COMPLEX CARBOHYDRATES IN BIOLOGY AND MEDICINE

Organizers: Jeffrey Winkelhake and Raymond Dwek

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Structural Determination of Complex Carbohydrates-Defining Microheterogeneity

U 001 NEW APPROACHES TO EXOGLYCOSIDASE-BASED SEQUENCING OF PROTEIN-ASSOCIATED OLIGOSACCHARIDES, R B Parekh, J Dearnley, S Prime, A Ventom, Oxford GlycoSystems Ltd, Hitching Court, Blacklands Way, Abingdon, Oxon. OX14 1RG. England.

Highly purified exoglycosidases of defined substrate specificity constitute a powerful set of reagents for probing oligosaccharide primary sequence. Usually, these reagents have been used in a sequential manner, which is an essentially *ad hoc* and extremely time-consuming process. A method has been developed and described for the primary sequence analysis of N-linked oligosaccharides (Edge *et al*, PNAS (USA) Vol. 89 p. 6338) in which an array of exoglycosidases consisting of different combinations of a chosen set of exoglycosidases is allowed to act on equal aliquots (~ 1-10 picomoles per aliquot) of an unknown oligosaccharide, pre-labelled only at its reducing terminus. Each mixture in the array hydrolyses the oligosaccharide until a glycosidic linkage is reached which requires for its hydrolysis an exoglycosidase which is absent from that mixture. In this way, each mixture generates a 'stop-point' fragment of the original oligosaccharide. All such 'stop-point' fragments are combined and analyzed in a single chromatographic step. A software program has now been developed which reconstructs, without any reference to a database (but assuming conservation of the trimannosyl chitobiosyl core), the primary sequence of the unknown oligosaccharide from a knowledge of the exoglycoisdase array and the chromatographic signature generated from the unknown oligosaccharide by that array. Both this original process and its extensions, in which (i) oligosaccharides labelled with various reporter groups, and (ii) sialylated N-linked oligosaccharides are analyzed, will be described. Efforts to adapt the process for analysis of O-linked oligosaccharides will also be summarized.

U 002 DETAILED STRUCTURAL ANALYSIS OF GLYCOPROTEINS AND GLYCOLIPIDS. Vernon N. Reinhold, Steve Chan, Rene Mora, and Bruce B. Reinhold, Division of Biological Sciences, Harvard University School of Public Health, Boston, MA 02115.

The functional roles being unraveled in the study of cell-cell interactions point frequently to anointed glycosylated molecules on their surfaces. In part, N-, and O-linked glycans have been shown to influence protein folding, have a directing control during intra-, intercellular trafficking, stabilize a proteins conformation to the extremes of pH and temperature, protect against proteolysis, provide an effective foil against immune surveillance, and serve as components in signal transduction. As numerous and compelling as these functional problems are, the analytical effort required to fully understand carbohydrate structural detail can be overwhelming.
To resolve isomers and profile a total glycoform distribution, we have combined pre-analysis chemistry and chromatography with electrospray ionization, collisional activation and tandem mass spectrometry. The molecular specificity imparted to branched glycans by the chemical lability of cis-glycols yields a molecular weight profile altered by linkage position and glycose monomer. Collision induced dissociation (CID) and tandem mass spectrometry provide major sequence and branching fragments that are differentially exposed by permethylation. Minor CID fragments, increments of cross-ring double cleavage at major sequence ions, can be directly related to the linkage position on adjacent reducing-end residues. The electrospray technique makes these minor fragments detectable due to the absence of a matrix background. Multiple charging yields a lower instrumental mass readout with patterns that allow data reduction by computational processing. In addition, these chemically modified glycans are very lipophilic following derivatization, and hence, further purified by extraction. Moreover, they ionize with high efficiency during electrospray. These general strategies have been applied to N-, and O-linked glycans, glycosphingolipids, protein anchors, and oligosaccharides and provide a sensitive, efficient approach to glycoconjugate complexity. (supported by NSF and NIH).

U 003 OLIGOSACCHARIDE CHAIN-CLEAVING ENZYMES FOR DEGLYCOSYLATION OF ASPARAGINE-LINKED GLYCANS, Anthony L. Tarentino, and Thomas H. Plummer, Jr., New York State Department of Health, Wadsworth Center for Laboratories and Research, Albany, NY 12201.

Flavobacterium meningosepticum expresses a variety of oligosaccharide chain-cleaving hydrolases which are useful for clarifying the structure/function of asparagine-linked glycans. Among these are three distinct endo-β-N-acetylglucosaminidases designated Endo F_1, Endo F_2, and Endo F_3, and two amidases, peptide N^4-(N-glycanyl)asparagine amidase (PNGase F) and N^4-(N-glycanyl)asparagine amidase (glycosylasparaginase). Endo F_1, Endo F_2, Endo F_3, and PNGase F, which are secreted into the medium, have been purified to homogeneity in two simple steps involving hydrophobic interaction chromatography and high-resolution sulfopropyl chromatography. Glycosylasparaginase which was purified to homogeneity from intracellular lysates is composed of two non-identical subunits and appears to be an evolutionary ancestor of the mammalian lysosomal glycosylasparaginases. All three Endo-enzymes and PNGase F have been cloned and their nucleotide sequences and catalytic properties determined. Endo F_2 and Endo F_3 are different from the other hydrolases in that they are post-translationally modified. Using mass spectrometry we verified that Endo F_2 contains 3 unique sites with an additional mass of 1,241 daltons/site, while Endo F_3 has 1 such site.
PNGase F is widely used for removal of intact oligosaccharide chains of asparagine-linked glycans. It binds primarily to the polypeptide backbone and the inner di-N-acetylchitobiose moiety of the glycan chain and hence will hydrolyze most asparagine-linked oligosaccharides. Endo F_1, Endo F_2, and Endo F_3, however, show differing specificities for the oligosaccharide moiety. Endo F_1 is very similar in substrate specificity to <u>S. plicatus</u> Endo H. Both enzymes hydrolyze high-mannose asparagine-linked glycans at approximately the same rate. They differ mainly in their ability to hydrolyze swainsonine-type hybrids because the presence of a core-bound Fuc1→6GlcNAc impedes Endo F_1 hydrolysis, but has no effect on Endo H. Endo F_2 preferentially hydrolyzes bi-antennary glycans, but high-mannose oligosaccharides are hydrolyzed at a greatly diminished rate. Endo F_3 hydrolyzes both bi- and tri-antennary glycans but the rates of hydrolysis are slower than those of Endo F_1 or Endo F_2 on their preferred substrates. The presence of core-bound Fuc1→6GlcNAc on bi- and tri-antennary oligosaccharides, greatly augments Endo F_3 activity. The results of these substrate specificity studies suggests a unique binding site on each Endo which recognizes structural determinants on the oligosaccharide moiety. X-ray crystallographic analysis of Endo F_1 at 1.8Å by Dr. P. VanRoey of this institution show a modified α/β barrell motif with a cleft that binds the glycan chain and positions the di-N-acetylchitobiose moiety into a catalytic site at the top of the barrel. Extension of these analyses to include Endo F_3 should serve to explain the catalytic differences of these related, but different, enzymes.

Experimental Synthesis of Complex Carbohydrates

U 004 PREPARATION OF GLYCOPEPTIDES AS OLIGOSACCHARIDE MIMETIC. *Klaus Bock and Morten Meldal, Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark.*

The complex oligosaccharides on the glycoproteins or glycans of glycolipids have shown to be important ligands for protein receptors mediating signals for numerous essential events in cells or cell-cell interactions. The oligosaccharide part of glycopeptides has furthermore been demonstrated to influence the physical and pharmacological properties of glycoproteins or glycolipids as well as serum lifetime, secretion and targeting. The complex carbohydrate ligands may either be obtained by tedious and laborious isolation from natural sources, but their low abundance and microheterogenity often presents severe problems for the isolation of reasonable amounts for further biological studies. Furthermore, the chemical or enzymatic synthesis of large oligosaccharides is still a tremendous undertaking, which is only possible in few laboratories around the world. However, the biologically active compounds may be obtained by substituting the structural part of the complex oligosaccharides with a peptide scaffold. Thus, glycosylated peptide templates can be designed to spatially arrange the small fragments of the complex carbohydrate which interact with the receptor in such a way that the interaction between the parent ligand and the receptor is simulated.
A building block strategy involving fully protected glycosylated Fmoc-amino acid-O-Pfp esters has been developed for the stepwise assembly using solid phase glycopeptide synthesis of a large range and for different types of glycopeptides and glycopeptide libraries. An example of this approach to address the question of interactions with important biological receptors will be described and it will be demonstrated that glycopeptides carrying 6-O-phosphorylated mannose units can be used to prepare compounds, which will interact strongly with the mannose 6-phosphate receptors (MPR).

U 005 FLUORESCENT DERIVATIVES OF GLYCOPEPTIDES, OLIGOSACCHARIDES, AND GLYCOSIDES. Y. C. Lee[1], Ludwig Brand[1], Kevin Rice[2], Kyung Bok Lee[1], Reiko T. Lee[1], Koji Matsuoka[1,3], Michael Quensenberry[1], and Pengguang Wu.[1] [1]Johns Hopkins University, Baltimore, MD 21218, [2]Ohio State University, Columbus, OH 43210, [3]Hokkaido University, Sapporo, Japan.

Fluorescent derivatives of glycopeptides, oligosaccharides and glycosides are useful in many areas of glycobiology. Simple fluorescent glycosides are useful for sensitive assay of glycosidases. Glycopeptides modified with Dansyl (5-dimethylamino-1-naphthylsulfonyl) group at the N-terminus can be used for assaying endo-type glycosidases (e.g., endo-H) with RP-HPLC. Fluorescent-labeled glycosides, such as 6-dansylaminohexyl α-D-mannopyranoside, can be used for measurement of carbohydrate-protein interactions. FMOC-hydrazide is a useful reagent for analysis of monosaccharides by RP-HPLC. Reductive amination of reducing oligosacchrides with 2-aminopyridine has been used to produce fluorescent alditols which can be efficiently separated by RP-HPLC and amino- or amide-silica columns. This approach has been useful in structural elucidation of oligosaccharides of both N- and O-glycosides especially when the sample availability is limited. Fluorescent-labeled oligosaccharides or glycopeptides in lieu of their radiolabeled counterparts can be used for binding studies. In recent years, we prepared doubly fluorescent-labeled glycopeptides for energy transfer experiments to study conformational features of a glycopeptide of triantennary structure derived from bovine fetuin. By selecting appropriate donor and acceptor pair of fluorescent probes (e.g., naphthyl and dansyl), the long range average distance between the donor-acceptor pair can be determined. Measurements of the fluorescent decay (life time) can also yield some interesting information. Doubly fluorescent-labeled compounds for energy transfer can be used for endo-type enzymes (Supported by NIH Research Grant DK09970).

U 006 ENZYMES IN THE SYNTHESIS OF BIOLOGICALLY ACTIVE OLIGOSACCHARIDES, Monica M. Palcic, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

The identification of specific oligosaccharides as biologically active ligands, particularly in cell adhesion, has generated a growing demand for a supply of structurally characterized molecules both as the free oligosaccharides and as their glycoconjugates. These molecules are used as inhibitors of carbohydrate-protein binding, enzyme substrates, tags to direct targeting, ligands for affinity chromatography and for receptor identification/localization.

The oligosaccharides in question are usually between two and five sugar residues in size. Such molecules are amenable to synthesis by organic chemists, but this can require many months and often over a year for the first of a new class of structure. In addition to being difficult and lengthy, the methods of organic synthesis are too harsh to be applicable to the modification of isolated glycoproteins, glycolipids or cells.

We are using glycosyltransferases, both isolated and cloned, to assist in the synthesis of the required oligosaccharides. We categorize their use into the following areas and this lecture will report developments in each :

1. "Biomimetic" oligosaccharide synthesis, where natural sequences are built up using the correct enzymes. The required glycosyltransferases must either be isolated or cloned, the sugar sugar nucleotide must be available (through recycling if large quantities are required), the reaction conditions must be defined and take into account substrate and product inhibition, and the reactions must be monitored. The synthesis of hexasaccharide using four enzymes has been devised.
2. Enzymatic synthesis of oligosaccharide analogs requires the glycosyltransferases to cross-react with unnatural substrates, both donor and acceptor. Over 30 analogs of oligosaccharides have been prepared using modified sugar nucleotides demonstrating that these enzymes are remarkably tolerant in the context of preparative synthesis.
3. Enzyme assisted glycopeptide synthesis: here two approaches are being investigated. In the first approach, Fmoc-protected glycosylated serine is being used as a glycosyltransferase acceptor. The product formed is then a glycopeptide building block with an oligosaccharide attached which can be used directly in a peptide synthesizer. In the second approach, transfer of sugar residues to glycopeptides immobilized directly on the resin used for peptide synthesis is being examined and a resin has been developed where the immobilized glycopeptide substrates are accessible to glycosyltransferases in aqueous buffers.

Environmental Effects and Control of Glycosylation

U 007 GLYCATION AND OXIDATION OF PROTEINS IN DIABETES AND AGING.
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Proteins in physiological systems are modified by both enzymatic and non-enzymatic glycosylation reactions. Both of these processes modulate the structural and functional properties of proteins. Non-enzymatic glycosylation, or glycation, is a common posttranslational modification of primary amino groups in proteins. The major product formed is fructoselysine (FL), the adduct of glucose to the ε-amino group of lysine residues. The extent of glycation of proteins achieves a steady state with respect to ambient blood glucose concentration and is increased in diabetes in response to hyperglycemia. Although it affects the structural and functional properties of proteins and enzymes, glycation *per se* appears to be of little pathological consequence. Research is now focused on events occurring after the glycation reaction, during the so-called browning stages of the Maillard reaction. Advanced glycosylation end-products (AGEs) formed after glycation cause permanent, cumulative chemical modification and crosslinking of proteins. AGEs may be detected by immunoassays using antibodies to proteins browned by incubation with glucose *in vitro* and increase in lens proteins and collagen with age. Thus far, N^{ε}-(carboxymethyl)lysine and the fluorescent crosslink, pentosidine, are the only AGEs which have been chemically characterized. Both accumulate in long-lived proteins, such as lens proteins and tissue collagens, with age and the rate of their accumulation is accelerated in diabetes. These AGEs require oxygen for their formation, and have been termed glycoxidation products (GOPs) since they are formed as a result of both glycation and oxidation reactions. Studies *in vitro* indicate that oxidation is required not only for formation of GOPs, but also for crosslinking and insolubilization of collagen by glucose. While glycation is reversible, the formation of GOPs and crosslinking of collagen are irreversible reactions. Thus oxygen may be considered a fixative of protein damage *via* the Maillard reaction. Antioxidants, chelators, reducing agents and the dicarbonyl trap, aminoguanidine (AG), are potent inhibitors of the formation of GOPs and the crosslinking of collagen by glucose, suggesting alternative therapeutic strategies for limiting chemical modification of proteins by glucose in diabetes. AG also inhibits the formation of AGEs and the development of neural, retinal, renal and vascular pathology in diabetic animals. Variations in endogenous oxidative stress may modulate the chemical consequences of hyperglycemia and the AGEing of protein in diabetes, explaining, in part, differences in susceptibilities of patients with similar duration of disease and long-term glycemic control to the development of complications. Treatment with antioxidant vitamins and non-steroidal anti-inflammatory agents, which modulate oxidative stress and inhibit AGE formation in diabetic animals, may be useful for limiting the development of diabetic complications. in humans. (DK-19971)

U 008 SULFATED PROTEOGLYCANS AND PROTEIN SORTING IN THE REGULATED SECRETORY PATHWAY.
Anna Castle and David Castle, University of Virginia, Charlottesville.

Sorting for regulated secretion involves targeting of secretory proteins into membrane-bounded storage granules that form at the *trans* -Golgi network. Recent studies suggest that specific structural domains of the secretory proteins serve as sorting signals by promoting selective aggregation of proteins destined for storage. Basic proline-rich proteins (bPRPs) are a family of salivary proteins that are efficiently stored in secretion granules in parotid acinar cells. When their expression in these cells is induced to high levels by β-adrenergic agonists, there is also enhanced expression of proline-rich proteoglycans (PRPgs). The PRPgs have a polypeptide backbone that is structurally related to that of bPRPs, and they are modified by addition of short chondroitin sulfate chains. Coordinate expression and costorage of PRPgs with bPRPs suggests that sulfation of glycosaminoglycan chains may facilitate aggregation as a part of the sorting process for regulated secretion. When a bPRP and a PRPg are each expressed separately from cDNAs in pituitary corticotrophs (AtT-20 cells), they are sorted for regulated secretion. Coexpression of PRPg and bPRP has two effects. First the presence of PRPg increases substantially the sorting of bPRP for regulated secretion. Second, the presence of bPRP increases the level of sulfation on the chondroitin sulfate chains of PRPg. These effects implicate proteoglycan sulfate groups as participants in the heirarchy of intermolecular interactions that comprise aggregative sorting for regulated secretion. Current studies are addressing directly the dependence of sorting on the presence of chondroitin sulfate and the storage-related sulfation mechanism.

Biochemistry-Complex Carbohydrate Structure: Activity Relationships on Circulating Macromolecules

U 009 SEX, SUGARS, AND SULFATE,
Jacques U. Baenziger, Shylaja M. Dharmesh, Dorothy Fiete, Brenda Mengeling, and Lora V. Hooper, Department of Pathology, Washington University Medical School, St. Louis, MO 63110.

The glycoprotein hormones LH (lutropin), FSH (follitropin), TSH (thyrotropin), and CG (chorionic gonadotropin) are a family of glycoproteins. Their β subunits, while highly homologous, arise from separate genes and are hormone specific whereas their α subunits arise from a single gene and have the identical amino acid sequence. As a result the secondary and tertiary structures of these hormones are closely related and two of these hormones, LH and CG, activate the same receptor, the LH/CG-Receptor, which is a G-coupled protein kinase. The Asn-linked oligosaccharides on these hormones are by contrast distinct. Those on LH and TSH terminate predominantly with SO_4-4GalNAcβ1,4GlcNAcβ1,2Man whereas those on FSH and CG terminate with Sialic acidα2,3/6Galβ1,4GlcNAcβ1,2Man. A glycoprotein hormone-specific GalNAc-transferase and a GalNAc-4-sulfotransferase account for the synthesis of these sulfated oligosaccharides. The GalNAc-transferase recognizes a tripeptide motif, ProXaaArg/Lys (PXR/K), which is located 6-9 residues N-terminal to Asn glycosylation sites on LHβ, CGβ, and α but is absent from FSHβ and TSHβ due to amino terminal truncations in the β subunit genes. CG, which is synthesized by placental trophoblasts, does not bear sulfated oligosaccharides because neither the GalNAc nor the sulfotransferase is expressed in human placenta. FSH does not bear sulfated structures because combination of FSHβ with the α subunit in some fashion masks the recognition site on the α subunit. We have used site-directed mutagenesis to examine the features of this motif which are essential for recognition by the GalNAc-transferase. The sulfated oligosaccharides on LH are recognized by a receptor in hepatic endothelial cells and as a result control the rate of LH clearance from the blood. Furthermore, GalNAc- and sulfotransferase levels in the pituitary are modulated by estrogen *in vivo* in the same manner as LH, thus assuring that the oligosaccharides on LH are always fully sulfated. LH levels rise and fall in a pulsatile manner in the blood, with the amplitude and frequency of the pulses increasing during the preovulatory surge. Rapid clearance is necessary to obtained this pulsatile pattern and we propose intermittent activation is essential to produce maximal stimulation of the LH/CG-Receptor which is down regulated when continuously occupied. The sulfated oligosaccharides on LH are therefore essential for the expression of its biologic activity *in vivo* during the ovulatory cycle, whereas sialylated oligosaccharides and a long circulatory half life are required during pregnancy for the expression of CG bioactivity.

U 010 MUCIN ANTIGENS IN CANCER, Young S. Kim, James R. Gum, Jenny J.L. Ho, Samuel B. Ho, Bader Siddiki, Gastrointestinal Research Laboratory, Department of Veterans Affairs Medical Center, San Francisco, CA 94121 and the Department of Medicine, University of California, San Francisco, CA 94143.

Mucins are high molecular weight glycoproteins that are heavily glycosylated with many oligosaccharide side chains linked to the protein backbone. Two main types of mucins have been characterized to date. One is a membrane bound mucin and another secretory mucin. An example is MUC1, a membrane associated mucin predominantly expressed in mammary and pancreatic tissues which has a central tandem repeat region consisting of 40 to 80 of 20 amino acid peptide repeating units. This region contains OH amino acids and therefore is highly glycosylated. This is a membrane glycoprotein with a transmembrane domain and a cytoplasmic tail which interacts with actin cytoskeletal components. The second type which includes MUC2, a secreted intestinal mucin is much larger and has a central tandem repeat region which is much more heavily glycosylated than that of MUC1. Furthermore, it has high cysteine content in the non-repetitive regions flanking the tandem repeats which may be involved in polymerization. In this cysteine rich region there is considerable homology to von Willebrandt's factor. Finally, this type of mucin does not have a membrane spanning domain. To date, seven different human mucin genes have been identified; MUC1 through MUC7. The predominant feature of mucin is the central region consisting of repeat peptide sequences. Each mucin gene has distinct amino acid sequence, but they all have a high content of threonine and/or serine, potential O-glycosylation sites, and mucins are expressed in an extremely tissue specific fashion by highly specialized cell types. The structure of mucin may differ in normal and cancerous epithelial tissue. In normal mucin glycoprotein, the tandem repeats are heavily glycosylated, there are many carbohydrate side chains per molecule and each chain is long. With malignant transformation, the tandem repeats are more sparsely glycosylated, carbohydrate chains may be much shorter and/or modified in the outer region. These changes may be due to altered carbohydrate metabolism or to changes in glycosyltransferases or to *altered processing of* mucin in cancer cells. Thus, the modified sugar structures, or exposed inner sugar core structures, or protein backbone moiety may serve as tumor markers and also may be involved in various biological properties of cancer cells. In addition, there is altered regulation of various mucin genes in cancer cells which is not well understood at the present time.

Biochemistry-Complex Carbohydrate Structure: Activity Relationships on Cellular Receptors and Matrix Components

U 011 LAMININ GLYCOSYLATION, CELLULAR ADHESION AND SPREADING, **Marvin L. Tanzer**, Martin S. Giniger, and S. Chandrasekaran, University of Connecticut Health Center, Farmington, CT 06030-3705.

Uncoupling of cell adhesion and spreading occurs in a model system using B16 murine melanoma cells on a laminin substratum. Selective blocking of laminin oligomannosides with ConA or use of unglycosylated laminin permits normal cell adhesion but the adherent cells fail to spread. Laminin glycoforms enriched in oligomannosides are more effective in restoring cell spreading than other laminin glycoforms. Reconstitution of spreading occurs with pronase-digested glycosylated laminin but not with its unglycosylated counterpart. Selective removal of oligomannoside-peptides from the pronase digest abolishes cell spreading activity. Mannan restores cell spreading whereas other polysaccharides of glucose or glucosamine are ineffective. Titration with microgram levels of mannan yields an absorption isotherm profile; cells commence spreading within 5 minutes and reach maximal spreading within an hour. Mannose is an antagonist, inhibiting mannan-initiated cell spreading, but is not an agonist. Cells did not adhere to neoglycoprotein surfaces except in the presence of soluble laminin protein; then only a mannosylated surface was effective. Only murine melanoma cells, of the cells tested, showed oligomannoside-dependent spreading on an unglycosylated laminin substratum. Micromolar levels of Man6 or Man9 readily restore maximal cell spreading whereas Man3 is ineffective. Man9 binds to the cells in a bimodal fashion at micromolar levels, reaching saturation, whereas Man3 shows linear binding in the same and higher levels. Comparison of Man9 binding and Man9-initiated spreading shows that maximal spreading occurs at about half saturation. Mannose or mannan effectively compete for Man9 binding whereas Gal or Fuc are ineffective. Cell surface labelling is seen with mannan-conjugated beads and with soluble mannan, using intact cells. Soluble mannan does not co-distribute with β1 integrin which is seen in focal adhesions. Rather, mannan shows a capping pattern which transiently disappears as cells spread and it returns after full spreading. The composite results indicate that: (1) oligomannosides are necessary to initiate murine melanoma cell spreading on glycosylated laminin but are not sufficient for cell adhesion; (2) occupancy of both an integrin and a cell surface lectin, recognizing oligomannosides, are required for melanoma cells to spread on glycosylated laminin. A model is proposed in which the cell surface lectin recognizes triantennary laminin oligomannosides simultaneously with integrin recognition of laminin polypeptide determinants. *Supported by NIH, AR-17220.*

Molecular Biology, Synthetic Pathways and the Control of Glycosylation Machinery

U 012 MOLECULAR GENETICS OF MAMMALIAN FUCOSYLTRANSFERASES. John B. Lowe[1,2], Daniel J. Legault[3], Robert J. Kelly[1], Peter L. Smith[1], Shunji Natsuka[1], Yuko Natsuka[1], Petr Maly[2], Bronia Petryniak[1], Dominique Giorgi[4], Sylvie Rouquier[4], Greg Lennon[4], Nozomu Hiraiwa[2]. [1]Howard Hughes Medical Institute, and Departments of [2]Pathology, and [3]Medicine, University of Michigan Medical School, Ann Arbor, MI, and the [4]Human Genome Center, Lawrence Livermore National Laboratory, Livermore, CA.

Cell surface fucosylated oligosaccharides have been implicated in cell adhesion events in mammalian organisms. These events include immune cell adhesion to vascular endothelium, and cell adhesive events operative during early mammalian embryogenesis. To explore these processes in greater detail, and to determine mechanism that regulate them, we have begun to define the structures and functions of several families of mammalian fucosyltransferase genes. In humans, there exist at least five distinct α(1,3)fucosyltransferases, encoded by five α(1,3)fucosyltransferase genes. Sequence comparisons and chromosomal localization studies allow these genes to be segregated into three distinct families. Three members of one of these families share more than 90% amino acid sequence identity, yet exhibit distinctive acceptor substrate specificities. Sequence comparisons indicate that these enzymes differ most substantially in a region of the protein beginning on the golgi luminal side of the transmembrane segment, and extending in the carboxy terminal direction for approximately 150 amino acids. To more precisely localize peptide segments within this domain that dictate acceptor substrate specificity, a series of chimeric fucosyltransferases were constructed using restriction fragment interchange procedures. These structure/function analyses indicate that acceptor substrate specificities idiosyncratic to each fucosyltransferase are dictated in large measure by a localized peptide sequence within the larger variable region domain. Biochemical analyses indicate that these enzymes are posttranslationally modified by asparagine linked glycosylation, and by proteolytic events that release soluble forms of these enzymes from their host cell. Analysis of tissue-specific expression patterns of murine homologues of these genes indicate that they are expressed in epithelial cells lining the gastrointestinal tract, in hematopoietic cells, and in the uterus in association with embryo implantation events. Gene ablation experiments are in progress in an attempt to define the functions of the oligosaccharide products of these genes in these tissues. Humans also express at least two distinct α(1,2)fucosyltransferase activities. One of these enzymes is encoded by the human H blood group locus. Recent molecular cloning studies have identified a second human gene with primary sequence similarity to the human H blood group α(1,2)fucosyltransferase gene. *Analysis of this* gene's structure, and the function of its polypeptide product, indicate that it represents a likely candidate for the human Secretor blood group locus. Mapping studies indicate that this sequence and a structurally similar pseudogene, are very closely linked to the human H blood group locus on chromosome 19.

U 013 MOLECULAR BIOLOGY OF PROCESSING MANNOSIDASES, Kelley W. Moremen[1], Anita Lal[1], John S. Schutzbach[2], W. Thomas Forsee[2], Peter J. Neame[3], J. Schneikert[4], A. Athanassiadis[4], and A. Herscovics[4], [1]University of Georgia, Athens, GA 30602, [2]University of Alabama, Birmingham, AL 35494, [3]Shriners Hospital for Crippled Children, Tampa, FL 33612, [4]McGill Cancer Center, Montreal, Quebec, Canada

At least three processing α-mannosidases are involved in the trimming of Asn-linked oligosaccharides on newly synthesized mammalian glycoproteins: a dMNJ-resistant α-mannosidase in the ER, a dMNJ-sensitive α-mannosidase in the ER or Golgi, and an α1,3/1,6-mannosidase in the Golgi complex. Recent cloning and expression studies on several of the processing α-mannosidases will be summarized with an emphasis on the relationships in sequence and function between the different gene families.

Clones have been isolated encoding the dMNJ-sensitive α1,2-mannosidase from rabbit and mouse cDNA libraries by a method employing mixed oligonucleotide-primed amplification of cDNA. Peptide sequences derived from the purified rabbit liver enzyme were used design primers and generate a 1011 bp rabbit α1,2-mannosidase-specific cDNA probe by PCR. A full length murine cDNA clone was isolated by hybridization of the cDNA probe and found to encode a 655 amino acid type II transmembrane protein with a 43 amino acid cytoplasmic tail, a single transmembrane domain, and a large COOH-terminal catalytic domain containing two potential N-glycosylation sites. Comparison of the murine cDNA sequence with the NH_2-terminal sequence of the purified rabbit enzyme indicates that the enzyme was proteolytically clipped during purification. The region removed by the proteolysis comprises the cytoplasmic tail, transmembrane domain, and 107 residues of lumenally-oriented polypeptide indicating that this region, comprising 26% of the open reading frame, is not essential for catalytic activity. Stable transfection of the murine α1,2-mannosidase cDNA into mouse L cells resulted in a ~22-fold overexpression of α1,2-mannosidase activity. Three transcripts were detected in rabbit tissues whereas two were found in rat and mouse tissues. The transcripts were consistent with the sequences obtained from the cDNA clones indicating that they differ in the length of their 3' sequences as a result of the use of multiple polyadenylation signals. Immunolocalization of the L cell transfectants using a rabbit polyclonal antibody to a rat α1,2-mannosidase detected cross-reactive material in a juxtanuclear pattern consistent with the Golgi complex. The catalytic portion of the murine α1,2-mannosidase, corresponding to the proteolytically-clipped purified rabbit enzyme, bears a strong similarity (37% identity) to the processing α1,2-mannosidase from *Saccharomyces cerevisiae* [1]. These regions of similarity between the murine cDNA and the yeast processing α-mannosidase were used to design degenerate oligonucleotides and isolate an additional α1,2-mannosidase PCR product from murine sources. This novel α-mannosidase cDNA was found to be 65% identical to the previously isolated murine α1,2-mannosidase with a high degree of similarity within the transmembrane and catalytic domains but little similarity within the cytoplasmic tail and the putative "stem" region. The relationships between the multiple murine α1,2-mannosidase forms, the rabbit and yeast α1,2-mannosidases, and similar α1,2-mannosidases from human and pig sources will be discussed. [Supported by NIH grants GM47533 and RR05351 (K.W.M.), GM47492 (J.S.S.), AR 35322 and the Shriners of N. America (P.J.N.) and the MRC of Canada (A.H.)]

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U 014 *THE SIALYLTRANSFERASE GENE FAMILY: HOW BIG?* *Hiroshi Kitagawa, Arun Datta and James C. Paulson, Cytel* Corporation, San Diego, CA 92121.

The sialyltransferase family of glycosyltransferases has been estimated to have 10-12 members in order to account for the variety of sialic acid containing linkages in carbohydrate groups of glycoproteins and glycolipids. Sequence information obtained from the cloned cDNAs of several sialyltransferase genes revealed a highly conserved "sialylmotif" representing 10-15% of their total sequence. The high conservation at the ends of this homologous domain suggested that a polymerase chain reaction (PCR) using primers to these sequences might be used to obtain cDNAs of sialylmotifs of other sialyltransferase genes. This approach has been used successfully to obtain additional cDNA sequences for novel members of the sialyltransferase gene family. Four sialyltransferase cDNAs have been cloned using this approach, bringing the total to 7 members of this gene family that have been cloned to date. Although the homology of these genes is largely limited to the sialylmotif when their sequences are directly aligned, when small insertions and deletions are allowed, extensive homology throughout the genes is revealed, suggesting that they may have arisen from a common ancestral gene. The challenge presented by having cloned a novel sialyltransferase gene by homology has been to identify the activity and corresponding function of protein product of that gene. One successful approach has been to express the sialyltransferase protein as a soluble secreted form and to assay the expressed protein for sialyltransferase activity using a variety of potential acceptor substrates. Analysis of the genes cloned to date reveal striking tissue and developmentally regulated expression patterns which, combined with the sialyltransferase activities demonstrated for the cloned enzymes, suggest that the number of genes in this family is larger than the estimated 10-12 sialyltransferases predicted based on an analysis of known carbohydrate structures.

U 015 REGULATION OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE V (GlcNAc-T V) BY ONCOGENE EXPRESSION Michael Pierce[1], Philip Buckhaults[1], Mohamed Shoreibah[1], Guang-Shing Perng[2], and Nevis Fregien[2], [1]Department of Biochemistry and Molecular Biology and Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602 and [2]Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101.

N-acetylglucosaminyltransferase V from rat sources is an unusual glycosyltransferase in several respects. The enzyme is the largest lumenal Golgi glycosyltransferase reported: 740 amino acids, 6 potential *N*-linked glycosylation sites, and an apparent molecular weight after SDS-PAGE of 95 kDa. In addition, even in tissues in which it is expressed in relatively high amounts, it is present in very low copy number: over 400,000-fold purification is necessary from rat kidney acetone powder, and cDNA libraries from tissues with high activity contain positive clones at the very low frequency of about 1 in 10^6. Relative to other glycosyltransferases in the *N*-linked pathway, its expression significantly varies among rat tissues, with thymus, intestine, lung, and kidney tissues showing high expression of GlcNAc-T V mRNAs. These transcripts are about 7.5 kbase in size, while cultured cells often express another transcript of >9 kbase. Interestingly, brain tissue shows the highest mRNA levels by Northern analysis, although enzymatic activity assays of this tissue show relatively low activity. Therefore, there may be several tissue-specific mechanisms for regulation of GlcNAc-T V activity. The oncogenic transformation of cells by tumor viruses and several isolated oncogenes, notably Rous sarcoma and polyoma viruses and H-*ras*, has been shown to cause a specific increase in GlcNAc-T V activity and cell surface expression of its β(1,6) product. Northern analysis of BHK, Rous sarcoma virus- and v-*src*-transformed BHK cells demonstrates that v-*src* expression causes at least a 7-fold increase in GlcNAc-T V mRNA levels (both 7.5 and 9 kbase transcripts), but no effect on GlcNAc-T I levels. Incubation of cells for 24 hr. with increasing amounts of herbimycin, a *src*-specific tyrosine kinase inhibitor, causes a dose-dependent, specific inhibition of GlcNAc-T V activity in the v-*src*-expressing cells down to the basal level of activity found in non-transformed BHK cells. No effect of the inhibitor was observed on the GlcNAc-T V activity in BHK cells or on GlcNAc-T I activity in either transformed or non-transformed cells. A decrease in GlcNAc-T V mRNA levels was also observed after herbimycin treatment (1 μg/ml, 24 hr.). In addition, over-expression of the *neu*/*her*-2 oncogene in 3T3 cells also causes a specific increase in GlcNAc-T V mRNAs of over 5-fold, but has no effect on GlcNAc-T I transcript levels. These results demonstrate that expression of at least two oncogenes with plasma membrane-associated tyrosine kinase activity causes specific, significant increases in the levels of GlcNAc-T V mRNAs.

Cell Biology-Glycoconjugates and Cell: Cell and Cell: Substrate Interactions

U 016 THROMBOSPONDIN INTERACTIONS WITH SULFATED GLYCOCONJUGATES REGULATE TUMOR CELL ADHESION, MOTILITY, AND PROLIFERATION, David D. Roberts[1], Neng-hua Guo[1], Tikva Vogel[1], Vivian S. Zabrenetzky[1], Diane A. Blake[2], John K. Inman[3], and Henry C. Krutzsch[1]. [1]Laboratory of Pathology, NCI, NIH, Bethesda, MD 20892, [2]Department of Ophthalmology, Tulane University School of Medicine, New Orleans and [3]Laboratory of Immunology, NIAID, NIH.

Thrombospondins are a widely distributed family of extracellular matrix glycoproteins. Thrombospondin-1 (TSP) is released from activated platelets and is synthesized by many cell types in tissue culture. TSP is both a positive and negative modulator of cell adhesion, proliferation, and motility and is an inhibitor of angiogenesis in vitro and in vivo. The complex responses of cells to TSP reflect the complex ligand binding properties of the protein. TSP contains binding sites for integrins, CD36, fibronectin, fibrinogen, collagens, some extracellular proteases, TGFβ, heparan sulfate proteoglycans, and sulfated glycolipids. Proteolytic or recombinant domains of TSP and synthetic peptides derived from TSP have been used to map binding sites for some of these ligands on TSP and to examine their role in specific biological activities of the protein. Using this approach, we have characterized two heparin-binding sites on TSP and examined their effects on tumor and endothelial cell behavior. One heparin-binding site is located in the amino-terminal globular domain of TSP. Antibodies to this domain inhibit melanoma spreading and chemotaxis to TSP, and fragments lacking this domain support adhesion and haptotaxis but not spreading or chemotaxis. Using recombinant heparin-binding domain, we found that this domain inhibits mitogenic and proliferative responses of endothelial cells to basic fibroblast growth factor (bFGF). A novel heparin-binding sequence has been identified in the three type I repeats of thrombospondin containing the consensus sequence Trp-Ser-Xaa-Trp-Ser. Peptides containing this sequence promote melanoma, breast carcinoma, and endothelial cell adhesion and motility. A peptide from the second type I repeat containing this sequence and a flanking basic motif inhibits proliferation of tumor and endothelial cells stimulated by bFGF and mimics the biphasic effect of TSP on motility of endothelial cells in the presence of bFGF. Polymeric conjugates of the TSP peptides have been prepared that have enhanced antiproliferative activity in vitro and inhibit tumor growth in vivo. Both heparin-binding molecules act at least in part by inhibiting bFGF binding to the cell surface, presumably by competing for binding to heparan sulfate proteoglycans. These studies suggest that TSP may play a broader role in regulating cell responses to heparin-dependent growth factors by modulating their interactions with glycosaminoglycans that are required for presentation of the growth factors to high affinity signalling receptors.

U 017 CELL SURFACE β1,4-GALACTOSYLTRANSFERASE FUNCTION DURING MAMMALIAN FERTILIZATION, Barry D. Shur, David J. Miller, Helen J. Hathaway, Adel Youakim, and Xiaohai Gong, Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

β1,4-Galactosyltransferase (GalTase) is traditionally viewed as a biosynthetic component of the Golgi complex, but it is now clear that a portion of GalTase is also expressed on the surface of some cells. On the cell surface, GalTase associates with the cytoskeleton enabling it to function as a cell adhesion molecule by binding to its oligosaccharide ligand on adjacent cell surfaces or in the extracellular matrix. One of the best studied examples in which GalTase functions as a cell adhesion molecule is during fertilization in the mouse. GalTase is expressed on the dorsal, anterior aspect of the sperm surface, where it mediates gamete recognition by binding selectively to terminal *N*-acetylglucosamine residues on the egg coat glycoprotein ZP3. After initial binding, ZP3 aggregates a receptor, possibly GalTase, which activates G-protein-dependent exocytosis of the sperm acrosome, or acrosome reaction. As a consequence of the acrosome reaction, the area of plasma membrane containing GalTase is lost; however, GalTase is retained on acrosome-reacted sperm by migrating to the lateral surface of the sperm head, where it may stabilize the initial binding of acrosome-reacted sperm to the zona. The acrosome reaction results in the release of β-*N*-acetylglucosaminidase from sperm, which is required for penetration through the zona pellucida, presumably due to its ability to remove exposed GalTase-binding sites on zona glycoproteins that would impede zona penetration. Following fertilization, the sperm-binding activity of ZP3 is destroyed by β-*N*-acetylglucosaminidase released into the perivetilline space from egg cortical granules. This enzyme removes the GalTase-binding site on ZP3, preventing additional sperm from binding to the zona, thus blocking polyspermy. The cloning of GalTase cDNAs has allowed the production of transgenic mice that overexpress surface GalTase. Sperm from these mice have elevated surface GalTase activity and are hypersensitive to ZP3-induction of the acrosome reaction, rendering them unable to remain bound to the zona pellucida. Since GalTase is found on the surface of all mammalian sperm assayed thus far, it may function during fertilization in other species similar to that in mouse. Supported by grants from the National Institutes of Health to B.D.S.

Physiology-Normal and Abnormal Processes Involving Complex Carbohydrates

U 018 MAMMALIAN DEVELOPMENTAL POTENTIAL IN THE ABSENCE OF COMPLEX ASN(N)-LINKED OLIGOSACCHARIDE STRUCTURES, Martina Metzler[1], Anita Gertz[1], Mohan Sarkar[2], Harry Schachter[2], John W. Schrader[1] and Jamey D. Marth[1].
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To gain insight into the role that complex N-linked oligosaccharide structures play in mammalian ontogeny, the *Mgat-1* gene, encoding β-1,2-*N*-acetylglucosaminyltransferase I (GlcNAc-TI), was mutated by means of homologous recombination in embryonic stem cells. GlcNAc-TI activity in the medial Golgi apparatus is known to be required for the synthesis of complex N-linked oligosaccharides on the processed high mannose core. Mice heterozygous for the *Mgat-1* mutation were phenotypically normal and were interbred to produce homozygous *Mgat-1*-null embryos. Loss of *Mgat-1* function did not interfere with pre-implantation embryo development, yet was lethal in embryogenesis, thus establishing a requirement for complex N-glycans in mouse ontogeny. Remarkably, post-implantation development proceeded until day 10.5 with the differentiation of multiple structures and cell types. *Mgat-1*-null embryo extracts were devoid of GlcNAc-TI activity and were deficient in complex N-glycans. Binding of the lectin Ricin, which recognizes asialo- tri- and tetra-antennary oligosaccharide structures, was dramatically reduced and cells from *Mgat-1*-null embryos lacked the ability to aggregate in response to L-phytohemagluttinin, which binds strongly to Gal-ß-1,4-GlcNAc-ß-1,2-Man structures. Mouse embryos homozygous for the *Mgat-1* mutation were growth retarded and harbored abnormalities in neural tube formation and vascularization. Interestingly, a randomization of body plan asymmetry occurred in the absence of GlcNAc-TI activity as a *situs inversus* phenotype was also observed. While specific morphogenic processes were impaired, cellular differentiation appeared to occur normally and included the production of multiple hemopoietic cell types that responded appropriately to specific cytokines. These studies demonstrate that complex Asn-linked glycosylation plays a fundamental role in specific developmental events in mouse ontogeny, such as morphogenesis, and may not influence cellular differentiation *per se*.

Cell Biology -Glycoconjugates in Cellular Machinery Regulation

U 019 ROLES OF POLY-*N*-ACETYLLACTOSAMINES IN DEVELOPMENT AND CANCER: A MOLECULAR APPROACH
Minoru Fukuda, Ritsuko Sawada, Kentaro Maemura, Shigeru Tsuboi, Assou El-Battari, Shinobu Sueyoshi, and Marti Bierhuizen. Glycobiology Program, La Jolla Cancer Research Foundation, Cancer Research Center, La Jolla, California USA.

Structural studies on carbohydrates attached to proteins and lipids revealed extreme variety in those molecules. At the same time, another important finding is that specific sets of carbohydrates are characteristic to certain types of cells. Our assumption is that these cell-type specific carbohydrates play a role in cell-cell interaction. In the past years, we have been particularly interested in the structure, biosynthesis and possible roles of poly-*N*-acetyllactosamines. Because poly-*N*-acetyllactosamines have long side chains, they tend to provide a favorable backbone for cell-type specific modification. In fact, one such modification, sialyl Le^x structure originally discovered on granulocytes by us, was found to be a ligand for E- and P- selectin.

In order to understand the roles of poly-*N*-acetyllactosamines in cell recognition, we are taking two different research directions. The first is to focus on the glycoprotein carriers that contain poly-*N*-acetyllactosamines. We found that lysosomal membrane glycoproteins, lamp-1 and lamp-2, are the major carriers for poly-*N*-acetyllactosamines. We found that highly metastatic colonic cells express more lamp-1 and lamp-2 on the cell surface, which leads into higher efficiency in their adhesion to E-selectin. Moreover, our studies demonstrated that the increased amount of lamp-1, achieved by genetic manipulation of lamp-1 expression, leads into stronger adhesion to E-selectin-expressing cells and this adhesion can be inhibited by soluble lamp-1 containing sialyl Le^x structure.

The second approach is to change the amount of poly-*N*-acetyllactosamines by genetic manipulation of glycosyltransferases, which are critical for poly-*N*-acetyllactosamine formation. Recently, we have cloned cDNA encoding core 2 β-1,6-*N*-acetylglucosaminyltransferase, C2GnT, that forms core 2 branchings in O-glycans. When we express this enzyme in CHO cells, which *lack C2GnT, we found* that the CHO cells express not only branched O-glycans but also poly-*N*-acetyllactosaminyl O-glycans. Moreover, CHO cells expressing both C2GnT and leukosialin present T305 antigen, which is abundantly present in immature thymocytes. The increase of the T305 antigen is associated with leukemia, immunodeficiency syndromes such as the Wiskott-Aldrich syndrome and AIDS. These results suggest that the conversion of the hexasaccharide to tetrasaccharide is critical for thymocyte (T-lymphocyte) maturation. Further studies to knock-out the core 2 enzyme in thymus will be expected to yield critical information on the roles of *O*-glycans in thymus development. (Supported by grants CA33000, CA33895, CA48737, and AI33189).

Complex Carbohydrates in Disease Conditions-From Mouse to Man

U 020 SELECTIN BLOCKADE IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY, Allan M. Lefer, Andrew S. Weyrich and Michael Buerke, *Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107.*

The selectin family of adhesion molecules play an important role in the regulation of leukocyte adhesion to the coronary vasculature in disease states. Early rolling of polymorphonuclear (PMN) leukocytes appear to be regulated, particularly by P-selectin (upregulated) and expressed on the endothelial cell surface as well as by L-selectin constitutively present on PMN cell membranes. P-selectin is maximally upregulated 20 minutes following reperfusion whereas E-selectin requires 4 hours. We have studied a variety of selectin blocking agents (i.e., monoclonal antibodies [MAbs] to the selectins, sialyl Lewis^X oligosaccharide) in a feline model of myocardial ischemia (90 minutes) *followed by reperfusion (270 minutes). The MAbs directed against either P-selectin (PB1.3) or L-selectin (DREG-200) and the* oligosaccharide (OS) exerted significant cardioprotection and endothelial preserving effects as well as pronounced anti-adherence effects.

Variable	Non-blocking MAb or OS Control	PB1.3	DREG-200	OS
Necrosis Index	35 ± 3	15 ± 2**	12 ± 2**	8 ± 2**
Degree of Endothelial Dysfunction	72 ± 6	12 ± 3**	41 ± 3*	20 ± 4**
Adhesion Index	132 ± 10	40 ± 9**	52 ± 8**	21 ± 7**

Significance *p<0.05 **p<0.01 from control

None of the MAbs or OS produced a significant alteration in the pressure x rate product, indicating no significant change in myocardial oxygen demand, nor did any treatment significantly induce a leukopenia during the ischemia reperfusion protocol. MAb PB1.3 immunohistochemically localized only in the coronary arterial and venous endothelium, and DREG-200 bound only to leukocytes using flow cytometry and did not bind to the coronary vascular endothelium. These results indicate that interference with selectin-mediated PMN-endothelial cell interaction is an effective and appropriate therapeutic approach to attenuate reperfusion injury at the time of either coronary angioplasty or thrombolysis.

U 021 SELECTIN BLOCKADE IN MODELS OF ACUTE LUNG INJURY, Peter A. Ward and Michael S. Mulligan, Department of Pathology, University of Michigan, Ann Arbor, Michigan.

Three models of acute lung injury in rats include systemic (intravascular) activation of complement by cobra venom factor (CVF) and intrapulmonary deposition of IgG or IgA immune complexes. In the IgG model, neutrophil recruitment, together with cytokine production by lung macrophages, is a prominent feature, whereas in the IgA immune complex model the effector cells appear to be residential lung macrophages and the small numbers of neutrophils recruited into lung are irrelevant to injury. Mediator requirements have been defined by the use of antibodies to selectins, and by the use of selectin Ig chimeras and infusion of sialyl Lewis^X (SLX), which blocks selectin-dependent adhesion pathways both in vitro and in vivo. Using these approaches, requirements for selectins have been identified in each of these three models of lung injury. In injury following infusion of CVF, both injury (defined permeability increase and hemorrhage) as well as tissue increase in myeloperoxidase (MPO) have been shown to be P- and L-selectin dependent but independent of a requirement for E-selectin. This pattern would be consistent with the rapid and early termination (at 30 min.) of intravascular events leading to acute lung vascular injury. In the IgG immune complex model, requirements for both E- and L-selectin have been identified, whereas P-selectin is not required. In this model the endpoint of injury is 4 hr. and production of IL-1 and TNFα is prominent. Upregulation of endothelial E-selectin is linked to cytokine elaboration while L-selectin also appears to be necessary for neutrophil recruitment into lung. In the IgA immune complex model in which injury depends on effector functions of lung macrophages, the reaction is independent of any selectin requirement, in keeping with the fact that recruitment of blood leukocytes apparently does not occur. These studies suggest that, within the context of lung inflammatory injury, selectin requirements vary with the nature of the inciting stimulus and the type of inflammatory cells involved.

Complex Carbohydrates in Human Pathobiology and Clinical Studies

U 022 DETECTION OF GLYCOSYLATION ABNORMALITY IN RHEUMATOID IMMUNOGLOBULIN G USING *N*-ACETYLGLUCOSAMINE SPECIFIC LECTIN, Akira Kobata[1] and Tamao Endo[2], [1]Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173 and [2]Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, JAPAN.

Human IgG is composed of two types of polypeptide chains: heavy (H) and light (L) with a stoichiometry of H_2L_2. IgG is a glycoprotein and two complex-type sugar chains are linked at Asn^{297} residue of the two H chains. Structural study of the sugar chains of IgGs purified from sera of healthy individuals revealed that the biantennary sugar chains with high microheterogeneity are included. Despite this extremely high multiplicity, the molar ratio of each oligosaccharide of healthy individuals is quite constant. Compared to the carbohydrate structures of serum IgGs from healthy individuals, the IgGs from patients with rheumatoid arthritis (RA) have an abnormally high percentage of those lacking terminal galactose residues(1). Analysis of the sugar chains of IgG samples from sera of more than fifty arthritis patients revealed that the reduced galactosylation of the sugar chains is characteristic to this disease. Absence of galactose residues in the sugar chains of serum IgGs from RA patients are due to the altered galactosyltransferase activity in B cells(2). In order to clarify the relevance of IgG glycosylation change to the development of arthritis, it is required to develop a rapid and sensitive method to detect nongalactosylated IgG, because conventional structural analysis of the sugar chains of purified IgG is tedious, complicate and time consuming. A novel *N*-acetylglucosamine binding lectin (PVL) was isolated from *Psathyrella velutina*. This lectin preferentially reacts with the GlcNAcβ1→2Man group which is exposed at the termini of nongalactosylated IgG(3,4). A new simple method to detect the amount of agalactoIgGs in serum samples, which can be used in a clinical laboratory, was established with use of the lectin (5).

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U 023 EVOLUTION OF AN ANTI-TUMOR AGENT FROM A NEONATAL PATHOGEN, Carl G. Hellerqvist[1], Gary B. Thurman[1], Bruce A. Russell[1], Gerald E. York[1], Yue-Fen Wang[1], David L. Page[2], and Hakan W. Sundell[3], Vanderbilt University, Departments of Biochemistry[1], Pathology[2] and Pediatric Neonatology[3], Nashville, Tennessee.

GBS Toxin, or CM101, is a polysaccharide exotoxin produced by Group B Streptococcus (GBS). This organism causes sepsis and respiratory distress in human neonates (so called "early onset disease"). This disease is marked by a strong inflammatory response only in the lung, with pulmonary sequestration of granulocytes and extensive capillary endothelial damage, and occurs only during the first few days after birth. We have found that a similar inflammatory response can be induced by i.v. infusion of picomol quantities of GBS Toxin in the developing vasculature of transplanted tumors in mice which can significantly retard the tumor growth. When optimum treatment with GBS Toxin was started shortly after tumor implantation, a majority of tumors in the mice regressed and the mice remained tumor-free for over five months. Some tumors regressed in mice receiving short-term treatment with GBS Toxin, but recurred after the treatment was stopped. Median survival times were extended by all regimens and all doses of GBS Toxin tested. No evidence of toxicity to the vasculature of other tissues was observed. Mechanistically, data suggests that GBS Toxin binds to developing neovasculature and induces release of chemokines which engages both macrophages and granulocytes based upon the lymphokines released. In addition, C3 binds to bound GBS Toxin effectively opsonizing the tumor endothelium. GBS Toxin is being tested for cancer therapy in humans. (Sponsored by CarboMed, Inc.)

Late Abstracts

POTENTIAL FOR EXTRACELLULAR HYDROLYSIS OF GLYCOPROTEIN OLIGOSACCHARIDES BY CHINESE HAMSTER OVARY CELL SIALIDASE AND FUCOSIDASE, Charles F. Goochee[1], Michael J. Gramer[1], David V. Schaffer[1], and Mary B. Sliwkowski[2], [1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305-5025 and [2]Department of Cell Culture and Fermentation R&D, Genentech, Inc., South San Francisco, CA 94080

To assess the potential for extracellular hydrolysis of glycoprotein oligosaccharides by the sialidase and fucosidase activities of Chinese hamster ovary (CHO) cells, we have determined the substrate specificity, stability, and mechanism(s) of release for each of these enzymes. These data indicate that CHO sialidase is a cytosolic enzyme which is released through damage to the cellular membrane. The CHO sialidase readily hydrolyzes sialic acid from glycoprotein oligosaccharides. The CHO fucosidase is a lysosomal enzyme which can be released either by secretion or by damage to cellular membranes. The CHO fucosidase hydrolyzes fucose from a variety of oligosaccharide substrates including the oligosaccharides released from CHO-produced protein, gp120. However, CHO fucosidase was unable to release fucose from intact CHO-produced glycoproteins. These results indicate that the oligosaccharides of glycoproteins secreted by CHO cells can potentially be modified extracellularly by CHO sialidase. However, the CHO fucosidase is unlikely to significantly modify the oligosaccharides of secreted glycoproteins.

ONCODEVELOPMENTAL EXPRESSION OF COMPLEX CARBOHYDRATES. Suzanne Laferté[1], Nicole L. Prokopishyn[1], Terence Moyana[2] and Ranjana P. Bird[3], Departments of [1]Biochemistry and [2]Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0 and [3]Department of Foods and Nutrition, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Altered expression of ABH blood group substances is a common feature of human colorectal carcinoma, yet it remains unclear how these structural changes influence the biological properties of tumor cells. Azoxymethane-induced rat colon tumors display many features of the human disease, thereby providing a potentially useful model to study the role of blood group substances in colon cancer progression. We have prepared monoclonal antibodies to a microsomal fraction isolated from an azoxymethane-induced rat colon tumor and selected an antibody which detects cancer-associated changes. Monoclonal antibody (MAb) 3A7 recognizes a determinant on type 2 chain blood group A (GalNAcα1-3[Fucα1-2]Galβ1-4GlcNAc-R) and B (Galα1-3[Fucα1-2]Galβ1-4GlcNAc-R) oligosaccharides whose expression is ondevelopmentally-regulated in rat colon. Immunohistochemical staining and Western blotting analyses of azoxymethane-induced colon tumors revealed increased expression of the epitope in all of the 21 colonic tumors examined, including pre-neoplastic glands within transitional mucosa. The epitope was also expressed in blood group A positive human colon carcinoma cell lines, including HT29 and SW480 but not by SW620, a cell line derived from a lymph node metastasis isolated in vivo from the SW480 primary tumor, or in the blood group B cell line SW1417. The glycoproteins detected by MAb 3A7 in rat colon tumors and HT29 cells ranged in size between 50 and 200 kDa, including a major species of 140 kDa. Affinity chromatography of detergent lysates of normal rat colon on the blood group A specific lectin *Dolichos biflorus* (DBA)-agarose resulted in nearly quantitative binding of glycoprotein species detected by the antibody. In contrast, immunoreactive glycoproteins from rat colon tumors or HT29 cells bound poorly to DBA-agarose but were retained by another blood group A binding lectin, *Helix-pomatia* (HPA)-agarose. These results indicate that colon carcinogenesis results in quantitative as well as qualitative changes in oligosaccharides detected by MAb 3A7 and suggest that the combined use of MAb 3A7 and blood group A-specific lectins may provide a useful tool for early detection of colon cancer.

Complex Carbohydrates and Infection

U 100 Glycosylation Patterns of Human Proteins Expressed in Transgenic Goat Milk, Edward S. Cole, Elizabeth Higgins, Rick Bernasconi, Louise Garone Tim Edmunds, GENZYME Corp. 1 Mountain Rd., Framingham, MA, 01701-9322

Production of therapeutic proteins in the milk of transgenic animals can be achieved at very high (g/liter) expression levels compared to tissue culture (mg/liter). Using a combination of electrospray mass spectrometry, monosaccharide analysis and Fluorophore Assisted Carbohydrate Electrophoresis (FACE) we have compared the glycosylation profile of two human proteins, antithrombin III (ATIII) and an engineered form of tissue plasminogen activator (LA-tPA) expressed in transgenic goat milk. Both transgenic proteins were found to contain a significant amount of GalNAc present on complex N-linked oligosaccharides. From LC/MS and monosaccharide analysis it appears that a substitution of GalNAc for galactose residues occurs. This substitution appears to be a function of expressing the proteins in the mammary gland and not a species difference as goat plasma ATIII does not contain this substitution. Both transgenic proteins are more fucosylated and less sialylated than their recombinant (LA-tPA) or plasma (ATIII) counterparts. N-glycolylneuraminic (NGNA) as well as N-acetylneuraminic (NANA) acid were found on both transgenic proteins as well as goat plasma ATIII. The presence of NGNA therefore appears to be a function of expressing the protein in goats. High mannose and/or hybrid structures were found on at least one site in each transgenic protein where as only complex structures were observed in the recombinant and plasma proteins. The effect of these substitutions on activity and clearance in the proteins is being investigated.

U 101 ROLE OF THE TNF-ALPHA LECTIN-LIKE DOMAIN IN ITS TRYPANOLYTIC ACTIVITY IN VITRO.

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According to previously reported studies, recombinant cytokines like IL-1, IL-2 and TNF-alpha possess at least two unique binding sites, one directed at target cell surface receptors and a recently evidenced lectin-like binding site [1].
These two binding activities seem to be topographically distinct on the molecules of TNF-alpha as evidenced using specific antibodies and site directed mutagenesis.
We report here that the lectin-binding activity of TNF-alpha appears to be able to modulate specifically the trypanolytic activity in a way that seems independant from the TNF-alpha cell surface peptidic receptor binding site.
We have investigated some of the lectinic properties of this interaction using a panel of glycoconjugates. Chitobiose and N-acetylglucosamine terminating glycoproteins have the maximum effect as inhibitors of the trypanolytic activity towards *Trypanosoma brucei brucei* (T. br. br.), and no effect on the tumorilytic activity on murine L929 fibrosarcoma cells.
Preliminary results using partially purified WGA fractions from T. br. br. extracts reveal that material interacting with the lectin can efficiently elicit the trypanolytic effect of TNF-alpha in vitro.
We are curently investigating the possible endogenous structure(s) that are involved in the specific binding with the TNF-alpha lectin site.
These results may lead to the possibility of molecular engineering TNF-alpha molecules with improved trypanolytic activities and open a new avenue to the understanding of the mechanism of action of cytokines.
WGA : Wheat Germ Agglutinin .

[1] Muchemore et al. Cancer Res., 1990, 50, 6285-6290).

U 102 Tissue Specific and Species Differences in the Glycosylation Pattern of Antithrombin III, Tim Edmunds, Elizabeth Higgins, Rick Bernasconi, Louise Garone, Edward S. Cole,, GENZYME Corp. 1 Mountain Rd., Framingham, MA, 01701-9322

Antithrombin III contains four N-linked glycosylation sites at Asn 96, 135,155 and 192. In the human plasma protein the glycosylation at all four sites is identical with the predominant glycoform being a biantennary disialylated structure with minor amounts of monosialylated and asialo structures. We have carried out a detailed oligosaccharide analysis of human plasma ATIII (tgATIII) produced in the milk of transgenic goats and compared the glycosylation found to that of both human and goat plasma derived ATIII (pATIII). The extent and type of heterogeneity at each site was determined by LC/MS analysis of a Lysyl endopeptidase digest. Structural predictions have been made based on LC/MS, monosaccharide, and Fluorophore Assisted Carbohydrate Analysis (FACE). Significantly more heterogeneity at each glycosylation site was observed for the tgATIII than either of the two plasma proteins. The major difference observed is in the degree of sialylation with the transgenic protein being significantly less sialylated than plasma ATIII. Another difference is the presence of fucose in the transgenic protein. Biantennary complex structures were found at 3 of the 4 glycosylation sites in tgATIII with the fourth site (Asn 155) containing High mannose/Hybrid structures.

U 103 EXPRESSION OF DIFFERENTIALLY GLYCOSYLATED CD43 ISOFORMS IN MURINE GRAFT-VERSUS-HOST DISEASE. L.G. Ellies, A. Tomlinson Jones and H.J. Ziltener. The Biomedical Research Centre, University of British Columbia, Vancouver, Canada.

Two distinct isoforms of CD43 result from differential glycosylation of a single gene product. CD43L (light), the 115 kDa isoform carries mainly tetrasaccharides and is a pan specific T cell marker. CD43H (heavy), the 130 kDa isoform carries mainly hexasaccharides and is associated with T cell activation. CD43 has been shown to play a role both in enhancing and inhibiting cell adhesion, however, the function of the individual isoforms is unknown. To investigate the distribution and regulation of the CD43 isoforms in the $CD4^+$ and $CD8^+$ T cell subsets in an in vivo immune response, we have examined the expression of CD43L and CD43H in a murine model of acute graft-versus-host disease (GVHD). F1 hybrid (C57BL/6 x DBA-2) mice were injected i.v. with 10^8 C57BL/6 splenocytes. At time intervals up to 21 days, single cell suspensions of the spleens were prepared and stained with monoclonal antibodies (mAbs) to CD43L (S7 mAb) and CD43H (1B11 mAb), and a control mAb. FACScan analysis of T cells showed that resting $CD4^+$ T cells expressed CD43L but not CD43H while resting $CD8^+$ T cells expressed CD43L and low levels of CD43H. A marked increase in CD43H expression occurred during GVHD from day 2 onwards in both T cells subsets and coincided with splenomegaly and the up-regulation of the activation markers IL-2Rß and ICAM-1. Upon activation, both CD43L and CD43H were up-regulated in $CD4^+$ T cells. In contrast, in $CD8^+$ T cells CD43L was down-regulated while CD43H was dramatically up-regulated such that two distinct subsets were observed, $CD43H^{lo}$ and $CD43H^{hi}$. These data show different patterns of expression of the CD43 isoforms both in normal and activated $CD4^+$ and $CD8^+$ T cells and suggest that glycosylation differences between the CD43 isoforms may reflect participation in the different functions of these T cell subsets.

U 104 OLIGOSACCHARIDE PATTERN OF HIV-2 GP120: DEPENDENCE ON HOST CELLS AND VIRUS ISOLATES,
H.Geyer[1], S.Liedtke[1], M.Adamski[2], H.Rübsamen-Waigmann[2] and R. Geyer[1], [1]Biochemisches Institut, Universität Giessen, D-35392 Giessen, [2]Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus, D-60596 Frankfurt, FRG
Three HIV-2 strains (HIV-2_{ROD}, HIV-2_{ALT}, HIV-2_{194}) differing in their biological properties were propagated in different host cells (MOLT4, HUT78, U937, macrophages, periperal blood lymphocytes). Following metabolic radiolabeling with [^{3}H]glucosamine, viral glycoproteins were isolated and purified. Carbohydrate moieties were sequentially released from tryptic glycopeptides by endo H and *N*-glycanase and fractionated according to charge and size by several HPLC methods. Resulting chromatographic data revealed that gp120 from different virus isolates, propagated in the same host cells, yielded very similar glycan pattern, whereas cultivation of an isolate in different host cells led to divergent oligosaccharide profiles. Hence, HIV-2 gp120 glycosylation clearly depends on the glycosylation potential of the host cell used for virus multiplication.

U 105 STUDIES ON THE STRUCTURE AND FUNCTION OF A NOVEL CARBOHYDRATE ANTIGEN OF *TRYPANOSOMA CRUZI*.
Paul A. Haynes and George A.M. Cross, Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Ave, New York, New York, 10021

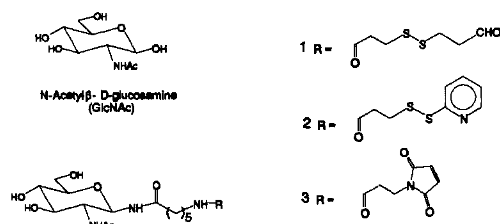
The monoclonal antibody WIC29.26 recognises a carbohydrate antigen, originally identified on GP72, a 72 kDa phosphoglycoprotein of *Trypanosoma cruzi*. The antigen is present in the epimastigote, insect vector life cycle stage, and may play a regulatory role as the antibody can inhibit the differentiation of epimastigotes to human-infective trypomastigotes. We have purified, from epimastigote lysates, a 20 kDa pronase-resistant glycopeptide, which carries the WIC29.26 determinant. Compositional analysis of the carbohydrates present on the 20 kDa glycopeptide confirms their unusual nature, with galactose and mannose as major constituents, and fucose, xylose and rhamnose also present. The galactose does not appear to be present in any non-reducing terminal position as glycopeptides show no reaction with peanut agglutinin lectin. Oligosaccharides that still contain the WIC29.26 epitope can be released from the glycopeptide by base-catalysed ß-elimination and reduction. The released oligosaccharides have monosaccharide composition very similar to the starting material, indicating most of the carbohydrate is present in base-labile linkages. Oligosaccharides that do not contain the epitope can be released from the glycopeptide by very mild acid treatment, suggesting they may be present in phosphodiester linkages. Further characterisation of oligosaccharides present on the glycopeptide, using chromatographic profiling and enzymic mapping, will be presented.

U 106 STRUCTURE-FUNCTION STUDIES ON CARBOHYDRATE LIGANDS OF THREE SELECTINS. MODIFICATIONS TO FUCOSE, SIALIC ACID AND SULFATE AS A SIALIC ACID REPLACEMENT.
Yuko Oda*, Makoto Kiso@, Saeed Abbas*, Pandarung Nikrad#, Om Srivasatava#, Carrol Foxall*, Akira Hasegawa@, Brian K. Brandley*
*Glycomed Inc. Alameda CA 94501, #Alberta Research Council, Alberta, Canada, @Gifu University, Gifu, Japan

The selectins are a family of carbohyrate binding proteins that have been implicated in the initial interaction between leukocytes and the vascular endothelium. The three members of this family will bind to the sialyl-Lewis X epitope(Siaα2-3 Galβ1-4(Fucα1-3) GlcNAc) and related oligosaccharides. In this report we examine the molecular details of that recognition using synthesized carbohydrates with specific modifications on the sialyl-Lewis X epitope. E- and L-selectin require hydroxyl groups at the 2, 3, and 4 positions of the fucose residue. P-selectin, however, requires only the 3-position hydroxyl group, while tolerating removal of the hydroxyl group at positions 2 or 4 of fucose residue. Modifications of the glycerol side chain or the N-acetyl group of the sialic acid have little effect on the binding of any of the selectins. All three selectins bind efficiently to an oligosaccharide with a sulfate replacement for the sialic acid. For E-selectin, binding to sulfo-Lewis X appears to be equivalent to binding to sialyl-Lewis X, while for L- and P-selectin binding to the sulfated structure show characteristics distinct from sialyl-Lewis X recognition. These data indicate that while all three selectins can recognize sialyl-Lewis X, E-, L-, and P-selectin each display distinct carbohydrate ligand preferences.

U 107 SITE SPECIFIC CHEMICAL MODIFICATION OF A STREPTOCOCCAL ANTIBODY BINDING SITE,
J. Bruce Pitner, C. Preston Linn, Patrick D. Mize†, and Douglas P. Malinowski, Becton Dickinson Research Center, P.O. Box 12016, Research Triangle Park, NC 27709. †Current address: Ensys, Inc. P.O. Box 14063, Research Triangle Park, NC 27709.

An IgG3 monoclonal antibody to *streptococcus* Group A polysaccharide antigen was modified using an antigen-based reagent (**1**) by reductive alkylation of a single lysine residue near the putative antigen binding site. After reductive cleavage of the disulfide bond incorporated in the resulting reagent-antibody complex, an antibody with a reactive thiol group near the binding site was obtained. Binding affinity of this modified antibody toward the immunodominant monosaccharide N-acetyl glucosamine (GlcNAc) is comparable to the native antibody but there is a significant increase in affinity for two derivatives (**2** and **3**) which can interact covalently with the modified antibody. These experiments suggest the affinity of non-catalytic protein binding sites for their respective ligands can be enhanced dramatically by adding covalent interactions to the existing non-covalent binding forces using site-specific chemical techniques.



U 108 α1,3 and α1,3/1,4 fucosyltransferase-dependent synthesis of L-selectin ligands, sialyl Lewis x and sialyl Lewis a, in cultured endothelial cells.
Risto Renkonen, Marja-Leena Majuri, Mari Pinola Ritva Niemelä, Sinikka Tiisala, Jari Natunen, Ossi Renkonen.
Departments of Bacteriology and Immunology, Pathology and Institute of Biotechnology, University of Helsinki

L-selectin, which is expressed only on leukocytes, recognizes α2,3 sialylated and α1,3/1,4 fucosylated ligands on endothelial cells. We and others have previously shown that lymph node high endothelial cells express sialyl Lewis x and sialyl Lewis a (sLex and sLea). Here we provide evidence that also cultured human umbilical vein endothelial (HUVE) cells express both sLex and sLea on their cell surface and that the expression of at least the latter can be further enhanced by cytokine stimulation. There are several human α1,3 and α1,3/1,4 fucosyltransferases participating in the generation of these L-selectin ligands and we show that mRNA of four of them is present in HUVE cells. In functional assays α1,3 or α1,3/1,4 fucosyltransferase activities were observed with both neutral and acidic acceptors, leading to the generation of Lex and sLex/sLea sequences, respectively. The level of mRNA expression of two α1,3 fucosyltransferases (FT V and FT VI), and the activity of α1,3 and α1,3/1,4 fucosyltransferases could be enhanced by TNF-stimulation of HUVE cells.

Taken together these data show that cultured endothelial cells express *sialylated* Lewis epitopes known to be recognized by L-selectin, they possess mRNA as well as enzyme activities of several α1,3 or α1,3/1,4 fucosyltransferases necessary in the final step of sLex/sLea synthesis, and inflammatory cytokines such as TNF can further enhance fucosyltransferase activities relevant in generating putative L-selectin ligands.

U 109 THE STRUCTURAL ANALYSIS OF SHORT CHAIN LIPOPOLYSACCHARIDE, Wayne B. Severn, Rosemary A. Z. Johnston, Robert J. Kelly and James C. Richards, Institute for Biological Sciences, National Research Council of Canada and the Canadian Bacterial Diseases Network. Ottawa, Ontario, K1A 0R6, Canada

The cell surface of gram-negative bacteria is composed of a number of complex outer membrane proteins, lipopolysaccharides (LPS) and extracellular polysaccharides. It has now been established that LPS represent one of the major cell surface antigens and they are critical components in the immune response to gram-negative bacteria. An understanding of the precise structure, biosysthesis and expression of the LPS is essential to identifying surface-exposed epitopes available for host pathogen interactions and for the development of a carbohydrate-based vaccines.

LPS are amphipathic molecules which are difficult to purify and characterize. Classical approaches to the characterization of core oligosaccharide (OS) samples have relied on the selective cleavage of the acid labile KDO linkage to produce lipid A and a free OS with KDO at the reducing terminus. Inherent in this procedure is the loss of sequence information concerning additional KDO residues and the location of phosphate groups.

In this report we describe our recent work and analytical strategies in the use of amino benzoate ethyl esters (ABEE) for the preparation, separation, and structural determination of glycolipids derived from bacterial LPS. To prepare purified oligosaccharides, chromatographic and chemical techniques have been developed that include high-pH anion-exchange chromatography and reverse-phase chromatography. Using nuclear magnetic resonance spectroscopy and mass spectrometry the structures of the LPS from *Pasteurella haemolytica* serotype A1 and *Klebsiella pneumonia* serotype O8 have been elucidated.

U 110 CHARACTERISATION OF CARBOHYDRATE MOEITIES OF EPITHELIAL CELLS IN THE MIDGUT OF BLOOD FEEDING DIPTERA. Simon Wilkins, Peter F. Billingsley and Richard P. Lane*, Dept. of Biology, Imperial College, London, UK, *Dept. of Entomology, Natural History Museum, London, UK.

Numerous parasite species are transmitted to the vertebrate host by blood feeding vectors; for example *Anopheles* and *Phlebotomus* are the insect vectors of malaria and leishmaniasis respectively. The recognition and attachment to the vector midgut are therefore essential for completion of the insect stages of the parasite life cycle. Studies have suggested that the motile *Plasmodium* ookinete can recognise carbohydrate moieties expressed on the mosquito midgut surface (Parasitol. Res. [1989] 75:268-279). Such complex carbohydrate molecules could be targeted as possible transmission blocking sites. This study sets out to examine the carbohydrates that may be involved in this interaction. Using SDS-PAGE and western style lectin blotting some of the oligosaccharides on midgut surface glycoproteins have been identified and partially characterised. A number of glycoproteins with high mannose oligosaccharides susceptible to PNGase-F and Endo-H treatments and complex-type oligosaccharides susceptible to PNGase-F were found on some glycoproteins. O-linked oligosaccharides susceptible to O-glycanase were present on a number of glycoproteins. The presence of terminal galactose and sialic acid has not been demonstrated.
Current studies involve i) the expression of midgut surface glycoproteins during digestion of the bloodmeal, and ii) comparisons with the *Phlebotomus* midgut, where vector-parasite interactions are of a different nature (Trop. Med. Parasitol. 37 [1986] 409-413).

U 111 CONSTRUCTION OF A SECRETED FORM OF RABIES VIRUS GLYCOPROTEIN. Boguslaw Wojczyk, Susan H. Shakin-Eshleman, William H. Wunner, and Steven L. Spitalnik. Dept. of Pathology and Laboratory Medicine, Univ. of Pennsylvania and Wistar Institute, Philadelphia, PA 19104.
Rabies virus glycoprotein (RGP) has 3 potential N-linked glycosylation sites. Asn247 and Asn319 are each efficiently glycosylated, while Asn37 is inefficiently glycosylated. RGP(sol), a soluble form of RGP, was produced by inserting a stop codon external to the transmembrane domain. When evaluated in a cell-free system, the glycosylation efficiency at each site was identical to that of full-length RGP. RGP(sol) was secreted by transfected CHO cells and immunoprecipitated by 8 conformation dependent monoclonal antibodies. Using chemical cross-linkers, we found that secreted RGP(sol) formed dimers and trimers. Secreted RGP(sol) was Endo H resistant, while the cell-associated glycoprotein was Endo H sensitive, indicating that secreted RGP(sol) has complex type N-glycans. Glycosylation inhibitors blocking initial processing, such as castanospermine and deoxynojirimycin, blocked secretion of RGP(sol). However, inhibitors of later processing steps, such as deoxymannojirimycin and swainsonine, did not affect secretion. No processing inhibitors altered cell surface expression of full-length RGP. By site-directed mutagenesis, we showed that in CHO cells a single N-glycan at either Asn37, Asn247, or Asn319 could individually support cell surface expression of RGP, while only an N-glycan at Asn319 could support secretion of RGP(sol). In summary, RGP(sol) is secreted by transfected CHO cells, it is structurally similar to the external domain of full-length RGP, and its *secretion* depends on the appropriate location, number, and type of N-glycan. This approach may be useful for producing a form of RGP amenable to analysis by structural methods such as x-ray crystallography.

U 112 CHARACTERIZATION OF THE ACTIVATION ISOFORM OF MURINE CD43, H.J. Ziltener, A. Tomlinson Jones, M.J. Williams, L.G. Ellies, B. Federsppiel, C. Anne Smith, and F. Takei. The Biomedical Research Centre and Terry Fox Laboratory, Vancouver B.C. Canada.

1B11, a rat IgG2a monoclonal antibody (mAb) recognizes a 130 kDa glycoprotein, expressed by myeloid cells and a small proportion of resting T-lymphocytes. We have identified the antigen as the high MW isoform of CD43 (Ly-48). The antigen is expressed by less than 25 % of resting $CD4^+$ T-lymphocytes and is present at low levels on 80-90 % of resting $CD8^+$ T-lymphocytes in the thymus and in peripheral lymphatic tissue. On activation, expression of the antigen is increased both on $CD4^+$ and $CD8^+$ T-lymphocytes, with maximal expression occurring after 72 hours of incubation in the presence of Con A. MAb S7, reactive with the low MW isoform of CD43 recognizes a 115 kDa molecule in immunoblots of splenic and thymic lysates and binds at high levels to both $CD4^+$ and $CD8^+$ T-lymphocytes. Expression of the 115 kDa isoform is not up-regulated in response to Con A activation, nor is there differential staining of the T-lymphocyte subsets. Both S7 and 1B11 mAb's bind to L-cells transfected with a cDNA encoding CD43. Interestingly, in immunoblots of lysates from transfected cells, mAb S7 shows reactivity with a broad band at 100-115 kDa, whereas mAb 1B11 recognizes proteins in the range of 115-135 kDa. Activation induced expression of a higher molecular weight isoform of CD43 has also been reported in the human system. Differences between the human CD43 isoforms have been attributed to alterations in O-linked glycosylation and increased expression of the activation associated CD43 isoform has been linked to diseases such as rheumatoid arthritis, AML, AIDS, graft versus host disease and the immune disorder Wiskott-Aldrich Syndrome. Our data show that expression of murine CD43 parallels the human system, enabling the establishment of animal models to study this molecule.

Complex Carbohydrates and Cancer

U 200 GDP-MAN-REQUIRING MANNOSYLTRANSFERASES FOR PROTEIN N-GLYCOSYLATION, Mudgapalli Ashok, Samir K. Roy and Inder K. Vijay, Department of Animal Sciences, University of Maryland, College Park, MD 20742

Five GDP-Man-requiring mannosyltransferases, localized in the ER, catalyze the transfer of Man to Dol-P-P-oligosaccharide intermediates during the assembly of $Glc_3Man_9GlcNAc_2$ precursor for protein N-glycosylation. Due to inherent instability and the lack of individual acceptor substrates, these enzymes have proven to be extremely difficult to purify. Preliminary observations indicated that one or more of these enzymes have a -SH group(s) at their active site. We have applied a novel strategy to identify these enzymes with the eventual goal of preparing molecular probes against them for studying their regulation. GDP-Man was added to a solubilized preparation of the rat mammary microsomes to load the active site of the individual enzymes. These groups were specifically labeled by 3-(N-maleimido-propionyl)biocytin tagging approach developed in our laboratory (Pukazhenthi *et al.* (1993) *J. Biol. Chem.* 268:6445-6452). Five polypeptides of M_r 27, 30, 45, 69 and 150 kDa were labeled. Among these, the 30 kDa polypeptide represents Man-P-Dol synthase, as identified by the product of its reaction and positive reaction to an anti-peptide antibody for the yeast Man-P-Dol synthase. The 69 kDa polypeptide represents $Man_2GlcNAc_2$-P-P-Dol synthase, as identified by the analysis of its product. This enzyme has been purified to homogeneity and monospecific polyclonal antibodies against this enzyme have been obtained. The enzymatic activities represented by other bands is currently under investigation. (Supported by N.I.H. grant DK19682 and the Maryland Agricultural Experiment Station).

U 201 IMMUNOGLOBULIN G GLYCOSYLATION CHANGES OCCUR IN RHEUMATOID ARTHRITIS AND PRIMARY SJOGREN'S SYNDROME BUT DUE TO DIFFERENT MECHANISMS. JS Axford, A Alavi, FC Hay, P Youinou* & A Bond. St George's Hospital Medical School, Division of Immunology, Cranmer Terrace, London SW17 ORE, UK & *Centre Hospitalier Regional et Universitaire de Brest, 29285 Brest Cedex, FRANCE

Oligosaccharides are of significant importance to glycoprotein function and variation in their composition has been postulated to be a mechanism whereby the fine tuning of glycoprotein function may be achieved, and furthermore, a number of disease associated oligosaccharide changes have been reported and their specific role in pathogenesis suggested. The galactose content of serum immunoglobulin G (IgG) is reduced in rheumatoid arthritis (RA) and similar findings have been suggested in systemic lupus erythematous (SLE) associated with Sjogren's syndrome (SS). This variation in immunoglobulin glycosylation could occur in two general ways: (1) by varying the number of glycosylation sites carried by the molecule and/or (2) by varying the oligosaccharide composition of the molecules attached to these sites. To determine whether serum IgG glycosylation changes are present in patients with primary SS and, if so, whether this is by the same process thought to occur in RA, serum IgG was purified using the DEAE method from 29 patients with active RA and 37 patients with active primary SS and the amount of terminal galactose (Gal) and N-acetylglucosamine (GlcNAc) determined by monosaccharide specific lectin binding.

IgG Gal level was significantly decreased in both the RA (-29% $p=0.001$) and in primary SS (-23% $p<0.005$) in comparison to a healthy population ($n=100$). In contrast, IgG GlcNAc level was significantly increased in RA (59% $p<0.001$), but there was no difference from controls in the primary SS population. The relationship between IgG GlcNAc and Gal levels was determined by regression analysis. A significant negative relationship was found in the RA population ($R=0.480$, $p<0.05$), which was not present in the healthy nor SS populations. The regression analyses were compared (95% confidence interval) and the RA population was found to be different from both the SS (0.211 to 0.983) and healthy (-0.682 to -0.190) populations, whereas the healthy and SS population were similar (-0.029 to 0.350).

These data imply that IgG glycosylation change in RA and SS occur by different mechanisms which may reflect the different biological processes in which they are involved. In RA, a reduction in immuno-globulin Gal level is associated with a concomitant increase in IgG GlcNAc level, whereas this is not demonstrated in primary SS.

U 202 MOLECULAR DYNAMICS SIMULATIONS OF HIGH MANNOSE OLIGOSACCHARIDES: POSSIBLE PATHWAYS FOR PROCESSING OF $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$. P.V. Balaji, P.K.Qasba & V. S.R.Rao, LMMB-NCI-NIH, 9000 Rockville Pk, Bethesda, MD 20892.
Molecular dynamics simulations of several high mannose type oligosaccharides were carried out on NCI-FCRDC's Cray Y-MP 8D/8128 supercomputer for 1ns with different initial conformations for an exhaustive search of the conformational space. Based on the preferred conformations of these oligomannoses, possible pathways for processing $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$ by α1,2-linkage specific mannosidases have been proposed which rationalizes the available experimental results. Unlike other oligomannoses, $Man_9GlcNAc_2$ prefers more than one distinct conformation around the core α1,6 linkage and plays an important role in determining the processing pathway. Results obtained suggest that the processing of the precursor oligosaccharide during N-linked complex and hybrid type glycan biosynthesis proceeds in a well defined pathway involving more than one α1,2-linkage specific mannosidase. These simulations show that the relative orientation of the Man-α1,2-Man fragment with respect to the previous residue(s) in each antenna of $Man_9GlcNAc_2$ is different and this may explain the differences in the specificities of α1,2-linkage specific mannosidases present in ER and Golgi. Thus in addition to the level of mannosidases in a cell, the conformation of the carbohydrate also will effect the production of a certain array of high mannose oligosaccharides. The specific intermediates thus generated are probably used for proper intracellular trafficking of glycoproteins. $Man_5GlcNAc_2$ simulation indicates that the addition of β1,2-GlcNAc to the α1,3-linked core mannose besides serving as a prerequisite for mannosidase II action, also prevents the removal of this core mannose. The conformations of the two α1,3 and two α1,6 linkages in any given oligomannose were found to be different suggesting that deriving oligosaccharide conformation based on the conformational preferences of the constituent disaccharide fragments will not always be correct. These simulations can be used in designing highly specific inhibitors of mannosidases which in turn have been shown to be potential therapeutic agents in several diseases.

U 203 CELL-CONTACT MEDIATED MODULATION OF CONTACTINHIBIN - SIALYLATION, C. E. Baumann, F. Oesch, R. J. Wieser, Institute of Toxicology, Obere Zahlbacher Str. 67, 55131 Mainz, Germany
Growth of non-transformed cells *in-vitro* is regulated by density-dependent mechanisms, leading to cessation of proliferation when cells reach confluency. Previously it has been shown that contactinhibin, a highly glycosylated plasma membrane protein with an apparent molecular mass of 60-70 kDa is involved in this contact-dependent inhibition of growth.
The biological active moiety resides exclusively within the N-glycosidically linked glycans. In addition, for an efficient inhibition of growth, unsialylated, terminal ß-glycosidically linked galactose residues have to be present on the N-glycans. The addition of only 10ng/ml of contactinhibin in immobilized form to sparsely seeded cells resulted in a reversible, 70-80% inhibition of growth. By culturing human fibroblasts in the presence of *anti* -contactinhibin-antibodies, cells grew to 2fold higher saturation density, together with an extensive focal and criss-cross, tumor - cell like growth pattern.
Here we show that in sparse cells the glycoprotein was present in biologically inactive, highly sialylated form both on the plasma membrane and intracellularily, while in confluent cells plasma membrane localized contactinhibin was expressed in biologically active, low sialylated form. Surprisingly, in confluent cells intracellular contactinhibin was still present in highly sialylated form. We have identified a novel cell surface sialidase which activates contactinhibin by unmasking of sialylated galactose residues. Our results point to a novel mechanism of cell contact-mediated *trans* - activation of sialylated cell surface glycoconjugates.

U 204 TYROSINE KINASE CONTROL OF *N* - ACETYLGLUCOSAMINYLTRANSFERASE V GENE EXPRESSION. Phillip Buckhaults, Nevis Fregien, Irene Margitich and Michael Pierce, Department of Biochemistry and Molecular Biology and Complex Carbohydrate Research Center, University of Georgia, Athens Georgia 30602 and Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, Florida 33101

N-Acetylglucosaminyltransferase V (GlcNAc-T V, EC 2.4.1.155) is the glycosyltransferase responsible for synthesizing the β(1,6) linked GlcNAc branch on tri and tetra-antennary asparagine-linked oligosaccharides. The specific activity of this enzyme is 7.5-fold higher in Rous sarcoma virus transformed BHK cells (RSBHK) when compared to untransformed BHK cells. Northern blots performed on total RNA and mRNA revealed a similar elevation of the steady state message levels for GlcNAc-T V. The tyrosine kinase activity of $pp60^{src}$ can be inhibited in vitro and in vivo by a fungal metabolite, herbimycin-A. Treatment of RSBHK cells with increasing amounts of herbimycin-A causes a dose dependent decrease in total cellular phosphotyrosine levels with maximum inhibition observed at 1.0 µg/ml. GlcNAc-T V specific activity is likewise decreased in a dose dependent manner in RSBHK cells with no effect seen on BHK cells. GlcNAc-T I specific activity is unaffected by either *src* transformation or herbimycin-A treatment. A similar reduction in GlcNAc-T V mRNA levels is observed as a result of herbimycin-A treatment in RSBHK cells while this effect is not observed on BHK cells. GlcNAc-T I mRNA levels are unaffected by *src* transformation or herbimycin-A treatment. An oncogenic cell surface receptor with tyrosine kinase activity, *neu*/*her-2* causes a 5-fold elevation in GlcNAc-T V mRNA levels when transfected into NIH 3T3 cells. These results indicate that a communication pathway exists between the phosphoaminoacid signalling network and GlcNAc-T V gene expression.

U 205 *Abstract Withdrawn*

U 206 INVESTIGATING THE DEVELOPMENTAL ROLE OF COMPLEX N-LINKED OLIGOSACCHARIDES USING HOMOLOGOUS RECOMBINATION, Robert M. Campbell, Martina Metzler and Jamey D. Marth, Biomedical Research Centre, U.B.C., 2222 Health Sciences Mall, Vancouver B.C., Canada, V6T 1Z3.

Although a diverse repertoire of N-linked glycans is expressed in mammals, the precise roles that particular oligosaccharide units play in development remain undefined. In this regard, we are using homologous recombination to inactivate specific enzymes that regulate this biosynthetic pathway. Previous work in our laboratory has revealed that mice lacking the enzyme N-acetylglucosaminyltransferase I (encoded by the *Mgat-I* gene) lack complex N-glycan structures and exhibit specific morphogenetic abnormalities. Such embryos fail to develop beyond embryonic day 9.5, the developmental stage at which we first detect complex N-glycan epitopes using lectin histochemical techniques in normal embryos. More recently, we are engaged in similar studies with the *Mgat-II* gene. Inactivation of the encoded enzyme N-acetylglucosaminyltransferase II (GNT-II) may permit the production of some mono-antennary complex N-glycan structures, since additions to the core β1-6 mannose could occur. Moreover, GNT-II deficiency is a marker of HEMPAS syndrome in humans, with associated anemia, hepatic cirrhosis, and diabetes. Although this would suggest that the minimal N-glycan structures required for mammalian development may be achievable in the absence of *Mgat-II* function, HEMPAS patients do appear to possess low levels of GNT-II. Since embryonic lethality in *Mgat-II* null mice is thus conceivable, we are using a recently developed technology to direct *Mgat-II* deletion to specific tissues as well as specific times in development. By including loxP sites in the targeting vector, gene inactivation events are dependent upon transgenic expression profiles of the Cre recombinase. In addition to avoiding embryonic lethality in homozygotes, this will allow the primary and secondary effects of GNT-II inactivation in particular tissues to be determined.

U 207 INTERFERON-α INDUCES L-SELECTIN EXPRESSION BY HUMAN B LYMPHOID CELLS, Sharon S. Evans, Michelle M. Appenheimer and Sandra O. Gollnick, Department of Molecular Medicine, Roswell Park Cancer Institute, Carlton and Elm Streets, Buffalo, NY 14263

The leukocyte adhesion molecule L-selectin plays a primary role in initiating normal and malignant lymphocyte migration from the blood into peripheral lymph nodes. L-selectin, a N-glycosylated surface protein, binds directly to sulfated glycoproteins on the luminal surface of high endothelial venules within peripheral lymph nodes. L-selectin expression is tightly regulated during lymphocyte development and maturation; however, little is known regarding the nature of the factors involved in the regulation of L-selectin synthesis and expression. In this report, the immunomodulatory cytokine interferon-α (IFN-α) was shown to markedly upregulate the surface density of L-selectin in (1) subpopulations of tissue-derived normal human B lymphocytes, (2) neoplastic B cells from chronic lymphocytic leukemia patients, and (3) the Burkitt's lymphoma-derived B lymphoid Daudi cell line. Other cytokines such as IFN-γ, tumor necrosis factor-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, and B cell growth factor did not affect L-selectin surface expression in the model Daudi B cell line. Upregulation of L-selectin surface density in IFN-α-treated Daudi B cells correlated directly with an increase in L-selectin mRNA steady state levels and enhanced L-selectin-dependent binding to a carbohydrate-based ligand, phosphomannan core polysaccharide (PPME). IFN-α induction of L-selectin mRNA and classical IFN-α-stimulated genes (ISG) exhibited similar characteristics including rapid kinetics of induction, high maintained levels in IFN-α-sensitive but not IFN-α-resistant Daudi cell lines, protein-synthesis-independent induction, and high sensitivity to inhibitors of tyrosine kinase activity. These data strongly implicate IFN-α and/or the IFN-α signal transduction pathway in regulating L-selectin synthesis and expression by human B lymphoid cells and provide insight into the mechanisms whereby IFN-α influences normal and malignant lymphocyte recirculation during therapy. (Supported by NIH grant CA46645).

U 208 REGULATORY MECHANISMS FOR THE EXPRESSION OF GANGLIOSIDES IN HUMAN CANCER CELLS, Koichi Furukawa, Shuji Yamashiro, Keiko Furukawa, Kenneth O. Lloyd[‡] and Hiroshi Shiku. Department of Oncology Nagasaki University School of Medicine, Nagasaki 852 Japan, [‡]Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

In order to analyze the mechanisms by which expression of gangliosides is regulated, GM2/GD2 synthase (β1,4 GalNAc transferase) cDNA was isolated. Using this cDNA, genetic and epigenetic regulatory mechanisms for ganglioside expression were analyzed. Using 20 human cancer cell lines, expression of GM2/GD2 synthase gene was analyzed in Northern blot analysis and RT-PCR (reverse transcription -polymerase chain reaction), and the results were correlated with actual enzyme activity and GM2/GD2 expression in each cell line. These results indicated that mRNA expression and the enzyme activity did not necessarily correlate in each line, and suggested that post-translational regulatory systems such as phosphorylation or glycosylation of the enzyme should be present. The amounts of the precursor structures which would be used as acceptors or the activities of glycosyltransferases synthesizing the precursor structures are also very important. In order to analyze the genetic regulation of the gene expression, the genomic structure of the gene was analyzed. It consisted of at least 11 exons and ranged more than 8 kb. The coding region started from 2nd exon. Promoter activity in 1.5 kb upstream of the transcription initiation site was confirmed in CAT assay. Addition of the first intron to the 1.5 kb 5' uncoding region and 1st exon resulted in marked enhancement of the promoter activity. Precise promoter/enhancer region including the regions associated with tissue specificity is now under investigation.

U 209 SYSTEMIC AND TISSUE-SPECIFIC ABLATION OF O-LINKED GLYCOSYLATION *IN VIVO*, Thierry Hennet, Elena Merletti, Lawrence A. Tabak#, and Jamey D. Marth, The Biomedical Research Centre, University of British Columbia, Vancouver V6T 1Z3, Canada; #Department of Dental Research, University of Rochester, Rochester, NY 14642

To define the role of O-linked protein glycosylation in mammalian development, we are generating mice with a null-mutation in the gene encoding UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase (GalNAc-transferase), which catalyzes the addition of N-acetylgalactosamine to serine/threonine residues of proteins. Additionally, our approach utilizes the the Cre-loxP transgene recombination system, which allows both systemic and tissue-specific gene ablation as a function of transgenic Cre recombinase expression. We have cloned the mouse GalNAc-transferase gene from a genomic DNA library and have created a targeting vector consisting of an exon of GalNAc-transferase flanked by loxP-sites and the neomycin resistance gene. The targeting vector is intervened by 8.7 kb (left) and 1.1 kb (right) of intron sequence. Embryonic stem cells are currently being transfected with the targeting vector and selected for neomycin resistance. Cell clones that have undergone homologous recombination at the GalNAc-transferase allele will be used to create a line of mice homozygous for the loxP-flanked GalNAc-transferase exon. The mating of these mice with those expressing a Cre recombinase transgene in thymocytes will yield animals with thymocyte-specific GalNAc-transferase inactivation. Additionally, the targeted and loxP-flanked GalNAc-transferase exon will be removed in ES cells by transient expression of Cre. The resulting subline of ES cells will also be used to create a mouse devoid of GalNAc-transferase activity in the classical pan-specific manner. Such animal models should provide important information regarding the role of O-linked glycosylation in mouse development, as well as in T cell development and function. Our current progress on this work will be presented.

U 210 IMMUNOCHEMICAL AND IMMUNOHISTOLOGICAL STUDY ON A MOUSE MACROPHAGE C-TYPE LECTIN,
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MMGL is a Gal/GalNAc specific calcium dependent (C-type) lectin detected on in vivo-elicited peritoneal macrophages. MMGL and its rat counterpart are known to function in the receptor-mediated endocytosis for galactose-terminated glycoproteins. MMGL may also participate in cell-cell interaction such as cell trafficking within tissue and macrophage-tumor cell interaction. In order to look into roles of MMGL in cell-cell recognition and other physiological functions, detailed examination of the sugar binding specificity and development of an antibody which can efficiently interfere with the lectin activity would be of great importance. For this end, a simple ELISA assay measuring lectin activity of MMGL was developed. A soluble recombinant form of MMGL (rML) was expressed and purified from E. coli transformed with a cDNA encoding extracellular portion of MMGL. Binding of galactosylated poly-lysine (termed as a ligand) to immobilized rML was measured quantitatively. The binding was calcium dependent and was inhibited by galactose and GalNAc. An antiserum against rML inhibited the ligand binding, demonstrating usefulness for screening of blocking antibodies. Using this assay, we found a significant interaction between MMGL and carrageenans, a group of sulfated polygalactan. The inhibitory effect of carrageenan was specific because other types of sulfated polysaccharides, such as glycosaminoglycans and fucoidin, did not interfere with the ligand binding. The present results suggest a possible involvement of MMGL in toxicity and other biological effects of carrageenans on macrophages.

We also developed a rat monoclonal antibody (LOM-14) against MMGL, and asked where MMGL was expressed in vivo. By immunoblotting analyses of tissue lysates, we demonstrated a significant expression in lymph nodes, thymus, skin, heart, skeletal muscle and lung. Liver was essentially negative, demonstrating lack of cross-reactivity of LOM-14 mAb to asialoglycoprotein receptors of hepatocytes. By immunohistochemical study on lymph nodes, cells in medulla, subcapsular sinus and marginal zone (between lymphoid follicle and paracortical zone) were positive. In thymus, cells localized beneath septa were stained.

U 211 UNMASKING OF SIALYLATED TUMOR ANTIGENS IN NORMAL AND CANCEROUS COLONIC TISSUES.
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Sialylated tumor-associated antigens such as STn, SLe^a, and SLe^x are often expressed by cancerous but not by normal colonic tissues. It is not clear whether the lack of expression of these epitopes is due to a lack of antigen synthesis or masking by other substances. Sialic acids (Sia) can undergo O-acetylation, and treatment with a base such as sodium hydroxide (NaOH) is known to liberate acetyl groups from sialic acids. The aim of this study was to determine whether treatment of tissues with NaOH would enhance immunohistochemical expression of STn (Siaα2,6GalNAc), SLe^a (Siaα2,3Type 1 chain) and SLe^x (Siaα2,3Type 2 chain).

METHODS: Ten pairs of normal and cancerous colonic tissues were stained by a conventional immunoperoxidase technique with monoclonal antibody TKH2* (anti-STn), SNH3* (anti-SLe^x), and CA19-9 (anti-SLe^a). MAb 1E3* (anti-Tn) served as a negative control. De-acetylation was accomplished by pretreating slides with 0.1N NaOH at r.t. for 25 minutes prior to incubation with primary antibody. Control slides received PBS instead of NaOH.

RESULTS: (% specimens with enhanced antigen expression after NaOH)

	TKH2	CA19-9	SNH3	1E3
Normal colon (NC)	80%	90%	60%	10%
Transitional mucosa	66%	78%	78%	11%
Colon cancer	33%	44%	56%	0

Sialylated antigen expression was enhanced by NaOH treatment in the vast majority of NC and transitional mucosa (immediately adjacent to cancer) specimens. STn was unmasked mainly in the lower crypts, SLe^a in the upper crypts, and SLe^x throughout the crypt. Unlike NC, cancer tissue phenotype changed relatively little after NaOH.

CONCLUSIONS: (1) Lack of sialomucin antigen expression, particularly in NC, is due, in part, to epitope masking rather than lack of synthesis. (2) Sialic acid epitopes of α2,3 or α2,6 linkage on different carbohydrate chains can be unmasked by this treatment. (3) This method should help to elucidate the fine cellular localization of these cryptic epitopes.
(4) Regulation of Sia O-acetylation may be important for exposure of tumor antigens during malignant transformation in the colon.
(*A kind gift of Dr.Hakomori, The Biomembrane Institute, Seattle, WA)
Supported in part by NCI Grant CA52491.

U 212 STUDIES OF Galα4Gal-ANTIBODIES; BINDING SPECIFICITY AND UTILISATION FOR MAPPING OF GLYCOLIPIDS FROM BURKITT LYMPHOMA TUMOR AND OTHER TISSUES,
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The binding specificity of four different monoclonal antibodies were tested towards a number of Galα4Gal-containing glycosphingolipids on thin-layer chromatograms. One of the antibodies (MC2102) was shown to be strictly galabiose specific (Galα4Galβcer) and did not bind to any glycolipids with elongations of this structure. Two antibodies (BLA and pk002) bound best to CD77, globotriaosylceramide (Galα4Galβ4GlcβCer) but showed some cross reactivity with the P1 antigen (Galα4Galβ4GlcNAcβ3Galβ4GlcβCer). The fourth antibody tested (P001) reacted most strongly with P1 but also to some extent with globotriaosylceramide. None of the globotriaosyl- or P1-specific antibodies bound to galabiose.

The binding specificities of the above mentioned antibodies was used in a characterisation study of a Burkitt lymphoma tumour grown in nude mice. Tumour material (2g) was extracted with chloroform/methanol and the neutral glycosphingolipids were purified. As known before this tumour contains globotriaosylceramide as major glycosphingolipid. We found that this was the only neutral glycosphingolipid with terminal Galα4Gal present in the Burkitt tumour as judged from tests with the antibodies used in this study.

The strict requirement of MC2102 for galabiose was utilised in investigations of glycolipids from other tissues. We could in this way show that e.g. dog intestine, human stomach, and chicken erythrocytes contain galabiose.

U 213 CLONING OF NEW MEMBERS OF β-1,6-N-ACETYLGLUCOSAMINYLTRANSFERASE GENE FAMILY
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Formation of β-1,6-*N*-acetylglucosaminyl linkages are important in both *N*- and *O*-glycans since these branches lead into further modification of oligosaccharides such as poly-*N*-acetyllactosminyl extension and the formation of sialyl Le^x structure. Recently we have cloned two β-1,6-*N*-acetylglucosaminyltransferases which form those critical branches. One enzyme is core 2 β-1,6-*N*-acetylglucosaminyltransferase that forms branches in *O*-glycans (*Proc. Natl. Acad. Sci. USA*, **89**, 9326-9330, 1992). The other enzyme is I β-1,6-N-acetylglucosaminyltransferase that forms I-antigenic branches in poly-*N*-acetyllactosamines (*Genes & Development*, **7**, 468-478, 1993). These two enzymes are highly homologous in three regions of the catalytic domains and the genes for two enzymes reside at the same locus in chomosome 9 q21. These results suggest that there may be more members of the β-1,6-*N*-acetylglucosaminyltransferase gene family.

By utilizing polymerase chain reaction, we amplified sequence homologous to I or C-2 GnT. The analysis of these amplified sequences revaled two distinct sequences. By using these newly identified sequences, we have isolated a cDNA, that is highly homologous to the I-gene, from cDNA library constructed from melanoma cells.

Work is also in progress to isolate a full-length cDNA for another new β-1,6-*N*-acetylglucosaminyltransferase which is related to C2 GnT. These results suggest that there are at least four members belong to the β-1,6-*N*-acetylglucosaminyltransferase gene family.

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U 214 IMMUNIZATION WITH STn ANTIGEN PROLONGS SURVIVAL IN A RAT MODEL OF COLON CANCER.
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Sialosyl-Tn (STn) is a mucin-associated carbohydrate antigen which is associated with poor survival in patients with colon, ovarian, and breast cancers. Establishment of an animal model would greatly facilitate investigation of the role of STn antigen in cancer biology. We have developed a highly lethal rat colon cancer cell line, called LMCR(-), which expresses STn and Tn antigens *in vivo*. In this study, we have tested the ability of a synthetic STn vaccine to protect animals from death due to this tumor.
METHODS: Rats were immunized s.c. and boosted one week later with STn conjugated to keyhole limpet hemocyanin (KLH) in Detox B adjuvant at low (1 μg) or high (100 μg) dose. Control animals received KLH alone (1 μg), Tn-KLH (1 μg;100 μg) or "crossover" STn-KLH (1 μg) followed by Tn-KLH (1 μg) boost. All animals were then challenged 1 week later with 10^6 LMCR(-) cells i.p. and survival was monitored.
RESULTS: Low dose STn-KLH and low dose Tn-KLH produced survival rates of 60% and 40%, respectively. Rats given KLH alone, high dose STn-KLH, high dose Tn-KLH, or the crossover regimen all succumbed to tumor. Adoptive transfer of splenic lymphocytes from rats immunized with STn-KLH resulted in survival rates of 45-55% compared to rats receiving naive lymphocytes.
CONCLUSIONS: (1) Immunization with STn-KLH, a synthetic carbohydrate antigen, prolongs survival in a rat colon cancer model. (2) Protection can also be achieved by transfer of lymphocytes sensitized to STn antigen, indicating a cell-mediated immune mechanism. (3) This model should be useful for studies on the role of carbohydrate mucin-associated antigens in tumor biology.
[STn-KLH, Tn-KLH, KLH, and Detox B were a generous gift of Dr. Mark Reddish, Biomira, Inc., Edmonton, Canada]
Supported in part by NCI Grant CA 52491.

U 215 STUDIES ON THE STRUCTURE AND FUNCTION OF THE STEM DOMAIN OF THE MURINE α1,3-GALACTOSYLTRANSFERASE,
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The enzyme UDP-Gal:Galβ1,4GlcNAc α1,3-galactosyltransferase (α1,3GT) is a Golgi resident type II transmembrane protein which catalyzes the addition of α1,3-linked galactose residues to oligosaccharide chains on glycoproteins and glycolipids. The structure and function of the stem region of α1,3GT, a polypeptide segment between the membrane spanning and catalytic domain, is not well defined. A series of nested deletions were generated within the putative stem region of the murine α1,3GT and the biological effects of these deletions tested in expression studies in CHO and COS-7 cells. We demonstrate that deletion of 47 amino acids (residues 67 to 113) from the putative stem region of α1,3GT results in a decrease in enzymatic activity but does not result in complete enzyme inactivation or in alteration of the enzyme subcellular localization. Deletion of 57 amino acids from this region (residue 67 to 123) however resulted in enzyme inactivation without alteration of its subcellular localization. Larger deletions within this region resulted in enzyme inactivation and the retention of these inactive deletion mutants within the ER. We also demonstrate that qualitative differences exist in the pattern of α-galactosylation of secreted glycoproteins between CHO cells stably expressing the wild type α1,3GT and the stem deletion mutants. These studies suggest that while the putative stem region does not contribute to catalytic activity *in vitro* it does influence activity toward secreted substrates *in vivo*. [Supported by NIH grants GM47533 and RR05351 (K.W.M.)]

U 216 GLUCOSYLTRANSFERASE INVOLVED IN PROTEIN N-GLYCOSYLATION, Samir K. Roy, Mudgapalli Ashok and Inder K. Vijay, Department of Animal Sciences, University of Maryland, College Park, MD 20742
The enzyme UDP-Glc:Dol-P glucosyltransferase or glucosyltransfer that transfers Glc residues for the biosynthesis of Dol-P-P-oligosaccharide precursor, $Glc_3Man_9GlcNAc_2$, for protein N-glycosylation, was previously photoidentified by our laboratory as an ER-localized, 35 kDa polypeptide in the rat mammary gland (Shailubhai *et al.* (1990) *J. Biol. Chem.* 265:14105-14108). In this study, we solubilized the enzymes from bovine liver with Emulgen 911 and partially purified it by ion exchange chromatography on DEAE- and CM-Trisacryl. After identifying an aliquot of the partially purified enzyme with 5-N_3-[β-^{32}P]UDP-Glc, the gel segment adjoining the labeled band was cut out, electroeluted and verified for purity by 2D-PAGE. Monospecific polyclonal antibodies were raised against the gel-purified enzyme. These antibodies identified a single polypeptide of 35 kDa on the Western blots of solubilized microsomal proteins from the rat mammary and the bovine mammary gland, and a 37-kDa polypeptide from the mung bean microsomes. The antibodies also immunoinhibited the enzyme activity in solubilized microsomes of these tissues by > 80%. Several kinetic parameters of the bovine liver glucosyltransferase were characterized. Also, the enzyme shows a substrate protectable -SH group at its active site, as identified by the biotin-avidin tagging approach recently developed in our laboratory (Pukazhenthi *et al.* (1993) *J. Biol. Chem.* 268:6445-6452). Currently, efforts are underway to clone the enzyme by oligonucleotide-antibody screening procedures in preparation for studying its regulation. (Supported by N.I.H. grants GM44651, DK19682 and Maryland Agricultural Experiment Station).

U 217 EXPRESSION CLONING OF A NOVEL Galβ(1-3/1-4)GlcNAc α2,3-SIALYLTRANSFERASE USING LECTIN-RESISTANCE SELECTION, Katsutoshi Sasaki, Etsuyo Watanabe, Kaoru Kawashima, Susumu Sekine, Taeko Dohi§, Mieko Oshima§, Nobuo Hanai, Tatsunari Nishi, and Mamoru Hasegawa, Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Machidashi, Tokyo 194, Japan, and the §Division of Clinical Biochemistry, Clinical Research Institute, National Medical Center, Tokyo 162, Japan
The sialyl Lewis x determinants serve as ligands for selectins. To isolate glycosyltransferases involved in biosynthesis of these determinants, we have developed an expression cloning approach using lectin resistance selection and cloned a novel human Galβ(1–3/1–4)GlcNAc α2,3-sialyltransferase (ST-4). A cDNA library of human melanoma cell line WM266-4 was constructed in an Epstein-Barr-virus-based cloning vector. Selection of the B-cell line Namalwa expressing transfected cDNAs in the presence of a cytotoxic lectin *Ricinus communis agglutinin* 120 gave a cDNA encoding a protein having conserved regions shared by all cloned sialyltransferases. The use of this lectin which is specific to galactose residues (especially Galβ1-4GlcNAc structure), originates from our prediction that the modification of Galβ1-4GlcNAc structure (a backbone of the sialyl Lewis x structure) by glycosyltransferases may increase the levels of resistance to this lectin. Expression of the COOH-terminal catalytic domain of this protein showed α2,3-sialyltransferase activity with substrate-specificity different from that of Galβ1-3(4)GlcNAc α2,3-sialyltransferase (ST-3; EC 2.4.99.6). ST-4 sialylates the type II chain more preferentially than the type I chain. Furthermore, expression of this cDNA in Namalwa cells increased the level of sialyl Lewis x antigens, but not that of sialyl Lewis a antigens. Using this approach, we also cloned human ST-3. Comparison of the detailed properties of ST-3 and ST-4 is currently underway and will help to identify the sialyl- and fucosyltransferases involved in biosynthesis of selectin ligands. In addition, the cloning approach based on lectin-resistance selection may be useful to isolate cDNAs encoding other mammalian glycosyltransferases.

U 218 THE PECTIC SUBSTANCES - COMPLEX CARBOHYDRATES OF PLANTS IN THE HUMAN PATHOLOGY, Ninel P. Shelukhina, Institute of Organic Chemistry, Academy of Science Kyrghyzstan, Bishkek, Chui prosp. 265A, Kyrghyzstan Republic, USSR.

The pectic substances is a group designation for those complex, colloidal carbohydrate substances which occur in or prepared from plants and contain a large proportion of anhydrogalacturonic acid units, which are thought to exist in a chain like combination [1]. Pectin and pectin preparations are extensively used in national economy and medicine [2, 3]. The application in surgical dressing is one of most striking developments among the uses of pectin. Pectin is useful homeostatic agent. It has been applied at great variety of bleeding, internal and external including tooth extraction, in haemorrhages of all sorts, as well as in gynaecological treatments. The fact, that pectin solution is a suitable plasma substitute, or more correctly replacement solution, is clearly established. Pectin has been used to lower the toxicity some drugs and to reduce the rate of absorbtion of antibiotics, adrenaline, ephedrine, sex hormones. Pectic substances are good antidotes for heave and radioactive metals poisoning.

Author [4] investigated medical and prophylactic effect of pectate an antimony intoxication. Comparison of results of toxicological and immunological investigations give evidence about reduction of resorption of antimony from intestine, increase rate (to 10-15 days) moving antimony away blood, liver, hair, skeleton. An addition pectate is improved indexes of cell immunity.

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4. Unpublished results.

U 219 ISOLATION AND CHARACTERIZATION OF EPIGLYCANIN-LIKE GLYCOPROTEINS FROM ASCITES FLUID OF A CANCER PATIENT.

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Epiglycanin is a mucin-type glycoprotein which first was isolated from the TA3-Ha mouse mammary carcinoma ascites cell (Codington, J.F. and Haavik, S. (1992) *Glycobiology*, **2**, 173-180) Mono- and polyclonal antibodies developed against epiglycanin bind an epiglycanin-like antigen (HC-antigen) in ascites fluid and sera from patients with carcinomas. This human carcinoma (HC) antigen was isolated from ascites fluid of a patient with ovarian carcinoma by a combination of gel filtration followed by affinity chromatography using an immobilized monoclonal anti-epiglycanin antibody. HC-activity was determined by an enzyme competitive binding assay using an immunoplate coated with epiglycanin and a monoclonal antibody raised against epiglycanin. The majority of the epiglycanin cross-reacting material had M_r greater than 1000 kDa and buoyant density of about 1.4 g/mL. Treatment of the isolated glycoproteins or epiglycanin with TPCK-Trypsin, Pronase, neuraminidase, O-glycanase or periodate followed by testing of antigenic activity indicated that the monoclonal anti-epiglycanin antibodies tested bind to Galß(1→3)GalNAc-containing epitopes on the glycoproteins. The affinity purified HC-antigen was subjected to SDS-PAGE and blotted onto a PVDF-membrane. The blots were stained for carbohydrate with the DIG Glycan Differentiation Kit (Boehringer Mannheim) and HC-activity by incubation with a monoclonal anti-epiglycanin antibody. The HC-antigen band was subjected to hydrolysis, amino acid analysis and determination of monosaccharide composition by HPAEC-PAD. The HC-antigen was also tested for co-expression of epitopes for other anti-carcinoma antibodies

U 220 GLYCOSAMINOGLYCAN N-ACETYLGLUCOSAMINYL N-DEACETYLASE/N-SULFOTRANSFERASE FOR THE BIOSYNTHESIS OF HEPARIN AND HEPARAN SULFATE

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Heparin and heparan sulfate are highly sulfated carbohydrate polymers that bind to and modulate the activities of numerous proteins. The formation of these binding domains in heparin and heparan sulfate is dependent on a series of biosynthetic reactions that modify the polysaccharide backbone in which the N-deacetylation and N-sulfation are the initiating and rate limiting steps. We have shown that in rat liver, heparan sulfate biosynthesis utilizes a single protein that carries on both the N-deacetylation and N-sulfation (Wei, et al, 1993 PNAS 99, 3885). We now report that in a heparin producing mastocytoma cell line, N-deacetylation and N-sulfation is also catalyzed by a single protein. This is demonstrated by showing that a soluble chimeric protein which includes the Golgi luminal domain of the enzyme possesses both the activities independent of other cofactors. Kinetics studies of both enzymes reveal that despite the high homology of their predicted amino acid sequences (Orellana et al, 1994 JBC in press), the mast cell enzyme displays ratios of N-deacetylase to N-sulfotransferase activities that are 4-8 fold higher than those observed for its rat liver counterpart. The difference in the catalytic properties of these two enzymes may account for the fact that heparin has a higher content of N-sulfated glucosamine and L-iduronic acid than heparan sulfate which confer their differentiated biological significance. The reaction mechanisms shared by these two enzymes in relation to the formation of N-sulfated blocks in these polymers are also discussed.

Complex Carbohydrates and Reperfusion Injury

U 300 Glycolipid Ligands For Selectins Support Leukocyte Rolling Adhesions Under Physiologic Flow Conditions

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Leukocyte-surface glycolipids, decorated with the sialyl lewis ^{a} or ^{x} carbohydrate motifs serve as ligands for all three selectins. Selectin-glycolipid interactions have been previously studied in direct binding assays using soluble selectin-chimeras or in cell adhesion assays, performed under static conditions, while physiological adhesive interactions mediated by selectins appear to take place mainly under flow conditions. We now report that lipid-linked oligosacharides, recognized by E- and L- selectin chimeras, support cell attachment of E- and L-selectin expressing cells under physiological flow conditions, studied in a parallel-plate flow chamber. The cell-adhesive reversible interactions mediate selectin-specific cell rolling on the glycolipid substrate which persist at applied shear stresses of 1-30 dynes/cm^2, are Ca++ dependent and can be completely inhibited by function-blocking anti-selectin mAb's. Rolling velocities and resistance to detachment are reciprocally related to the glycolipid site density and fall within the range measured for myeloid cell rolling on purified E selectin substrates.

These observations are, to our knowledge, the first indication that glycolipids expressed on leukocytes may have a significant role in E-selectin mediated cell rolling. The finding that several types of lymphocytes can attach under flow and roll on glycolipids decorated with carbohydratre ligands for L selectin suggest that analogous glycolipids expressed on specialized endothelial tissues *in vivo*, may play some role (along with glycoprotein addressins) in lymphocyte homing. Thus, we propose that glycolipids may participate in selectin-mediated cell attachment and rolling, under physiologic shear conditions.

U 301 PURIFICATION AND PARTIAL CHARACTERIZATION OF A HEPARIN PROTEOGLYCAN FROM A HUMAN INTESTINAL CELL LINE - POTENTIAL ROLE IN CHOLESTEROL ABSORPTION,

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Pancreatic cholesterol esterase (PCE) is a pivotal enzyme involved in cholesterol absorption. Prior treatment of CACO-2 cells or intestinal vesicles with heparinase I markedly reduces cholesterol absorption even in the presence of PCE. PCE binds heparin on the surface of enterocytes in a receptor-like manner. We have purified and partially characterized this proteoglycan (PG) under dissociative conditions using anion-exchange, size exclusion and affinity chromatography. S^{35} radiolabelled chaotrophed CACO-2 cells passed over DEAE resin resulted in a step-gradient elution of a defined peak (0.4-0.65M NaCl). This was passed over a CL-2B gel filtration column. Treatment of the single CL-2B peak with heparinase I demonstrated digestion of a 180kd species but no degradation was seen with chondroitinase ABC, heparitinase or keratanase. Chemical cleavage with nitrous acid and cetylpyridinium chloride precipitation confirmed the heparin nature of this PG. The CL-2B peak bound avidly to PCE affinity resin requiring 5M urea to elute the purified PG. This 180kd species stained with alcian blue and the core protein size following digestion with heparinase I was 62kd. Heparin PG purification was 450-fold over S^{35} macromolecules derived from chaotrophed CACO-2 cells. A lower molecular weight PG is released into the supernatant by heparinase I treatment. Thus we have isolated a 180kd heparin PG for PCE by a simple chromatographic purification procedure which will assist us in elucidating the molecular mechanisms of cholesterol absorption in man.

U 302 GLYCOSYLATION OF TWO RECOMBINANT HUMAN UTERINE TISSUE PLASMINOGEN ACTIVATOR VARIANTS CARRYING AN ADDITIONAL *N*-GLYCOSYLATION SITE IN THE EPIDERMAL GROWTH FACTOR-LIKE DOMAIN,

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Recombinant human uterine tissue plasminogen activator (tPA) glycosylation mutants carrying an additional *N*-glycosylation site in the epidermal growth factor-like domain due to the replacement of either Tyr-67 by Asn (**YN-tPA**) or Gly-60 by Ser (**GS-tPA**) were expressed in mouse epithelial (C127) cells. Glycopeptides comprising individual glycosylation sites were isolated. Oligosaccharides attached were liberated by treatment with endo H or *N*-glycanase and were studied, after reduction and HPLC subfractionation, by methylation analysis, liquid secondary-ion mass spectrometry and, in part, by exoglycosidase digestion. Corresponding deglycosylated peptides were identified by amino acid analysis and N-terminal amino acid sequencing.

The results revealed that Asn-117 of **YN-tPA** carried exclusively high-mannose type glycans with five to nine mannose residues similar to wild-type tPA expressed in this cell line [Pfeiffer, G., Schmidt, M., Strube, K.-H. & Geyer, R. (1989), *Eur. J. Biochem. 186*, 273-286]. In contrast, Asn-117 of **GS-tPA** carried only small amounts (about 25 %) of high-mannose and hybrid type species and predominantly complex type sugar chains (about 75 %) which were partially incomplete and mostly devoid of fucose. Newly introduced *N*-glycosylation sites at Asn-67 (**YN-tPA**) or Asn-58 (**GS-tPA**) as well as those at Asn-184 and Asn-448 were solely substituted by complex type glycans, carrying, in part, Galα3-residues, sulphate groups, intersecting GlcNAc and lactosamine repeats.

Our study clearly demonstrates that creation of a new glycosylation site at Asn-58 influenced the oligosaccharide processing and, hence, the glycosylation pattern at Asn-117, whereas introduction of a new site in position 67 did not. The relative amounts of complex type glycans at Asn-117 of **GS-tPA** correlated with the degree of carbohydrate substitution of Asn-58. Therefore, it can be concluded that the presence of a sugar chain at that position and not the Gly to Ser mutation *per se* is responsible for the observed alteration of **GS-tPA** glycosylation.

U 303 USE OF BACULOVIRUS - DERIVED ICAM-1 AS A TOOL FOR THE CHARACTERISATION OF THE GLYCOSYLATION STRUCTURE : FUNCTION RELATIONSHIP OF HUMAN TISSUE-DERIVED ICAM-1,

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The human intercellular adhesion molecules, ICAM-1, ICAM-2 and ICAM-3, and their counter-receptors, the β2 integrins, mediate a variety of leukocyte and endothelial interactions central to the immune response. These ICAMs exhibit close homology in their sequences, especially in the two most N-terminal domains, which contain the binding site for the primary receptor, LFA-1. ICAM-1 also binds a second β2 integrin, Mac-1, and is subverted as a receptor for rhinoviruses and malaria-infected erythrocytes.

The differences in distribution and expression of the ICAM species within the body suggests that they each have a unique role in the immune response. Heterogeneity observed in the molecular masses of the individual ICAMs is primarily due to a differential glycosylation of the polypeptide core, and this may play an important role in defining their biological functions.

The role of glycosylation on ICAM function is being studied using a variety of techniques, including surface plasmon resonance, mass spectroscopy and adhesion assays. Functionally active recombinant ICAM-1 has been expressed and purified from a baculovirus-infected *Spodoptera frugiperda* insect cell line. This has been used to develop analytical techniques prior to the construction of a glycosylation structure-function map for ICAM-1 isolated from human tissue. Data from the oligosaccharide characterisation of baculovirus derived ICAM-1 will be presented.

U 304 ALKYLMALTOSIDES SERVE AS EXOGENOUS SUBSTRATES FOR GLUCOSYL AND XYLOSYL TRANSFER BY GLYCOGENIN, Elias Meezan, Stephen Manzella and Lennart Rodén, Department of Pharmacology, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294

Glycogenin is a self-glycosylating enzyme which can catalyze the transfer of glucose or xylose residues from UDP-glucose or UDP-xylose, respectively, into an oligosaccharide chain which provides the primer for the initiation of glycogen synthesis. Because the protein serves as both enzyme and acceptor in glycosyl transfer and can exist in several states of glycosylation, the assay of its activity and the study of its mechanism of action have been complicated. These circumstances have led to a search for suitable exogenous acceptors with a high affinity for the enzyme. In this study, we show that several alkylglucosides and alkylmaltosides are active as acceptor substrates for both glucose and xylose transfer from their respective UDP-sugar donors by beef kidney glycogenin. Dodecyl-ß-D-maltoside (DBM) was the most effective acceptor examined having a K_m for the enzyme of about 100 μM. A comparison of the acceptor activity of several alkylglycosides indicates that the active site of glycogenin recognizes oligosaccharides with α-(1--->4) linked glucose units as the best acceptors in preference to glucose attached in α- or ß-linkage to various aglycones. Preliminary experiments indicate that DBM can also serve as a substrate for rabbit muscle glycogen synthase, yielding a series of dodecylmaltooligosaccharide products in which up to seven or more glucose residues are attached to the alkyl chain. The ability of DBM and other alkylglycosides to serve as substrates for glycosyl transfer by glycogenin makes these compounds useful for both the assay and study of the mechanism of action of this enzyme; it also opens up the possibility that these compounds may be active in vitro and in vivo as artificial initiators of glycogen synthesis in a manner analogous to the known initiating properties of xylosides in the biosynthesis of sulfated proteoglycans.

U 305 CAPILLARY ELECTROPHORESIS IN PROTEIN GLYCOSYLATION ANALYSIS, Roger A. O'Neill, Claudia Chiesa, Daotian Fu, Shaheer Khan, Sylvia Yuen, Iqbal Zaidi, Lynn Zieske, Carbohydrate R&D, Perkin Elmer Applied Biosystems Division, Foster City, CA 94404.

Analytical separations of glycoprotein oligosaccharides are now routinely performed using gel permeation , reverse-phase and high pH anion exchange chromatographies, as well as more recently, gel electrophoresis. A limited amount of work on electrophoretic separations of oligosaccharides in narrow bore (25m to 75m) capillaries (capillary electrophoresis or CE) is now being reported. However, a majority of this work is being performed using large, structurally simple polysaccharides, or smaller oligosaccharide ladders that are derived from such polymers. We are developing methods for high resolution CE-based separations of complex carbohydrates derived from mammalian and other glycoproteins. Both sialylated and neutral oligosaccharides are being resolved using chromophores and fluorophores that impart charge and detectability to the carbohydrates. Glycoprotein-derived oligosaccharide standards that have been well characterized by NMR and mass spectrometry, as well as complex oligosaccharide mixtures released from glycoproteins, have been examined in our CE investigations. We have been able to resolve linkage isomers that in some cases have been unresolved by other powerful oligosaccharide separation methods. In related work, we have followed the course of reactions of carbohydrate modifying enzymes in real time using the automation features available in capillary electrophoresis systems. Development of CE technologies for carbohydrate applications should provide tools for glycoprotein oligosaccharide analysis that offer high resolution, excellent sensitivity and quantitation, and attractive opportunities for automation of the analytical process.

U 306 GROWTH-PROMOTING ACTIVITY OF HUMAN TSH AND PRL GLYCOFORMS. Catherine Ronin, Tania Hoffmann, Marie-Jeanne Papandréou and Gabriella Medri, Laboratoire des Hormones Glycoprotéiques, Faculté de Médecine-Secteur Nord, 13916-Marseille Cedex 20, France.

TSH (Thyroid-Stimulating Hormone) and PRL (Prolactin) are pituitary hormones for which endocrine functions are modulated through obligatory or alternate glycosylation respectively. Since both hormones may be involved in autoimmune diseases through their action on cell proliferation, we investigated how carbohydrates (CHO) can modulate their duration in blood and their mitogenic action on target cells.

TSH circulating in patients with primary or central hypothyroidism, with pituitary or brain tumors differ from euthyroid controls in core branching or sialic acid content (Papandréou et al, J. Clin.Endocr. Met. 1993, 77, 393-398). Isoelectric focusing separated pituitary-derived TSH in two main glycoforms according to their sialic acid content. Both displayed similar recognition at the receptor site but alkaline (pI: 8.5-7.5) TSH showed a higher potency in inducing either cAMP production or thymidine incorporation in FRTL-5 cells than the neutral (pI: 7.5-6.0) forms (ED_{50}= 0.25 vs 0.66 nM and 0.29 vs 0.70 nM respectively). When added in combination, neutral TSH proved to antagonize the action of alkaline forms on DNA replication without a significant change in cAMP, suggesting that both glycoforms are necessary to regulate thyroid cell growth besides other post-receptor events.

PRL, secreted by human prolactinoma cells, was separated by Con A chromatography as non-glycosylated (NG)-unbound, biantennary weakly-bound (WB-) or mannose-rich firmly-bound (FB-) glycoforms.WB- and FB-PRLs were found 2-3 fold less active than NG-PRL on rat lymphoma cell growth, indicating that CHO down-regulate hormone mitogenic activity. When injected in rats, WB- and FB-PRLs were cleared in a biphasial fashion with a similar rapid phase of 2 ± 0.3 min followed by a slow phase of 12 ± 8 and 27± 9 min respectively while NG-PRL was eliminated with a half-time of 5 ± 0.1 min followed by a very slow disappearance over several hours.The metabolic clearance rate was of 0.13 ± 0.07ml/min for NG-PRL to 0.47± 0.12ml/min for WB-PRL and 0.8 ± 0.2ml/min for FB-PRL.Increasing the percentage of G/NG-PRLs from 10 to 50% gradually augmented hormone clearance. NG-PRL and FB-PRL were preferentially taken up by the liver and WB-PRL eliminated by the kidney, indicating that PRL glycosylation also governs organ targeting.

In conclusion, a variable ratio of glycoforms was shown to occur in blood for TSH and PRL and to account for a coordinate action in inhibiting cell proliferation at the target organ.

U 307 EXPRESSION OF A NEW MOUSE α1,2-MANNOSIDASE AND CHARACTERIZATION OF ITS ENZYMATIC PROPERTIES. Jean Schneikert* and Annette Herscovics, McGill Cancer Center, McGill University, 3655 Drummond street, Montreal, H3G 1Y6, Quebec, Canada.

α1,2 mannosidases play an essential role in the biosynthetic pathway for *N*-glycoproteins. Taking advantage of regions of identity between the amino acid sequences of a yeast α1,2-mannosidase that removes only one mannose residue from $Man_9GlcNAc$ and a rabbit liver α1,2-mannosidase that trims $Man_9GlcNAc$ to $Man_5GlcNAc$, PCR was used to clone a novel mouse cDNA. The deduced amino acid sequence predicts a type II transmembrane topology with an *N*-terminal cytoplasmic tail, a single transmembrane domain and a lumenally-oriented catalytic domain. Two variants of this cDNA were obtained, clone 4 and clone 416, that specify two proteins differing by only three amino acids in the C-terminal domain. Epitope tagging followed by indirect immunofluorescence of transiently transfected COS7 cells showed that both proteins are localized to a perinuclear region corresponding either to the Golgi apparatus or to the intermediate compartment between ER and Golgi (1). The C-terminal domain from both clones, lacking the transmembrane region was transiently expressed in COS cells as a secreted protein A fusion protein. The enzymatic properties of the secreted clone 416- derived fusion protein bound to IgG-Sepharose were investigated. It is an α1,2 mannosidase that trims $Man_9GlcNAc$ to $Man_5GlcNAc$. The activity requires divalent cations since it is inhibited by EDTA. The enzyme may use Mn^{+2}, Mg^{+2} or Co^{+2}, but Ca^{+2} is the most effective consistent with the occurence of a consensus Ca^{+2} binding domain in the sequence. The enzyme is inhibited by 1-deoxymannojirimycin, but not by swainsonine. In contrast, the clone 4-derived fusion protein is poorly secreted, resulting in undetectable enzymatic activity. Mutagenesis of each one of the three polymorphisms revealed that they contribute to the low secretion and enzyme activity of the clone 4-derived fusion protein.

Ref.: (1) Herscovics A., Schneikert J., Athanassiadis A. and Moremen K.W., submitted.

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U 308 THE EFFECTS OF SULFATED OLIGOSACCHARIDES ON EXPERIMENTAL PULMONARY DAMAGE.

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Several research groups have indicated that selectins clearly function as carbohydrate-binding proteins which recognize oligosaccharides terminating with Sialyl-Lewis^x like structures. These selectins have a crucial role in the initial stages of recruitment of leukocytes to sites of inflammation. It has been shown that Sialyl-Lewis^x and its analogs can block the adhesion to selectins and soluble recombinant selectins. We believe that soluble low molecular weight carbohydrates will be the next generation of anti-inflammatory drugs. Evidence will be presented from our research demonstrating that several soluble carbohydrates including Lewis^x-3' sulfate are capable of inhibit experimentally LPS induced lung injury in a mouse model system.

Carbohydrate: Protein Interactions-S-Type Lectins

U 400 BIOSYNTHESIS SURFACE EXPRESSION AND FUNCTION OF THE FIBRONECTIN RECEPTOR DURING RAT HEPATOCYTES TUMORIZATION

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Zajdela hepatoma cells are poorly-adherent cells derived from an undifferentiated tumor and transplanted into rat. We compared the biosynthesis, structure and function of the fibronectin receptor in normal rat hepatocytes with that in Zajdela hepatoma cells. The rat hepatocyte fibronectin receptor has been isolated in hepatocytes; it is composed of an α_5-155 kDA and a β_1-115 kDa subunits (Johansson et al., 1987). Using polyclonal antibodies raised against each of the subunits of the receptor, we observed that the $\alpha_5$subunit was synthesized also as a 155-kDa protein in the tumor cells. In contrast, the molecular mass of theβ_1-subunit was 130 in the tumor cells versus 115 kDa in the normal cells. Pulse-chase experiments showed that the apparent transition time from the 100-kDaβ_1-precursor to the 130-kDa mature form was abnormally prolonged in the tumor cells since the latter was not detected until 24h, while the transition from the 100 kDa precursor to the 115 kDa-mature form began within 3h in the normal hepatocytes. Digestion of both the normal and tumor 100-kDa β_1–precursors with Endo H and N-Glycanase yielded products of 84 and 82 kDa, respecively, suggesting that the same polypeptide chain is synthesized in normal rat hepatocytes and Zajdela hepatoma cells. Incubation of the mature hepatocyte 115-kDa subunit with N-Glycanase reduced its molecular mass to 82 kDa, while the molecular mass of the abnormal subunit decreased from 130 to 110 kDa.
Thus, in addition to alterations in the Asn-linked oligosaccharide processing, "ascitic growth" induced other post-tranlational changes which modulate the biological function of the fibronectin receptor. Indeed, Zajdela hepatoma cells were found to not bind well fibronectin.

U 401 ABNORMAL GLYCOSYLATION OF MAG IN THE DYSMYELINATING, QUAKING MUTANT

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The myelin-associated glycoprotein (MAG) is a highly glycosylated member of the immunoglobulin superfamily which appears to mediate interactions between myelin-forming glial cells and axons. It occurs in two isoforms (L-MAG and S-MAG) generated by alternative splicing of its mRNA. Using antisera specific to L-MAG and S-MAG, respectively, we have shown that both isoforms have higher apparent molecular weights in quaking mice than in controls. Unlike controls where L-MAG predominates at early ages, S-MAG is the principal isoform in quaking mice at all ages between 13 and 72 days. However, L-MAG was just detectable by western blotting in young quaking mice. Studies involving lectin binding and glycosidase treatment revealed an increase of NANAα2-3Gal-oligosaccharide structures and subterminal galactose residues in quaking MAG, indicating a more extensive branching of oligosaccharides in the mutant which accounts for its higher apparent molecular weight. The total amount of NANA released from immunopurified quaking MAG determined by HPAE-chromatography was 40% higher than that in control MAG. The MAG in quaking mice also contained less of the adhesion-related, HNK-1 carbohydrate eptiope. In addition, a lower molecular weight form of MAG with only high mannose oligosacchrides was prominent in young quaking mice but not in controls. The abnormal glycosylation of MAG in quaking mice may alter its function and thereby contribute to the myelin pathology in this mutant.

U 402 EXPRESSION OF GLYCOSYLTRANSFERASE ENZYMES IN T CELL DEVELOPMENT: A ROLE IN CELL-CELL RECOGNITION IN THE THYMUS, Linda Baum[1], Mabel Pang[1], Nancy Perillo[1], Kelly Derbin[1], Terry Wu[1], Christel Uittenbogaart[2], Minoru Fukuda[3], and Jeffrey Seilhamer[4], Depts of Pathology[1] and Pediatrics[2], UCLA School of Medicine, Los Angeles, CA, La Jolla Cancer Research Foundation[3], La Jolla, CA, Incyte Pharmaceuticals[4], Palo Alto, CA

Developing T cells undergo a variety of changes in cell surface glycosylation during maturation in the thymus. Neither the mechanisms governing the expression of developmentally-associated glycoforms, nor the biologic function of these cell surface oligosaccharides, are well understood. Using in situ hybridization, we have found distinct developmental patterns of expression of three glycosyltransferase enzymes in human thymus. Expression of the Galβ1,3/4GlcNAc α2,3 sialyltransferase appears to be constant throughout thymocyte development, while expression of the Galβ1,3GalNAc α2,3 sialyltransferase is higher in mature medullary thymocytes than in immature cortical thymocytes (J. Biol. Chem. 268:3801-3804, 1993). Conversely, expression of the GalNAc β1,6 GlcNActransferase (core 2 enzyme) is high in cortical cells, and low in medullary cells.

The high level of expression of the core 2 enzyme in immature cortical thymocytes appears to relate to the ability of these cells to bind to an endogenous S-type lectin, L-14, produced by human thymic epithelial cells. Inhibition studies demonstrated that binding of immature T cells to thymic epithelial cells was inhibited by antibodies both to L-14 and to core 2 O-glycans on a T cell surface molecule CD43. These results suggest that the regulated expression of core 2 O-glycans, which contain lactosamine sequences, may promote the adhesion of immature T cells to thymic epithelial cells via L-14. Interaction between thymic epithelial cells and developing thymocytes is essential for selection and maturation of immunocompetent T cells. The interaction between core 2-based O-linked oligosaccharides and L-14 may play a role in the adhesion or selection of immature T cells during thymocyte development.

U 403 CYTOKINE-INDUCED β-GALACTOSIDE α2,6-SIALYLTRANSFERASE IN HUMAN ENDOTHELIAL CELLS.
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Sialic acids that decorate blood and cell surface proteins are thought to play important roles in a variety of biological processes. The inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), as well as bacterial lipopolysaccharide (LPS), are known to activate vascular endothelium, increasing the expression of several cell surface glycoproteins. In this study, treatment of cultured human endothelial cells (HEC) with TNF-α, IL-1, or LPS resulted in increased expression of the enzyme β-galactoside α2,6-sialyltransferase (α2,6-STN). TNF-α was most effective, inducing a 3.5-fold enhancement of cell-associated sialyltransferase activity by 72 h. In addition, activated endothelial cells shed a large portion of their induced sialyltransferase activity into the conditioned medium.

Analysis of [^{3}H]glucosamine-labeled HEC showed both a relative and an absolute increase of α2,6-linked sialic acid on N-linked oligosaccharides after TNF-α stimulation, all of which is N-acetylneuraminic acid (Neu5Ac). These effects coincided with increased expression of endothelial glycoproteins bearing N-linked glycans containing α2,6-linked sialic acid, as determined by blotting with the lectin *Sambucus nigra* agglutinin (SNA). SNA blotting of selected glycoproteins revealed that the cytokine-inducible endothelial cell adhesion molecules E-selectin, ICAM-1 and VCAM-1 are substrates for α2,6-STN. These changes also correlated with a substantial increase in binding sites for CD22β, a mammalian lectin known to recognize oligosaccharides carrying multiple copies of α2,6-linked Neu5Ac. Northern analysis revealed increased levels of mRNA encoding α2,6-STN. These results suggest that activation of endothelial cells during inflammatory and immunological processes induces α2,6-STN, which participates in sialylation of other activation-dependent molecules. In addition, α2,6-STN released from activated endothelial cells may contribute to the elevated serum level of this enzyme during an acute phase response.

U 404 ALTERED LEVELS OF GALACTOSYLTRANSFERASE AFFECT SPERM:EGG BINDING IN TRANSGENIC MICE, Helen J. Hathaway, Adel Youakim, David J. Miller, Xiaohai Gong, and Barry D. Shur, Department of Biochemistry & Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

β1,4Galactosyltransferase (GalTase) on the surface of mouse sperm mediates sperm:egg binding by interacting with the zona glycoprotein ZP3. Transgenic mice have been created that overexpress surface GalTase. Sperm from these transgenic mice bind more ZP3 than do wild type sperm. Surprisingly, transgenic sperm have a significantly reduced ability to bind eggs in *in vitro* assays. This results from a perturbation of sperm GalTase function during capacitation and during induction of the acrosome reaction. During capacitation, polylactosamines are spontaneously released from wild type sperm, unmasking sperm GalTase, making it available to interact with its oligosaccharide ligand in ZP3. On transgenic sperm, increased levels of surface GalTase results in increased binding and/or a decreased release of polylactosamines, resulting in fewer sperm that are capable of initial binding to eggs. After binding to the zona pellucida, ZP3 induces the acrosome reaction, apparently by cross-linking sperm GalTase. Transgenic sperm undergo a faster rate of acrosome reaction in response to soluble zona, and transgenic sperm bound to the egg zona pellucida have a higher proportion of acrosome-reacted sperm, compared to wild type. Since acrosome-reacted sperm are thought to bind more weakly to the zona, these sperm are more easily lost when loosely adherent sperm are removed after the assay. These results provide further evidence for the role of GalTase in sperm:egg binding and subsequent induction of the acrosome reaction. Supported by HD23479.

U 405 cDNA SEQUENCE AND GENE ORGANIZATION OF CONGLUTININ, A C-TYPE MAMMALIAN LECTIN CONTAINING A COLLAGEN-LIKE DOMAIN, Toshisuke Kawasaki, Nobuyuki Itoh, and Nobuko Kawasaki*, Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University and *College of Medical Technology, Kyoto University Kyoto 606, Japan.

Bovine conglutinin is a Ca^{2+}-dependent serum lectin specific for N-acetylglucosamine, which is suggested to be involved in host defense. In the present study, we report the full-length cDNA (1548bp) structure and the gene organization of conglutinin. The coding region of the conglutinin cDNA encoded the sequences of the signal peptide of 20 amino acids and the mature protein of 351 amino acids. The latter was identical to the sequence determined by Lee et al. using protein chemistry (1) except for one amino acid residue. The coding region of conglutinin mRNA was encoded by seven exons. Exon I encoded the signal peptide, a cysteine-rich NH_2-terminal region and six repeats of a Gly-X-Y triplet with one irregular triplet. The remaining long collagen-like domain (49 Gly-X-Y repeats with one irregular triplet) was encoded by four separate exons (Exon II~V). Exon VI encoded the neck region and Exon VII encoded the carbohydrate-recognition domain. The overall exon-intron organization of conglutinin was very similar to that of mannan (mannose)-binding proteins. However, the presence of 108 bp exons in the gene encoding the collagen-like domain was a characteristic of conglutinin. In the promoter region of the gene, several putative consensus sequences that may be involved in the expression of conglutinin were identified.

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U 406 THE GLYCOSYLATION OF RHODOPSIN; INFLUENCE OF GLcNAc-TRANSFERASE II, Edward L. Kean and Jermin Ju, Department of Ophthalmology, Case Western Reserve University, Cleveland, Ohio 44106

The oligosaccharide chains of rhodopsin are unique in terms of their abridged size. The major isomer found in all species which have been examined is a mono-antennary oligosaccharide of structure: GlcNAcβ1 → 2Manα1 → 3(Manα1 → 6)Manβ1 → 4GlcNAc β1 → 4GlcNAc. In an attempt to explore control mechanisms that give rise to this situation, we have examined preparations from the retina for the activity of N-acetylglucosaminyltransferase II (Gm Tase II). This enzyme would catalyze the transfer of GlcNAc to the Manα1 → 6 arm producing GnGn and thus could initiate the assembly of more highly branched structures. Bovine rhodopsin (R), opsin (O), and the oligosaccharide (OL) cleaved from rhodopsin were used as potential acceptors of GlcNAc from UDP-[^{3}H]GlcNAc as substrate. The enzyme sources were Golgi-enriched fractions (GEF) from bovine and human retinas, and from rat liver, and a partially purified Gm Tase II from rat liver. While the V_{max}/K_m ratios using R, O and OL as acceptors were similar using the GEF from bovine and human retinas, 100-250 fold higher ratios were obtained with rat liver. Using Gm II Tase, the ratios were 20-100 fold higher. When the products were examined on con A-Sepharose, the major component eluted in a manner expected for GnGn, although the formation of more highly branched structures was also suggested. Dionex chromatography also showed that the major radioactive product was GnGn. Consistent with previous observations, prior galactosylation of rhodopsin extensively inhibited GmTase II activity. These studies indicate that the retina in vitro, has the capacity to assemble bi-antennary, and perhaps more highly branched, oligosaccharide chains, but, as evidenced by the low V_{max}/K_m, with greatly reduced efficiency as compared to rat liver GEF or partially purified Gm Tase II. Supported in part by EY00393 and the Ohio Lions Eye Research Foundation.

U 407 CONFORMATIONAL CLUES TO THE SUPERIOR INHIBITORY ACTIVITIES OF SOME SIALYL- AND NON-SIALYL-FUCO-OLIGOSACCHARIDES TOWARDS E-SELECTIN, Heide Kogelberg, Chun-Ting Yuen, Jacqui O'Brien and Ten Feizi, Glycoconjugates Section, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

The selectins (E-, P- and L-selectin) are cell-adhesion molecules which have crucial roles at the initial stages of leucocyte recruitment and extravasation in inflammation, and are implicated in haematogenous spread of tumor cells. They are members of a group of modular proteins characterised by the presence of C-type lectin domains, and as expected, their ligands have been identified as carbohydrates. Sialyl Le^x and the isomeric sequence sialyl Le^a are bound by all three selectins.[1] E-selectin can also bind to the asialo analogues, although less strongly.[2] Recently, sulphated Le^a/Le^x type sequences have been shown to be strongly bound by E-selectin[3] and L-selectin[4]. Correlation of three-dimensional features of carbohydrate ligands with their potencies as inhibitors for selectin binding will help to identify ligand groups which are involved in the binding process and open ways to the design of drugs which serve to manage disorders of inflammation and prevent the blood borne spread of tumor cells. It is generally accepted that strong binding activity is conferred by a negatively charged group in proximity to the C3 of the outer galactose. Favorable electrostatic interactions between the charged group on the oligosaccharide and corresponding charges on the protein are therefore most likely responsible for the strong binding. The present study is focussed on sialyl and non-sialyl trisaccharides which differ in their inhibitory activities towards E-selectin. The superior binding observed for some of them can be explained by distinct conformational features of these oligosaccharides.

HK is supported by the Leukaemia Research Fund.

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U 408 DIFFERENTIAL RECOGNITION BY CONGLUTININ AND MANNAN-BINDING PROTEIN OF HIGH-MANNOSE TYPE OLIGOSACCHARIDES PRESENTED ON THE COMPLEMENT GLYCOPROTEIN C3 AND DERIVED GLYCOPEPTIDES

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Conglutinin and mannan-binding protein are serum lectins which have similar but not identical carbohydrate-binding specificities (R.W.Loveless *et al.* 1989. *Biochem.J.* **258**, 109-113; T.Mizuochi *et al.* 1989. *J.Biol.Chem.* **264**, 13834-13839). However, only conglutinin binds to the complement glycoprotein iC3b, which like the parent glycoprotein C3 contains high-mannose type Man8 or Man9 oligosaccharides at Asn 917 of the α chain and Man5, Man6 or Man7 at Asn 63 of the β chain. Binding studies were performed with neoglycolipids prepared from oligosaccharides released from the isolated α and β chains of human C3 and also with various C3-derived glycoproteins and fragments. The results clearly indicate that although conglutinin and mannan-binding protein bind to the high-mannose type oligosaccharides free of protein, the binding of each lectin is influenced differently by the state of the carrier proteins. We conclude that the marked differences in conglutinin binding to the various C3 forms are a reflection of the mode of presentation of the Man8 or Man9 oligosaccharide on the α chain, and second, that neither this oligosaccharide nor the Man5 or higher oligosaccharides on the β chain are recognised by mannan-binding protein on the various physiologically derived forms of C3.

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U 409 NOVEL PEPTIDES THAT INHIBIT E-, L- AND P-SELECTIN MEDIATED ADHESION *IN VITRO*, AND NEUTROPHIL-MEDIATED INFLAMMATION *IN VIVO*. Bruce A. Macher[1,2], John B. Briggs[2], Yuko Oda[2], and James H. Gilbert[2], [1]Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA 94132, and [2]Glycomed, Inc. Alemeda, CA 94501

A series of peptides have been found to inhibit the interaction between E-, L- and P-selectin and their ligands. The peptides inhibited the binding of myeloid cells to recombinant forms of E- and P-selectin. The binding of myeloid cells to HUVEC cells stimulated to express E-selectin was also inhibited by the peptides. Finally, the peptides blocked the binding of lymphocytes, expressing L-selectin, to high endothelial venules in lymph nodes which contain the ligand for L-selectin. A clear structure/activity relationship was established when peptides of different amino acid chain lengths were tested in these assays. Peptides with six or seven amino acids were also found to inhibit neutrophil-mediated inflammation in a thioglycollate induce peritonitis mouse model system by as much as 70%.

U 410 RELATIVE SUBSTRATE ACTIVITIES OF AN α-L-FUCOSIDASE FROM *HALIOTIS RUFESCENS*,
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Information about the substrate specificity of glycosidases is essential to their use for structural determination of glycoconjugates or for modification of glycoconjugates to determine effects on biological activity. We have investigated an α-L-fucosidase which has α-1,2, α-1,3, α-1,4 and α-1,6 activities. Substrates tested indicate that the characteristics determining the relative activities include the anomeric linkage and the molecular weight of the substrate as well as the particular adjacent sugar residue. The relative activity against various substrates in decreasing order is the following: *p*-nitrophenyl-α-L-fucopyranoside > 2'-fucosyllactose > 6-O-α-L-fucopyranosyl-N,N'-diacetylchitobiose> 3-fucosyllactose > lacto-N-fucopentaose II (LNFP II) > lacto-N-fucopentaose I (LNFP I) > Lewis X trisaccharide > lacto-N-fucopentaose III (LNFP III) > Lewis a trisaccharide > lacto-N-fucopentaose V (LNFP V). Other substrates which the enzyme is active against include 6-α-fucosyl GlcNAc, and NGA2F(asialo-, agalacto-, bi-antennary, core-substituted with fucose). Some of the differences in relative activities were unexpected, particularly in the lacto-N-fucopentaose series. The enzyme is capable of removing fucose from some native glycoproteins such as horseradish peroxidase and bovine submaxillary mucin. The enzyme has low activity against the fucose containing lipophosphoglycan and TF_1 Glycolipid from *Tritrichomonas foetus*. The enzyme is not active against fucoidan, an α-1,2-fucan which is sulfated in the 4 position.

U 411 ROLE OF THE CARBOHYDRATE CHAINS OF HUMAN CHORIONIC GONADOTROPIN IN BIOLOGICAL ACTIVITY AND RECEPTOR BINDING
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The biological role of the N- and O-linked glycans of human chorionic gonadotropin (hCG) was studied using several hCG preparations of which the carbohydrate chains were enzymatically remodeled. The testosterone stimulating ability of these preparations in mouse Leydig cells, as well as the affinity for the LH/CG receptor in rat testicular membranes was assayed in vitro. It was concluded that the presence of a sialic acid residue on the Manα1→3Man branch of the N-linked diantennary glycans of hCG is essential for full expression of its biological activity. However, the presence of α2→6-linked sialic acid or α1→3-linked Gal on the Manα1→6Man branch of these glycans interferes with hormone-receptor binding, and concomitantly leads to a decrease of its bioactivity. Sialic acid residues on both subunits of hCG were found to be equally important for the bioactivity of the hormone. However, resialylation of the O-glycans of the β-subunit of desialylated hCG (as-hCG) did neither influence its bioactivity nor its affinity for the receptor. Degalactosylation of as-hCG decreased its bioactivity, however, its affinity for the receptor was similar to that of the native hormone. Replacement of the terminal Gal residues of the N-glycans of as-hCG with β1→4-linked GalNAc residues, and extension of the N-glycans of native and desialylated hCG with α1→3-linked fucose, resulted in a decrease of both bioactivity and receptor affinity of these preparations.

U 412 THE HEAT-LABILE ENTEROTOXIN OF *ESCHERICHIA COLI* BINDS TO TERMINAL GALACTOSYL(ß1,4)-N-ACETYLGLUCOSAMINYL-CONTAINING RECEPTORS IN CACO-2 INTESTINAL EPITHELIAL CELLS,
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The heat-labile toxin (LT) of *E. coli* shares considerable functional, structural, and immunological homology with cholera toxin (CT). Both toxins are composed of an A subunit and a pentameric B subunit. The A subunits irreversibly activate adenylylcyclase by ADP-ribosylation of the stimulatory G protein while the B subunits bind to specific cell-surface receptors. G_{M1} ganglioside is considered the sole receptor for CT. LT, which can also bind to G_{M1}, appears to utilize additional, unique receptors not recognized by CT. In this study we examined the nature of LT and CT receptors on the human intestinal epithelial cell line, CaCo-2 to identify those alternate receptors recognized by LT and to characterize the carbohydrate determinants necessary for LT binding and adenylylcyclase activation. In binding studies with these cells, LT bound to approximately 20-fold more sites than CT. Competitive binding studies with cell monolayers or membrane preparations indicated that the B subunits of CT and LT were equally effective in inhibiting the binding and action of CT. In contrast, CT-B only partially inhibited LT binding. Similarly, CT-B concentrations which inhibited CT stimulation of cyclic AMP formation only partially affected the action of LT. Mild trypsin treatment of intact cells for extended periods of time up to 1 h however, abrogated this difference. While thin layer chromatograms of total lipid extracts overlayed with ^{125}I-toxin showed LT and CT bound only to G_{M1} ganglioside, western blot analysis of membrane proteins probed with ^{125}I-LT, as well as immunoprecipitation of the LT-receptor complexes from cells labeled with [^{3}H]galactose revealed a series of six membrane galactoproteins which specifically bound LT. LT binding was abolished when membrane proteins were treated with *D. pneumoniae* ß-galactosidase or endo-ß-galactosidase prior to toxin exposure in binding studies and toxin overlays. In contrast, ß-galactosidase from bovine testes had no effect. When analyzed by toxin overlay, LT cross-reacted with those glycoproteins immunoprecipitated from crude membrane extracts with antibodies specific for SSEA, the Ii blood groups, and the lysosomal membrane glycoprotein LGP120. The glycoproteins immunoprecipitated from this array of antibodies encompassed those recognized in total by LT, and in each case was found to contain varying levels of the galactosyl(ß1-4)-N-acetylglucosaminyl-determinant. LT binding could be significantly enhanced with neuraminidase treatment of these immunoprecipitates. These results indicated that at a minimum, terminal ß1-4-linked galactosyl residues were required for alternate LT binding and further suggested that the galactosyl(ß1-4)-N-acetylglucosaminyl- component of polylactosylated membrane glycoproteins were the alternate receptor for LT in CaCo-2 cells.

U 413 SYNTHESES OF MODIFIED OLIGOSACCHARIDES OF N-GLYCOPROTEINS FOR SUBSTRATE SPECIFICITY STUDIES OF GLYCOSYLTRANSFERASES,
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We have developed a strategy of modified building blocks which allows any combination of different glycosylation reactions to synthesize a large number of modified tri- and tetrasaccharides. These compounds can be used for substrate specificity studies of the glucosaminyltransferases (GlcNAcT) of the biosynthesis of N-glycoproteins. The modified oligosaccharides of the typ Manα1→3[Manα1→6]ManβOR and GlcNAcβ1→2Manα1→3-[Manα1→6]ManβOR contain deoxy or O-methyl functions at different positions. With the help of this oligosaccharides it was possible to determine the substrate specificities of GlcNAcT-I and GlcNAc-II. The results give a good idea of the binding site of the two enzyms with the oligosaccharide substrate. In a further series of compounds reactive groups or groups for photolabeling which can react with the enzyms were introduced into the oligosaccharide substrates. Some of these substances show reversible or irreversible inhibitory properties for GlcNAcT-I and for GlcNAcT-II. The irreversible inhibitors can be possibly used for labeling of the active side of the enzyms.

U 414 THE INFLUENCE OF PROLACTIN, PROGESTERONE AND INSULIN ON THE OLIGOSACCHARIDE MOIETY OF IGG & IGA SECRETED FROM CULTURED LYMPHOCYTES

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Post-translational modification of antibody proteins occur in the Golgi of secreting cells. Some antibody-dominant autoimmune diseases are not gender neutral but favor expression in women. Pregnancy often offers a repreve from symptoms of autoimmunity. The hypothesis is that there is some hormonal regulation of oligosaccharide synthesis and attachment to IgG and IgA. Two secreting, myeloma cell lines (IM-9 and J558) were grown in the presence of differing concentrations of one of the following hormones: prolactin (0.132 I.U.,/ml and 0.198 I.U./ml), progesterone (5nM and 20nM) and Insulin (50 *ug* /ml and 200 *ug* /ml). The secreted IgG or IgA was purified by ammonium sulfate precipitation followed by DEAE Affi-Gel Blue column purification. (BioRad) Each sample was tested for the presence of terminal galactose and/or terminal GlcNAc. Methods of detection included dot blot analysis using two biotinylated lectins *Bandeiraea simplifilia* and *Ricinus communis*. (Sumar, N. et al. 1990, J. Immunol Method. 131:127-136), SDS PAGE and isoelectric focusing. IgG and IgA isolated from untreated cultures were used as controls. Another control was prepared by treating the cultures with tunicamycin (1ug/ml) to obtain an oligosaccharide-free antibody. The results are subject to several different interpretations, including a discussion relating to rhematoid arthritis.

U 415 RECOGNITION OF α-L-Fuc(1→6)-β-D-GlcNAc-OMe, α-L-Fuc(1→4)-β-D-GlcNAc-OMe and α-L-Fuc-OMe BY ALEURIA AURANTIA AGGLUTININ - DIFFERENT BINDING MODES INFERRED FROM TRANSFER NOE EXPERIMENTS.

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Aleuria aurantia agglutinin (AAA) is a lectin from orange peel mushroom which specifically recognizes fucose residues α(1→6) linked to N-acetyl glucosamine (1). Albeit this linkage specificity, it has been qualitatively shown by means of 2D transfer NOE experiments that AAA binds multiple conformations of the disaccharide α-L-Fuc(1→6)-β-D-GlcNAc-OMe **1** (2). This finding prompted for more quantitative experiments, especially to pin-point the disturbances of the conformational equilibrium about the (1→6) linkage upon complexation. Here, we will present (transfer) NOE curves obtained from selective one dimensional NOE experiments with shaped pulses for the free and liganded forms of α-L-Fuc(1→6)-β-D-GlcNAc-OMe **1**, α-L-Fuc(1→4)-β-D-GlcNAc-OMe **2** and α-L-Fuc-OMe **3**. A quantitative comparison of the NOE curves for free **1** with the transfer NOE curves for liganded **1** allows to estimate the conformational changes induced at the (1→6) linkage upon binding. A comparison with corresponding NOE data for the more rigid disaccharide **2**, part of the Lewis A blood group determinant, and monosaccharide **3** reveals characteristic structural features required by the lectin for specific recognition of **1**.

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U 416 OLIGOSACCHARIDE BINDING SPECIFICITIES OF CD22β, A SIALIC ACID-SPECIFIC LECTIN OF B CELLS

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CD22β is a B cell surface glycoprotein involved in cell adhesion and activation. Utilizing the recombinant soluble form CD22βRg, we previously reported that natural glycoprotein ligands from lymphoid cells bear complex-type N-linked oligosaccharides with α2-6 linked sialic acid residues that are required for recognition (Powell et al, J.Biol.Chem. 268: 7019-7027). Here, we have defined the oligosaccharide binding specificity of this lectin by examining a number of naturally and enzymatically sialylated oligosaccharides and sialoglycoproteins. The minimal structure recognized by CD22β is Siaα2-6Galβ1-4Glc(NAc). Reduction of the lactose residue of Siaα2-6Galβ1-4Glc diminishes the interaction, while truncation of the sialic acid side chain by periodate oxidation abolishes it. Branched oligosaccharides with two α2–6-sialyl residues bind better, regardless of whether they were derived from *N*- linked oligosaccharides, *O*-linked oligosaccharides, or gangliosides. Alpha 2-3-sialyl residues have no effect on binding, whereas increasing the numbers of α2-6-sialyl residues on multiantennary *N*- linked oligosaccharides progressively improves binding, up to four. No specific feature of the core regions of *N*-linked oligosaccharides studied (including bissection) affected binding. However, the localization and spacing of the α2-6-sialyl residues on triantennary chains appears to have a significant effect. Of several model sialoglyco-proteins examined, fetuin and transferrin had an apparent affinity no greater than that observed with free sialylated *N*-linked oligosaccharides. Some subfractions of these proteins displayed unexpectedly weak binding, suggesting that the protein backbone can exert a negative effect. In contrast, a subfraction of α_1-acid glycoprotein was identified as having a substantially higher binding affinity than free oligosaccharides derived from it, indicating that multiply glycosylated glycoproteins can have improved affinities. Thus, CD22βRg contains a lectin activity specific for the minimal motif Siaα2-6Galβ1-4Glc(NAc), and branched, multi-sialylated oligosaccharides are better ligands, regardless of the core sequences. Intact sialoglycoproteins can also interact, although with a variable affinity not directly predictable from the structure of the sialylated oligosaccharides that they carry. These data may help to explain why certain T- and B-cell surface sialoglycoproteins with the Siaα2-6Galβ1-4GlcNAc motif are superior ligands capable of mediating CD22β-mediated adhesion and activation events.

U 417 STRUCTURE-GLYCOSYLATION RELATIONSHIP IN YEAST INVERTASE,

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Mannose oligosaccharides are added in yeast to the consensus glycosylation sites Asn - Xxx - Ser/Thr of the nascent polypeptide, cotranslocationally or shortly after translocation. However some potential glycosylation sites are not glycosylated for reasons that are poorly understood. Yeast invertase contains 14 glycosylation sites of which 13 are either partially or fully glycosylated. Site 5 (Asn93) which overlaps site 4 (Asn92) in the sequence Asn92-Asn93-Thr-Ser, is not glycosylated. To examine whether oligosaccharide on Asn92 is sterically blocking the addition of oligosaccharide to Asn93 , we have abolished site 4 by converting Asn92 to Gln using site-directed mutagenesis. The mutant enzyme was digested with trypsin and a glycopeptide encompassing glycosylation sites 3,5 and 6 was purified by reverse phase HPLC. Sequence analysis of the glycopeptide showed that site 3 (Asn78) and site 6 (Asn99) were glycosylated as expected, but site 5 (Asn93) was not. This observation was confirmed by sequencing the glycopeptide following incubation with PNGase F (which converts N-linked Asn to Asp). The results of the mutation study eliminate the possibility that oligosaccharide on Asn92 sterically hinders the addition of oligosaccharide to Asn93. Asn93 is probably inaccessible to glycosylating enzyme because it is sequestered in a discreetly folded region. Support for this proposal comes from an *in vitro* glycosylation study using a 17 aminoacid invertase peptide encompassing sites 4 and 5, and the same peptide with the Asn92Gln mutation. Preliminary evidence indicates that the mutant peptide is glycosylated in contrast to the mutated invertase, but less extensively than the native peptide, suggesting that the latter is glycoylated at both sites. (Supported in part by a grant from the National Cancer Institute CA44355)

U 418 THE CBP35-CBP70 INTERACTION IS MODULATED BY THE BINDING OF LACTOSE TO CBP35 IN HL60 CELL NUCLEI. Annie-Pierre Sève[1], Murielle Felin, Yasmina Hadj-Sahraoui, Marie-Agnès Doyennette-Moyne, Michèle Aubery and Jean Hubert Laboratoire de Glycobiologie et de Reconnaissance Cellulaire, U180 INSERM UFR Biomédicale des Saints-Pères (Université René Descartes, Paris V), 45 rue des Saints-Pères, 75270 PARIS Cedex 06, France.

Two carbohydrate-binding proteins, CBP35 which recognizes lactose and CBP70 which recognizes glucose, can under appropriate conditions of affinity chromatography, be isolated as a complex from HL60 cell nuclear extracts. Moreover, we have demonstrated that the CBP70-CBP35 are associated by a protein-protein interaction which can be modulated during affinity chromatography when CBP35 binds to lactose (Sève *et al.*, 1993, *Glycobiology*, **3**, 23-30.). To know whether such a lactose-modulated interaction occurs in the nucleus, we have followed he behaviour of CBP70 and CBP35 in membrane depleted-nuclei of HL60 cells, incubated or not incubated with lactose, taking the opportunity of a cross reaction of an anti-serum between CBP35 and CBP70 and of the availability of an antiserum specifically raised against CBP70. A speckled fluorescence staining was observed in nuclei not incubated in the presence of lactose whatever the antibody used. The observations performed by fluorescence microscopy added to the results of immunoblotting analysis of proteins present in the nuclei after incubation with or without lactose and of the proteins released in the incubation medium show that: i) CBP35 and CBP70 are colocalized in the nuclei which were not incubated with lactose. ii) CBP35 still stays inside the nucleus while most CBP70 is released from nuclei upon lactose incubation.

These results strongly suggest that, in membrane-depleted nuclei, CBP35 and CBP70 are associated by interactions which can be modulated by the binding of lactose to CBP35. These data are discussed with regard to the possibility that the CBP35-CBP70 association might be modulated *in vivo* by the binding of a not yet identified nuclear glycoprotein to CBP35.

U 419 CARBOHYDRATE - MEDIATED INTERACTIONS OF IgA WITH CELLS, Milan Tomana and Jiri Mestecky, Departments of Medicine and Microbiology, The University of Alabama at Birmingham, Birmingham, AL 35294.

To explore the role of the carbohydrate moiety in the *in vivo* distribution of IgA, monomeric (m) myeloma IgA1 proteins were modified by sequential treatment with neuraminidase and galactose oxidase and/or β-galactosidase, and then subjected to affinity chromatography on agarose-bound Jacalin, a lectin with specificity for gal β1-3 galNAc glycans, which occur in the hinge region of the IgA1. IgA protein which did not bind to Jacalin and Jacalin-binding controls were radioiodinated and injected i.v. into mice. The distribution of radioactivity among organs 1,2,4, and 8 days later revealed that a significantly larger amount of IgA which did not bind to Jacalin deposited in the kidneys and a smaller quantity in the liver, compared to Jacalin-binding IgA. Furthermore, mice injected with galactose-deficient IgA had higher protein-associated radioactivity in serum. Results of *in vivo* experiments were confirmed by studies of the interactions of mIgA1 proteins having modified carbohydrate structures with human and mouse cells and cell lines. Radioiodinated mIgA1 which binds to Jacalin, bound efficiently to the hepatoma cell line, Hep G2 , which expresses the asialoglycoprotein receptor. In contrast, the human monocyte-like cell line, U937, glomerular cells isolated from mouse and human kidney, and cultured human mesangial cells bound significantly more IgA in which was modified or removed galactose. These data suggest that removal of galactose resulted in diversion from the usual degradation pathway of IgA in the liver. Instead, this IgA is deposited in the kidney by an as yet unknown mechanism, or else remains in the circulation, thus increasing the circulating pool of this Ig. Supported by NIH grant #DK28537.

U 420 BIOTINYLATED DIAMINOPYRIDINE (BAP): ADVANCES IN THE USE OF A NOVEL FLUORESCENT TAG FOR OLIGOSACCHARIDES. Derek Toomre and Ajit Varki, Glycobiology Program, UCSD Cancer Center, and Division of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093

We recently described a novel fluorescent compound, 2-amino,6-amidobiotinyl-pyridine (BAP), that allows the tagging of oligosaccharides, their fractionation by reversed phase HPLC with picomole scale detection, and the formation of functional neoglycoprotein equivalents with (strept)avidin for the detection of receptors and the generation of mono-specific antibodies (Rothenberg et al., PNAS; in press). Here, we describe the enhancement of this approach by the following improvements:

(1) A simple one-step purification of BAP from its synthetic precursors and other reactants.
(2) The use of BAP for sensitive compositional analysis of monosaccharide mixtures, with excellent resolution.
(3) Development of HPLC sizing column methods to fractionate distinct species of BAP-coupled oligosaccharides away from free BAP, and from one another.
(4) Development of anion exchange HPLC procedures for the fractionation of BAP-oligosaccharide adducts.
(5) Investigation of the off-rates of BAP-oligosaccharide adducts from streptavidin, confirming the formation of stable complexes.

These advances make available a multitude of techniques for the fractionation of BAP-coupled oligosaccharides based on different physical parameters. Distinct species of BAP-coupled oligosaccharides can be isolated and subjected to detailed structural analysis. Such defined molecules form stable complexes with streptavidin that are effectively neoglycoproteins which can be used in a variety of functional applications. Notably, all of these approaches require relatively inexpensive materials and conventional equipment available in most laboratories. The major remaining drawback is that the efficiency of coupling varies with the oligosaccharide (this is a problem common to <u>all</u> reported tagging methods involving reductive amidation). Issues such as oligosaccharide size, charge, and hydrophobicity may be important. Also, there is significant complexity in the coupling reaction, due to adduct formation by alternative pathways other than Schiff base formation. We are currently investigating these issues to optimize and standardize the coupling reaction.

U 421 Characterization of the Okra Mucilage by Interaction with Gal, GalNAc and GlcNAc Specific Lectins*

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A bio-active polysaccharide, which was the major component of the aqueous extract of the commom okra, *Hibiscus esculentus*, was isolated from the extract by ethanol precipitation between 28.5 to 45%. This fraction contained 40% D-galactose, 27% rhamnose, 24% galacturonic acid, less than 4% protein estimated by the Lowry method, and trace or none of hexosamine and sialic acid. According to the previous report (Whistler, R.L. and Conrad, H.E. [1954] J. Amer. Chem. So. 76 1673-1674), this polysaccharide contains the Galα1→4Gal sequence, which is the receptor for the uropathogenic *E. coli* ligand and toxic lectins. Thus, its binding property was analyzed by precipitin assay with Gal, GalNAc and GlcNAc specific lectins. The results showed that this okra mucilage reacted best with *mistoletoe toxic* lectin - I (ML-I) which is specific for the galactobiose (Galα1→4Gal) sequence. This polysaccharide precipitated over 80% of the ML-I nitrogen (5.1 μg N) added and only 3.5 μg of polysaccharide was required for 50% precipitation. It also precipitated well with *Abrus precatorius* (APA), *Momordica charantia* (MCA) and *Ricinus communis* (RCA_1) agglutinins, but poorly with other lectins. Except the reaction with MCA, the binding reactivity of this polysaccharide was enhanced slightly by mild acid treatment (pH 2, 80°C for 90 min). Treatment of the polysaccharide with BH_4^- (0.1 M $NaBH_4$ at room temp for 20 h) gave a sharp decrease of its reactivity toward ricin, while its binding to RCA_1, APA, abrin-a and MCA was slightly reduced. The results obtained suggested that this polysaccharide can be used as a valuable reagent to differentiate Gal specific lectins from GalNAc specific and/or GlcNAc series.

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U 422 IMMUNOLOCALIZATION OF THE LONG FORM OF β1,4 GALACTOSYLTRANFERASE TO THE CELL SURFACE AND GOLGI OF 3T3 AND F9 CELLS, Adel Youakim and Barry D. Shur, Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, Houston, TX 77030.

β1,4 Galactosyltransferase (GalTase) is found in the Golgi complex and on the cell surface of most tissues and cells examined. The gene for GalTase encodes two proteins with identical lumenal/extracellular and transmembrane domains but with distinct cytoplasmic domains. The short form of GalTase has an 11 amino acid cytoplasmic tail, whereas the long form of the protein has an additional 13 amino acids attached to its cytoplasmic domain. Previous studies from our laboratory using cells transfected with various forms of GalTase cDNA have shown that both the long and the short form of GalTase are found in the Golgi complex and that the long form of the protein is also preferentially targeted to the plasma membrane, relative to the short form. Furthermore, it is the long GalTase that functions as a cell adhesion molecule during fertilization and development. In the current study, we have determined the subcellular distribution of the endogenous long GalTase in untransfected 3T3 and F9 cells using antibodies that specifically recognize the unique 13 amino acid domain of the long form of GalTase. This antiserum immunoprecipitates GalTase activity and also immunoprecipitates and western blots a protein of the appropriate size. This antiserum was used to immunolocalize the long form of GalTase in F9 and 3T3 cells using confocal microscopy. In 3T3 cells, there is staining in the presumptive Golgi complex and at the tips of lamellipodia in migrating cells. In F9 cells, there is also perinuclear staining, but staining at the cell surface is more uniformly distributed than in 3T3 cells, and is particularly intense between adherent cells. Thus, in contrast to previous studies that have used transfected cells, and that have suggested that the long GalTase is localized primarily to one subcellular compartment, these results show that the endogenous long form of GalTase is found in the Golgi and at the cell surface. (Supported by NIH grant HD22590)