 weeks, in tumor growth, as compared to non-treated cells. Injection of treated cells into irradiated mice resulted in immediate progressive tumor growth, at similar patterns as non-treated cells. Histological evaluation of the site of injection of tumor cells has revealed that 10 days post-injection of non-treated cells, microscopic tumors are observed, whereas, only small tumor cell deposits were observed following treatment with sialidase. Immunohistochemical studies have revealed more infiltrating macrophages at sites of injection of treated cells, as compared to non-treated cells. These results indicate that SA residues might play a role in interaction between tumor cells and immune effector.

(288) **Chondroitin Sulfate Intake Inhibits the IgE-mediated Allergic Response by Down-regulating Th2 Responses in Mice**

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Chondroitin sulfate (CS) was administered orally to BALB/c mice immunized intraperitoneally with ovalbumin (OVA) and/or dinitrophenylated OVA. The titers of antigen-specific IgE and IgG1 in mouse sera were determined. The antigen-specific IgE production by mice fed *ad libitum* with CS was significantly inhibited. We also examined the effect of feeding CS on immediate-type hypersensitivity. One hour after antigen stimulation, the ears of mice fed with CS swelled less than those of the control mice. Furthermore, the rise in serum histamine in the mice fed with CS under active systemic anaphylaxis was significantly lower than that in the controls. We next examined the pattern of cytokine production by splenocytes from mice followed by re-stimulation with OVA *in vitro*. The splenocytes from the mice fed with CS produced less interleukin (IL)-5, IL-10 and IL-13 than those from the control group. In contrast, the production of interferon-γ and IL-2 by the splenocytes of mice fed with CS was not significantly different from those in the control mice. In addition, the production of transforming growth factor-β from the splenocytes of mice fed with CS was significantly higher than that of the control mice. Furthermore, we showed that the percentages of $CD4^+$ cells, $CD8^+$ cells and $CD4^+CD25^+$ cells in the splenocytes of mice fed with CS are significantly higher than those of the control. These findings suggest that oral intake of CS inhibits the specific IgE production and antigen-induced anaphylactic response by up-regulating regulatory T cell differentiation, followed by down-regulating the Th2 response.

(289) **Anti-human Immunodeficiency Virus Type 1 (HIV-1) Activity of Lectins from Ascidian Didemnum ternatanum**

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GlcNAc-specific lectin (DTL) and GlcNAc/GalNAc-specific lectin (DTL-A) were isolated from ascidian Didemnum ternatanum. DTL agglutinates trypsinized human erythrocytes, whereas DTL-A agglutinates native and trypsined human ones. The results of hemagglutination inhibition assay indicate that DTL does not exhibit any preference for anomeric configuration of preferred N-acetyl-D-glucosamine, whereas DTL-A preferred the □-anomers of N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. The lectins showed anti-HIV-1 activity in vitro. As was shown DTL and DTL-A inhibited cytopathic effect induced by HIV-1 and the production of viral p24 antigen. The EC50 values of DTL were 0.002 and 0.006 μg/mL, and that of DTL-A were 0.36 and 0.59 μg/mL, respectively. DTL and DTL-A could blocked the cell-to-cell fusion process of HIV infected and uninfected cells with EC50 values 1.37 μg/mL for DTL and 6.97 μg/mL for DTL-A.

(290) **Anti-Carbohydrate IgY Antibodies Elicited by Display on a Polyvalent Viral Scaffold**

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The development of anti-glycan antibodies has gained considerable interest in recent years as a diagnostic and therapeutic tool. Profiles of various cancer cell lines have confirmed the aberrant overexpression of specific glycans on the cell surface. Anti-glycan antibodies are useful tools in the detection, characterization and possibly destruction of such cancer cells based on the recognition of this aberrant expression. However, anti-carbohydrate antibodies are not easy to obtain, as it is generally difficult to break immunological tolerance with carbohydrates. Substantial effort has been made in recent years to find an appropriate scaffold for the attachment and presentation of carbohydrates to elicit an immune response that generates anti-glycan antibodies. It has long been recognized that presentation of an antigen on viral scaffolds often generates a strong immune response, but this strategy has apparently yet to be reported for carbohydrates. Here we describe the use of cowpea mosaic virus (CPMV) as a polyvalent scaffold for the purpose. Sophisticated carbohydrates with known relevance to cancer were chosen for this study and were conjugated to exposed lysine residues on the viral surface using copper(I) catalyzed azide/alkyne cycloaddition (CuAAC).

(291) **A β-galactose-specific Lectin Isolated from Green Alga Monochrome nitidum Wittr**

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A lectin, Monochrome nitidum Wittr (MNL), was isolated from green alga Monochrome nitidum Wittr by ion-exchange on DEAE-52 and purified by gel filtration on Sephadex G-200. On SDS-PAGE the purified lectin ran a single band at 66 kDa. Gel filtration of purified lectin on Sephadex G-200 indicates that it exists as a 66 kDa protein in its native state. The total carbohydrate content of MNL was 2.31%. MNL was found to agglutinate human A, B, AB, O erythrocytes, and agglutinate erythrocytes of rabbit. Significant MNL activity was observed between pH 5 and 8. The lectin isn't sensitive to temperature. Heating of the lectin solution at 100 oC for 30 min, their activity remains 25 %. Carbohydrate-binding specific of MNL was examined by hemagglutination-inhibition test. Hemagglutination activity of MNL was inhibited by D-galactose and lactose.

(292) **Control of CD8+ T Cell Homeostasis by ST3Gal-I Protein Sialylation**

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Elimination of post-activated CD8+ T cells by apoptosis is essential in maintaining homeostasis yet the molecular interactions initiating this process remain poorly defined. A candidate mechanistic component involves the ST3Gal-I sialyltransferase, which catalyzes sialic acid addition to unsialylated Core 1 O-glycan structures among mature CD8+ T cells. Following T cell receptor stimulation, this sialic acid linkage is significantly diminished on cells that also express the pre-apoptotic marker phosphatidylserine (PS) and are eliminated in vivo, while viable memory cells re-express the ST3Gal-I-dependent sialic acid and are PS-negative. Absence of ST3Gal-I in mice induces PS expression among CD8+ T cells coincident with their depletion. We now show that diminished Core 1 O-glycan sialylation occurs by a post-transcriptional mechanism and is linked to the contraction phase of post-activated wild-type CD8+ T cells in vivo. Moreover, pharmacologic agents that activate CD8+ T cells with continued ST3Gal-I sialylation fail to induce PS expression. PS induction and CD8+ T cell depletion in vivo do not depend upon either CD43, a major substrate of ST3Gal-I, Core 2 O-glycan linkages, or diminished Bcl-2 levels. Remarkably however, rescue of normal CD8+ T cell homeostasis occurs in the absence of Bim function. Deficiency of ST3Gal-I further attenuates the accumulation of Bim-deficient CD8+ T cells. These data reveal a physiologic mechanism of CD8+ T cell homeostasis independent of Bcl-2 in which Bim function is either epistatic to, or antagonized by, signals propagated by an O-glycoprotein in the absence of ST3Gal-I Core 1 O-glycan sialylation.

(293) **Immunoglobulin G As A Biomarker For Multiple Sclerosis**

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Background: Multiple sclerosis (MS) is a chronic, inflammatory condition of the central nervous system, in which the myelin sheath is attacked by the antibody Immunolglobulin G (IgG). The resultant plaques disrupt messages travelling along nerve fibres. IgG exists in normal blood as a population of glycoforms which is altered in disease and is excessively produced within the CNS during MS. Altered IgG glycosylation has already been shown to cause the excessive and uncontrolled autoimmune response in rheumatoid arthritis. This study seeks to determine whether the IgG mediated attack on myelin in MS is due to the production of unique IgG glycoforms. This may have diagnostic application particularly as there is no current laboratory test that is specific for MS. The IgG variants may also contribute to the development of MS by increasing the affinity of IgG for myelin.

Methods: IgG was isolated from the serum and CSF of patients with MS using DEAE ion exchange chromatography. The glycosylation pattern, in terms of monosaccharide and oligosaccharide composition, of each glycoform was determined using high pH anion-exchange chromatography.

Results: IgG glycosylation differs between MS and a normal population in terms of monosaccharide and oligosaccharide composition. Additionally there were also differences in the glycoform populations present in the serum and CSF from the same person.

Conclusions: Variations in IgG glycosylation could be utilised to detect the presence of MS. Additionally differences in glycosylation could be the basis of the extra oligoclonal bands observed in the CSF of MS patients.

(294) **Analysis of N-linked Carbohydrates on Recombinant Human IgA1 and IgA2 by Mass Spectrometry**

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Immunoglobulins (Ig) are glycoproteins consisting of two heavy (H) and two light (L) chains. IgA is the most abundant class of Ig produced in humans and plays a critical role in providing immunologic protection at mucosal surfaces. Humans possess two IgA isotypes, IgA1 and IgA2, with IgA2 having three allotypes. The level of glycosylation differs among the isotypes and allotypes of IgA. IgA1 but not IgA2 contains a hinge region with three to five O-linked carbohydrate addition sites. In addition, human IgA contains two to five N-linked carbohydrate addition sites on each H chain depending on the isotype and allotype. The carbohydrates on IgA have been shown to influence effector functions, binding to receptors and pharmacokinetic properties, and alterations in glycosylation are associated with immune pathology.

To study the differences in N-glycan structure, recombinant human IgA1 and three allotypes of IgA2 (IgA2m(1), IgA2m(2) and IgA2n) were produced in the Sp2/0 murine myeloma cell line. The N-linked glycans for recombinant IgAs were analyzed by HPLC and MALDI-TOF and compared with those of human serum IgA1 and myeloma derived IgA. These studies revealed that the N-glycans on recombinant IgA are extensively processed and that there are differences in the level of sialylation. In addition, a significant proportion of recombinant IgA produced in the murine cell line contained glycans structures with the β-Gal-(1→3)-β-Gal epitope, which is not present in humans and is highly immunogenic, suggesting that a murine expression system is not optimal for production of therapeutic human IgAs.

(295) **ST6Gal-I Restrains CD22-Dependent Antigen Receptor Endocytosis and Shp-1 Recruitment in Normal and Pathogenic Immune Signaling**

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The ST6Gal-I sialyltransferase produces Siglec ligands for the B-cell-specific CD22 lectin and sustains humoral immune responses. Using multiple experimental approaches to elucidate the mechanisms involved, we have found that ST6Gal-I deficiency induces immunoglobulin M antigen receptor (BCR) endocytosis in the absence of immune stimulation. This coincides with increased BCR co-localization with CD22 in both clathrin-deficient and clathrin-enriched membrane microdomains concurrent with diminished tyrosine phosphorylation of Igα/β, Syk, and phospholipase C-γ2 upon immune activation. Co-deficiency with CD22 restores BCR half-life at the cell surface in addition to reversing alterations in membrane trafficking and immune signaling. Diminished immune responses resulting from ST6Gal-I deficiency further correlate with constitutive and Lyn tyrosine kinase-independent recruitment of Shp-1 to CD22 in unstimulated B cells. Moreover, loss of ST6Gal-I activity prevents autoimmune disease pathogenesis in the Lyn-deficient model of systemic lupus erythematosus, resulting in a significant extension of life span. Protein glycosylation by ST6Gal-I restricts access of CD22 and Shp-1 among unstimulated BCRs and operates by a CD22-dependent mechanism that decreases the basal rate of IgM antigen receptor endocytosis in altering the threshold of B-cell immune activation. Additional and recent progress will be presented.

(296) **O-Glycosylation by Polypeptide GalNAcT-1 Directs Tissue-Specific Lymphocyte Retention, Enables Normal Thrombosis, and Sustains Both**

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Humoral and Innate Immunity

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Multiple polypeptide glycosyltransferases initiate O-glycan formation among various cell types and may thereby differentially modulate development and physiology. We show that the polypeptide ppGalNAcT-1 glycosyltransferase is dispensable for development but is a key determinant in the synthesis of L-selectin ligands among peripheral lymph nodes as well as the majority of E- and P-selectin ligands produced by neutrophils. As a result, ppGalNAcT-1 deficiency in mice reduces B lymphocyte lymph node residency and attenuates the majority of neutrophil accumulation in early inflammation assessed in the acute peritonitis assay. ppGalNAcT-1 further supports thrombosis as deficiency of O-glycoslyation normally contributed by this glycosyltransferase markedly decreases blood coagulation in assays of bleeding time. O-glycan formation by ppGaNAcT-1 severely impairs immunoglobulin-G production in pre- and post-immunization, apparently by a selectin ligand-independent mechanism. These findings reveal that the initiation of O-glycan formation by ppGalNAcT-1 plays multiple and essential physiologic roles and is a key determinant in sustaining normal thrombosis as well as humoral and innate immune responses. Further study on the mechanisms of ppGalNAcT-1 function in these physiologic activities will be presented.

(297) **PNA Binding on Antigen-specific Memory T Cells**

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The glycosylation pattern of T cell surface glycoproteins changes with the activation status and development of T lymphocytes. Previously, it was reported that after viral infection, peanut agglutinin (PNA) binding increases dramatically on splenic CD4 and CD8 T cells compared with naïve T cells, and viral specific activity was retained in the PNAhigh binding population (Galvan et. al 1998). In the current study, we assayed PNA binding directly on antigen-specific CD8 T cells in various anatomical sites (PBMC, spleen, lung, MLN, CLN, ILN, BAL and O-NALT) at different time-points post infection with LCMV. We used an adoptive transfer model of TCR transgenic T cells specific for the LCMV glycoprotein peptide GP33-41. Our results determined that once activated, antigen-specific CD8 T cells, irrespective of their tissue localization, retained high PNA binding long after clearance of viral infection. Moreover, antigen-specific effector CD8 T cells had higher PNA binding than memory antigen-specific CD8 T cells, despite the same TCR specificity and similar expression of ST3 Gal I, a glycosyltransferase whose expression in naïve T cells decreases binding to PNA (Priatel et. al 1999). Thus, following antigen experience, PNA binding increases dramatically on virus specific CD8 T cells and these cells populate lymphoid, non-lymphoid, and mucosal tissues, retaining high/intermediate PNA binding. So while PNA binding cannot distinguish T central (TCM) or effector (TEM) memory subsets, these results indicate that similar to CD44, PNA binding remains high on antigen experienced T cells, regardless of tissue location, or time since antigen-experience.

(298) **Fucosylation-Dependent Thymocyte Development and Related Notch Signaling**

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Over the past several years, experimental evidence has been gathered in many systems to indicate that cell surface glycans are involved in the regulation of development. These glycans are branched structures composed of monosaccharides including glucose, mannose and fucose. Fucose exists in glycans structures as mostly terminal modification or directly linked to Ser/Thr. The biological functions of fucose during ontogeny and cellular differentiation have been suggested by using genetically engineered animal models including FX null mice with a mutation of the 3',5'-epimerase/4'-reductase locus, in which the last synthetic step was abolished in the conversion of GDP-Mannose to GDP-fucose (the only de novo fucose donor) in the construction of fucosylated oligosaccharides. FX null mice are therefore conditionally deficient in all fucosylated glycans. These mice display many phenotypes including global fucosylation deficiency, neutrophilia, and notably, thymic atrophy. The thymus of FX null mice in the fucose depletion environment is characterized by a dramatic T cell hypoplasia phenotype. T cell development in these mice can be restored by feeding the animal with dietary fucose, which restores the production of GDP-fucose through an endogenous salvage pathway. The hypoplastic FX null thymus has been characterized with identification of the critical functions of fucose in T cell development and related signaling pathways. These studies include an effort to identify different contributions among the five Notch ligands in the T cell development. Forthermore, the Notch1 receptors on cell membrane are down-regulated in fucose-depletion condition.

(299) **The Expression of Bisecting Type N-Glycans and Ligands for DC-SIGN on Human Sperm**

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Human sperm are foreign cells that evoke leukocyte release from the cervix immediately following coitus. Sperm also eliminate MHC class I expression during their development, thus avoiding histocompatibility based responses, but potentially increasing their sensitivity to NK cell lysis. MHC class I negative cell types can evade NK cell responses by displaying sufficient levels of bisecting type N-glycans on their surfaces. Purified sperm were subjected to glycoproteomic analysis using ultrasensitive MS methods to confirm the expression of bisecting type N-glycans. These studies indicate that sperm present both high mannose and bisecting type N-glycans on their surfaces. However, sperm also show substantial expression of unusual biantennary, triantennary and tetraantennary type N-glycans terminated with Lewis$^{x/y}$ sequences. One novel tetraantennary N-glycan is capped on each of its four antennae by a Lewis^{y} sequence. Thus sperm are also highly decorated with specific carbohydrate ligands for DC-SIGN. Recent studies with *H. pylori* suggest that the interaction of its Lewis type glycoconjugates with DC-SIGN redirects antigen processing in dendritic cells to induce tolerance to this bacterial pathogen. Therefore high mannose and Lewis$^{x/y}$ type N-glycans could play a major role in abrogating antigen driven responses to foreign sperm proteins in the female reproductive system. Pathogens and tumor cells that express Lewis$^{x/y}$ sequences to evoke tolerance could be employing this natural system that protects human sperm to their great advantage. Thus these glycan profiling studies provide additional support in favor of the eutherian fetoembryonic defense system (eu-FEDS) hypothesis linking pathogenic and tumorigenic subterfuge to the human reproductive imperative.

(300) **Visualization of Galectin-3 Oligomerization on the Surface of Neutrophils and Endothelial Cells using Fluorescence Resonance Energy Transfer (FRET)**

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Galectin-3, a member of the galectin family, is expressed in cells involved in the immunity. Galectin-3 plays a role in various activities, ranging from cell repression to cell activation and adhesion, and has been recognized as an immunomodulator. Our previous works suggest that galectin-3 activates neutrophils, inducing production of IL-8 and shedding of L-selectin. Galectin-3 also supports neutrophil adhesion to endothelium. Interactions of galectin-3 with neutrophils differ depending on their activation states. Galectin-3 forms lattices on naï¨ve neutrophil surfaces, while it is rapidly endocytosed in primed neutrophils. Although the activities and interactions are likely to be associated with ligand cross-linking, galectin-3 exists as a monomer. It has been proposed that oligomerization of the N-terminal domains of galectin-3, after ligand binding by the C-terminal domain, is responsible for this cross-linking. The oligomerization status of galectin-3 could thus control the majority of its extracellular activities. However, little is known about the actual action mode through which galectin-3 exerts its function. Here, we present data suggesting that oligomerization of galectin-3 molecules occurs on cell surfaces with physiological concentrations of the lectin. Using galectin-3 labeled at the C-terminal with Alexa488 or Alexa555, the oligomerization between galectin-3 molecules on cell surfaces was detected using FRET. We observed this FRET signal in settings representing the different action modes of galectin-3: ligand cross-linking leading to cell activation, cell-cell interaction/adhesion and lattice formation. Furthermore, our data using FRAP suggest that galectin-3 lattices are robust and could thus be involved, as previously proposed, in the restriction of receptor clustering.

(301) **Biological Activity Evaluation of α-Lactosylceramide**

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The NKT cells can be activated by glycolipids binding CD1d to secrete Th-1 and Th-2 cytokines, such as IFN-γ and IL-4, to regulate many critical biological conditions in vivo, including malignancy and infection, and autoimmune diseases. A synthetic α-galactosylceramide (α-GalCer), originally derived from a marine sponge, has been used in research as an exogenous ligand for CD1d to stimulate iNKT cells. The α-GalCer is the most active glycolipid discovered so far that can be presented by CD1d. It has remarkable activity to stimulate NKT cell proliferation and cytokine releasing, both in vitro and in vivo. α-GalCer has been demonstrated with anti-tumor effects on a variety of transplantable tumors, including melanomas, lymphomas, colon, lung, breast, and renal cancers. It has also shown promise in treating a variety of autoimmune pathologies in animal models, including multiple sclerosis, autoimmune diabetes and experimental encephalomyelitis. To probe the processing of oligosaccharide sphingolipids inside the cell and evaluate the activities of glycolipids with different sugar parts, we synthesized α-lactosylceramide (α-LacCer). Through our bio-assays, we have proved that the α-LacCer can also stimulate the iNKT cells to proliferate and release cytokines, both in vitro and in vivo, but, with different cytokine releasing profiles comparing to α-GalCer. Mice tumor model and experimental autoimmune encephalomyelitis (EAE) model also proved that the α-LacCer had similar in vivo clinical effects. The kinetic assay showed the processing by β-glycosidase was critical for α-LacCer activity. Finally, we have proved that the α-LacCer is a new glycolipid antigen with significant activities.

(302) **Mammalian N-Glycosylation Inhibits Innate Immune Mechanisms that Induce and Mediate Autoimmune Disease**

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Autoimmune diseases are a group of pathogenic syndromes that can engage both innate and adaptive immune systems in cellular activation responses that override normal mechanisms of self-tolerance. The interplay between innate and adaptive immunity in the initiation of autoimmune disease has been increasingly studied during the early phases of pathogenesis. Yet mammalian autoimmune diseases are thus far characterized as syndromes mediated by the adaptive immune system, and typically reflect pathogenic defects that can be transferred upon hematopoietic reconstitution by bone marrow grafts. In contrast, we find that the absence of the alpha-mannosidase-II (αM-II) enzyme induces an autoimmune disease diagnostic of systemic lupus erythematosus that originates from innate immune system activation by endogenous stimuli residing among radiation-resistant somatic cells. Hematopoietic reconstitution using αM-II null donors does not induce or transfer disease, nor does wild-type marrow inhibit disease pathogenesis. Remarkably, loss of the adaptive immune system in animals lacking both αM-II and RAG-1 amplifies tissue pathogenesis coincident with elevated macrophage recruitment, increased severity of glomerulonepthritis, and impaired kidney function. Intravenous IgG treatment attenuates macrophage recruitment and iNOS induction while maintaining normal kidney function. αM-II deficiency interferes with mammalian N-glycan branching thereby exposing mannose residues at the cell surface and modulating endogenous mannose receptor expression, implicating this lectin-based innate immune recognition system in autoimmune disease pathogenesis. These findings imply that the evolutionary acquisition of complex N-glycan branching in vertebrates afforded the innate immune system the ability to distinguish glycomes of pathogenic organisms from host N-glycosylation in promoting mechanisms of self-tolerance.

(303) **Heparan Sulphate Facilitates Endocytosis of Eosinophil Cationic Protein**

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Eosinophil cationic protein (ECP) belongs to human RNaseA superfamily. ECP has been used as bio-markers for the severity of asthma.

The cytotoxicity of ECP is closely associated with the efficient endocytosis of ECP to the target cells. In this study, we conducted a systematic analysis on the molecular basis of endocytosis of ECP. Here we report that cell surface-bound heparan sulfate proteoglycans (HSPGs) act as the major cellular receptor for internalization of ECP. Shedding cell surface heparan sulfate (HS) by treatment with heparinases or reducing sulfation of glycans by chlorate treatment strongly decreased ECP binding to Beas-2B cells. Uptake and cytoxicity of ECP in glycosaminoglycans-deficient cells were also significantly reduced. After associating with cell surface HS, ECP was found to rapidly internalize the cells through detergent-resistant lipid rafts in a clathrin-independent and caveolin-dependent fashion, followed by trafficking from early endosomes to late endosomes. Our results demonstrate for the first time that interaction between ECP and HSPG results in lipid raft-associated macropinocytic uptake of ECP, which is in turn routed to the degradative compartment.

(304) **Changes of Serum Glycans in Acute Inflammation**

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Although acute inflammatory response is generally a unique homeostatic mechanism, there are differences associated with the nature and site of inflammation. We examined changes of N-linked glycans released from serum of a patient with sepsis and a patient with acute pancreatitis during first eight days of disease and compared them to the control sample. Sera were taken from patients at time of reporting to hospital and then three more times. The blood from healthy individual was drawn on one occasion only. Glycans were released using N-glycosydase F and subjected to normal phase HPLC combined with exoglycosidase digestions and mass spectrometry. Levels of identified structures have been followed through the course of disease and compared to the control. Changes of serum glycans were found to occur very early in acute inflammation. Changes that are presumably part of regulatory processes during inflammation have been observed in tri- (A3G3S3 and A3G3S3F) and tetra-sialylated structures, mannose structures, level of fucosylation (both core and outer arm), and the degree of branching. The proportions of different glycans were changing daily and some differences were also observed between sepsis and pancreatitis, presumably as a reflection of the fact that in these two conditions the acute phase response is triggered by a different stimulus and is associated with different patterns of production of specific cytokines.

(305) **The Glycosylation of Myelin-oligodendrocyte Glycoprotein**

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Glycoproteins from pathogens and self-antigens can target dendritic cells (DC) through C-type lectin receptors (CLR) to induce tolerance. Myelin/oligodendrocyte glycoprotein (MOG) is a quantitatively minor glycoprotein in myelin carrying one N-linked glycan. Immunization of mice, rats, or marmosets with MOG results in the development of experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS). A more severe EAE is induced when non-glycosylated MOG is used as immunogen. Changes in the glycosylation of the self-antigen MOG could prevent its binding to CLR on DC resulting in the loss of peripheral tolerance and the induction of a chronic autoimmune reaction to myelin antigens.

To determine the glycosylation of native myelin we first analysed the expression of glycosylation-related genes in oligodendrocytes isolated from rhesus monkey. This gene profiling predicts the presence of triantennary glycans carrying I-type polylactosamine chains, the presence of the LDN (and LDNF) antigens, the expression of Lewis-type structures (3- and 6-O sulphated), and the prevalence of a2,3 over a2,6/a2,8 sialylation. Secondly, the analysis of the carbohydrate composition by HPLC and mass spectrometry of isolated MOG from myelin of healthy and non-healthy individuals (EAE marmosets and MS patients) will be investigated to elucidate the glycosylation of MOG in normal conditions.

Through the generation of well characterized MOG glycoforms we will study in vitro their effects on CLR binding and DC function. It is our ultimate goal to modify the tolerant or immunity active state of DC through glycan-modified self-glycoproteins to treat autoimmune diseases.

(306) **The Effects of Maturation on the Glycosylation of Dendritic Cells**

Marieke Bax; Juan J. García Vallejo; Yvette van Kooyk
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Dendritic cells (DCs) are the most potent antigen presenting cells in the organism. Immature DCs (iDCs) reside in peripheral tissue and sense for pathogens whereas mature DCs (mDCs) are able to activate T cells in the lymph nodes. This dramatic functional change is mediated by an important genetic reprogramming. Glycosylation has been implicated in multiple aspects of the immune response. To investigate the involvement of glycosylation in the changes that occur during DC maturation we have studied the differences in the glycans expressed by iDCs and mDCs as well as their glycosylation machinery. We show that maturation of DCs results in large changes in the expression of glycosylation-related genes. There is an upregulation of genes involved in the synthesis of LacNAc, core 1, Lewis-type and sialylated structures and a downregulation of the genes involved in the synthesis of core 2 glycans. A glycan fingerprint performed by MALDI-TOF corroborated this

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prediction. Tri- and tetra-antennary N-Glycans carrying sialylated and fucosylated poly-LacNAc were present only in mDCs and not in iDCs. To investigate the function of these upregulated structures the binding of galectins and siglecs to iDC and mDC was measured. These lectins bind with a higher affinity to mDCs. Further experiments are being set up to unveil the functional consequences of galectin and siglec binding to iDCs and mDCs.

(307) **Immune-modulation by an Unique Mixture of Prebiotic Oligosaccharides**

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Human milk oligosaccharides (HMOS) are prebiotic, anti-infective and modulate the immune system.

As alternative to complex HMOS a mixture of beta-galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) (ratio 9:1) have been identified as effective prebiotic ingredients.

In an influenza vaccination-model (C57Bl/6 mice) GOS/FOS induced an significant and dose-dependent increase in cellular immunity (DTH).

In a clinical study (281 infants) at an age of 12 months the cumulative incidences of diarrhoea (N=17 vs. 34, $p < 0.05$) and of recurrent upper respiratory infections (> 3 episodes) were significantly lower (N=19 vs. 35, $p < 0.05$) in the group fed the prebiotic formula versus the standard formula.

In a further clinical study (259 infants) with family history of atopy, at an age of six months the cumulative incidence of atopic dermatitis was significant lower in the GOS/FOS group (N=10 vs. 24, $p<0.03$). In a subgroup (N=98) analyses of the intestinal flora revealed a significant increase of the bifidobacteria in the prebiotic group (10.3 vs 8.65 log cfu/ g stool, $p<0.0001$). The plasma-IgE levels and the IgE/IgG4 ratios measured in a subgroup (N=42, $p<0.05$) were significantly reduced in the prebiotic group. There is accumulating evidence that T-regulatory cells are involved in the suppression of IgE and in the induction of IgG4, This regulatory effect may be at least partly contributing to the results obtained.

The data clearly indicate the potential role of prebiotic oligosaccharides as a new dietary tool for immune modulation during infancy.

(308) **Endogenous Galectin-1 Promotes Agonist Mediated and Antagonizes Partial Agonist Mediated Selection Events**

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CD8 T cell development occurs in the thymus and selects for potentially helpful CD8 cells, while deleting potentially autoreactive CD8 cells. Development includes setting CD8 T cell activation signaling thresholds to generate a repertoire of cells that does not respond to self-antigens. A protein expressed by thymocytes and the thymic stroma is the endogenous lectin, galectin-1 (gal-1). Our previous experiments using recombinant gal-1 indicate that gal-1 promotes apoptosis in developing CD4CD8 double positive thymocytes, and antagonizes TCR signaling. These findings predict that endogenous gal-1 might function in the selection of CD8 T cells and setting TCR signaling thresholds during T cell development.

To study the effects of gal-1 on the development of conventional CD8 T cells, we analyzed the effect of gal-1 gene ablation in wild-type mice, and in H-Y and OT-1 TCR transgenic mice. Gal-1-/- H-Y males negatively select 2.5 times fewer CD8 SP thymocytes and peripheral CD8 cells than wild-type littermates. Gal-1-/- H-Y females positively select twice as many CD8 thymocytes and peripheral CD8 cells compared to wild-type littermates. Similarly, gal-1-/- OT-1 TCR mice positively select twice as many CD8 thymocytes and peripheral CD8 cells.

Furthermore, our studies on CD8aa development indicate that gal-1 -/- H-Y male mice have two times fewer CD8aa IELs. In OT-1 mice, there is a 3 fold increase in the number of CD8aa IELs in gal-1-/- OT-1 mice. Taken together, these data suggest that gal-1 selectively promotes agonist mediated selection events, while antagonizing partial agonist mediated selection events.

(309) **Core 2 Branch-dependent Sialyl Lewis X Oligosaccharides on Mouse Natural Killer Cells**

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Natural killer (NK) cells are innate immune effectors that have the ability to directly lyse target cells such as virus-infected cells and tumor cells. They express a variety of calcium-dependent (C-type) lectin-like receptors and L-selectin. The latter has been shown to mediate the recruitment of NK cells into regional lymph nodes during an immune response (Chen et al., J. Exp. Med. 2005, 202:1679). In addition, activated NK cells also express high levels of sialyl Lewis X oligosaccharides recognized by monoclonal antibodies CSLEX-1 and HECA-452 in humans. mAb KM93, originally raised against human lung adenocarcinoma cells, bound CHO cells only after transfecting with fucosyltransferase-VII. It also strongly reacts with cell line HL60, and this reactivity was completely diminished after treatment of HL60 cells with an alpha2,3-specific sialidase. These results indicate that KM93 epitope is fucose- and sialic acid-containing sialyl Lewis X oligosaccharides. Only a small fraction of freshly prepared mouse spleen NK cells were positive for KM93; by contrast, all IL-2 activated spleen NK cells are positive. In vivo stimulation of C57BL/6 mice with poly-I:C also dramatically increased KM93-positive NK cells in the spleen. We further showed that majority of sialyl Lewis X oligosaccharides on NK cells are presented on core 2 O-glycans because KM93 reactivity was almost absent in core 2 GlcNAc-transferase-I-deficient NK cells. Finally, C2GnT-I-deficient mice were partially defective in NK cell infiltration in response to tumor challenge, suggesting that core 2 branch is important for NK cell recruitment.

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(310) **Glycan differences in Serum Immunoglobulin A1 from Healthy and Diabetes Type 2 Patients**

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Serum IgA1 is frequently elevated in type 2 diabetes mellitus (DM). Previous studies using enzyme-linked lectin assay and fluorophore assisted carbohydrate electrophoresis (FACE) has shown that O-glycans from IgA1 are hypersialylated and probably implicated in the serum elevation of this protein. Mass spectrometry (LC-ESI-MS) of oligosaccharides obtained by hydrazinolysis from serum IgA1 showed the presence of bi, three and oligosialylated Galβ1-3GalNAc motif, whereas Galβ1-3GalNAc with only two or three sialic acids were found in IgA1 from sex and age-matched healthy controls.

(311) **Isolation and Characterization of a Novel Hemocyte-Associated Galectin from the Protochordate *Clavelina picta***

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Galectins are a family of carbohydrate-binding proteins that are widely distributed in nature having been identified in vertebrates, invertebrates, and protists. Members of this family are defined by their binding affinity for ß-galactosides and the presence of a conserved sequence motif within their carbohydrate recognition domain (CRD). Recent studies in mammals have demonstrated that galectins participate both directly and indirectly in mediating immune responses such as host-pathogen interactions, inflammation, and autoimmunity. Given their evolutionary conservation galectins may be part of an ancient mechanism of host defense. To gain insight into the origin and evolution of galectin function in the innate immune response we have isolated four proteins using lactose affinity chromatography with relative mobilities of 37.5, 33.5, 15.8, and 14.8kDa from the protochordate, *Clavelina picta*. Western and northern analysis revealed that the 15.8kDa band (designated CpG16) is specifically localized and synthesized in the circulating hemocytes, the cells which carry out many of the immune functions in the tunicate. In addition, CpG16 appears to be a novel member of the galectin family in terms of its primary structure and carbohydrate specificity. CpG16 is upregulated in response to bacterial challenge, and we have further evidence that suggests this upregulation may be the result of a protein-carbohydrate interaction. Immunostaining with an anti-CpG16 antiserum revealed the presence of the galectin in hemocytes of challenged colonies. These results suggest that galectins have an evolutionarily conserved role in innate immunity. (Supported by Grants NIH R01 GM070589-01 and NSF MCB-00-77928 to GRV)

(312) **A Galectin-1-Like Protein from Striped Bass (*Morone saxatilis*): Expression in Macrophages, Leukocytes and Rodlet Cells**

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Like all aquatic vertebrates and invertebrates, teleost fish are subject to the constant pressure of bacterial, fungal and parasitic organisms present in the the environmental interface, and that can potentially cause disease. Numerous defense molecules have been isolated from the skin and gut mucus of various fish species. To provide new insights into the potential role(s) of galectins in the teleost fish innate immune system, we selected the striped bass (*Morone saxatilis*), a teleost fish species of environmental and economic relevance in the Chesapeake Bay, and amenable to biochemical, molecular, and histological approaches, particularly concerning skin and intestinal mucus. We identified in skin mucus, and subsequently purified from skin and muscle tissue a 15 kDa galectin-like protein which we designated MS-15. Analyses of its primary structure and carbohydrate specificity indicate that MS-15, is closely related to the mammalian galectin-1. The exon-intron boundary in MS-15 gene is conserved when compared with the mammalian galectin-1. MS-15 directly binds to various bacterial species and strains, such as *Vibrio anguillarum* and *V. mimicus*, which are potential pathogens of striped bass. On histological examination MS-15 was found expressed in connective tissue, resident macrophages, circulatory leukocytes, and rodlet cells. Electron microscopy with immunogold reveals numerous positive signals in the cytoplasm of cells that morphologically resemble intestinal macrophages, as well as clustering of MS-15 on the surface of intestinal microvilli. The possible role(s) of MS-15 in striped bass immunity will be discussed. (Supported by NIH Grant R01 GM070589-01 and NSF grant MCB-00-77928 to GRV)

(313) **Lectin Expressions in Hemocytes of Manila Clams (Ruditapes philippinarum) (Bivalvia: Mollusca) Infected with Perkinsus olseni**

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The hemocytes of invertebrates play key roles in both cellular and humoral immune reactions by phagocytosis or delivering immune factors such as lectin. Previously, we found that infection with the protozoan parasite, Perkinsus, increases lectin synthesis in hemocytes. In order to investigate the patterns of genes expressed in Manila clams (Ruditapes philippinarum) infected with the protozoan parasite P. olseni, we constructed a cDNA library and sequenced 1,850 clones (expressed sequence tags). A total of 79 ESTs, were related to 29 functional immune genes such as C-type lectin, lysozyme, and cystatin B, in Manila clams. Lectins were the largest group of immune-function ESTs found in our Manila clams library. Among 7 lectin clones, two full length cDNAs of lectins were cloned. MCL-3, which is a simple C-type lectin composed of 151 amino acids, has a relatively short signal sequence of 17aa and single carbohydrate-recognition domain (CRD) of ~130 residues. It is highly homologous to eel C-type lectin. The sequence of mc-sialic acid-binding lectin consists of 168 amino acid residues with molecular weight of 19.2 and shows high homology to sialic acid-binding lectin from the snail, Cepaea hortensis.

The expression of 7 different lectins in hemocytes was analyzed by RT-PCR using gene-specific primers. Hemocytes from Perkinsus-infected clam expressed different sets of lectins than with Vibrio infection. These results demonstrate that several lectins are involved in Manila clam innate immunity and different challenges induce expression of different lectins.

(314) **The Broad and Variable Spectrum of Circulating Anti-N-glycolylneuraminic Acid Antibodies in Normal Humans**

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Sialic acids are 9-carbon acidic sugars found on cell surfaces of vertebrates. N-acetylneuraminic acid (Neu5Ac) and its hydroxylated form, N-glycolylneuraminic acid (Neu5Gc), are the two major sialic acids in mammals. Humans, in contrast to great apes (our closest evolutionary cousins) cannot produce Neu5Gc, due to a specific irreversible gene mutation. Instead, humans express increased amounts of Neu5Ac. Despite this, Neu5Gc occurs in some normal human tissues at low levels, and is enriched in human tumors. We have reported that humans metabolically incorporate exogenous Neu5Gc from dietary sources, even while having variable amounts of anti-Neu5Gc antibodies, which can elicit complement-mediated cytotoxicity. Our original ELISA assay target was α-linked Neu5Gc. We have now ELISA-screened sera from healthy individuals against various sialoside structures containing terminal Neu5Gc, which are more similar to natural antigens on human cells. High specificity was achieved using the corresponding Neu5Ac-containing glycans as background controls, i.e., sialyl-glycan antigen pairs differing only in a single oxygen atom at the sialic acid 5-position. These included purified glycolipids, glycans conjugated to polyacrylamide, and α2-3- and α2-6-linked sialoside pairs that were chemically synthesized using an efficient one-pot three-enzyme chemoenzymatic synthetic system, and then conjugated to human serum albumin. Analysis of anti-Neu5Gc-glycan IgA, IgD, IgG and IgM from multiple human sera on eight such different sialyl-glycan pairs revealed that the recognition pattern is highly variable and complex, and not well correlated with values previously obtained with α-linked Neu5Gc alone. Moreover, the maximum levels of circulating Neu5Gc–dependent antibodies appear much higher than our previous estimates.

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