GLYCOBIOLOGY

Organizers: Joseph Welply and Gerald Hart

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Plenary Sessions Page

Poster Sessions

Emerging Analytical Technologies

P 001 MASS SPECTROMETRY: ENABLING TECHNOLOGY FOR GLYCOBIOLOGY, Anne Dell[1], Howard Morris[1], Maria Panico[1], Roy McDowell[1], Andrew Reason[1], Kay-Hooi Khoo[1], Anna Chan[1], Parastoo Azadi[2], Luc-Alan Savoy[2] and Mark Rogers[3]. [1]Department of Biochemistry, Imperial College London, [2]M-Scan Ltd, Ascot Berks, [3]M-Scan Inc, West Chester PA.

High field magnetic sector double focussing mass spectrometry has played a major role in biopolymer analysis in the 15 years since we introduced it, and high field instruments fitted with fast atom bombardment (FAB) sources have solved a wide range of structural problems thereby helping to foster the current explosion in glycobiology research. This talk will show how structural problems are being addressed using concerted derivatisation/FAB-MS strategies which have been optimised for high sensitivity production of both molecular and fragment ions which afford information on compositions, sequences, branching patterns, functional groups and anomeric stereochemistry.

Current achievements with these methodologies will be illustrated with data from amongst the following studies: (a) Identification of O-GlcNAc attachment sites in Serum Response Factor (a ubiquitous transcription factor); (b) Characterisation of N- and O-glycans from nematode glycoproteins; (c) Sequencing the sulphated O- and N- glycans in pro-opiomelanocortin (POMC); (d) Mapping the non-reducing structures in sulphated tetra and penta-antennary lactosaminoglycans; (e) Identification of novel structures in mycobacterial polysaccharides.

Despite the many successes achieved with current high field instrumentation, there are many research projects in the field of glycobiology that demand even greater sensitivity and mass range. We are addressing the problem of sensitivity with a new mass spectrometer, the ZAB-2SE FPD, which incorporates a wide angle (1.5:1) multichannel array focal plane detector for high sensitivity, low resolution survey analysis up to 15 kilodalton. A second array (1.05:1) permits narrow angle high resolution analysis. The performance of this instrument will be described.

For high mass studies in the field of glycobiology the new electrospray (ES) instrumentation offers considerable promise. ES-MS data on glycopeptides and glycoproteins will be presented and the complementarity of ES-MS and FAB-MS will be discussed.

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P 002 ELECTROSPRAY MASS ANALYSIS OF COMPLEX GLYCOCONJUGATES, Kevin L. Duffin and Joseph K. Welply, Monsanto Corporate Research, Monsanto Company, St. Louis, MO 63198

Electrospray mass spectrometry promises to complement the analytical capabilities for characterization of oligosaccharide structure by virtue of its sensitivity, its ability to detect underivatized carbohydrates, and its ability to accurately measure the masses of glycoproteins and resolve oligosaccharide heterogeneity on the glycoprotein. This paper presents experimental conditions for electrospray and tandem mass spectrometric characterization of complex, underivatized N-linked oligosaccharides. The N-linked structures have a common core comprised of Man2-Man-GlcNAc-GlcNAc which is extended with branched chains containing additional mannose, N-acetylglucosamine, galactose and sialic acid residues. In addition, data is presented on the analysis of the heterogenous mixture of N-linked oligosaccharides obtained from ovalbumin, a glycoprotein whose oligosaccharides have been extensively characterized by several techniques. The structural data obtained from electrospray mass analysis of N-glycanase-released oligosaccharides are compared to those generated by enzymatic sequencing and to those obtained from the electrospray mass spectrum of intact ovalbumin before the oligosaccharides are removed. Electrospray is also used as an interface for the on-line LC/MS characterization of tryptic digests of various glycoproteins. These LC/MS data yield the sites of glycosylation of the glycoproteins and also indicate the carbohydrate composition of individual glycopeptides. Comparison of the LC/MS data that are obtained before and after deglycosylation of the glycoproteins helps in determining the glycosylation sites.

P 003 RECENT DEVELOPMENTS IN CAPILLARY ELECTROPHORESIS, RANDY I. HECHT, SUSAN C. HOWARD, AND GARY S. JACOB, MONSANTO CORPORATE RESEARCH, GLYCOSCIENCES UNIT, 800 NORTH LINDBERGH BLVD., ST. LOUIS, MO. 63167.

Capillary electrophoresis (CE) is emerging as a powerful high resolution and rapid analytical technique for the separation and analysis of a wide array of biomolecules. Due to its dramatic resolution enhancement over traditional separation methodologies, CE has demonstrated the potential to separate individual glycoprotein glycoforms. We present an example of this capability with a study of ribonuclease B, a 124 amino-acid glycoprotein (MW= 13.7 kD) which contains a single N-glycosylation site, and which could be separated into glycoforms attributable to the Man_5-Man_9 series. We also demonstrate the utility of a particular CE buffer system to separate tryptic-digest derived glycopeptides of ribonuclease B. A second example of CE's utility will be demonstrated with the human protein interleukin-4, a 129 amino-acid containing 2 N-linked glycans per molecule, and the effect of enzymatic removal of N-linked oligosaccharides on the resulting CE electropherograms. Finally, we present initial information on our efforts to interface CE with an electrospray mass spectrometer.

P 004 FACE: FLUOROPHORE ASSITED CARBOHYDRATE ELECTROPHORESIS. A NEW TECHNOLOGY FOR THE ANALYSIS OF COMPLEX CARBOHYDRATES, Peter Jackson, Glyko, Inc., Novato, CA 94949

The carbohydrate moieties of glycoconjugates are involved in numerous important biological processes. Their isolation and structural analysis present considerable difficulties partly because of the relatively small quantities which are available from biological sources and partly because of the inherent difficulty of analyzing their complex structures. In order to address these problems a new method of analysis, FACE, Fluorophore Assisted Carbohydrate Electrophoresis, has been developed in which saccharides having a reducing end group are labelled covalently and quantitatively with a fluorophore and the derivatives separated with high resolution by polyacrylamide gel electrophoresis (PAGE). The resulting electrofluorograms can be viewed with high sensitivity using an imaging system based on a cooled charge - coupled - device (CCD). Subpicomolar quantities of individual saccharides can be detected. Two fluorophores, 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) and 2-aminoacridone (AMAC) have been used successfully to label a variety of oligo and monosaccharides (1,2,3). Both fluorophores enable the electrophoretic separation of a variety of positional isomers, anomers and epimers. After labeling with AMAC all of the six monosaccharides found commonly in mammalian glycoproteins and also glucose could be separated in a single gel lane thus enabling compositional analysis of hydrolysates of complex saccharides. AMAC can also be used to distinguish unequivocally between acidic and neutral oligosaccharides.

Complex mixture of oligosaccharides obtained by enzymatic cleavage from a variety of glycoproteins have been labeled with the alternative fluorophore, ANTS, and separated by high resolution PAGE to yield an oligosaccharide profile of each protein. The method enabled rapid, simultaneous, parallel analyses of multiple samples. Preparative electrophoresis was used for the isolation of the individual labelled oligosaccharides for further analysis for composition and saccharide sequence. Methods for determining the saccharide sequence in the isolated oligosacchardes have been developed using a combination of enzymatic degradation and FACE technology. Data demonstrating the application of these methods to the analysis of structure/function relationships in specific biological phenomena will be presented.

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P 005 THE APPLICATION OF NEW NMR TECHNIQUES FOR THE STRUCTURAL AND CONFORMATIONAL CHARACTERIZATION OF GLYCOPROTEIN CARBOHYDRATES.

Herman van Halbeek. *Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602, U.S.A.*

We developed an NMR spectroscopy strategy for detailed characterization of the solution conformation and dynamics of oligosaccharides. Our strategy generates a large number of inter-glycosidic spatial constraints. In addition to the commonly used {^{1}H,^{1}H} NOEs between aliphatic protons, we use as constraints (a) homonuclear NOEs of OH protons in H_2O to other protons in the oligosaccharide [1], (b) heteronuclear {^{1}H,^{13}C} NOEs [2], and (c) long-range heteronuclear scalar couplings across glycosidic bonds [3]. This approach generated over 25 spatial constraints for the trisaccharide NeuAcα(2→6)Galβ(1→4)Glc [4]. In combination with Metropolis Monte-Carlo simulations, the trisaccharide in aqueous solution was shown to exist in two families of conformers, the predominant one stabilized by interresidue hydrogen bonding [4].

However, a large number of interglycosidic constraints in itself may not be sufficient to determine the conformation of an oligosaccharide. Multiselective NOE measurements on sucrose in aqueous solution yielded 10 interglycosidic contacts, compatible with a single (apparently rigid) conformation. Measurements of cross-relaxation rates (NOE and ROE) for certain pairs of protons at different temperatures and magnetic field strengths revealed, however, a remarkable flexibility of the glycosidic bond in sucrose [5].

The results of applying the combined NMR and molecular modeling approach to a glycoprotein-derived octasaccharide, *i.e.*, Galα(1→4)Galβ(1→4)Galβ(1→4)GlcNAcβ(1→6)[NeuAcα(2→3)-Galβ(1→3)]GalNAcα(1→3)GalNAc-ol, will be presented [6]. We conclude that flexibility similar to that established for sucrose may occur for glycosidic linkages in larger oligosaccharides as well, but this flexibility is transparent to most types of NMR measurements.

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CHO Binding Proteins: Lectins and Glycosyltransferases

P 006 CELL ADHESION INVOLVING OLIGOSACCHARIDE RECOGNITION MOLECULES: ß-GALACTOSIDE BINDING LECTIN L-14 AND P-SELECTIN. Richard D. Cummings[1], David F. Smith[1], Ajit Varki[2], Kevin L. Moore[3], and Rodger P. McEver[3], [1]Dept. of Biochemistry, University of Georgia, Athens, [2] Department of Medicine and Cancer Center, University of California at San Diego, La Jolla, [3]Oklahoma Medical Research Foundation, Oklahoma City.

Interactions of cells with matrix or other cells requires the specific recognition between a number of extracellular molecules. Among the known oligosaccharide recognition molecules (ORMs) that animal cells synthesize are proteins that facilitate several types of cellular interactions. Many of the ORMs are lectins that have been classified as S-type and C-type. Members of both types are known to interact with oligosaccharides in animal glycoconjugates containing the underlying N-acetyllactosamine -Galß1-4GlcNAcß1- sequence. Our recent studies have focused on lectins interactive with oligosaccharides containing this sequence.

L-14 is a member of the S-type lectin family and is a soluble dimeric protein found in extracellular matrix. We have shown that L-14 has high affinity binding to oligosaccharides containing the poly-N-acetyllactosamine sequence [3Galß1-4GlcNAcß1-]$_n$. Consequently, this lectin binds to a select group of animal glycoproteins that contain this sequence, and these include laminin and the lysosome-associated membrane proteins LAMP-1 and LAMP-2. Our studies demonstrate that L-14 can influence cellular interactions with laminin via its calcium-independent interactions with poly-N-acetyllactosamine sequences.

A member of the C-type lectin family that is expressed by activated platelets and endothelial cells has been termed GMP-140, CD62, PADGEM and most recently P-selectin. Our studies demonstrate that P-selectin binds to cells expressing the sialyl-Lewis x antigen NeuAcα2-3Galß1-4(Fucα1-3)GlcNAcß1-R (Sialyl Le^x). We have found, however, that while this determinant is necessary it may not be sufficient for high affinity interactions of cells with P-selectin. Using immobilized P-selectin in affinity chromatographic procedures, we have identified a sialylated 120 kD glycoprotein expressed by HL-60 cells that binds specifically and with high affinity to P-selectin. The 120 kD glycoprotein is only one of many different glycoproteins in HL-60 cells that contain sialyl Le^x antigens. The 120 kD glycoprotein appears to contain both N- and possibly O-linked oligosaccharides; the specific oligosaccharide determinants in the 120 kD molecule recognized by P-selectin are being investigated. Together these studies illustrate the recognition specificity of ORMs and the restricted expression of important oligosaccharide determinants on cellular glycoconjugates.

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P 007 MOLECULAR BIOLOGY OF SIALYLTRANSFERASE EXPRESSION, Joseph T.Y. Lau, Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263.

Correct attachment of sialic acids to glycoforms are apparently requisite for a diverse number of physiologic events, including lymphocyte homing and targeting, longevity of serum glycoproteins in circulation, and invasiveness of some pathogens. It is suspected that up to a dozen different sialyltransferases are required to elaborate the complete complement of sialic acid linkages in mammals. However, next to nothing is known about the molecular pathways regulating the expression of these enzymes. As a first step, we have initiated an in-depth analysis of one of these enzymes, the β-galactoside α2,6-sialyltransferase. This enzyme is responsible for the synthesis of the predominant sialic acid linkage in serum glycoproteins. Expression of this enzyme is subject to tissue, developmental, and hormonal signals. The exons encoding β-galactoside α2,6-sialyltransferase is distributed along more than 60 kb of human genomic DNA. This gene appears to be a unique sequence and resides in human chromosome 3 between 3q21 and 3qter. Comparison between the human and rat homologs reveals exact intron/exon conservation between the two species. Functional sialyltransferase transcripts can be generated from two physically distinct promoter regions. Transcription activities from the two promoters are also subject to differential modulation by specific cellular factors. Thus it appears that the β-galactoside α2,6-sialyltransferase, an enzyme that has widespread, but differential tissue specificities in expression, specific regulation of expression is governed at least on one level by multiple promoters that are responsive to different cellular signals.

P 008 **PHYSICAL SHAPE OF CLUSTERED LIGANDS AND RECOGNITION BY ANIMAL LECTINS,** **Y. C. Lee, Reiko T. Lee, and Kevin Rice, Biol. Dept., Johns Hopkins Univ., Baltimore, MD, 21218**

Some animal lectins prefer multibranched ligands over linear ligands ("glycoside clustering effect"). This is most notable in hepatic lectins. Although mammalian and avian lectins have different sugar specificities (Gal/GalNAc for the mammalian and GlcNAc for the avian), both prefer branched oligosaccharide chains. Another C-type lectin, alveolar macrophage Man-specific receptor, also shows the same trend.

Mammalian hepatic lectins bind proteins modified with simple sugars (Gal, GalNAc) avidly, and the binding affinity increases logarithmically as the sugar density on protein increases linearly. The results with synthetic oligosaccharides and natural glycopeptides also show a dramatic enhancement of binding as the number of branches increases.

The "glycoside clustering effect" is believed to be the consequence of subunit organization. The rat hepatic lectin was labelled by photoaffinity technique using three isomeric glycopeptide probes, each bearing a photolyzable group at a different terminal Gal residues. The results showed that Gal on the GlcNAcβ4Manα3Manβ4GlcNAc-branch is bound only by the 52/60kDa (minor) subunits, and Gal's on the other two branches are bound only by the 43kDa subunit (major).

To investigate further the physical shape of the ligand, a triantennry glycopeptide was modified with naphthyl group at the N-terminus amino acid and with Dansyl group at the C-6 of a specific Gal residue to perform fluorescence energy transfer. experiment. By steady state fluorescence energy transfer measurement, the distances between the N-terminal amino acid and the three terminal Gal residues were found to be 186, 213, and 204 nm. Moreover, time-resolved fluorescence energy transfer measurements revealed that while the branch that contains the Gal residue specifically bound by the minor subunits contains only one distance population (i.e., inflexible), the other two showed two distance populations (i.e., more flexible). The population distribution of the flexible branches is found to be temperature dependent. ΔH for these branches are 3.1 and 7.1 Kcal/mol/K and ΔS are 10.8 and 25.8 Kcal/mol/K, respectively. The inflexibility of the one of the branches in the triantennary structure can explain other binding data. (Supported by NIH Research Grant DK99-70).

Pharmaceutical Interests in Glycobiology

P 009 STRUCTURAL ANALYSIS OF PERIODATE-OXIDIZED HEPARIN, H. Edward Conrad and Yuchuan Guo, Glycomed, Inc., Alameda, CA 94501.

For the development of many of the interesting biological activities of heparins as pharmaceuticals, it is desirable to destroy the anticoagulant activity of the heparin. One way in which this can be accomplished is to destroy the antithrombin III binding region of the heparin by periodate oxidation. In order to follow the effect of the periodate oxidation, it is necessary to follow the loss of the residues in heparin that are susceptible to periodate. This is accomplished by treatment of heparin with HONO at pH 1.5 to cleave the polymer at N-sulfated, but not at N-acetylated glucosamine residues, thus converting the polymer to di- and tetrasaccharides. The GlcNS residues at the sites of cleavage are converted into anhydromannose residues. Reduction of heparin cleavage products with NaB^3H_4 yields mixtures of di- and tetrasaccharides with reducing terminal [^{3}H]anhydromannitol residues. The identification and quantification of these oligosaccharides by HPLC procedures have been described[1,2]. These procedures have been used to determine the rates of periodate oxidation of the susceptible unsulfated GlcA and IdoA residues in heparin by measuring the disappearance of the di- and tetrasaccharides that contain GlcA and IdoA. Complete oxidation with IO_4^- results in the total loss of the unsulfated uronic acid-containing oligosaccharides, but kinetic studies show that IdoA is oxidized much more rapidly than GlcA under all reaction conditions. As the pH is lowered from 7 down to 3, the overall rate of the oxidation slows markedly, but the relative rates of GlcA and IdoA oxidation do not change. This result differs from a previouly reported result[3] showing that GlcA but not IdoA is oxidized at pH 3, whereas both uronic acids are oxidized at pH 7. The slow rate of oxidation of GlcA residues at all pH's yields intermediate oxidation products in which all of the unsulfated IdoA residues are destroyed while 70-80 % of the original ATIII-binding pentasaccharide is retained. However, the anticoagulant activity (APTT) of this product is reduced from 170 IU/mg to 38 IU/mg for the partially oxidized product. This suggests that the anticoagulant activity of heparin requires a more extensive sequence of monosaccharide units than that represented by the ATIII-binding pentasaccharide.

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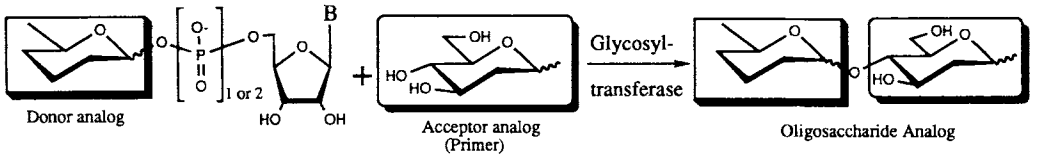
P 010 RAPID SYNTHESIS OF OLIGOSACCHARIDES BY COMBINED CHEMICAL ENZYMATIC APPROACHES, Monica M. Palcic and Ole Hindsgaul. Departments of Food Science and Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2.

The most interesting functions of the complex carbohydrate chains of mammalian glycoproteins and glycolipids involve their recognition and binding by receptors, usually protein molecules. The demonstration of a key recognition of an oligosaccharide in a biological process has a corollary - namely that the binding can be inhibited by that oligosaccharide structure. If such inhibition is observed to be sufficiently potent using either the isolated or chemically synthesized ligand then that ligand, or perhaps a simpler and more easily prepared analog, may have potential as a commercial drug. Inhibitiors of cell-adhesion molecules binding to sialylated oligosaccharides are recent candidates in this regard for the development of anti-inflammatory or anti-cancer drugs.

In a competitive arena, such as the pharmaceutical industry, the ability to rapidly produce small quantities of potentially inhibitory oligosaccharides and analogs is especially critical. The total chemical synthesis of complex oligosaccharides is, however, very time-consuming on top of being very difficult. In our academic laboratories a combined chemical-enzymatic approach has proven to be the most efficient manner of rapidly preparing small (ca. 1-5 mg) amounts of complex oligosaccharides which is generally sufficient to perform at least preliminary assays for biological activity. This approach involves chemically synthesizing small "primers", usually 2-3 sugar residues in size, which can often be assembled in only a few weeks. Addition of specific sugars to these primers is then performed using partially purified glycosyltransferases and a panel of synthetic sugar-nucleotides. By varying the structure of the primer, i.e. using acceptor analogs, as well as the structure of the donor (sugar nucleotide analogs) a large array of oligosaccharide analogs can be produced and evaluated in a short time.To date, we have examined this approach using readily available β(1,4)galactosyltransferase, α(1,3/4)fucosyltransferase and *N*-acetylglucosaminyltransferases I and II. These enzymes have been used in the preparation of over 60 oligosaccharide analogs having transferred 15 sugar analogs from their corresponding sugar nucleotides.



P 011 APPROACHES TO THE IDENTIFICATION, SYNTHESIS AND MODELLING OF NATURAL LIGANDS FOR ELAM-1, Raj B. Parekh[1], Thakor P. Patel[1], Colin C. Campion[1], Mark R. Wormald[2], and Christopher J. Edge [2], [1] Oxford GlycoSystems, Abingdon, UK, [2] Oxford Glycobiology Institute, Oxford, UK.

An early event in the recruitment of leukocytes to sites of inflammation is the adhesion of carbohydrate(s) on circulating leukocytes to the lectin ELAM-1, whose expression is induced on endothelial cells by inflammatory cytokines. The importance of the ELAM-1 dependent adhesion pathway has now been shown *in vivo*, and much interest centres around the identification of the ELAM-1 carbohydrate ligand. Many of the approaches taken towards identifying this carbohydrate have indicated that the blood group determinant sialyl Lewis X (NeuNAc α2 → 3 Gal β1 → 4 (Fuc α1 → 3) GlcNAc) can bind to ELAM-1. While the sialyl Lewis X (and sialyl Lewis a) structures may prove useful as pharmacophores for the development of more potent antagonists, relatively few attempts have been made to characterise the 'natural' set of ELAM-1 carbohydrate ligands. Towards this end, we have performed a systematic study of the N- and O-glycans associated with the total cell-surface glycoproteins of various cell lines, including human PMN, and correlated the expression of individual N- and O- glycan structures to the ability to bind cells expressing ELAM-1.

The results indicate that the 'natural' set of glycoprotein-associated glycan ligands for ELAM-1 consists of multi-antennary (tri- and tetra-) complex type N-glycans, one or more of whose outer-arms terminate in 3-sialyl poly Lewis X. Subsequent *de novo* chemical synthesis of 3-sialyl Lewis X, 3-sialyl Lewis a and related carbohydrates as well as a variety of myristic-acid conjugates has been performed. This is allowing some of the important details of ELAM-1-carbohydrate binding to be addressed, including the relative potency of individual carbohydrates as competitive inhibitors of ELAM-1 dependent adhesion, and the effects of multi-valent presentation of carbohydrate ligand on the potency of synthetic inhibitors. In addition, analysis of solution conformations (using 600 MHz 1- and 2-dimensional ^{1}H-NMR), and modelling studies on 3-sialyl Lewis X, 3-sialyl Lewis a, and related structures has allowed the identification of structural epitopes that appear to be conserved in carbohydrates that are able to bind ELAM-1. These epitopes should be useful in the design of non-carbohydrate, synthetic antagonists of ELAM-1 binding.

Glycosylation and Human Disease

P 012 OLIGOSACCHARIDE PROCESSING IN CANCER CELLS: EXPRESSION CLONING OF A REGULATOR FACTORS. **James W. Dennis, Carol Fode, Michael Heffernan and Shida Yousefi. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5 and Department of Medical Genetics, University of Toronto.**

Studies on tumor cell glycosylation mutants and drugs which inhibit oligosaccharide processing suggest that expression of sialylated and highly branched complex-type N-linked oligosaccharide is required for efficient tumor cell invasion and metastasis. Cell surface oligosaccharides function as ligands for mammalian lectins and as such, enhance the retention of blood-born tumor cell in the microvasculature of host tissues. However, a variety of cellular phenotypes appear to be affected by inhibiting oligosaccharide processing, including tumor cell invasion and proliferation. These observations prompted our recent investigation to determine whether the loss of sialylated complex-type N-linked oligosaccharide in tumor cells affects the expression of genes which in turn, could influence the malignant phenotypes. In both human and rodent tumor cells, we observed a selective increase in proto-oncogene (i.e., c-jun) and tissue inhibitor of metalloproteases (TIMP) mRNA levels under conditions where N-linked processing was inhibited either by the alkaloid swainsonine or by stable glycosylation mutations. This suggest that N-linked oligosaccharide processing may be an integral element of the cellular phenotype controlling expression of select genes.

To identify genes coding for processing enzymes and regulators of these reactions, we have developed a cDNA expression cloning system employing large T-expressing Chinese hamster ovary (CHO) cells and the pCDM8 shuttle vector. Wild-type CHO cells were transfected with a murine tumor cell cDNA library, and following 7-10 days of selection in wheat germ agglutinin (WGA), plasmids bearing cDNA inserts were recovered from surviving colonies of CHO cells. A 1.5kb cDNA in the antisense orientation was recovered from 4 independent WGA-resistant colonies. The cDNA was found to be a portion of a 4.1kb cDNA sequences which is expressed in CHO cells and has an open reading frame coding for 923 amino acids. Residues 1-270 show good homology (70-75% similar) and domain conservation with the ser/thr kinase family, and therefore we have designated the sequence as sialyltransferase kinase (SATK). Down-regulation of SATK mRNA levels in CHO cells is associated with decreased cell surface sialylation and decreased sialyltransferase activity but no change in several other glycosyltransferase activities. Experiments are underway to determine whether the putative kinase domain in SATK regulates sialyltransferase at the protein or gene level. These results show that expression-cloning methods coupled with lectin selection can be used to isolate cDNA encoding proteins involved in oligosaccharide processing in CHO cells.

P 013 TOWARDS UNDERSTANDING CARBOHYDRATE MEDIATED ADHESIVE INTERACTIONS IN INFECTION, INFLAMMATION AND MALIGNANCY, Ten Feizi, Medical Research Council Clinical Research Centre, Harrow, Middlesex, United Kingdom.

Oligosaccharides are abundant and prominent components at the surface of cells, on extracellular matrix components including biologically important molecules, e.g. numerous hormones, growth factors and receptors. They are also prominent components of infective agents. A major challenge in cell biology and pathology has been to understand the physiological roles of the diverse oligosaccharides on glycoproteins and glycolipids and their involvement in macromolecular interactions in disease processes.

Work with monoclonal antibodies has shown that programmed changes occur (positional and temporal) in the branching patterns and in peripheral substitutions of oligosaccharides during embryonic development and cellular differentiation, and that predictable changes occur in oncogenesis. This has led to suggestions that specific oligosaccharides may serve as 'area codes' determining cell migration pathways, or serve as ligands for proteins involved in cell signalling pathways[1,2]. Support for these suggestions has been forthcoming as information is accumulating on the existence of families of proteins (endogenous lectins) with carbohydrate binding specificities.

To facilitate elucidation of the roles of specific oligosaccharides (particularly those associated with glycoproteins) as recognition structures for these proteins, we are using the technology which enables oligosaccharide probes (neoglycolipids) to be generated either from mixtures of oligosaccharides released from glycoproteins or from desired chemically synthesized oligosaccharides for use in ligand binding studies. This allows concomitant microsequencing of the oligosaccharides, since the neoglycolipids have unique ionisation properties in mass spectrometry.

Thus a picture is emerging of the repertoires of oligsaccharide ligands for the human proteins, mannan binding protein, amyloid P component and endothelial-leukocyte-adhesion-molecule ELAM-1, which are variously implicated in molecular mechanisms of infection, amyloidosis, inflammation and metastasis.

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P 014 ALTERED GLYCOSYLATION CORRELATED WITH TUMORIGENESIS AND METASTASIS OF MALIGNANT CELLS, Akira Kobata[1], Tamao Endo[1], Kiyoshi Furukawa[1], T. -W. Tao[2] and Seiichi Takasaki[1], [1]Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan, [2]Stanford University, Stanford.

By comparative study of glycopeptides obtained from the surface of normal and transformed cells, increase in the size of *N*-linked sugar chains was found in cells transformed by viral, chemical and spontaneous methods. Since Warren and Glick thoroughly investigated this interesting phenomenon, its is now widely known by the name of Warren-Glick (W-G) phenomenon. By investigating in detail the structures of oligosaccharides released from BHK and Py-BHK cells by hydrazinolysis, we found that the enrichment of complex type sugar chains containing the Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ 1→2)Man group (2,6-branch) and the Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAc group is the structural background of W-G phenomenon[1]. This alteration is induced by the enhancement of *N*-acetylglucosaminyltransferase V (GnT V) in malignant cells[2]. Studies of the surface *N*-linked sugar chains of rat 3Y1 cells transfected with the genome and its fragments of adenovirus 12 revealed that the enhancement of 2,6-branch is positively correlated with the tumorigenicity of cells[3]. Dennis[4] reported that mouse mammary tumor cell lines with higher metastatic character are stained more strongly with L-PHA, which interacts with 2,6-branched *N*-linked sugar chains. Our recent study of mouse B16 melanoma cells and their mutant with various metastatic characters also indicated that the enhanced expression of 2,6-branch is correlated to the metastatic character of the cells[5]. All these data indicated that W-G phenomenon is not related to the basic character of malignant cells, such as abnormal growth, but is related to their unsocial behavior such as tumorigenicity and metastasis. Therefore, study of the mechanism of the enhanced expression of GnT V is important to pick up the tumor cells with high malignancy.

Several additional evidences related to W-G phenomenon have been obtained by our recent studies. NIH 3T3 cells, transformed by the *middle T* gene of polyoma, shows high tumorigenicity and marked W-G phenomenon. The surface glycoproteins of this cell was subjected to SDS-PAGE. When the gel was stained with *Datura stramonium* agglutinin (DSA), which specifically binds to the 2,6-branch, limited number of glycoproteins were stained. An interesting evidence is that the serine residues of only these DSA positive glycoproteins are phosphorylated by metabolic labeling with $^{32}PO_4$. Further study of this finding is expected to elucidate the physiological meaning of W-G phenomenon. Another interesting result was obtained by the comparative study of the *N*-linked sugar chains of lectin mutants of mouse B16 melanoma[6]. Both wheat germ agglutinin resistant variant (WGA-R) and *Ricinus communis* agglutinin variant (ricin-R) of this cell line showed dramatic decrease in metastasizing capacity . Studies of their surface *N*-linked sugar chains revealed that bi-, tri- and tetraantennary oligosaccharides are included in the WGA-R with almost the same molar ratio as those of parent cell. However, X-antigenic determinants are expressed in every antenna of the tri- and tetra-antennary sugar chains of the variant, resulting in the marked decrease of the sialylated sugar chains. On the contrary, the ricin-R variant showed extensive reduction in tetraantennary sugar chains in compensatory increase of biantennary sugar chains. No decrease in the amount of sialylated sugar chains was detected in the variant. For a tumor cell to metastasize, release from its home tissue, invasion into the surrounding tissues, invasion into and slip out of blood vessel, and adhesion to other tissues, can be considered as a series of process included. Among them, acquirement of the ability to invade into blood vessel might be the most important for a tumor cell to become really virulent. In view of the recent finding that sialylated X plays a role as a ligand for ELAM-1, it is probable that sialylated X formed on the 2,6-branch of *N*-linked sugar chains leads a tumor cells to invade into blood vessel and thus afford them high metastatic character.

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P 015 LECTIN-CARBOHYDRATE INTERACTION IN INFECTIOUS DISEASE: STUDIES OF LECTINOPHAGOCYTOSIS *IN VIVO*, Wolfgang Bernhard*, Awni Gbarah and Nathan Sharon, Department of Membrane Research and Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel.

Phagocytosis of bacteria requires their binding to the phagocytic cells. Such binding is mediated either by opsonins (antibodies and complement) resulting in opsonophagocytosis, or by direct interaction between the phagocyte and its prey. The latter may be mediated by lectins and the complementary carbohydrates on the apposing cells, resulting in activation and degranulation of the phagocytes, as well as uptake and killing of the bacteria, a process designated lectinophagocytosis [1]. It may represent conservation of a primitive host defense mechanism, that acts against many saprophytic or opportunistic microorganisms, especially in sites that are low in opsonins, or in a preimmune host. A well-studied system of lectinophagocytosis is that of type 1 fimbriated (mannose-specific) *Escherichia coli* and related enterobacteria that bind to oligomannose and hybrid units of glycoproteins on the surface of mouse [2] or human peritoneal macrophages [3] or human granulocytes [4]. Recently it has been shown that type 1 fimbriated *E. coli* bind to human granulocytes via carbohydrate units of the leucocyte adhesion molecules CD11/CD18 [5]. Since CD11b/CD18 and CD11c/CD18 serve as receptors for complement fragment iC3b, a link between lectino- and opsonophagocytosis has thus been established. However, no direct evidence for the occurrence of lectinophagocytosis *in vivo* has been available. To obtain such evidence, we injected type 1 fimbriated or nonfimbriated *E. coli* into the peritoneal cavity of mice (10^6-10^9 bacteria/animal) in the absence or presence of sugars, and quantified the phagocytic activity by assaying the release of lysosomal *N*-acetyl-ß-D-glucosaminidase [6] into the peritoneal fluid, up to 1h after injection. Methylumbelliferyl-*N*-acetyl-ß-D-glucosaminide was used as substrate for the enzyme. A significant release of the enzyme was observed following injection of type 1 fimbriated bacteria, which was time-dependent and increased with the number of bacteria injected. The nonfimbriated bacteria caused a negligible release. Methyl α-D-mannopyranoside (50 mM), but not methyl α-D galactopyranoside, inhibited the release by 70 - 100%. We conclude that lectinophagocytosis can occur *in vivo* and may thus contribute to host defence against type 1 fimbriated bacteria.

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Biology of Glycosaminoglycans

P 016 SYNDECAN, THE PROTOTYPE OF A FAMILY OF INTEGRAL MEMBRANE PROTEOGLYCANS, ACTS AS A HIGHLY REGULATED "RECEPTOR PARTNER", Merton Bernfield, Harvard Medical School, Boston, MA 02115

All adherent vertebrate cells have heparan sulfate, a heparin-like glycosaminoglycan (GAG), at their surfaces. These GAG can bind a wide variety of components in a cell's microenvironment, including extracellular matrix, growth factors, degradative enzymes and proteinase inhibitors. These components change during embryonic development and tumor invasion and can influence the adhesion, shape, growth, and differentiation of cells. Indeed, adding heparin to model systems developing in culture modifies their morphogenesis. Therefore, we hypothesized that changes in the amount and type of cell surface heparan sulfate could control the influence of these components during morphogenesis and tumorigenesis. A major source of cell surface heparan sulfate is an integral membrane heparan sulfate-containing proteoglycan, syndecan (from the Greek, *syndein*, to bind together), that is the *prototype of a proteoglycan gene family*. The expression of syndecan in embryos and neoplastically transformed cells is highly regulated. Syndecan appears soon after fertilization and localizes to the cells that will form the embryo. It is lost from the anterior mesenchyme following gastrulation and its subsequent expression follows morphogenetic rather than histologic patterns and is dictated by epithelial-mesenchymal interactions. It also shows cell-specific expression: syndecan is predominantly at the cell surface on epithelia, where it has smaller heparan sulfate chains, but is predominantly intracellular in mesenchymal cells, where its heparan sulfate chains are larger. Indeed, these heparan sulfate chains from epithelial and mesenchymal cells differ in the number and size of N-sulfated, iduronic acid-rich domains. Syndecan on epithelia associates with the actin cytoskeleton via its cytoplasmic domain and inducing syndecan deficiency in cultured epithelia alters their shape, ability to migrate within matrix and response to growth factors. Syndecan expression is reduced upon neoplastic transformation. Thus, because syndecan may interact with a variety of extracellular effectors, we propose that syndecan is a "receptor partner", acting in combination with more highly specific receptors, which mediates the actions of matrix components and growth factors on cells. Thus, change in expression of the syndecan family of proteoglycans at distinct times or sites may regulate the effects of the microenvironment on cells.

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P 017 BIOSYNTHESIS OF HEPARIN AND HEPARAN SULFATE, Ulf Lindahl[1], Kerstin Lidholt[1], and Lena Kjellén[2], [1]University of Uppsala, The Biomedical Center, Uppsala, Sweden, and [2]Swedish University of Agricultural Sciences, The Biomedical Center, Uppsala, Sweden.

Heparin and heparan sulfate are synthesized as proteoglycans. The polysaccharide chains initially formed, composed of alternating ß1,4GlcA and α1,4GlcNAc units, are subsequently modified through a series of reactions, involving N-deacetylation/N-sulfation of GlcN units, C5 epimerization of GlcA to IdoA units and O-sulfation at various positions (1). The structural heterogeneity of the final product reflects the course of polymer modification, most of the reactions being incomplete; at each reaction step a fraction of the potential target residues escape modification. The selection mechanism is only understood in part, but relies on the substrate specificities of the enzymes involved in the process.

Additional information has been obtained through studies on the concerted action of the various reactions in microsomal and whole-cell biosynthetic systems, as well as from attempts to purify the individual enzymes. Notably, some reactions that occur together appear to be catalyzed by the same enzyme species or by enzymes sharing common subunits. A presumably single mutation in CHO cells, resulting in inability to synthesize heparan sulfate (chondroitin sulfate formation being unimpaired), thus eliminated both the GlcA-transferase and the GlcNAc-transferase activities required to generate the heparan sulfate precursor polymer (2). Further, the N-deacetylation of GlcNAc units involves a 110 kDa protein (in mouse mastocytoma) that also harbors the active site for the N-sulfotransferase (3). The latter activity appears inherent to the 110 kDa protein, whereas the N-deacetylation reaction requires, in addition, a cationic protein cofactor. Purification (>100,000-fold) of O-sulfotransferases from mouse mastocytoma tissue (22% recovery of overall activity in initial extract) yielded a product that emerged as a distinct, symmetrical peak on gel chromatography (K_{av} ~0.30 on Sephacryl S-300 in the presence of 0.1% deoxycholate), corresponding to a M_r >100,000. SDS-PAGE of this material yielded silver-staining components in the M_r range of 20,000-30,000, suggesting that the native O-sulfotransferases may occur as an enzyme complex composed of two or more polypeptides (H. Wlad, I. Pettersson, L. Kjellén, and U. Lindahl, unpublished work). Some of these findings will be discussed in relation to a novel model of heparin proteoglycan biosynthesis, based on the consecutive action of discrete enzyme complexes along still nascent polysaccharide chains. The regulation of biosynthetic polymer modification will be considered in relation to the formation of specific saccharide sequences.

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P 018 STRUCTURE AND ACTIVITY OF GLYCOSAMINOGLYCANS, Robert J. Linhardt, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa, 52242

Glycosaminoglycans (GAGs) are among the most structurally complex of the glycoconjugates. Structural studies in our laboratory have relied on the use of polysaccharide lyases and hydrolases to breakdown GAGs into acidic oligosaccharides for studies aimed at understanding their structure activity relationship (SAR). Heparin is one of the most widely studied GAG because of its medical importance as an anticoagulant. Compositional analysis relies on capillary zone electrophoresis to fractionate disaccharides prepared from heparin using a mixture of three enzymes from *Flavobacterium heparinum*, heparin lyases (1, 2 and 3). Treatment of heparin with a single lyase, i.e. heparin lyase 1, results in a mixture of oligosaccharides. These can be fractionated on a discontinuous gradient polyacrylamide gel or by strong-anion exchange high performance liquid chromatography resulting in an oligosaccharide map. This approach, similar to the peptide mapping of proteins, has been used to compare heparins from a variety of tissues and species. The structure of a large number of heparin-derived oligosaccharides have been determined primarily through the use of sophisticated spectroscopic methods. Fast atom bombardment mass spectrometry (FAB-MS) is used when only microgram quantities of oligosaccharide is available. Underivatized samples gave both molecular ions and fragment ions resulting in saccharide sequence. Recently, we have used tandem MS to obtain both linkage position and sulfate position. High resolution nuclear magnetic resonance spectroscopy is used to determine the structure of milligram amounts of oligosaccharide samples. The ultimate challenge, however, remains developing methods to sequence heparin and other GAGs. In one approach, an end-label, in the form of a fluorescent probe, is introduced into a GAG chain or into a large GAG-derived oligosaccharide and with the use of polysaccharide lyases and hydrolases, a sequence is determined. Our laboratory has also examined computer and mathematical modelling to examine GAG sequence. Although this approach can be used to eliminate certain sequences it is not possible to definitively establish a GAG sequence by this method. Recently, our attention has been focused on proteoglycans (PGs). Although PGs often contain multiple GAG side chains they can also be prepared having a single GAG chain attached to core protein. Such PGs offer a unique possibility in understanding GAG sequence. The single GAG chain can be chemically or enzymatically removed from the protein and the sequence determined using its linkage region as a reading frame. A second aspect of our research involves the activity of GAGs and their SAR. The biological activites of GAGs are regulated through their ability to bind specifically to a wide range of proteins. These interactions require the presence of specific sequences within the GAG, i.e. heparin's *antithrombin pentasaccharide binding site, and specific sequences within* the protein, including small, contiguous consensus peptides. To understand this interaction, studies were begun to examine peptide binding of small peptides to GAG-derived oligosaccharides. These studies should help build the basis for understanding GAG interaction in the extracellular matrix, with growth factors, with nuclear proteins, with proteins involved in coagulation and complement activation, and with viral proteins and their receptors.

P 019 HYALADHERINS: HYALURONAN-BINDING PROTEINS INVOLVED IN EXTRACELLULAR MATRIX ASSEMBLY AND CELL-MATRIX INTERACTIONS, B.P.Toole, K.Deyst, E.Goedecke, R.Turner, Q.Yu, and S.D.Banerjee, Department of Anatomy and Cellular Biology, Tufts University Health Science Schools, Boston, MA 02111.

The hyaluronan-binding proteins, or hyaladherins, are a group of related proteins that serve as structural components of extracellular matrices and as receptors at the cell surface. We have recently characterized a monoclonal antibody, MAb IVd4, that recognizes hyaluronan-binding protein with the properties of a hyaluronan receptor in a wide variety of cell types (1). SDS-gel electrophoresis and Western blotting reveal that proteins of various sizes are recognized by MAb IVd4. The most common species are ~93, 90, 69, and 48kDa; the relationship between these proteins is not yet known but does not appear to arise from differing degrees of glycosylation. Antigen recognized by MAb IVd4 is found both at the surface of cells and in extracellular matrices, depending on the type and state of differentiation of the cells. MAb IVd4 blocks binding to isolated hyaluronan-binding protein and to intact SV-3T3 cells (1). The latter result implies that the MAb IVd4 antigen is related to the 85kDa HA receptor and to CD44 (2). The localization of antigen changes radically during development of many tissues. For example, in the very early cornea, intense immunoreactivity is seen in the extracellular matrix coincident with hyaluronan itself. Both hyaluronan and the antigen remain present throughout the time of invasion of mesenchymal cells into the stroma but disappear thereafter. In the limb, hyaluronan and the MAb IVd4 antigen are at first uniformly distributed throughout the mesoderm. The antigen becomes highly enriched in newly differentiating cartilage, whereas hyaluronan decreases in cartilage and remains at high levels in the subepithelial area that will eventually become dermis. MAb IVd4 blocks assembly of pericellular matrices produced by a wide variety of cells, e.g. embryonic limb mesoderm, chondrocytes, and fibrosarcoma cells. The antibody also blocks cell migration, e.g. of endothelial cells and neural crest cells. The antigen is localized to lamellipodia at the leading edge of migrating cells. The effects of the antibody on pericellular matrix assembly and on cell migration are mimicked by hyaluronan hexasaccharide, a competitive inhibitor of the interaction between polymeric hyaluronan and its receptor. It is not yet known whether the effects of MAb IVd4 and hexasaccharide on matrix assembly and migration are directly related, but our results clearly indicate that hyaluronan and hyaladherins are important to both processes.

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Glycosyl Phosphatidylinositols

P 020 BIOSYNTHESIS OF GLYCOSYL PHOSPHATIDYLINOSITOLS IN AFRICAN TRYPANOSOMES: FATTY ACID REMODELING AS A POTENTIAL TARGET FOR ANTI-PARASITIC CHEMOTHERAPY T. L. Doering, L. U. Buxbaum, J. Raper, E. F. Hoff, D. Raben, J. I. Gordon*, G. W. Hart & P. T. Englund, Johns Hopkins Med. School, Baltimore, MD, 21205, *Washington Univ. Med. School, St. Louis, MO 63110

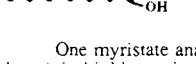
Glycosyl phosphatidylinositol (GPI) structures anchor diverse eukaryotic surface proteins and glycoconjugates to the plasma membranes of organisms ranging from yeast to mammals (review, 1). The best-studied GPI-anchored protein is the abundant variant surface glycoprotein (VSG) of *Trypanosoma brucei*, the protozoan parasite responsible for African sleeping sickness. Its GPI anchor is preconstructed as a compound termed glycolipid A, to which VSG is transferred within one minute of protein synthesis.

To define and study the steps involved in trypanosome GPI biosynthesis, we developed a cell free system which incorporates radioactivity from sugar nucleotides or fatty acids into an array of glycolipids, including glycolipid A. We (2) and others (3) characterized these compounds, and described a pathway for GPI biosynthesis consisting of sequential addition of sugars and phosphoethanolamine to phosphatidylinositol.

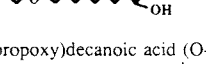
The VSG anchor is unique among GPIs in containing exclusively myristic acid. This is not incorporated until the final part of GPI biosynthesis, when the longer fatty acids of the endogenous phosphatidylinositol (which was initially glycosylated) are sequentially replaced with myristate. We identified intermediate *lyso* species and singly myristoylated compounds consistent with this fatty acid remodeling pathway (4).

The strict myristate specificity is more striking because *T. brucei* is unable to synthesize fatty acids *de novo* and must import them from the infected host bloodstream, where myristate comprises only 1.5% of the fatty acids. This suggests that trypanosomes employ efficient mechanisms for the uptake and use of myristate, which we are currently investigating. We studied the utilization of heteroatom-containing myristate analogs in intact cells and in the cell free system. Our results indicate that the specificity of incorporation depends on chain length rather than hydrophobicity. Also, although the fatty acid remodeling process does not discriminate betwen analog and myristate, other cellular processes (e.g. uptake or localization) do (5).

myristate



O-11



One myristate analog, 10-(propoxy)decanoic acid (O-11; see above) is highly toxic to bloodstream trypanosomes, but not to mammalian cells (5). We are now screening similar compounds which offer a potential approach to anti-trypanosome chemotherapy.

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P 021 STRUCTURE AND FUNCTION OF GLYCOSYLATED-PHOSPHATIDYLINOSITOLS IN *LEISHMANIA* PARASITES. Malcolm J. McConville, Department of Biochemistry, University of Dundee, Dundee, U.K.

In common with other protozoa, the majority of the glycoproteins on the cell surface of *Leishmania* parasites are anchored to the plasma membrane by glycosyl-phosphatidylinositol (GPI) membrane anchors (1). In addition to these protein-GPI anchors, *Leishmania* also synthesize two novel classes of GPI which are not linked to protein; the lipophosphoglycans (LPGs) and the glycoinositolphospholipids (GIPLs). The LPGs appear to be the major cell surface molecules of the insect (promastigote) stage (5×10^6 molecules/cell), while their level of expression is down-regulated ($<10^3$ molecules/cell) in the amastigote stage, which infects mammalian macrophages. The essential nature of these molecules to promastigote infectivity suggests that they may have evolved as part of the adaptation of these organisms to their parasitic life cycle. Structural studies on the LPGs from *L. major* (2),*L. donovani* (3) and *L. mexicana* have shown that all these molecules contain a linear chain of phosphorylated oligosaccharide repeat units, which is capped at the non-reducing terminus by a neutral, mannose-containing oligosaccgaride and is attached at the reducing terminus to a phosphorylated hexaglycosyl-*lyso*alkyl-PI anchor. The oligosaccharide repeat units may bear side chains of one to four monosaccharides, which are different in each species and which may also vary as promastigotes change from a dividing, non-infective stage to a non-dividing infective stage. The current ideas on the structure-function relationships of the LPGs will be discussed, with regard to (a) the role of the cap structures in the invasion of mammalian macrophages and (b) the role of the oligosacharide side-chains in facilitating adhesion of promastigotes to the gut wall of the sandfly vector.

The second class of *Leishmania*-specific GPIs, the GIPLs, are expresed in high copy number in all developmental stages of the parasite. Two families of GIPLs have been characterized; those that share homology to the GPI anchor of LPG (i.e. the GIPLs of *L. major*, 4) and those that share homology with the protein-linked GPIs (i.e. the GIPLs of *L. donovani* (5) and *L. mexicana*). Several lines of evidence suggest that these glycolipids are mature products rather than precursors for the membrane anchors: (i) the levels of GIPL expression do not correlate with the levels of either LPG or GPI-anchored proteins, (ii) they are expressed in high copy number on the cell surface, (iii) the structures of the GIPLs, particularly with regard to the lipid moieties, differ from the anchor GPIs and (iv) the kinetics of incorporation of [^{3}H]-labelled monosaccharides are consistent with the GIPLs having only a minor role as precursors. Finally, the rate of turnover of the GIPLs and LPG have been determined after metabolically labelling promastigotes with ^{13}C-labelled glucose. The results indicate that there are two metabolic pools of GPI; one which is turned over rapidly, corresponding to cell surface material and an intracellular pool which turns over very slowly. The possible function of this intracellular pool will be discussed.

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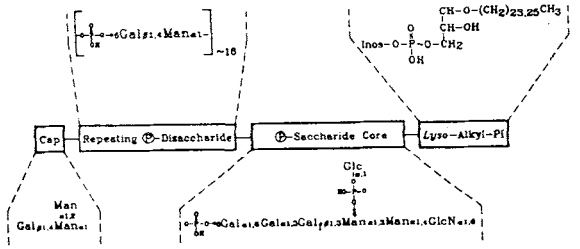
P 022 FUNCTION AND BIOSYNTHESIS OF THE LIPOPHOSPHOGLYCAN OF LEISHMANIA DONOVANI, Sam Turco, Albert Descoteaux, and Mary Carver, Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40536

Lipophosphoglycan (LPG) is the major cell surface glycoconjugate of the promastigote form of *Leishmania* parasites. Structurally, the LPG from *Leishmania donovani* is a capped carbohydrate polymer of repeating PO_4-6Gal(β1,4)Manα1 disaccharide units attached via a phosphosaccharide core to a novel *lyso*-1-O-alkylphosphatidylinositol anchor as follows:



The uniqueness of the structure of LPG would suggest that it may have one or more important functions for the parasite. A striking characteristic of the parasites is their ability to avoid destruction within phagolysosomes of host macrophages. We hypothesize that LPG plays an important protective role for the parasite by acting as an inhibitor of the macrophage's protein kinase C during the early stage of infection.

Very little is known regarding the enzymatic synthesis of LPG. To begin an investigation of the polymerization of the repeating units of LPG, we have developed a membrane preparation from *L. donovani* as a source of glycosyltransferases catalyzing the transfer of radioactive galactose and mannose from their respective nucleotide donors to an endogenous LPG acceptor. The results indicated that both galactose and mannose of the LPG repeating units are added sequentially and directly to LPG. Since mannosylphosphoryldolichol is known to be involved as an intermediate in the transfer of mannose to certain glycoconjugates in eukaryotic cells, the potential mannolipid intermediate in the assembly of the repeating units of LPG was investigated. In experiments in which the effect of exogenously added dolichylphosphate and the effect of amphomycin were examined, no evidence was found for the possible participation of mannosylphosphoryldolichol in repeating unit assembly. The development of an in vitro glycosylating system capable of LPG biosynthesis should facilitate efforts to elucidate the enzymatic details of this assembly process.

Banquet Address

P 023 CARBOHYDRATE-RECOGNTION DOMAINS: DECODING INFORMATION IN COMPLEX OLIGOSACCHARIDE STRUCTURES. Kurt Drickamer, Columbia University, New York, NY 10032.

Calcium-dependent recognition of complex carbohydrates is mediated by a variety of C-type animal lectins, which share a common structural motif, designated a C-type carbohydrate-recognition domain (CRD). Proteins containing such domains include membrane receptors which mediate endocytosis and phagocytosis of selected saccharide-bearing structures, receptors responsible for specific interactions between leukocytes and endothelia, soluble opsonins found in serum, and proteoglycans of the extracellular matrix. Different C-type CRDs are also distinguished by their unique spectrum of affinities for various monosaccharides and complex sugar structures. These domains have thus evolved to target a variety of biological functions based on selective recognition of diverse saccharide structures.

One approach to understanding selective sugar recognition is to develop a molecular picture of the interactions between individual CRDs and their saccharide ligands. To this end, we are investigating the mechanism of saccharide recognition by a prototype C-type CRD, from rat serum mannose-binding protein, using a combination of evolutionary comparisons, mutagenesis, and crystallography. The overall structure of the CRD consists of a region of regular structure and a series of loops which are stabilized by two calcium ions. The structure reveals the role of amino acid residues common to all the C-type CRDs in creating the CRD fold by forming disulfide bonds, a hydrophobic core, and ligands for the divalent cations. In addition, the mechanism of selective, calcium-dependent saccharide ligation is being deduced. Extensive mutagenesis of this CRD has provided confirmatory evidence for the importance of evolutionarily conserved residues in the formation of an active domain. It has also demonstrated the importance of the hydrophobic core of the CRD in positioning the calcium-binding loops.

Selective ligand binding by C-type animal lectins is often a result of mutliple CRDs interacting with multivalent ligands. This phenomenon is being explored by investigating the macrophage mannose receptor expressed *in vitro* and *in vivo*. This receptor contains eight repeated CRD-like motifs. The binding of the intact receptor to monosaccharides can be mimicked by the fourth CRD, but this CRD expressed in isolation fails to bind complex ligands with the high affinity characteristic of the intact receptor. At least two other CRDs are necessary to achieve selective recognition of high mannose structures. The presence of multiple CRDs within a single polypeptide is one of several ways in which CRDs are clustered in C-type lectins to achieve high affinity recognition of complex oligosaccharides.

Finally, since the C-type CRD motif appears to be widespread in extracellular proteins, the evolutionary basis for its association with various effector domains through the process of exon shuffling is also being studied.

Glycoconjugates in Cellular Recognition and Adhesion

P 024 DO LOW AFFINITY EXTRACELLULAR MATRIX PROTEOGLYCAN SITES MEDIATE HIGH AFFINITY, HIGH SPECIFICITY CELLULAR RECOGNITION AND ADHESION? Max M. Burger[1,2], Dorothee Spillmann[1,3], and Gradimir Misevic[2,4], [1]Friedrich Miescher Institut, Basel, POBox 2543, CH-4002 Switzerland, [2]MBL Woods Hole, USA, [3]Turkku University, Finland, [4]ZLF University Hospital, Basel, Switzerland.

Many cell-cell and cell-matrix interactions were suspected to be influenced or mediated by carbohydrates. Functional tests like aggregation, adhesion, fertilization, electrical coupling etc. were set up. Membrane and extracellular matrix glycopeptides and oligosaccharides were isolated and failed to inhibit these functional tests in high concentrations. It was then generally concluded that carbohydrates were most likely not involved in the particular biological phenomenon studied. Based on the following observations such conclusions may have been premature.

In the oldest cell-cell recognition system the species-specific recognition and adhesion of marine sponge cells (Microciona prolifera) a proteoglycan-like factor (MAF) of M_r = $2x10^7$ was shown to mediate specificity and aggregation. Two functional domains could be distinguished, one responsible for binding MAF to the sponge cell surface in a Ca^{++} independent manner and one for aggregating the cells via MAF-MAF interaction in a CA^{++} dependent manner. Sponge cell aggregation could not be inhibited even by highest concentrations of monosaccharides or oligosaccharides isolated from MAF. A Gal, GlcNAc, Uronic acid, Fuc and Man containing glycopeptide fraction of approx. M_r = 10^4 poorly inhibited the binding of the proteoglycan-like MAF and had a 13'000 times lower binding affinity than MAF. When these small glycopeptides were crosslinked with diepoxybutane or glutaraldehyde to the size of the native MAF, they regained essentially the binding constant of native MAF (K_a = 10^{10} M^{-1}). A mixed glycan fraction isolated with pronase was unable to bind to itself or MAF. When crosslinked and reconstituted to MAF size, it bound to itself and to MAF in Ca^{++}. A purified glycan of M_r = $2x10^5$ was also unable to selfassociate in Ca^{++}. If reconstituted to MAF size it did, and if aminopropyl beads were coated with this glycan, they aggregated in Ca^{++} as well. Immunological studies supported the conclusion that multiple low-affinity binding sites do generate the biologically relevant forces for sponge cell-cell recognition and aggregation, a concept which may explain why carbohydrates may have been overlooked in many other cell-cell and cell-matrix interactions studied so far. The biological advantage of such low affinity sites in multivalent high affinity macromolecules may be attributed to the fact that they provide high overall adhesion and specificity but do permit caterpillar-like lateral movement due to the poor affinity of single or a small group of attachment sites.

P 025 ANIMAL GLYCOLIPIDS AS RECEPTORS FOR MICROBES. EFFECTS OF INTERNAL BINDING AND MEMBRANE PROXIMITY IN THE RECOGNITION OF CARBOHYDRATE EPITOPES. Karl-Anders Karlsson, Maan Abul Milh, Jonas Ångström, Jörgen Bergström, Boel Lanne, Maria Ölwegård Halvarsson, and Susann Teneberg, Department of Medical Biochemistry, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden.

Glycolipids are membrane-associated and do not appear in secretions and this may explain why several microbes have selected glycolipids as specific receptors. Moreover, some sequences are relatively glycolipid-specific (lactose, globo and ganglio sequences), making such a selection more likely to occur. There are two properties of more conceptual interest as experienced so far (1,2). One is the binding internally in the saccharide chain and one is the potential membrane proximity of the binding.The internal binding, that has been shown for viruses, bacteria and bacterial toxins, is the basis for isoreceptors with different neighboring sequences to the common and minimally required sequence. A group of microbes may show slightly different binding preferences to isoreceptors when suspended on thin-layer plates, which is a sensitive way of detecting variant binders. Such variants are known for lactosylceramide-binding bacteria and fungi, for *E. coli* and Shiga-like toxins requiring Galα4Gal for binding, for GM1-recognizing cholera toxin and LT, for viruses that bind to GalßCer or GlcßCer, and others. It is very likely that the complementary binding sites of the adhesins or toxins within one variant group differ only slightly to explain this difference in binding to isoreceptors. In case of *E. coli* and urinary tract infection such a change in binding specificity between two adhesins is enough to shift the host selectivity from human to dog, which have separate isoreceptors in their kidneys, globoside and Forssman glycolipid respectively (3). It is therefore likely that the internal binding has been chosen to minimize adhesin changes to shift tropism. For this *E. coli* infection system it is of interest that the specificity of binding is higher when the receptor glycolipid is inserted in the membrane than when exposed on technical assay surfaces as thin-layer plates. Molecular modeling (2,4) indicates that the proximity of the binding epitope to the membrane monolayer (Galα4Gal are sugars no. 3 and 2 from the ceramide) in combination with the extended saccharide chain may impose sterical hindrance which improves the selectivity. Other effects of membrane proximity may be penetration or invasion by the ligand (cholera toxin, lactosylceramide binders, monoglycosylceramide binders). The binding by HIV-1 (5) and other viruses (2,6) to HexßCer may be to gain proximity for fusion and genome entrance into the host cell.

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Glycoconjugates and Cellular Metabolism

P 026 THE ROLE OF POLYLACTOSAMINOGLYCANS IN THE CYTOPLASM AND ON THE CELL SURFACE
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Lysosomal membrane glycoproteins, lamp-1 and lamp-2, are the major carriers for polylactosaminoglycans in nucleated cells. The glycoproteins consist of polypeptides with Mr~40kDa and a large part of the molecule is located in the lumenal side of lysosomes. This lumenal part of the molecule has four loops connected by disulfide bonds and can be divided into two internally homologous domains, separated by a hinge-like structure. The hinge-like structure is enriched with proline and serine or threonine and free to move (for review see J. Biol. Chem. 266, 21327-21330, 1991). The lumenal portion of lamps is characterized by its extensive *N*-glycans, some of which are polylactosaminoglycans. More recently, we also identified *O*-glycans in lamp molecules, a small portion of which are polylactosaminoglycans. The *O*-glycans are clustered at the hinge-like region, which is otherwise exposed to the proteases in the lysosomal lumen. As a whole, these *N*- and *O*-glycans constitute 55-65% of the total mass of the molecules.

The polylactosaminoglycans apparently protects the molecules from proteolytic digestion in lysosomes. When HL-60 cells are differentiated into granulocytic cells, the content of polylactosaminoglycans in lamp molecules is increased with the concommitant increase of their half-lives. On the other hand, the lack of polylactocaminoglycans in differentiation-deficient HL-60 sublines results in the shorter life of lamps. However, the loss of polylactosaminoglycans in *N*-glycans can be overcome by the increased amount of *O*-glycans in some sublines, resulting in almost the same half-life of lamps as in wild type HL-60 cells.

Secondly, we have compared the surface expression of lamp molecules among cell lines derived from a human colon cancer and found that more lamp molecules are expressed on highly metastatic cell lines than on poorly metastatic cell lines (Saitoh *et al.*, J. Biol. Chem., in press). These results suggest that lamps on metastatic tumor cells provide ligands for selectins on endothelial cells and platelets, which may play a role in adhesion of tumor cells to a metastatic site. To test this hypothesis, cells were made to overly express lamp-1 molecules by gene transfer and those cells were assayed for adhesive property. The results indicate that more lamp-1 is expressed on the cell surface, more adhere to ELAM-1 presenting cells.

These results indicate that polylactosaminoglycans attached to lamp molecules play significant roles both in the cytoplasm and on the cell surface. (Supported by grant CA48737 from the National Cancer Institute).

P 027 NUCLEAR AND CYTOPLASMIC GLYCOPROTEINS, Robert S. Haltiwanger, William G. Kelly, Melissa A. Blomberg, Dennis Dong, Elizabeth Roquemore and Gerald W. Hart, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; FAX: (301) 955-0215

Numerous laboratories have reported the existence of nuclear and cytoplasmic glycoproteins. One of the best characterized examples is O-linked N-acetylglucosamine (O-GlcNAc) which consists of GlcNAc residues in O-linkage to the hydroxyl side chains of serines and threonines. It is a major form of post-translational modification found almost exclusively in the nuclear and cytoplasmic compartments of the cell and has been found in all eukaryotic cells examined to date. Several proteins modified with O-GlcNAc have been identified by our group and others, including RNA polymerase II and several RNA polymerase II transcription factors, viral proteins, cytoskeletal proteins and nuclear pore proteins.

Our laboratory has been studying the biosynthesis and function of O-GlcNAc. Recent results suggest that the modification is dynamic, much like phosphorylation. When T cell hybridomas are stimulated with phorbol esters and ionophores, there are rapid and transient changes in the levels of O-GlcNAc on individual proteins. These rapid changes suggest the presence of glycosyltransferase(s) and N-acetylglucosaminidase(s) responsible for the addition and removal, respectively, of the GlcNAc moiety from proteins. We have recently identified and purified a glycosyltransferase responsible for the addition of GlcNAc to proteins and identified and partially purified a neutral, soluble N-acetylglucosaminidase. The O-GlcNAc transferase is a soluble heterodimer with subunit molecular weights of 110,000 and 80,000. The 110,000 dalton subunit appears to contain the active site. We are currently examining the minimal peptide sequence requirements for activity. Interestingly, only peptides which contain proline residues serve as substrates, and the spacing of the hydroxy amino acid from the proline appears to be important for recognition by the enzyme.

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P 028 CARBOHYDRATE BINDING PROTEIN 35 (MAC-2): MULTIMERIC STRUCTURE AND POSSIBLE BIOLOGICAL FUNCTIONS OF A LAMININ BINDING LECTIN, Arthur M. Mercurio, Hee-Jong Woo, Margaret M. Lotz, Edward C. Lee, and Siew Ho. Laboratory of Cancer Biology, Deaconess Hospital, Harvard Medical School, Boston, MA.

Carbohydrate binding protein 35 (CBP35), also known as Mac-2 is an N-acetyllactosamine specific lectin found primarily in the cytoplasm of many cell types, though it is also evident in the nucleus and on the cell surface. Similar to IL-1 and FGF, the extracellular localization of CBP35 is difficult to explain because it lacks a defined signal sequence. Although CBP35 has been well-studied, its cellular functions are not known. Possible functions are suggested by recent findings. CBP35 binds avidly to the basement membrane glycoprotein laminin and in many cell types it is the major, non-integrin laminin binding protein. These observations suggest that it may function in concert with integrins in extracellular matrix interactions. Wang et al. have postulated a nuclear function for CBP35. These seemingly disparate functions have prompted us to examine CBP35 structure in more detail, to study the mechanism of its surface localization, and to make use of molecular genetic approaches to gain insight into specific cellular functions.

The first approach we have taken is to study the multimeric nature of CBP35. Macrophage CBP35 purified by laminin affinity chromatography exists as several distinct species (Mr 35,000, 67,000, and 80,000) when analyzed under non-reducing conditions. Upon reduction, only the 35,000 species is seen. rCBP35 expressed in E. coli forms disulfide linked homodimers (Mr, 67,000). Site-directed mutagenesis indicated that cysteine 186, the single cysteine residue in this protein is required for dimerization. The dimeric form of CBP35 binds to laminin with higher affinity than does monomer and by a lactosamine dependent mechanism. The larger 80kD form of CBP35 probably represents a heterodimer whose structure is being investigated. These structural studies suggest that the apparently disparate functions that have been proposed for CBP35 may be regulated by its ability to form intermolecular associations with nuclear, cytoplasmic, and cell surface proteins.

The mechanism of extracellular localization of CBP35 is being studied using TG-elicited mouse macrophages. Compared to most other cell types, these cells express copious amounts of extracellular CBP35. Extracellular CBP35 is found on the cell surface and in the culture medium. Only 20% of CBP35 can be released from the cell surface by 0.2M lactose washing. Because CBP35 is not a membrane protein, this observation suggests that the majority of CBP35 is attached to the surface by a non-carbohydrate dependent mechanism. The possibility that CBP35 is attached to the macrophage surface by other mechanisms (e.g., lipid anchor, salt bridge) is being examined. In other cell types such as colon carcinoma cells, most of the surface CBP35 is removed by lactose washing indicating that carbohydrate dependent mechanisms can be important for its surface localization.

CBP35 is expressed as a function of both monocyte differentiation into macrophages and intestinal crypt cell differentiation into mature enterocytes. In the absence of defined function, it is not known whether this expression is a cause or consequence of differentiation. To address these issues and to gain insight into specific functions, we are transfecting sense and anti-sense CBP35 cDNA into both monocytes and intestinal crypt cells.

P 029 **NUCLEAR AND CYTOPLASMIC LECTINS**, John L. Wang, Department of Biochemistry, Michigan State University, East Lansing, MI 48824.

Carbohydrate Binding Protein 35 (CBP35) is a galactose-specific lectin belonging to the L-30 subgroup of the S-type family of animal lectins. The polypeptide chain (M_r ~ 35,000) consists of two distinct domains: a proline- and glycine-rich domain at the amino-terminal half and a carbohydrate recognition domain at the carboxyl-terminal half. The amino acid sequence information also indicates that CBP35 is identical to (within a given species) or homologous with (between species) proteins isolated and studied under other names: (i) L-34, a tumor cell lectin; (ii) human and rat lung lectins, HL-29 and RL-29; (iii) IgE-binding protein, ϵBP; (iv) a non-integrin type laminin-binding protein, LBP; and (v) Mac-2, a cell surface marker of thioglycollate-elicited macrophages.

In mouse 3T3 fibroblasts, the majority of CBP35 is intracellular. Quiescent cultures of 3T3 cells expressed a low level of CBP35; the polypeptide was almost exclusively in the phosphorylated form (pI 8.2) and was located predominantly in the cytoplasm. The addition of serum to these cells increased the expression of CBP35, in terms of elevated transcription rate of the gene, increased accumulation of the mRNA, and increased amount of the protein. These proliferating cells had an increased level of the phosphorylated polypeptide, both in the cytosol and the nucleus. More striking, however, was the dramatic increase in the level of the unmodified form (pI 8.7), which was confined to the nucleus.

Analysis of CBP35 in the cytoplasm and in the nucleoplasm suggests that the lectin is associated with a ribonucleoprotein complex, as indicated by its position of sedimentation on sucrose and cesium sulfate gradients and by the sensitivity of this position of migration to treatment with micrococcal nuclease. Using a cell-free assay for the splicing of intervening sequences from pre-mRNA, we have found that lactose, thiodigalactoside, and an A-tetrasaccharide human serum albumin conjugate inhibited the formation of the spliced mRNA product, whereas control reagents such as sucrose, glucose, and bovine serum albumin failed to yield the same effect. Moreover, a rat monoclonal antibody reactive with CBP35 (anti-Mac-2) also showed inhibition, whereas an isotype-matched control monoclonal rat antibody did not. These results provoke new views on the binding activities and physiological role of an intracellular lectin.

Applications of Technology for Characterization of Physical and Structural Properties of Oligosaccharides, Lectins and Glycosyltransferases

P 100 TWO TRANSCRIPTS THAT DIFFERS IN THE 5' UNTRANSLATED REGION OF THE GENE ENCODING β-GALACTOSIDE α 2,6-SIALYLTRANSFERASE, Hans-Christian Åsheim, Anne Aas-Eng, Arne Deggerdal, Steinar Funderud, Heidi K. Blomhoff and Erlend B. Smeland, Lab for Immunology, The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway.

The B lymphocyte antigen CD75 has been cloned and found to be a β-galactoside α 2,6-sialyltransferase. This enzyme is generally thought to be localiced to the Golgi apparatus. The gene encoding this enzyme transcribes different messenger RNAs due to different exon usage. We will here present data regarding two of the transcripts differing in the 5' untranslated region, but with the same open reading frame. Mature B cells express both transcripts, while pre-B cells and the hepatoma cell line HepG2 do not express the B cell spesific transcript as demonstrated by RNAse protection assay. The proteins translated from both transcripts are capable of reaching the plasma membrane as shown by immunostaining on enzyme on transfected COS cells. Although pre-B cells do not express the enzyme on the cell surface, both mature B cells and HepG2 do. Resting B cells express less of the transcripts than activated cells in accordance with a increased level of CD75 on the surface of the cells upon activation. We will also present data of the relative relationship between the two transcripts in resting and activated B cells using an approach involving DNA amplification.

P 101 PURIFICATION OF THE SECRETOR-TYPE β-GALACTOSIDE α1→2 FUCOSYLTRANSFERASE AND β-N-ACETYLGLUCOSAMINIDE α1→3 FUCOSYLTRANSFERASE FROM HUMAN SERUM, Magdalena Blaszczyk-Thurin, Jan Thurin, Annikki Sarnesto, Ole Hindsgaul*, Torvald Köhlin, and, Katherine Vogele, The Wistar Institute, Philadelphia, Pennsylvania 19104 and *Department of Chemistry, University of Alberta, Edmonton, Canada T6G 2G2. Two human serum β-galactoside α1→2 fucosyltransferases, presumably the H and secretor-type and the β-glucosaminide α1→3 fucosyltransferase, were purified from the human serum by hydrophobic chromatography, ion exchange chromatography on sulphopropyl-Sepharose, and affinity chromatography on GDP-hexanolamine-Sepharose. The Se type α1→2 fucosyltransferase as well as α1→3 fucosyltransferse were separated from H blood group α1→2 fucosyltransfere by being absorbed onto ion exchange chromatography matrix. The H and Se type α1→2 fucosyltransferases are a multi-subunit molecules of M_r 150,000 and a monomer of M_r 50,000, as determined upon reduction. The substrate specificity and K_m values showed that Se type α1→2 fucosyltransferase shows significantly lower affinity than the H enzyme for phenyl-β-D-galactopyranoside and GDP-fucose as well as for type 2 oligosaccharide acceptors. On the contrary, type 1 and 3 oligosaccharide acceptors are preferentially utilized by the secretor-type enzyme as compared with the H enzyme. The enzymes also differ in several physicochemical properties, implying structural differences between the two molecules. The native β-N-acetylglucosaminide α1→3 fucosyltransferase is a monomer with a M_r of 45,000. The specificty of the purified α1→3 fucosyltransferase is restricted to type 2 structures, as revealed by its reactivity with various oligosaccharide acceptors. The enzyme has the ability to utilize the N-acetyl-β-lactosamine determinant and its sialylated and fucosylated derivatives and thus is distinct from both the human Lewis gene encoded enzyme and the α1→3 fucosyltransferase of the myeloid cell type.

P 102 OLIGOSACCHARIDES FROM RAT BRAIN MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) Zbigniew Bartoszewicz, Carl Lauter and Richard Quarles, Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH, Bethesda, MD 20892

The myelin-associated glycoprotein (MAG) is a highly glycosylated (30% by weight) member of the immunoglobulin superfamily that appears to mediate interactions between myelin-forming glial cells and axons in the central and peripheral nervous system. Fractionation of N-linked oligosaccharides released from immunopurified rat brain MAG by hydrazinolysis or N-glycanase treatment on the Dionex HPLC-PAD system revealed a complex mixture of neutral, sialylated and sulfated components. A prominent neutral oligosaccharide was purified on the PA-1 column and characterized by TFA hydrolysis, serial incubations with glycosidases and FAB-MS. The results showed that it was a high mannose structure containing only mannose and N-acetylglucosamine. Digestion of purified MAG with Arthrobacter ureafaciens or Newcastle disease virus neuraminidases and binding of digoxigenin-labeled Sambucus nigra and Maackia amurensis agglutinins revealed that the oligosaccharides contain both α2-6- and α2-3-linked NANA. Many of the oligosaccharides of MAG appear to contain more than one sulfate moiety and bind very strongly to the PA-1 anion exchange column necessitating modified methods of separation. Information about the structure of MAG oligosaccharides may be relevant to its function in glia-axon interactions during myelination.

P 103 STRUCTURAL STUDIES OF AN OLIGOSACCHARIDE-LECTIN CROSS-LINKED COMPLEX. Fred Brewer[1], Lokesh Bhattacharyya[1], Wen Cheng[2], and Lee Makowski[2], [1]Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461; [2]Dept. of Physics, Boston University, Boston, MA 02215.

Certain cell-surface oligosaccharides have been shown to be multivalent and capable of binding, cross-linking and precipitating with specific lectins (cf. Bhattacharyya, L. and Brewer, C.F. (1989) Eur. J. Biochem. 178, 721-726). This leads to an important new source of specificity in carbohydrate-protein interactions: namely, the formation of unique, homogeneous cross-linked lattices (precipitates) between each carbohydrate and lectin. Many of these lattices have recently been shown to be crystalline and suitable for structural analysis. In the present study, the structure of a lattice made up of L-fucose specific isolectin A from Lotus tetragonolobus (LTL-A) cross-linked by difucosyllacto-N-neohexaose, a biantennary fucosoyl carbohydrate derived from milk, has been characterized by a combination of electron microscopy, x-ray diffraction, computer simulation of electron micrographs and molecular model building. Molecular models for the cross-linked lattice were constructed from the known molecular structure of concanavalin A, based on the high degree of sequence homology between LTL-A and concanavalin A. X-ray diffraction patterns from unoriented pellets of the lattice were used to determine lattice dimensions and filtered electron micrographs were used to determine the symmetry of the lattice. The orientation of LTL-A in the lattice was determined by simulation of the electron micrographs of negatively stained lattices. Molecular model building of the lattice reveals a remarkable self-complementarity of the protein in the vicinity of the cross-linking sites. The self-complementarity suggests that LTL-A is designed to foster and stabilize cross-links, indicating a functional role for cross-linking by certain branched chain carbohydrates. A detailed description of the interactions and the binding properties of such lectin-carbohydrate complexes may provide a basis for understanding the activity of certain branched carbohydrates and lectins and provide direct insight into the molecular recognition properties of both classes of molecules.

P 104 GLYCOPROTEIN ANALYSIS USING LC-ELECTROSPRAY AND TANDEM MASS SPECTROMETRY, A.L. Burlingame, S.C. Hall, D.A. Maltby, K.F. Medzihradszky and R.R. Townsend, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446

We have investigated the advantages of microbore and packed capillary reversed-phase HPLC coupled to a mass spectrometer via an electrospray ionization source [Carlo-Erba Phoenix 20-VG Bio-Q triple quadrupole instruments] for the analysis of digests of glycoproteins. In a single chromatographic run the molecular weights of all components of a 40 pmol aliquot of a tryptic digest of bovine fetuin (a 341 amino acid glycoprotein with three *N*- and four *O*-linked glycosylation sites) were measured. In contrast to results from our previous studies using LSIMS (1), the molecular ions for the large, relatively hydrophilic glycopeptides from both *N*- and *O*-linked sites were readily observed (5000-8000 Da range). All three *N*-linked sites gave molecular ions which were consistent with triantennary oligosaccharides bearing three and four sialic acids and their respective amino acids. Only the "Asn-Asp" site gave molecular ions indicative of disialylated biantennary oligosaccharides. Interestingly, molecular ions attributable to partially desialylated glycopeptides are not observed. The *O*-linked glycopeptides were consistent with either two or three sialylated Hex-HexNAc residues. Taken together with earlier results of Carr and co-workers involving smaller *N*-linked glycopeptides in the 3000-4000 Da range from $rsCD_4$ (2), LC/ES-MS will most likely become the method of choice for quickly determining the location of glycopeptides in tryptic maps providing an initial global assessment of the types of oligosaccharides and heterogeneity at each glycosylation site prior to any detailed structure elucidation. Further delineation of the *N*-linked structures themselves can be established by strategies previously described (3). The *O*-linked structures and their site specificity can subsequently be determined by high energy collision-induced dissociation analysis obtained from high performance tandem mass spectrometry (4) using suitable further enzymic or chemical cleavages of multiply *O*-linked glycopeptides such as the 61-mer observed in this case. Further details and results on protein glycosylation will be presented.

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P 105 PURIFICATION AND GENE CLONING OF β-N-ACETYLGLUCOSAMINIDASE FROM *STREPTOCOCCUS PNEUMONIAE*.

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β-N-acetylglucosaminidase from *Streptococcus pneumoniae* is an important reagent for sequencing N-linked oligosaccharides. Usually the secreted form of this enzyme is purified, but we have taken the novel approach of purifying the membrane bound form, released by autolysis after subjecting cells to high pressure using a French press. The enzyme, purified to apparent homogeneity using anion exchange and hydrophobic interaction chromatographies, gives the monomeric molecular weight of 120kD.

A stable genomic DNA library isolated from *S. pneumoniae* cells was made using the λ-ZAP® II cloning protocol. Clones were screened using oligonucleotide probes designed from protein sequence information of tryptic peptides, and by enzyme activity of positive plaques using 4-methylumbelliferone substrate.

P 106 CHARACTERIZATION OF CARBOHYDRATE ENZYMATIC REACTIONS BY ELECTROSPRAY MASS SPECTROMETRY, Joseph K. Welply and Kevin L. Duffin, Monsanto Corporate Research, Monsanto Company, St. Louis, MO 63198

Mass spectrometry was used to monitor changes in the molecular weights of carbohydrates that were subjected to various enzymatic reactions. The negative ion electrospray mass spectra of sialylated oligosaccharides that were cleaved from fetuin with N-glycanase and fucosylated with milk-derived α1-3/4 fucosyltransferase showed additions of varying numbers of fucose units to the oligosaccharides. The total number of fucose units that could be attached to an oligosaccharide was dependent upon the sequence and linkage positions of the carbohydrate units that comprise the oligosaccharide. Oligosaccharides with the sequence SAα2-3Galβ1-4GlcNAc were fucosylated to form the Sialyl-Le^x structure. Fucosylation of intact fetuin and a tryptic digest of fetuin with fucosyl transferase resulted in the same number of fucose additions to the oligosaccharide portion of the molecule. Electrospray mass spectrometry was also used to monitor enzymatic digestions. The carbohydrate portion of ovalbumin was digested with α-mannisidase and with endoglycosidase H, which resulted in a change in mass of the different glycoforms of ovalbumin that could be correlated to digestion of the N-linked oligosaccharide. The combination of mass spectrometry and exoglycosidase digestion for determining carbohydrate structure will be presented and compared to methods in which carbohydrate structure is determined by fragmentation patterns in the mass spectra of the carbohydrate.

P 107 ABNORMAL SUBCELLULAR COMPARTMENTALIZATION OF O-LINKED OLIGOSACCHARIDE EPITOPES AND GOLGI RESIDENT PROTEINS IN COLON CANCER CELLS. G. Egea[1,2], C. Francí[1], G. Gambús[1], T. Lesuffleur[3], A. Zweibaum[3], and F. X. Real[1].[1] Inst. Mun. Invest. Med., Barcelona (Spain); [2] Facultat de Medicina, U. Barcelona; INSERM U178, Villejuif (France).

Cellular transformation is frequently associated with alterations in the glycosylation pattern of membrane glycolipids and glycoproteins. Mucins from tumors derived from glandular epithelia show incomplete glycosylation that leads to the accesibility of carbohydrate (i.e., Tn) (GalNAc α-O-Ser/Thr) and peptide epitopes that are normally cryptic. To elucidate the mechanisms responsible for altered mucin glycosylation we have analysed the expression of Tn (mAb Cu-1), and a peptide epitope of MUC2 intestinal mucin tandem repeat (PTPTGT) (mAbLDQ10) in cultured colon cancer cells and in colonic tissues using immunogold techniques. In normal colon, the PTPTGT epitope was only detected in the RER, whereas Tn was always detected in the cis-Golgi. In cultured colon cancer cells, the RER was enlarged and the Golgi complex was disorganized. These morphological alterations were accompanied by the abnormal colocalization of PTPTGT, Tn, and a 130 kD cis-Golgi integral protein in the RER of cultured colon cancer cells. These findings were not related to in vitro culture since miscompartmentalization of Tn and cis-Golgi resident proteins in the RER was also demonstrated in colon cancer tissues. The identity of the RER was demonstrated by visualization of membrane-bound ribosomes, the distribution of a PDI - a resident RER protein - and the distribution of the PTPTGT epitope. Based on these results, we propose that in colon cancer cells a rearrangement of molecules normally present in the Golgi apparatus takes place, leading to the co-distribution of resident RER and Golgi proteins and possibly contributing to the abnormal glycosylation of proteins and lipids.

P 108 CHARACTERIZATION OF OLIGOSACCHARIDE STRUCTURES SYNTHESIZED BY THE INSECT CELL LINE SF9, Åke P. Elhammer and Paul A. Aeed, Biochemistry, Upjohn Laboratories, Kalamazoo, MI 49001

In vivo [^{3}H]-mannose labeled oligosaccharides isolated from total Sf9 proteins and from baculo virus expression of recombinant human prorenin were characterized, using a combination of chromatographic techniques and digestion with specific glycosidases. The majority of the oligosaccharide structures on the secreted prorenin molecule were of truncated high-mannose, Man_3 type, all of which appeared to be core-fucosylated. The molecule also contained significant amounts of regular high-mannose type structures composed of 7 to 9 mannose residues. In contrast to prorenin expressed in mammalian cells, none of the high-mannose type oligosaccharides on the Sf9 synthesized molecule was phosphorylated. Characterization of the oligosaccharide structures on total Sf9 protein resulted again in the majority of the structures being of truncated Man_3 type or of high-mannose type containing 6 to 9 mannose residues. We found no evidence of structures containing outer galactose residues or terminal sialic acid.

P 109 ISOLATION OF A GENE ENCODING A NEW HUMAN S-LAC LECTIN, Michael A. Gitt, Stephen M. Massa, Hakon Leffler, and Samuel H. Barondes, Dept. of Psychiatry, University of California, San Francisco, CA 94143. We have isolated a gene, LGALS2, encoding a new human S-lac lectin (L14-II) by PCR amplification out of a HepG2 cDNA library, and confirmed its identity by genomic sequencing. Expression of the coding sequence utilizing the putative translational initiation site produces a functional protein of 14 kDa which binds lactose and exists as a dimer in solution. Like the L14-I gene, the L14-II gene contains four exons, with similar intron placement. The upstream genomic region contains several sequences characteristic of regulatory elements, including two tandem TATA boxes. LGALS2 does not cross-hybridize with any other genes on a genomic Southern blot, including other members of the S-lac lectin gene family (L14-I and L29 genes). The gene is also detectable in a Southern blot of bovine genomic DNA.

P 110 ANALYSIS OF SIALYL OLIGOSACCHARIDE-ALDITOLS DERIVED FROM GLYCOPROTEINS

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O-linked oligosaccharides are usually released from glycoproteins by reductive β-elimination. The alditols thus produced are not amenable to labelling with UV or fluorescent chromophore for HPLC analysis. However, alditols can be readily detected with high sensitivity by pulsed electrochemical detectors (PED or PAD).

High-pH anion exchange chromatography (HPAEC) is a powerful tool for analysis of N-linked oligosaccharides from glycoproteins. To develop an analytical method for O-linked oligosaccharides, separation of sialyl oligosaccharide-alditols by HPAEC was investigated. Fourteen sialyl oligosaccharide-alditols isolated from bovine submaxillary mucin (BSM) and bovine fetuin, having type 1, type 2, type 3, or type 5 (GalNAcα3GalNAc) core structures and containing either NeuAc or NeuGc, can be analyzed at 0.1 nmol level by HPAEC with PED.

Analysis of oligosaccharide-alditol mixtures released from BSM and bovine fetuin by this method demonstrated seven major peaks for BSM and three for bovine fetuin coincident with the reference oligosaccharide-alditols. Thus HPAEC-PED was shown to be a rapid and sensitive mapping technique for O-linked oligosaccharides. The application of this technique to a comparative study of several glycoproteins will also be presented.

P 111 **THE 2D-MAPPING BY HPAEC AND CE OF THE GLYCANS OF HUMAN α_1-ACID GLYCOPROTEIN, RELEASED BY HYDRAZINOLYSIS** P. Hermentin, R. Witzel, R. Doenges, H. Haupt, T. Patel #, and D. Brazel, Research Laboratories of Behringwerke AG, D-3550 Marburg, FRG, and #: Oxford GlycoSystems, Abingdon, England.

Human α_1-acid glycoprotein (AGP) is a polymorphic glycoprotein carrying five N-glycosylation sites (1). Eight neutral N-glycans of AGP have thus far been characterized (2). However, due to the presence of sialic acid in α2,3- and α2,6-linkages to terminal βGal residues (2), several acidic glycan isomers should be expected that have yet to be characterized. Nashabeh and El Rassi (3) have recently analyzed by capillary electrophoresis (CE) the pyridyl-aminated (PA) oligosaccharides gained from AGP via PNGase F treatment of a tryptic digest and subsequent derivatization with 2-aminopyridine. Their CE mapping revealed six well defined peaks and a few minor peaks (3).

We have analyzed the reducing oligosaccharides, released from AGP by hydrazinolysis, in a two-dimensional mapping technique, using CE with UV detection at 190 nm as the first and high-pH anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) as the second dimension. Approximately 40 distinct peaks could be detected by either technique, suggesting multiple N-glycan isomers and/or partial degradation during hydrazinolysis. Superposition of the CE and HPAEC patterns obtained from the Mono Q-fractionated N-glycans allowed resolution of the peaks according to charge. The asialo N-glycans, generated by sialidase treatment, revealed two major, three intermediate and a few small size peaks by HPAE-PAD.

Ref.: (1) Schmid et al. (1977) Biochim. Biophys. Acta 492, 291-302. (2) Yoshima et al. (1981) J. Biol. Chem. 256, 8476-8484. (3) Nashabeh and El Rassi (1991) J. Chromatography 536, 31-42.

P 112 SYNTHESIS OF 4 NOVEL TRISACCHARIDES BY INDUCTION OF LOOSE ACCEPTOR SPECIFICITY IN GAL(ß1->4) TRANSFERASE (EC 2.4.1.22). Roger A. Laine and Ensun Yoon, Departments of Biochemistry and Chemistry, Louisiana State University and The LSU Agricultural Center, Baton Rouge, LA 70803.

Synthesis of linkage epimeric sets of trisaccharides for development of tandem mass spectrometric methods of carbohydrate structural analysis required long lead times because of lengthy organic procedures. Trisaccharides are the minimum size oligosaccharides containing an internal sugar and both reducing and non-reducing terminal linkage parameters. Using two sets of trisaccharides having internal GlcNAc and either terminal Galactose or Fucose, we observed signficant and useful-for-interpretation differences in the low energy collision induced dissociation mass spectra for both intact (Laine, et al., JACS 110: 6931, 1988) and permethylated (Laine, et al. Biomed. Mass Spectrom. 20: 505, 1991) heterotrisaccharides. Interpretation of the results included a hypothesis that collision energy dissipation was dependent on stereochemical degrees of freedom of motion in the trisaccharides and was partly responsible for the mass spectral differences observed. This hypothesis was supported by molecular modelling. However, questions arose regarding participation of the central aminosugar in electronic mechanisms of glycosidic bond breakage. We therefore needed linkage isomeric sets of neutral trisaccharides for test purposes, of the general formula Sugar(α or ß1->X)Sugar(α or ß1->Y)Sugar where X was the same and Y was 2,3,4 and 6, or where Y was the same and X varied. Organic synthesis would have needed 1 man-year for any such set. We therefore set about to utilize enzyme-assisted synthesis and examined the acceptor specificity of GAL(ß1->4) TRANSFERASE (EC 2.4.1.22 or EC 2.4.1.38 or EC 2.4.1.90) under a number of reaction conditions. We found that low pH induced a loose acceptor specificity such that a linkage isomeric set of glucose disaccharides could be used as low efficiency acceptors. Using this method, we obtained 5-10 mg each of 4 novel trisaccharides as follows: Gal(ß1->4)Glu(ß1->3)Glu; Gal(ß1->4)Glu(ß1->4)Glu; Gal(ß1->4)Glu(ß1->6)Glu; and Gal(ß1->4)Glu(α1->4)Glu. (Supported, in part by NIH Grants DK33755 and GM32594 to RAL.)

P 113 THE BOVINE SERUM LECTIN CONGLUTININ BINDS TO A HIGH-MANNOSE TYPE OLIGOSACCHARIDE ON THE COMPLEMENT PROTEIN iC3b. S.B.Laursen, Y.Wang, S.Thiel, U.Holmskov, B.Teisner & J.C.Jensenius. Institute of Medical Microbiology, Aarhus University, Denmark and Institute of Medical Microbiology, Odense University, Denmark.

Bovine conglutinin is a serum lectin with specificity for high mannose type oligosaccharides, and N-acetyl-D-glucosamine (GlcNAc) -terminated complex oligosaccharides. It has previously been shown that conglutinin selectively binds to physiologically generated degradation products of complement factor C3, which become bound to antibody-coated erythrocytes on incubation with serum. More specifically it has been shown that it binds to iC3b deposited on the erythrocytes (Lachmann and Müller-Eberhard, 1968). In contrast, it was by ELISA found that plastic-bound conglutinin binds all the examined liquid phase "C3" molecules containing the C3c structures, i.e. C3b, iC3b and C3c. The binding of C3 was not examined (Hirani, Lambris and Müller-Eberhard, 1985). We have reinvestigated this discrepancy between the results obtained between liquid phase and solid phase bound C3 degradation products.
An ELISA was constructed with bovine conglutinin immobilised on microtiterplates. The wells next received consecutive samples taken from serum incubated with Sephadex G25 to induce complement activation. Bound C3 components was detected using anti-C3c and anti-C3d monoclonal and polyclonal antibodies. We observed maximum binding of C3 components to bovine conglutinin with serum activated for 45 minutes. There was no binding when the complement activation had proceeded for 24 hours. At this time there was complete conversion to C3c. To further investigate the nature of the bound C3 split product, we eluted the protein bound to conglutinin in the wells. This was done by adding 2mM GlcNAc or 10 mM EDTA. The eluted proteins were analyzed by SDS-PAGE followed by Western blotting and analysis of the proteins with different anti-C3 antibodies. It could be concluded that conglutinin only binds to fluid phase iC3b. The ligand for conglutinin could be demonstrated in the NH2-terminal domain of the α-chain of iC3b, which is known to possess a high mannose type oligosaccharide. There is also a high mannose type oligosaccharide on the ß-chain of iC3b. The discrepancy between these two oligosaccharides is three α1-2 bound mannose residues. It thus appears that the preferred ligand for conglutinin is α1-2 bound dimannose units. The results could also be explained by different accessibility of conglutinin for the two oligosaccharides.

P 114 STRUCTURE AND FUNCTION RELATIONSHIP OF BOVINE CONGLUTININ: A CASE OF HYDROXYLYSINE GLYCOSYLATED ANIMAL LECTIN. Young Moo Lee and Thomas B. Okarma. Applied Immune Sciences, Inc. Menlo Park, CA 94025-1109

Conglutinin is a bovine serum protein with calcium dependent carbohydrate binding properties. Our structural studies showed that conglutinin consists of a collagenous domain and carbohydrate binding region. The collagenous sequence (171 amino acids) separates a short non-collagenous NH_2-terminal region of 25 amino acids from a 155 residue long COOH- terminal carbohydrate binding domain (J. Biol. Chem. 266, 2715-2723, 1991). In the course of our studies we identified 8 sites in the collagenous domain of conglutinin as hydroxylysine residues. To prove the implication that these hydroxylysines are glycosylation sites, protease fragmented peptides (15 to 17 residues long) were analyzed by mass spectrometry. Data are consistent with the notion that disaccharide moiety is bound to each of the hydroxylysine in conglutinin. In addition, it showed the existence of two major molecular ion clusters in the spectrum due to an adduct of oxyanion with something like sulfate.

In functional studies, carbohydrate binding properties of conglutinin were analyzed by employing immune complexes (IC). Our standardized conglutinin based IC assay ELISA system showed that conglutinin can detect IC with a sensitivity of less than 100 ng/ml. Such IC binding was competitively inhibited most efficiently by N-Acetyl-D-Glucosamine. Intact collagenous domain of conglutinin may be a required entity in efficient carbohydrate binding, since CL1 peptide, which contains the entire non-collagenous lectin domain plus five repeats of Gly-X-Y, failed to capture IC in solid phase assay. For further structural characterization of IC, an IC-affinity column was constructed by oriented coupling of conglutinin to agarose. The column showed very efficient capturing of IC. Therefore, structure-function relationship studies showed that conglutinin has potential clinical application in detection and capture of IC from a wide spectrum of disease states.

P 115 SEQUENCE AND SPECIFICITY OF A SOLUBLE 16 KD LACTOSE-BINDING LECTIN FROM XENOPUS LAEVIS. Philippe Marschal, Jorg Herrmann, Samuel H. Barondes, and Douglas N.W. Cooper. University of California, San Francisco, CA 94143

The mucus exudate of frog skin includes remarkably high levels of a 16 kD lactose-binding lectin synthesized in granular gland cells. The characteristics of that lectin are very similar to those of a mammalian 14 kD lectin, L-14, which is expressed in a wide range of tissues. Localization of L-14 in extracellular matrix in many tissues, such as muscle, has been taken to imply some function in cell-cell or cell-matrix interactions. However, the abundance of an apparently similar lectin in frog skin and its externalization onto the skin surface seem at odds with those presumed functions. Instead, the specialization of granular gland cells for high level expression of a range of toxins and antibiotics suggests a similar defensive role for the lectin. Before testing L-14 for such activity, we had to consider the possibility that the frog skin lectin might be distinct from L-14 expressed in other tissues or species. For this reason and to open a new experimental model for studying L-14 function, we have compared the sequence and binding specificity of the lectins from Xenopus skin and muscle. The 16 kD lactose-binding lectins were purified, partial peptide sequences were obtained, and deduced oligonucleotides were used to isolate full-length cDNA. We also confirmed the striking abundance of the skin lectin (1 mg/gm wet weight compared to 4.6 µg/gm in muscle). Based on Northern blotting, ribonuclease message protection, and sequencing of selected peptides, Xenopus skin and muscle express the same lectin (actually two closely related isolectins in each case). It is also clear that the Xenopus lectin is, indeed, homologous to mammalian L-14, based on similarity of sequence and relative affinity for a panel of saccharide structures. Thus, it seems likely that either prior assumptions regarding L-14 function are incorrect or L-14 performs multiple functions.

P 116 CHARACTERIZATION OF THE OLIGOSACCHARIDES OF HUMAN IGM FROM A HUMAN-HUMAN-MOUSE TRIOMA PRODUCED IN CELL CULTURE AND ASCITES

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We have characterized the oligosaccharides of a monoclonal human IgM antibody secreted by a mouse-human-human trioma cell line cultured in both ascites and cell culture using lectin blots and high pH anion exchange chromatography with pulsed amperometric detection (HPAE-PAD). The data indicate that the ascites and cell culture samples contain identical sets of structures, but the cell culture material, which has an order of magnitude higher clearance rate, has more tetra-charged oligosaccharides. Both samples have high mannose, hybrid, and complex N-linked oligosaccharides with α(2,6) linked sialic acid, and mono-, di-, tri- and tetra-charged structures. This is the first report of hybrid and of tetra-charged oligosaccharide structures on human IgM. The presence of tetra-charged structures and the absence of α(2,3) sialic acid linkages suggests that this cell line expresses a mixed repertoire of human and mouse post-translational modification processing enzymes.

P 117 EXPRESSION CLONING OF β-1,4 N-ACETYL-GALACTOSAMINYLTRANSFERASE cDNA.

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Gangliosides play important roles in cell differentiation, development and malignant transformation. Such biological roles for gangliosides are considered to be controlled by the cell type-specific acitivity of the glycosylation machinery. It is therefore important to isolate glycosyltransferase genes and to analyze the regulation of their expression for further analysis of the biological roles of gangliosides. In order to clone the cDNAs of such glycosyltransferases, we used a eukaryotic cell expression cloning system with a modified mouse melanoma cell line (which expresses GM3 ganglioside) derived from B16 cells by transfection with the polyoma T antigen gene and the pCDM8 vector developed by Seed et al. By using this system, 2 clones of cDNA which determined the expression of both GM2 and GD2 gangliosides were isolated from a cDNA library prepared from the mRNA of the YT cell line. The length of the inserts were 2.2 and 2.5 kb, respectively and both showed the same open reading frame of 1683 bp. The deduced amino acid sequence showed a type 2 transmembrane structure for the protein, typical of other glycosyltransferases. Northern blot analysis revealed 3 different transcripts with lengths of 5.2, 3.0, and 2.0 kb. Analysis of the expressed gangliosides in transfected cell lines indicate that these cDNA code for the gene of UDP-GalNAc: GM3\GD3 β1,4 N-acetylgalactosaminyl transferase.

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P 118 EXPRESSION OF AMINO-TERMINAL DELETION-MUTANTS OF β1-4GALACTOSYLTRANSFERASE IN COS-7 AND *E.COLI* CELLS,

Pradman K. Qasba, Arni S. Masibay and Elizabeth Boeggeman, Laboratory of Mathematical Biology, DCBDC, NCI, NIH. For the studies on structure-function relationship of β1-4galactosyltransferase the cDNA was manipulated and expressed both in mammalian and *E. coli* cells. In Cos-7 cells expression of the cDNA, manipulated in an Okayama-Berg vector, was driven by the SV40 promoter. Production of protein was monitered both by measuring enzymatic activity and Western blot analysis. In *E. coli*, using pGEX vectors, the protein was expressed as a fusion protein with glutathione S-transferase as an affinity tail. Enzymatic activities were measured after purification and clevage of the affinity tail. The results show that the full-length 402 residue long β1-4galactosyltransferase that contains the amino-terminal membrane-anchoring domain has enzymatic activity when produced either in mammalian or *E. coli* cells. The binding sites for UDP-galactose, N-acetylglucosamine, glucose and α-lactalbumin are intact and operative in the expressed protein. The deletion constructs coding for the amino-terminal truncated-proteins that lack the first 70 residues of the protein which corresponds to the secreted form of the enzyme, or constructs missing only the membrane-anchoring domain, do not produce an enzymatically active protein in Cos-7 cells. On the other hand in *E. coli* the secreted form of the protein which lacks the first 70 residues, including the membrane-anchoring domain is enzymatically active. The galactosyltransferase enzyme without the amino-terminal membrane-anchoring domain when synthesized within the mammalian cells is either unstable or inactivated. The role of this domain may be to target the protein to a proper site within the cell thereby physically blocking the inactivation process.

P 119 IDENTIFICATION AND DERIVATIZATION OF 1-AMINO-1-DEOXY-OLIGOSACCHARIDES OBTAINED BY TREATMENT OF ASN-GLYCOPEPTIDES WITH N-GLYCANASE® ENZYME,

James R. Rasmussen, Jeffrey Davis, Genzyme Corporation, Cambridge, MA and John M. Risley and Robert L. Van Etten, Department of Chemistry, Purdue University, West Lafayette, IN The initial steps in the structural analysis of the asparagine (Asn)-linked oligosaccharides of a glycoprotein generally consist of chemical or enzymatic hydrolysis of the N-acetylglucosaminyl-asparagine bond between each oligosaccharide chain and the polypeptide, derivatization of the released oligosaccharide chains with a radioactive or fluorescent tag, and chromatographic fractionation of the labeled oligosaccharides. The purified oligosaccharides can be further characterized by ^{1}H-NMR spectroscopy, mass spectroscopy, methylation analysis and exoglycosidase digestions. Current methods for labeling the released oligosaccharides involve derivatization of the hemiacetal moiety at the reducing terminus of the sugar chain, for example, by reduction with sodium borotritide or reductive amination with a chromophore such as 2-aminopyridine. We have developed an alternative approach for labeling Asn-linked oligosaccharides that is based on our observation that treatment of glycopeptides with a peptide-N^4-(N-acetyl-β-glucosaminyl)-asparagine amidase (PNGase, EC 3.5.1.52) generates a set of 1-amino-1-deoxyoligosaccharide intermediates that can be derivatized with electrophilic reagents. Reaction with an electrophilic reagent containing an appropriate chromophore provides a simple route to stable oligosaccharide derivatives suitable for chromatographic fractionation and subsequent structural characterization.

P 120 STRUCTURE OF THE MURINE MANNOSE-BINDING PROTEIN-C GENE, Kedarnath N. Sastry and Alfred I. Tauber, Department of Pathology and Medicine, Boston University School of Medicine, Boston, MA 02120.

Mannose-binding proteins are Ca^{2+} dependent (C-type) lectins synthesized exclusively by hepatocytes. Previous studies on functions of human mannose-binding protein (MBP) have shown that it can play an important role as an opsonin and that its mRNA level is elevated as a part of the acute phase response of inflammation. Little is known about the functions of mouse and rat MBP-C. Of the two mannose-binding proteins in rodents, called MBP-A and MBP-C, only the structure of gene encoding rat MBP-A is known. In the present study, the structure of the gene encoding murine mannose-binding protein-C has been characterized to obtain some clues about its evolution and regulation. Two full length genomic clones, one of which has more than 3 kb 5' flanking region have been isolated by screening a NIH3T3 cell line genomic library in Lambda Fix II vector with a cDNA probe. The gene is encoded by six exons separated by five introns and spans about 7 kb. The first two exons encode the 5' untranslated regions. The third exon encodes the signal peptide, the region up to the first interruption in the collagen-like domain and is rich in cysteines important for inter-chain crosslinking. The fourth exon encodes rest of the collagen domain probably derived by exon-shuffling. The fifth exon encodes for a short stalk, containing the motif Arg-Gly-Asp, a characteristic sequence recognized by integrins. The entire carbohydrate-recognition domain and the 3' untranslated regions are encoded by the sixth exon. Primer extension studies show three start sites. The structure of the mouse MBP-C gene is thus similar to that of rat MBP-A, human MBP and pulmonary surfactant apoprotein genes which encode the carbohydrate-recognition domain in a single exon.

P 121 CARBOHYDRATE CHAINS OF FETAL α_1-ACID GLYCOPROTEIN. S.D. Shiyan, V.V. Nasonov, N.V. Bovin, Shemyakin Institute of Bioorganic Chemistry, Moscow. N.Y.Belogortseva, A.A.Bulgakov, A.F. Pavlenko, Pacific Institute of Bioorganic Chemistry , Vladivostok.

α_1-Acid glycoprotein isolated from abortive human blood (f-AGP) had immunochemical properties and amino acid composition similar to AGP from plasma of adult normal donors (n-AGP).

We studied the structure of carbohydrate chains of f-AGP using a sequential splitting : of O- followed by N-oligosaccharides from GP. Three N-linked and four O-linked sugar chains were found.

O-linked OSs were selectively splitted off by treatment with alkaline sodium borohydride in presence of 6 mM $CdCl_2$ and released by HPLC on Si-lasorb-NH_2. Structure of major OS was determined by methylation analysis:

Galβ1→4GlcNAcβ1→6(3)[Galβ1→3(6)]GalNAc-ol

N-linked OSs were obtained by $LiBH_4$ - LiOH digestion. Fluorescent labelling of the mixture with 7-amino-4-methylcoumarine followed by HPLC separation (Ultrasphere ODS) gave rise to 18 components. Structure of labelled glycans was studied by comparison with known AMC-OS and exoglycosidase digestion [1].

It was found that: i)f-AGP differs from n-AGP by ratio of tetra-, tri-, and biantennary glycans; ii) about 30% of amount of f-AGP glycans are represented by repeating lactosamine blocks Galβ1→4GlcNAcβ1→3; iii) f-AGP has increased amounts of fuco-OS.

[1] S.D.Shiyan, V.V.Nasonov, N.V.Bovin, L.I.Novikova, V.A.Aleshkin, Bioorganicheskaya Khimia, 17(5), p.663-670, (1991).

P 122 MAMMALIAN α(1,3)FUCOSYLTRANSFERASES: HOW MANY ARE THERE? Pamela Stanley and Barry Potvin, Department of Cell Biology, Albert Einstein College of Medicine, New York, NY 10461.

The α(1,3)fucosyltransferases (α(1,3)Fuc-T's) responsible for synthesizing α(1,3)fucosylated lactosamine units in glycoconjugates are of interest because the expression of these carbohydrates is developmentally regulated and several related structures of this type have been shown to bind to LEC-CAM ligands. In order to identify different α(1,3)Fuc-T enzymes that might be responsible for synthesizing particular types of α(1,3)fucosylated lactosamine structures, CHO mutants that express an α(1,3)Fuc-T activity have been isolated and characterized. Previous studies have shown that the dominant CHO mutants LEC11, LEC12, LEC29 and LEC30 express different arrays of surface carbohydrates that reflect the α(1,3)fucosylation of different lactosamine units on N-linked carbohydrates. This is most easily summarized by a comparison of their abilities to bind monoclonal antibodies that recognize specific α(1,3) fucosylated lactosamines.

MAb	anti-Le^X	anti-$SALe^X$	anti-VIM-2
Structure Recognized	Δ ↑ ●-■	Δ ↑ ▲-●-■	Δ ↑ ▲-●-■-●-■
LEC11	+++	++++	++++
LEC12	++++	—	++++
LEC29	+++	—	—
LEC30	++++	—	++

▲, α(2,3)sialic acid; ●, galactose; ■, GlcNAc; Δ, fucose. In addition, the α(1,3)Fuc-T expressed by each mutant exhibits distinctive properties in in vitro assays. These mutants will be compared to two new CHO mutants that express α(1,3)Fuc-T activity termed LEC31 and LEC32. Both these isolates exhibit a unique pattern of lectin resistance reflecting the expression of distinctive arrays of cell surface carbohydrates. Their abilities to bind specific monoclonal antibodies and the properties of their α(1,3)Fuc-T enzymes in in vitro assays will be presented.

P 123 THERMODYNAMIC AND CONFORMATIONAL ANALYSIS OF LIGAND BINDING TO WINGED BEAN (PSOPHOCARPUS TETRAGONOLOBUS) ACIDIC LECTIN (WBA II) REVEALS ITS SPECIFICITY FOR THE TERMINALLY FUCOSYLATED H-ANTIGEN & THE ANTIGENIC LOCI INVOLVED IN INTERACTION, Avadhesha Surolia, U.Sajjan, S.Acharya, S.R. Patanjali and K.D.Puri, Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, INDIA

Thermodynamic studies of ligand binding to WBA II together with their conformational analysis underscore some remarkable features of its interaction with fucosylated oligosaccharides. Despite L-fucose being non-inhibitory the lectin strongly prefers terminally monofucosylated sugars with fucose linked α1-2 to the nonreducing end galactose with 2'-fucosyllactose being the most complementary ligand. Type I (LNF I) & III H-antigenic structures are poorly recognized. L-Fucose in α1-3 linkage to the penultimate glucose as in 3-fucosyllactose & difucosyllactose & α1-3 and α1-4 linked fucose to N-acetylglucosamine as in LNF II and LNF III, respectively, sterically prevent the access of these sugars to the binding site. WBA II interacts with 2'-fucosyllactose from 3 surfaces in contrast to Ulex and Evonymus lectins which bind it from one side alone. Positive entropy change for the binding of 2'-fucosyllactose highlight for the first time the predominance of non-polar forces in protein-sugar recognitions. Relative contributions of the polar and non-polar interactions in this stabilization and the loci invovled threin highlight the subtelities of predominant forces, hitherto not appreciated, in protein-sugar recognitions. Its exquisite ability to distinguish between terminally monofucosylated sugars from internally fucosylated and difucosylated compounds combined with its better recognition of type II structures than type I structures confers upon the lectin a unique position as a tool in characterization of glycoconjugates.

1. Patanjali et al (1988) Biochem. J. 252, 625-631
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P 124 SPECIFICITIES OF THE RAT LIVER ßGal (1-4)ßGlcNAc α(2-6)- AND THE ßGal (1-3/4)ßGlcNAc α(2-3)- SIALYLTRANSFERASES TOWARDS CHEMICALLY MODIFIED SYNTHETIC ACCEPTOR SUBSTRATES.

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Many sialylated and fucosylated N-acetyllactosaminyl structures that occur as terminal structures of glycoproteins or glycolipids have been found to be involved in cellular adhesion processes. Although sialyltransferases are known to possess some specificity for extended structures (branch specificity), it remains that the terminal structure of the carbohydrate moiety of the acceptor is critical for the recognition, binding and transfer of Neu5Ac by these enzymes.

The kinetic parameters for the transfer of Neu5Ac by the α(2-6)- and the α(2-3)- sialyltransferases to a large number of chemically modified disaccharide acceptors have been determined. The results indicate that only a few functional groups of the acceptor are likely to be highly involved in the binding site of the enzyme. Correlation of the enzyme activity with the alterations of the basic structures of the acceptors explains why the α(2-6)sialyltransferase is specific for terminal N-acetyllactosaminyl structures, whereas the α(2-3)sialyl-transferase can transfer to both ßGal(1-4)ßGlcNAc- and ßGal(1-3)ßGlcNAc- terminal structures.

P 125 A PSEUDOGENE AND AN ALTERNATIVELY SPLICED TRANSCRIPT: THE ß-GAL α 2, 6-SIALYLTRANS-FERASE GENE, Jasminder Weinstein+ and James Paulson°, +Amgen Center, 1840 Dehavilland Drive, Thousand Oaks, CA 91320, °Cytel Corp., 11099 Torrey Pines Road, LaJolla, CA 92307. The ß-gal α 2, 6-STase has been cloned and its gene described. We postulated that the many members of this family of enzymes may share some homology. All the sialyltransferases use CMP-sialic acid as a donor substrate and it is probable that some conservation of structure would be observed at the binding site for this activated sugar molecule. Accordingly, we set out to isolate clones with homology to the ß-galα 2, 6-STase cDNA. We first targeted a rat genomic DNA 2Kb EcoR1 fragment, since this was visible by low stringency Southern analysis. Characterization of the cloned genomic fragment revealed: 1) Several frame shift and nonsense mutations within the putative coding region which would truncate the polypeptide. 2) This was not a processed pseudogene since an exon/intron boundary was observed. We had observed the possibility of alternative splicing as a mechanism for generating isoforms of sialyltransferase. Northern analysis of a number of rat tissues using a variety of ß-galα 2, 6-STase cDNA probes demonstrated that alternative exon usage was occurring in the kidney. An alternatively spliced clone was isolated from a rat liver cDNA library. The 1500 bp of cloned sequence lit up a unique message of 1.7 Kb on Northerns. This transcript was only detectable in rat livers at various developmental stages. The amino acid sequence revealed complete identity to the ß-galα 2, 6-STase for the first 199 amino acids, then the sequence became unique and continued for an additional 94 amino acids. In vitro and in vivo studies showed that this transcript is translated into a 38 kD protein and that the two glycosylation sites are utilized. This protein localizes to the golgi apparatus and is capable of binding to CDP-hexanolamine, implying that the CMP-sialic acid binding site may reside in exon 2. Chinese hamster ovary cells transfected with this clone do not demonstrate any ß-galα 2, 6-STase activity. However, there is an observable change in the pattern of sialylation in this cell line. The role of this protein as a potential sialyltransferase or a modulator of sialyltransferase activity is under investigation.

P 126 DIRECT CARBOHYDRATE ANALYSIS OF GLYCOPROTEINS ELECTROBLOTTED ONTO PVDF MEMBRANES, **Michael Weitzhandler, Douglas Kadlecek and Nebojsa Avdalovic, Dionex Corp., Sunnyvale, CA. 94088 and R. Reid Townsend, Dept. of Pharmaceutical Chemistry, University of California, San Francisco 94143-0446**

Three glycoproteins differing in size, extent, and type of glycosylation were purified by SDS-PAGE and electroblotted to PVDF membranes. The electroblotted glycoproteins (bovine fetuin, mouse recombinant erythropoietin, bovine ribonuclease B) were stained with Coomassie Blue, excised, and subjected to either 2M TFA hydrolysis (monosaccharide analysis) or to endoglycosidase treatment (oligosaccharide profiling). Monosaccharide quantitation using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), revealed similar kinetics of monosaccharide release for the blotted and soluble glycoproteins. Recoveries of individual monosaccharides from electroblotted glycoproteins were assessed and found to be quantitative. Additionally, the release of oligosaccharides from the individual glycoprotein bands by treatment with endoglycosidases was studied. This release was facilitated by detergent (0.05% triton X100). The chromatographic profiles of oligosaccharides from "electroblotted" and "in solution" endoglycosidase treated glycoproteins were identical. Endoglycosidase release of oligosaccharides from electroblotted glycoproteins was monitored by HPAEC-PAD monosaccharide analysis. It was also shown that after endoglycosidase cleavage, electroblotted glycoproteins remained largely bound to the PVDF membrane. The above described method should prove useful for the direct monosaccharide and oligosaccharide analysis of glycoproteins which have been separated by SDS-PAGE.

P 127 DEFINING A GLYCOSYLTRASFERASE GENE FAMILY: CLONING AND EXPRESSION OF A GENE ENCODING A GDP-FUCOSE:N-ACETYLGLUCOSAMINIDE 3-α-L-FUCOSYLTRANSFERASE HOMOLOGOUS TO BUT DISTINCT FROM KNOWN HUMAN α(1,3)FUCOSYLTRANSFERASES. Brent W. Weston, Robert J. Kelly, and John B. Lowe, the Howard Hughes Medical Insitute and the Departments of Pediatrics and Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109. At least four distinct categories of human GDP-fucose:*N*-Acetylglucosamide 3-α-L-fucosyltransferase (FT) activities have been described on the basis of tissue-specific expression patterns, oligosaccharide acceptor preferences, divalent cation requirements, and analysis of pedigrees. Molecular correlates for these loci are now sought. Human genes encoding three of these activities have been previously isolated: the Lewis blood group α(1,3/1,4)FT (Fuc-TIII); a homologous α(1,3)FT expressed in the myeloid lineage (Fuc-TIV); and another homologous α(1,3)FT resembling the human "plasma type" FT (Fuc-TV). We report here the cloning and expression of a fourth homologous but distinct human α(1,3)FT gene (Fuc-TVI). The predicted polypeptide has type II transmembrane topology characteristic of mammalian glycosyltransferases. Sequence divergence from known α(1,3)FT genes occurs in 5' and 3' untranslated regions, and in the putative "stem" region and amino terminus of the catalytic domain. This protein's 359 amino acids show 85% sequence identity with Fuc-TIII and 89% sequence identity with Fuc-TV. When expressed in transfected mammalian cells, recombinant Fuc-TVI directs surface expression of the sialyl Lewis x and Lewis x epitopes, but does not construct sialyl Lewis a or Lewis a structures. Absence of α(1,4)FT activity is confirmed by biochemical analyses, and distinct type II acceptor substrate preferences are noted which separate this α(1,3)FT from previously described enzymes. Preliminary PCR analyses indicate that this gene is syntenic to the Fuc-TIII and Fuc-TV loci on human chromosome 19, suggesting that regulation of FT gene cluster(s) may account for the precise array of fucosylated glycans expressed in various human cells and during mammalian developmental processes.

P 128 CRYSTAL STRUCTURE OF A CROSSLINKED WHEAT GERM AGGLUTININ-SIALOGLYCOPEPTIDE RECEPTOR COMPLEX: A MODEL FOR ITS BEHAVIOR IN VIVO, Christine S. Wright, Department of Biochemistry and Molecular Biophysics, Medical College of Virginia, Richmond, Va. 23298

Wheat germ agglutinin(WGA) is known to interact with sialylated cell surface receptors in eukaryotic cell systems. Its specific receptor in red cells is the transmembrane protein glycophorin A. The structure of WGA complexed with the octa-sialoglycopeptide T-5 of glycophorin A, containing a divalent branched tetrasaccharide unit (NeuNAc-α2,3-Gal-β1,3-GalNAc (α6,2-NeuNAc)-α1-O-Thr37), was determined and refined at 2.0Å resolution. Two spatially unique binding modes are observed. The dominant binding mode, exhibiting high specificity, consists of an infinite open-ended array of tetrasaccharide interlinked WGA dimers. This type of aggregation along one crystal dimension is a result of the multi-valent nature of both WGA and oligosaccharide ligand: The branched tetrasaccharide, spanning the distance between WGA dimers possesses an extended rigid conformation. Each of its terminal NeuNAc residues is bound to a chemically different site on opposing WGA dimers. Only one of these sites has been characterized previously. This binding mode provides a structural basis for the cooperative *in vivo* cell binding behavior of WGA, considering critical factors such as unequal binding site affinities, asymmetric dimer substitution and lectin and receptor multivalency.
A less well-defined second binding mode that utilizes a third type of binding site, exhibits isologous sugar/sugar contacts across a 2-fold crystal symmetry axis. All three types of binding sites are related by internal structural symmetry (four-fold sequence repeat). Binding site specificity is conferred through hydrogen bonding and van der Waals' stacking interactions with three aromatic amino acid residues in all sites.

P 129 *STUDIES ON THE BINDING PROPERTY OF ABRUS PRECATORIUS* AGGLUTININ REVEALS SPECIFIC FOR **T** [Galβ1→3GalNAc] **> I/II** [Galβ1→3(4)GlcNAc].
Albert M. Wu[1], Shing-Ru Lin[1], L.K.Chin[1], Lu-Pin Chou[2] and Jung-Yaw Lin[2]. [1]Glyco-Immunochemistry Research Lab., Dept. of Molecullar & Cellular Biology, Chang-Gung Medical College, Kwei-San, Tao-Yoan 33332, Taiwan, and [2]Institute of biochemistry, College of Medicine, National Taiwan University, Taipei,Taiwan.

Binding property of a nontoxic carbohydrate binding protein (*Abrus Precatorius* agglutinin, APA) purified from the seeds of *Abrus precatorius* (Jequirity bean), was studied by quantitative precipitin and precipitin-inhibition assays. Of twenty-six complex carbohydrates tested. All, except for sialic acid containing glycoproteins and desialized ovine salivary glycoproteins, reacted strongly with the lectin and precipitated over 70% of the lectin added indicating APA has a broad range of affinity and recognize (internal) Galβ1→sequence of carbohydrate chains. The strong reaction with desialized porcine and rat salivary glycoproteins and pneumococcus type XIV polysaccharides suggested that APA is affinity to one or more of the following : **T**,Galβ1→3GalNAc; **I** and/or type **II**, Galβ1→3(4)GlcNAc disaccharide determinants on the carbohydrate moiety of complex carbohydrates. Among the oligosaccharides tested, the **T** structure was the best inhibitor which is 2.6 and 3.7 times as active as type **II** and type **I** sequences respectively. Blood group I Ma active trisaccharides, Galβ1→4GlcNAcβ1→6Gal, was about as active as its disaccharide (II) sequence.

This work aided by Grants from the Chang-Gung Medical Research plan (CMRP No. 293), Kwei-san, Tao-Yuan, Taiwan and National Science Council (NSC 80-0412-B-182-35 and NSC 81-0418-B 182-02), Taipei, Taiwan.

P 130 ALTERATION OF CARBOHYDRATE-BINDING SPECIFICITY OF *BAUHINIA PURPUREA* LECTIN THROUGH THE CONSTRUCTION OF CHIMERIC LECTIN, Kazuo Yamamoto, Yukiko Konami, Toshiaki Osawa, Tatsuro Irimura, Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, JAPAN

The carbohydrate-binding domain of *Bauhinia purpurea* lectin (BPA), a galactose and lactose binding lectin, was investigated from Asp-N endoproteinase digests of BPA. A peptide which interacted with lactose was purified by means of lactose-Sepharose column chromatography. It consisted of 9 amino acids and its amino acid sequence was Asp-Thr-Trp-Pro-Asn-Thr-Glu-Trp-Ser. A tryptic fragment which interacted with lactose-Sepharose was also purified and found to contain the same sequence. This nonapeptide was aligned to the metal-binding region conserved in all of legume lectins (Asp-135-Ser-143). The chemical synthesis of this nonapeptide was carried out by the solid-phase method and the synthetic peptide was found to exhibit lactose-specific binding activity in the presence of calcium.

Similar studies were carried out on several anti-H(O) lectins using Fuc-Sepharose for *Lotus tetragonolobus* lectin (LTA) and *Ulex europeus* lectin I (UEA-I), and oligo-GlcNAc-Sepharose for *Ulex europeus* lectin II (UEA-II) and *Laburnum alpinum* lectin I (LAA-I). The peptides which showed affinity were also found as a part of the metal-binding region of these lectins. The results lead us to tentatively conclude that these peptides represent the carbohydrate-binding and the metal ion-binding of legume lectins.

To further confirm this hypothesis, we constructed a chimeric lectin gene by using a cDNA coding BPA in which nonapeptide sequence was replaced by the corresponding region of the α-mannose binding *Lens culinaris* lectin. The chimeric lectin expressed in *E. coli* was found to bind α-mannosyl-bovine serum albumin and this binding was inhibited by mannose. These results are the first to describe the presence of a "variable binding region" in legume lectins that determine their carbohydrate-binding specificity.

P 131 NOVEL OLIGOSACCHARIDE STRUCTURES ON RECOMBINANT HUMAN PROTEIN C EXPRESSED IN HUMAN KIDNEY 293 CELLS. S. B. Yan[1], Y. Chao[1], & H. Van Halbeek[2]. [1]Cardiovascular Res., Eli Lilly & Co., Indianapolis, IN 46285; [2]CCRC, Univ. of Georgia, Athens, GA 30602.
Human Protein C (HPC), an antithrombotic factor, is a serine protease with 4 types of post-translational modifications. Glycosylation is one of them. There are 4 N-glycosylation sites on HPC. Recombinant HPC (rHPC) was expressed in human kidney 293 cells. Glycosyl composition analysis revealed a five fold higher fucose and a two fold lower NeuAc content in HK 293 cell-derived rHPC than in plasma HPC. In addition, there was 2.6 moles of GalNAc per mole of rHPC that was absent in plasma HPC. The GalNAc in rHPC was all in N-linked oligosaccharides. All the charges on the oligosaccharides were removed by neuraminidase treatment suggesting the absence of sulfated sugars. The oligosaccharides on the rHPC were enzymatically released by N-Glycanase and were separated and purified with the Dionex HPAE-PAD II system. Novel oligosaccharide structures involving GalNAc were identified by composition and linkage analyses, and 1-D ^{1}H-NMR spectroscopy. One of the GalNAc-containing novel structure has no charge. The novel oligosaccharides may contribute to the higher anticoagulant activity observed for the rHPC as compared to the plasma HPC.

P 132 VARIABILITY ANALYSES OF THE AMINO-ACID SEQUENCES OF LEGUME LECTINS, AND THE ARCHITECTURE OF THEIR BINDING-SITES.
N. Martin Young and Raymond P. Oomen, Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6 CANADA.
Twelve plant lectins from different tribes within the Papilionoideae subfamily were selected to represent a range of carbohydrate specificities, and their amino-acid sequences were aligned. To quantitate the sequence differences among the lectins, two variability indices were calculated for the aligned sequences, the index used by Wu and Kabat (J. Exp. Med. **132**, 211-250, 1970) for antibody sequences and the pair-wise index Jores *et al.* (Proc. Natl. Acad. Sci. USA **87**, 9138-9142, 1990) used for T-cell antigen receptor sequences. The results were analyzed using the three-dimensional structures of concanavalin A and the pea lectin, to investigate the distribution of variable residues over the protein surface. It was found that the areas of greatest variability were located in the carbohydrate-binding site region, forming a perimeter around a well-conserved core. These variable residues are inferred to be specificity determining, in the manner of antibodies. The most variable position corresponded to Tyr100 in concanavalin A, a known ligand contact residue. The conserved core includes an aspartic acid and an asparagine residue that are common to all these lectins. In addition to the five peptide segments seen to interact with carbohydrate ligands in the X-ray determined structures, an additional variable segment is suggested by this analysis. The overall compositions of the lectin sites resembled those of the sugar-transport proteins rather than antibodies.

P 133 RNA POLYMERASE II IS A GLYCOPROTEIN.
William G. Kelly, Michael E. Dahmus, and Gerald W. Hart.* Dept. of Biological Chemistry, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205; *Dept. of Biochemistry and Biophysics, University of California, Davis, CA 95616. N-acetylglucosamine attached to protein via the hydroxyl of a serine or threonine (O-GlcNAc) is a post-translational modification found on the nuclear and cytosolic faces of many intracellular proteins in all eukaryotes (for review, see **Ann. Rev. Biochem.**, 58:841). O-GlcNAc-modified proteins include nuclear pore components, endoplasmic reticular membrane residents, cytoskeletal proteins, viral proteins, and numerous chromatin proteins, including, but not restricted to, transcription factors. Interestingly, this modification is found on RNA polymerase II- specific transcription factors, but it has not been found on the polymerase I or III-specific factors that have been examined.

We have found that the large catalytic subunit of RNA Polymerase II itself is also modified by O-GlcNAc. The O-GlcNAc is restricted to the C-terminal domain (CTD) of the protein, a region distinguished by the presence of a heptapeptide repeat (consensus sequence YSPTSPS) that appears 52 times in mammalian Pol II. The CTD of Pol II is known to be rapidly and extensively phosphorylated during its transition from an initiating to an elongating transcription complex. Only the unphosphorylated form of the large subunit (Pol IIA) contains O-GlcNAc, whereas this modification is not detectable on the phosphorylated form (Pol IIO), suggesting that the glycosylation and phosphorylation of Pol II may be mutually exclusive events. Reverse-phase HPLC peptide mapping studies suggest that the O-GlcNAc is found at a unique site on the CTD, in contrast to the multiple (>30) sites of phosphorylation. These data imply that O-GlcNAc may play a role in regulating the initiation of the rapid phosphorylation cascade that accompanies transcription elongation. Supported by HD13563, GM33300, and The March of Dimes Predoctoral Fellowship Program.

The Role of Glycosylation in Human Disease: Possibilities for Therapeutic Intervention

P 200 ANTI-GALACTOSYLTRANSFERASE ANTIBODY: A POTENTIAL CONTROL MECHANISM OF GLYCOSYLATION CHANGES IN HEALTH AND DISEASE.
Azita. Alavi, John.S.Axford Academic Rheumatology Unit, Division of Immunology, St.George's Hospital Medical School, Cranmer Terrace, London SW17 ORE UK.
Reduced peripheral lymphocytic galactosyltransferase (GTase) activity reflects decreased galactosylation at the Cγ2 domain of serum IgG in patients with rheumatoid arthritis (RA), Crohn's disease (CD), pulmonary tuberculosis (PTB) and systemic lupus erythematosus (SLE). To investigate whether there is a humoral component associated with these changes, a direct binding ELISA technique was used to measure the level of anti-GTase antibodies (Abs) in sera of patients with active RA (n=25), active CD (n=22), PTB (n=10) and SLE (n=30). Comparisons were made to healthy individuals (n=21) and a non autoimmune disease control group, comprising ankylosing spondylitis (AS, n=8) and ulcerative colitis (UC, n=6). In RA and CD, IgG and IgA anti-GTase Abs were significantly increased when compared to the healthy population: RA (IgG: p=0.006, 36% > healthy mean + 2SD , IgA: p=0.018, 20% > 2SD), CD (IgG: p=0.000, 54% > 2SD, IgA: p=0.008, 27% > 2SD). In SLE and PTB, only IgG anti-GTase Ab levels were found to be significantly increased when compared to the healthy population: SLE (p=0.009,17% > 2SD), TB (p=0.001, 20% > 2SD). No increase in serum anti-GTase Ab levels was found in the non autoimmune disease control group . IgM anti-GTase Ab levels, in contrast, were significantly decreased (18% - 55%, p<0.02) in all disease groups, when compared to the healthy population. To determine the significance of these findings, a longitudinal study of 13 patients with active RA was made. A strong association between reduced GTase activity and increased IgG anti-GTase Ab levels was found. Where there was an increase in IgG anti-GTase Ab levels, 82% showed a corresponding decrease in B cell and 54% in T cell GTase activity. No correlation was found with other disease associated parameters (disease activity score, ESR and RF). The specificity of these polyclonal anti-GTase Abs has been further confirmed by immunoblotting the affinity purified IgG isotype against purified bovine and human GTase. These data suggest that there may be a humoral component to the glycosylation changes associated with this restricted group of diseases. Naturally occurring IgM anti-GTase Ab may be part of a normal physiological immune regulatory mechanism, whereas IgG and IgA Abs may result from isotype switching in disease and be associated with aberrant immune regulation.

P 201 The Glycosylation of Recombinant Soluble CD4 Variants Expressed in CHO Cells.
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The T-cell-surface glycoprotein CD4 is involved in MHC class II responses and in human T-helper lymphocytes is the receptor for human immunodeficiency virus (HIV). CD4 consists of an extracellular region of 371 (human) or 367 (rat) amino acids, which form four Ig-like domains, followed by a typical transmembrane sequence and cytoplasmic domain of 38 amino acids [1]. Human CD4 contains two glycosylation sites at Asn-270 and Asn-297. The rat sequence has three potential glycosylation sites; Asn-159, Asn-270 and Asn-365. The conservation of the Asn-270 site suggests that N-glycosylation may be structurally and/or functionally important. Soluble CD4 (sCD4) has been produced by introduction of a termination codon into the CD4 sequence adjacent to the transmembrane region. A "half" molecule was formed by a similar technique. Variants of rat sCD4 with deletions of the glycosylation sites were made by changing the Asn-159 to a Thr and the Asn-270 to a Ser [2]. Comparison of the glycosylation of rat and human sCD4 showed that the major component in each case was a core-fucosylated, biantennary, complex sugar. Differences were seen in the relative abundance of hybrid and oligomannose oligosaccharides in rat sCD4 and in the presence of a fucosylated, biantennary sugar carrying one α-galactose residue in human sCD4. Analysis of the oligosaccharide populations present at each glycosylation site showed that the complex oligosaccharides were restricted to the Asn-270 site. Much less processing was seen associated with the Asn-159 site where oligomannose and hybrid structures predominate. Comparison of these patterns with those of the glycosylation variants and the "half" molecule shows a qualitative similarity, indicating site-specific and predominantly independent processing occurred at each glycosylation site. However, onto this is superimposed a subtle and complicated modulation of glycosylation which is seen particularly at the Asn-159 site.
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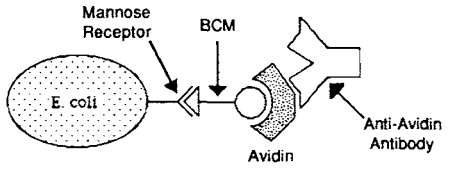
[2] Davis, S.J., Ward, H.A., Puklavec, M.J., Willis, A.C., Williams, A.F. & Barclay, A.N. (1990) J.Biol.Chem. **265**, 10410-10418.

P 202 SYNTHETIC GLYCOCONJUGATES CONTAINING CARBON-LINKED GLYCOSIDES TARGET ANTIBODIES TO BACTERIAL CELLS AND PRIME THEM FOR KILLING BY COMPLEMENT PROTEINS AND MACROPHAGE CELLS, Carolyn Bertozzi and Mark Bednarski, Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720

A series of carbon-linked glycosides of mannose have been synthesized and their inhibitory activity towards the receptor-mediated adhesion of *E. coli* to yeast cells has been tested. *C*-glycosyl compounds with hydrophobic side chains inhibit yeast cell agglutination at lower concentrations than those with hydrophilic or charged side chains. The multivalent complex of a biotinylated *C*-glycoside of mannose (BCM) with avidin binds to the bacterial receptors with the highest avidity of all the compounds tested. The BCM-avidin conjugate can target anti-avidin antibodies to the surface of the bacterial cell as determined by TEM studies with whole cells. Antibodies directed to the mannose receptors by the synthetic glycoconjugate activate both complement and macrophage-mediated processes that result in cell death. The conserved binding domain of cell-surface lectins can, therefore, be utilized to direct antibodies to pathogens and prime them for killing by host defense mechanisms.



P 203 COMPARISON OF THE N-LINKED OLIGOSACCHARIDE STRUCTURES OF THE TWO MAJOR HUMAN MYELIN GLYCOPROTEINS: MAG AND P_0. Danielle Burger and Andreas J. Steck. Laboratoire de Neurobiologie, CH-1011 CHUV/Lausanne, SWITZERLAND.

Myelin-associated glycoprotein (MAG) and P_0 are myelin cell adhesion molecules that belong to the immunoglobulin superfamily and express the L2/HNK-1 carbohydrate epitope. MAG ($M_r \approx 100'000$) possesses 9 potential N-glycosylation sites. P_0 ($M_r \approx 30'000$) possesses 1 N-glycosylation site. The L2/HNK-1 epitope on human P_0 and MAG is borne by N-oligosaccharides (Burger et al. (1990) *J. Neurochem.* **54**, 1569-1575). By serial lectin affinity chromatography (SLAC) of MAG and P_0 [^{14}C]-glycopeptides, 16 and 15 fractions were obtained, respectively. Assuming that all [^{14}C]-glycopeptides were labelled to the same specific radioactivity, MAG and P_0 bear respectively 80.4% and 94.9% of complex type N-oligosaccharides. Two types of structures are found in large amount in both MAG and P_0: i) N-oligosaccharides with a bisecting GlcNAc residue accounting for 60.1% and 65.3% of radioactivity, respectively; and ii) N-oligosaccharides with an α(1-6)fucose residue in the core accounting for at least 49.3% and 86.2% of radioactivity, respectively. The analysis of the glycopeptide fractions by dot-TLC plate overlay shows that the L2/HNK-1 epitope is present in MAG and P_0 glycopeptide fractions bearing N-oligosaccharides with an α(1-6)fucose residue in the core. These results show that in addition to their amino acid homology both these myelin glycoproteins bear similar N-oligosaccharide structures. This finding supports the hypothesis that carbohydrates play a role in the function(s) of these neural cell adhesion molecules.

P 204 SOLUTION STRUCTURE OF THE LEWIS^X OLIGOSACCHARIDE DETERMINED BY NMR SPECTROSCOPY AND MD SIMULATIONS. C. A. Bush, K. E. Miller, C. Mukhopadhyay and P. Cagas

Department of Chemistry & Biochemistry, University of Maryland Baltimore county, Baltimore, MD 21228

The Lewis^X (Le^x) determinant has been implicated as a specific differentiation antigen, as a tumor antigen and as a key component of the receptor for the endothelial leukocyte adhesion molecule, ELAM-1. Since the Le^x determinant is believed to be of pathologic importance, knowledge of its structure may be useful in predicting its bioactive conformation, interpreting its binding specificity with different receptors, designing drugs to act as inhibitors of the binding and activity of Le^x, and aiding in the structure determination of related antigenic carbohydrates. We have determined the three-dimensional solution structure of the Le^x trisaccharide by two independent techniques. High resolution nuclear magnetic resonance (NMR) spectroscopy was used to completely assign the ^{1}H and ^{13}C spectra for the human milk pentasaccharide, lacto-N- fucopentaose-3 (LNF-3) which contains the Le^x determinant. Comparison of ^{1}H NOESY data with a recently developed method for quantitative NOESY simulation yields a relatively rigid structure for the Le^x determinant in that only a small range of glycosidic dihedral angles in the trisaccharide produce simulated nuclear Overhauser effect (NOE) spectra agreeing with measured data. The same average structure for the Le^x determinant arises from *in vacuo* molecular dynamics (MD) simulations. Notably, the proposed conformation of the Le^x trisaccharide is very similar to that recently determined for the closely related Le^a trisaccharide. In agreement with the recent finding that both sialylated Le^a and Le^x react with ELAM-1, the results in this study show that the Le^a and Le^x determinants contain very similar carbohydrate domains.

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P 205 ***A FULLY SYNTHETIC HAEMOPHILUS CONJUGATE VACCINE,*** *Pele Chong, Ali Kandil, Brian Tripet, Neville Chan and Michel Klein, Connaught Centre for Biotechnology Research, 1755 Steeles Ave. West, Willowdale, Ontario, Canada, M2R 3T4.*

Recent studies have indicated that antibodies directed against Haemophilus influenzae (Hi) outer membrane proteins (OMP) P1, P2 and P6 are protective in the infant rat model of bacteremia. Therefore, a promising strategy to design synthetic conjugate Hi vaccines with enhanced protective ability would be to use OMPs' immunodominant epitopes both as additional protective antigens and carriers for the oligosaccharide polyribosyl-ribitol-phosphate (PRP). Such vaccines have specific advantages over the existing vaccines. Firstly, the use of OMPs as carriers will reduce the possibility of over-immunisation with diphtheria toxoid (D), or tetanus toxoid (T) in any future DTP-Hib combination vaccine. Secondly, conserved OMP epitopes may induce cross-protection against non-typeable Hi strains that do not produce PRP and are a common cause of otitis media for which there is no current vaccine. A synthetic PRP trimer hapten was prepared from commercially available ribose using a solid-phase carbohydrate synthesis methodology with an 8% overall yield. To study the possibility of preparing a fully synthetic PRP-peptide conjugate vaccine, the PRP trimer was conjugated to a multiple antigenic peptide (MAP) OMP2-8 (residues 193-219 of P2 protein) and a linear peptide HIBP1-4 (residues 165-193 of P1 protein) by site-directed conjugation. PRP-(P2-8)$_4$ and PRP-HIBP1-4 conjugates adjuvanted with alum were both capable of eliciting strong anti-PRP IgG responses, and either anti-P2 or anti-P1 antibody responses, respectively. These results suggest that T-cell epitopes can provide T-cell help when conjugated to a synthetic carbohydrate hapten. The fully synthetic peptide-carbohydrate conjugates will serve as model structures to study the factors affecting the immunogenicity of carbohydrate antigens and their presentation and processing.

P 206 GLYCOSYLATED AND NON-GLYCOSYLATED FORMS OF RECOMBINANT HUMAN PROLACTIN HAVE DIFFERENT BIOACTIVITIES IN THE Nb2 LYMPHOMA CELL PROLIFERATION ASSAY, Edward S. Cole, Albert E. Price, Pamela A. Peterson, Vicki M. Giuggio, Richard J. Bernasconi, Kimberly B. Logvinenko, Genzyme Corporation, Framingham, MA 01701

Both glycosylated and non-glycosylated forms of recombinant human prolactin were expressed in murine C127 cells. The isoforms fractionated with molecular weights corresponding to Mr 29,000 and Mr 24,000 respectively in SDS-PAGE 10-20% gradient gels. Based on laser densitometry of coomassie blue-stained gels, the glycosylated band accounted for 20% of the total prolactin produced, assuming equivalent staining of the two forms. Prolactin contains a single site available for N-linked glycosylation. Determination of the total carbohydrate composition by monosaccharide analysis suggested that 37% of the prolactin molecules were glycosylated with a minimal complex oligosaccharide. Additionally, 32% of the available terminal galactose residues were sialylated. We have separated the two forms using a combination of gel filtration, metal chelate chromatography, and affinity chromatography. Bioactivity was assessed in the Nb2 lymphoma cell proliferation assay. The results indicated that the glycosylated prolactin is 50-100 fold less active compared to non-glycosylated prolactin in this assay.

P 207 LECTIN BINDING PATTERN IN HUMAN TONSILS, Gilbert C. Faure, Philippe Perrin, Denis Mayot, Michèle Kessler, Marie C Béné Laboratoire d'Immunologie & Cliniques ORL et Néphrologique, Faculté de Médecine & CHU de Nancy, 54500 Vandoeuvre les Nancy, France

Palatine tonsils are the best individualized lymphoid organ of the Waldeyer ring in man. They appear to belong to the common mucosal immune system and share similarities with Peyer's patches. Lymphoid cells proliferate in this area upon local antigenic challenge or as a consequence of cell activation and migration from the Peyer's patches. Human tonsils contain an intricate network of venules and only efferent lymphatics. High endothelial venules (HEV) must thus be involved in homing phenomena allowing activated lymphocytes to colonize and proliferate in the tonsils. We used a panel of 15 fluorescein-conjugated lectins to investigate the glycosylation pattern of tonsillar structures. All lectins were used in direct immunofluorescence on 4 micrometer-thick frozen cut sections of human tonsils. *Pisum sativum, Lens culinaris* and *Ulex europeanus* were the most efficient in allowing the visualization of HEV. Endothelial cells and the center of lymphoid follicles were characteristically stained with *Soybean*. Most corona and interfollicular lymphocytes were brightly stained by wheat germ agglutinin. The other lectins used stained the extracellular matrix, occasional endothelial cells or no specific structure.

We used this method to compare tonsils from patients with common recurrent tonsillitis (n=6) and from patients with IgA nephropathy (n=6). In the latter, clinical evidence of kidney damage currently follows infectious episodes of the upper respiratory tract. Previous studies have shown that palatine tonsils from patients with IgA nephropathy contain larger numbers of plasma cells, and preferentially produce IgA. In this study, lectin staining allowed to evidence a significant increase in the number of HEV and endothelial lectin binding activity in tonsils from IgA nephropathy patients. These data suggest that homing phenomena involving highly glycosylated endothelial cells are activated in the Waldeyer ring of patients with IgA nephropathy.

P 208 INTERACTION OF ANTI-GAL WITH GALα1-3GALß1-4GlcNAc-R EPITOPES ON PORCINE THYROID CELLS, MIMICS EFFECT OF TSH, U. Galili and R.J. Winand, The Medical College of Pennsylvania, Philadelphia, PA. and University of Liege, Belgium.

Anti Gal is a natural antibody constituting 1% of circulating IgG in man. It interacts specifically with the carbohydrate epitope Galα1-3Galß1-4GlcNAc-R (α-galactosyl epitope) which is abundant on thyroid cells and other cells of nonprimate mammals and New World monkeys. Studies on anti-Gal effect on thyroid cells are of interest since the titer of this antibody is markedly increased in patients with Graves' disease (an autoimmune thyroid disorder). Thus the in vitro effect of anti-Gal on mammalian thyroid cells may provide information of the possible in vivo effect of this antibody on human thyroid cells aberrantly expressing the α-galactosyl epitope. The α-galactosyl epitope was found to be expressed on porcine TSH (thyroid stimulating hormone) receptors, and the interaction of anti-Gal with these epitopes partially inhibits TSH binding to the receptor. Assays performed with cultured porcine thyroid cells have indicated that anti-Gal can mimic TSH effect in stimulation for cAMP synthesis, ^{125}I incorporation and cell proliferation. Furthermore, depletion of anti-Gal from sera of patients with Graves' disease (by adsorption on a column with Galα1-3Galß1-4GlcNac-R epitopes) resulted in elimination of most of TSI (thyroid stimulation immunoglobulins) activity, as measured by stimulation for cAMP synthesis in porcine thyroid cells. These studies suggest, therefore, 1) The α-galactosyl epitope is expressed on mammilian TSH receptors and binding of anti-Gal to these epitopes mimics the effect of TSH. 2) A large proportion of TSI activity observed in Graves' sera assayed with mammalian thyroid cells may be attributed to increased anti-Gal activity in these patients. 3) Aberrant expression of α-galactosyl epitopes on human thyroid cells may result in chronic TSH-like stimulation of these cells by the natural anti-Gal antibody.

P 209 POTENTIAL FOR DEGRADATION OF GLYCOPROTEIN OLIGOSACCHARIDES BY EXTRACELLULAR GLYCOSIDASES

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Fine details of oligosaccharide structure can have a significant impact on the properties of a human therapeutic glycoprotein. For example, the absence of terminal sialic acid on the antennae of complex-type oligosaccharides can lead to drastically reduced glycoprotein circulatory half-life and solubility. Enzymes that hydrolyze oligosaccharide structures (glycosidases) are present within most mammalian cells. There has been no previous study addressing the probability that these enzymes could be released into the extracellular medium and attack glycoprotein oligosaccharides during routine cell culture. In fact, there is no previous evidence that Chinese hamster ovary cells even possess the enzyme neuraminidase (sialidase) that hydrolyzes sialic acid from glycoprotein oligosaccharides. We have confirmed the existence of neuraminidase activity in CHO cells, have determined that significant neuraminidase activity can accumulate in the extracellular medium, and have determined properties of the CHO neuraminidase relevant to the potential for neuraminidase attack of secreted glycoproteins (e.g. thermal stability, activity as a function of pH, and Km). We have also confirmed the presence of several other CHO glycosidases in the extracellular medium. We conclude that extracellular glycosidases can potentially pose a serious threat to the integrity of secreted glycoproteins.

P 210 INTERACTIONS OF HUMAN SERUM MANNAN-BINDING PROTEIN WITH MANNOSE-CONTAINING OLIGOSACCHARIDES FROM *LEISHMANIA MAJOR* AND *LEISHMANIA DONOVANI*, Paula J. Green[1], Malcolm J. McConville[2], Mark S. Stoll[1], Steffen Thiel[3] and Ten Feizi[1], [1]MRC Clinical Research Centre, Harrow, [2]Dundee University, UK and [3]Institute of Medical Microbiology, Aarhus, Denmark.

Mannan-binding protein in human serum (MBP-I), 650-700 kDa, is composed of subunits of 32 kDa each containing a Ca^{2+} dependent carbohydrate recognition domain at the carboxy-terminal end. Previous studies[1] with a series of structurally defined oligosaccharides, conjugated to the lipid phosphatidylethanolamine dipalmitoate (PPEADP), have shown that MBP-I binds to oligosaccharides terminating with the sequence Manα1-3(or -2)Man or GlcNAcβ1-2Man e.g. high *mannose or complex type chains;* GlcNAcβ1-4GlcNAc: chitin oligosaccharides; Glcα1-6(or -3)Glc: dextran oligosaccharides; Fucα1-4(Galβ1-3)GlcNAc and Fucα1-3(Galβ1-4)-GlcNAc as in a variety of glycoproteins and glycolipids. The natural *in vivo* ligands for MBP-I are uncertain but the protein clearly can bind the high mannose type oligosaccharides on the envelope glycoprotein of HIV-1 produced in human lymphocytes and chinese hamster ovary cells; the biological significance of this interaction requires further studies[2]. MBP-I also binds to yeast mannan and to several bacterial species and since this binding can mediate complement activation[3], and individuals with a genetic defect giving low concentrations of MBP-I are susceptible to bacterial infections[4], the protein is considered to have a protective role. Other potential targets for MBP-I are the cell surface lipophosphoglycans (LPGs) of *Leishmania major* and *donovani*, the causal agents of cutaneous and visceral leishmaniasis in man, respectively. These LPGs have mannose-rich sequences capping their non-reducing termini[5] (and unpublished). *Some of these "cap structures" are bound with varying intensities by serum MBP-I, when conjugated to PPEADP.*

[1]Childs R A *et al* (1989) *Biochem J* **262**:131; [2]Larkin M *et al* (1989) *AIDS* **3**:793; [3]Kawasaki N *et al* (1989) *J Biochem* **106**:483; [4]Sumiya M *et al* (1991) *Lancet* **337**:1569; [5]McConville M J *et al* (1990) *J Biol Chem* **265**:19611

P 211 ANTI-TUMOR EFFECTS OF GBS TOXIN: A POLYSACCHARIDE EXOTOXIN FROM GROUP B β-HEMOLYTIC STREPTOCOCCUS. Carl G. Hellerqvist, Gary B. Thurman, David L. Page, Yue-Fen Wang, and H.W. Sundell, Department of Biochemistry, Pathology and Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232.

Group B Streptococcus (GBS) isolated from human neonates diagnosed with sepsis and respiratory distress ("early onset disease") produces a polysaccharide exotoxin (GBS Toxin) that, when infused in sheep, causes lung pathology similar to that seen in human. Histological studies have demonstrated that GBS Toxin induces a strong inflammatory response in the lung, with pulmonary sequestration of granulocytes and extensive capillary endothelial damage. The susceptibility of humans to GBS Toxin is age-dependent and limited to about four days after birth. It is rarely evident thereafter. This suggests that the binding of GBS Toxin to the target endothelium occurs via specific receptors in the developing lung endothelial cells of the newborn that are later lost.

We report here that GBS Toxin can also bind to developing endothelium associated with neoplasia and induce an inflammatory response. GBS Toxin was shown by immunohistochemistry to bind to capillary endothelium of human large cell carcinomas. In nude mice bearing human tumor xenografts, intravenously administered GBS Toxin caused tumor necrosis and hemorrhagic lesions, and substantially inhibited the rate of growth of the tumors. In BALB/c mice bearing Madison Lung Tumors, GBS Toxin induced an inflammatory response resulting in marked changes in tumor morphology, including vasodilation, endothelial and tumor cell necrosis, invasion of lymphocytes and macrophages, and capillary thrombosis. In these tumor models, no evidence of toxicity to the vasculature of other tissues was observed.

The reported pathophysiology of GBS in human neonates, the lack of disease in adults and these results suggest that GBS Toxin has potential as a non-toxic anti-tumor agent in human cancer therapy.

P 212 *DOES SIALYL LEWIS-X ANTIGEN DETERMINE HUMAN* COLORECTAL CARCINOMA METASTASIS? Tatsuro Irimura, Yoshifumi Matsushita, Shoji Nakamori, Karen R. Cleary, David M. Ota, Elizabeth A. Seftor, and Mary J. C. Hendrix: Chemical Toxicology and Immunochemistry, University of Tokyo, Faculty of Pharmaceutical Sciences, Tokyo 113, Japan; *Departments of Tumor Biology, Pathology, General Surgery,* University of Texas M. D. Anderson Cancer Center, Houston, TX 77030; and Department of Anatomy, University of Arizona, College of Medicine, Tucson, AZ 85724

We have previously shown that higher levels of sialyl-dimeric Lewis-X antigen (SLX) are expressed in human colorectal carcinoma metastases than corresponding primary tumors. The post-operative survival of colon carcinoma patients who had high content of SLX-positive carcinoma cells in their primary tumors were poorer than that of the other patients with low SLX tumors. Stable human colon carcinoma SLX variant cells were selected from the KM12C cell line. The high SLX cells (KM12-HX) produced SLX associated with a M_r 900 kD mucin and >40 discrete glycoproteins (M_r 40-200 kD), whereas none of these components were observed in low SLX cells (KM12-LX). Northern blotting analysis with α(1-3/4)-fucosyltransferase cDNA showed that the content of transcripts for this enzyme in KM12-HX cells was greater than that in LX cells. KM12-HX cells were more metastatic when injected intrasplenically into nude mice, more adherent to human umbilical cord endothelial cells previously treated with TNF-α, and more invasive in vitro, than KM12-LX cells. The TNF-α-induced adhesion was significantly reduced by pretreatment of endothelial cells with anti-ELAM-1. KM12-HX and -LX cells did not differ in their contents of collagenases, plasminogen activators, laminin binding proteins, nm23 transcript, and p53 transcript. These results strongly suggest that SLX directly promotes human colorectal carcinoma metastasis.

References: (1) Cancer Res, 48:6883, 1989; (2) Arch Surg, 125:206, 1990; (3) Lab Invest, 63:780, 1990; (4) Seminars in Cancer Biology, 2: 129, 1991; (5) Clin Exp Metastasis, 9: 283, 1991; (6) Exp Cell Res 196: 20, 1991.

P 213 GLYCOPROTEIN MODULATION BY THE GLUCOSIDASE I INHIBITOR *N*-BUTYLDEOXYNOJIRIMYCIN Gunilla B. Karlsson and Frances M. Platt*, Glycobiology Institute and *Searle Research Group, Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK.

The imino-sugar *N*-butyldeoxynojirimycin (NB-DNJ) inhibits the isolated glycoprocessing enzyme glucosidase I and exhibits anti-HIV activity *in vitro*. There is no direct experimental evidence indicating whether or not glucosidase inhibition results in the anti-viral properties of this compound. We have investigated *in vitro* the effects of this compound on cell surface glycoproteins and have observed selective reduction in the cell surface expression of the human transferrin receptor (TfR) in treated cultures.

We have demonstrated, by SDS-PAGE analysis and endoglycosidase H sensitivity, that a population of TfR molecules with altered glycosylation is generated following NB-DNJ treatment. Pulse-chase analysis with the HL-60 cell line reveals that 50% of TfR molecules remain in an immature, totally endo H sensitive, form in treated cells after a 4 hour chase. In contrast, TfR from untreated cells are fully processed within this time period. Furthermore, TfR from treated cells is sensitive to glucosidase digestion demonstrating definitively that NB-DNJ is acting as a glucosidase inhibitor in this system.

Finally, we are investigating the effects of NB-DNJ on gp120 in a mammalian expression system. We wish to determine whether NB-DNJ acts as a glucosidase inhibitor in this system and if so, does this have a biological consequence.

P 214 CHEMICAL AND ENZYMATIC SYNTHESIS OF OLIGOSACCHARIDES TERMINATING IN THE SIALYL DIMERIC LEWIS^x (Sialyl Le^x i) AND THE CORRESPONDING INTERNALLY MONOFUCOSYLATED DERIVATIVE (CD-65 or VIM-2 EPITOPE).

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The availability of pure and well characterized oligosaccharides is a necessity in the determination of the binding specificities of the selectins involved in cellular adhesion processes.

The heptasaccharide (I):

αNeu5Ac(2-3)βGal(1-4)βGlcNAc(1-3)βGal(1-4)βGlcNAc-OR
↑(1-3) ↑(1-3)
αFuc αFuc

and the hexasaccharide (II):

αNeu5Ac(2-3)βGal(1-4)βGlcNAc(1-3)βGal(1-4)βGlcNAc-OR
↑
αFuc

were obtained from the synthetic tetrasaccharide:

βGal(1-4)βGlcNAc(1-3)βGal(1-4)βGlcNAc-OR

by using sialyltransferases from rat liver and the Lewis fucosyltransferase from human milk. A specific enzymatic sequence was used in order to obtain the hexasaccharide II selectively. The structures of these oligosaccharides were confirmed by ^{1}H-n.m.r.

P 215 SIALOADHESIN BINDING MEDIATES SELECTIVITY IN CELLULAR INTERACTIONS OF MACROPHAGES

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Macrophage subpopulations in bone marrow, lymph nodes, and spleen express sialoadhesin, a receptor which recognizes the structure Neu5Acα2→3Galß1→3GalNAc on glycoproteins and glycolipids[1]. Specific localization of this receptor at contact sites between myelomonocytic progenitor cells and resident bone marrow macrophages and the distribution of sialoadhesin expression in lymph nodes suggested a role of this receptor in specific cellular interactions during hematopoietic development and lymphocyte trafficking.

In order to study whether sialoadhesin alone can differentiate between cell types, the number of cells required to bind the same amount of purified sialoadhesin was estimated for erythrocytes, erythroblasts, lymphocytes, macrophages, neutrophils, and density fractionated bone marrow cells. Data will be presented showing that the affinity of sialoadhesin towards these cells varied by two orders of magnitude between those with the lowest affinity, like mouse erythrocytes, and those with the highest affinity binding as strongly as human erythrocytes, which were used as reference. In addition, the same order of preference towards cell types was found in rosetting assays with macrophages expressing sialoadhesin. Sialic acids on mouse cell surfaces often carry 9-O-acetyl groups. Removal of these groups led to an increased binding of sialoadhesin to a degree which varied between cell types. For example, after influenza C esterase treatment, mouse erythrocytes bound sialoadhesin almost as well as human erythrocytes. In conclusion, our experiments suggest that recognition of Neu5Acα2→3Galß1→3GalNAc by sialoadhesin can mediate specific interactions between macrophages and cells carrying this structure and that this selectivity can be regulated by 9-O-acetylation of sialic acid.

[1]Crocker, P.R. *et al* (1991), EMBO J. 10, 1661-1669

P 216 ROLE OF N-LINKED GLYCOSYLATION IN INFLUENZA VIRUS HOST RANGE, Ian D. Manger and Irene T. Schulze, Dept. of Microbiology, St Louis University Medical School, St Louis, Mo. 63104.

Previous work in this laboratory has led to the isolation of a number of variants of influenza A/WSN/33 which are distinguished by point mutations in the hemagg -lutinin (HA) gene which either introduce or eliminate glycosylation sequons. These strains exhibit host-dependence in their growth properties; strain N129N184 grows well in CEF cells but not in MDBK cells, whilst strains N129H184 and D129N184 grow well in both. The N184 mutation disrupts a highly conserved H-H-P-S motif and creates an N-P-S sequon. We have conflicting evidence (based on Endo-F band shift and direct glycopeptide isolation) that this site is used, although Pro N+1 sequons are generally not glycosylated.

According to the 3-D model of the HA, this sequon is immediately adjacent to the receptor binding pocket. Comparison of the growth properties of D129N184 and N129N184 suggests that host-cell restriction is unlikely to result from simple steric occlusion of the binding pocket. Instead, combined occupancy of the glycosylation sites at N129 and N184 and the nature of oligosaccharide processing in MDBK appear to be responsible for the profound changes in function of the HA.

To investigate the proposed basis for the growth restriction of N129N184, we are 1) attempting to compare the folding, assembly and transport properties of the variant HAs 2) examining utilisation of the NPS sequon in different cell types and 3) constructing mutants to evaluate the biological consequences of a non-proline containing sequon at site 184.

P 217 GLYCOSYLATION OF AN ANTIBODY (Ab) FRAMEWORK REGION MAY INHIBIT ANTIGEN BINDING. D. M. Marcus, L-y. Yu-Lee, Q. Dinh, T. Endo*, A. Kobata*, S. Morrison** and J.G. Snyder. Baylor College of Medicine, Houston, TX 77030, *Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan, **Univ. California, Los Angeles, CA 90024

The murine Ig heavy chain gene family, X24, contains only two members, VH441 and VHX24. Although the two genes are 98% similar, they are not utilized with comparable frequencies. The VH441 gene encodes Ab against a number of carbohydrate antigens (Ag), including levan, galactan, 3-fucosyllactosamine, and galactosylgloboside (GalGb4), but VHX24 is rarely used. We are investigating the molecular basis for the preferential use of VH441 over VHX24 in the BALB/c Ab response. Biased use of the VH441 gene could be due to differences in the frequency of VH rearrangement, or to the structures of the peptides encoded by these genes. Both genes encode an N-glycosylation site in CDR2, and VHX24 encodes an additional site in FR1. The anti-GalGb4 Ab 3A9, which is encoded by VH441, exhibits several somatic mutations, including one that eliminates the CDR2 glycosylation site. Mutagenesis techniques were used to examine the effect of variable region glycosylation on the binding of Ag by 3A9. Recombinant Ab were obtained by ligating wild-type and mutated VDJ gene segments into a mammalian expression vector that contains a human IgG1 constant region, and transfecting them into a mutant 3A9 hybridoma that synthesizes only light chains. Replacement of the 3A9 VH segment with germline VH441 sequence produced an Ab that is glycosylated in CDR2 and binds antigen comparably to 3A9. In contrast, replacement of the 3A9 VH segment with the VHX24 germline gene produced an Ab with no detectable Ag binding activity. Additional mutagenesis experiments on the wild-type 3A9 Ab indicate that a single amino acid change in FR1, which introduces the glycosylation site found in the VHX24 gene, is sufficient to abrogate Ag binding activity. Preliminary carbohydrate analysis indicates that this site is glycosylated. The rare use of the VHX24 gene may be due to glycosylation of the FR1 site.

P 218 BINDING OF HUMAN HYBRIDOMA IgG BUT NOT IgM RHEUMATOID FACTORS TO IgG Fc IS INFLUENCED BY CARBOHYDRATE, Marianna M. Newkirk and Joyce Rauch, Department of Medicine, McGill University, Montreal General Hospital Research Institute, Montreal, Que., H3G 1A4, Canada.
Rheumatoid Factors (RFs), which are anti-gamma antibodies, are one of the hallmarks of patients with rheumatoid arthritis (RA). The precise nature of the epitope on the Fc portion of the IgG molecule to which RFs bind has not been identified. Because patients with RA have been found to have abnormal glycosylation of the Fc portion of IgG, we investigated the impact of the sugar present in the Fc on the binding of IgG RFs. We have previously shown, in studies of monoclonal IgM RFs that changes in the structure or removal of the carbohydrate had little effect on RF binding. In this study, we report on studies with five human hybridoma-derived monoclonal IgG RFs. These IgG RFs, derived from patients with RA or systemic lupus erythematosus, were detected using an immunoblot RF assay. We demonstrate that the binding of all five RFs was strongly influenced by the presence of carbohydrate, binding maximally to the Fc when the carbohydrate molecule was intact. Even small changes in the nature of the carbohydrate appeared to adversely affect the ability of the IgG RFs to bind. No direct binding to the carbohydrate moiety itself was observed, suggesting that conformational changes in the polypeptide chain induced by the carbohydrate are responsible for the changes in the binding patterns observed. We propose possible reasons for the observed differences in the effect of the carbohydrate on IgG versus IgM RF binding and suggest that changes in the secondary structure of the Fc may explain why IgG RFs but not IgM RFs appear to correlate better with some clinical features of RA.

P 219 SYNTHESIS, PURIFICATION AND ANALYSIS BY 2-D NMR OF SIALYL-LEWIS X TETRASACCHARIDE (NeuAc-α-2,3-Gal-ß-1,4-[Fuc-α-1,3]-GlcNAc) AND THE RELATED LEWIS X-O-ALLYL GLYCOSIDE. Roger A. O'Neill[1], Graham Ball[2], Theodora de Vries[3], Joanne E. Schultz[4], Jon O. Nagy[5], Chris Hobbs[5], John B. Lowe[6], Brent W. Weston[6], Dirk H. van den Eijnden[3], and Mark D. Bednarski[2,5]. [1]Applied Biosystems, Inc. Foster City, CA 94404, [2]Dept. of Chem., Univ. of California, Berkeley, CA 94720, [3]Dept. of Medical Chemistry, Vrije University, Amsterdam, the Netherlands, [4]Cytel Corporation, San Diego, CA 92121, [5]Lawrence Berkeley Laboratory, Berkeley, CA 94720, [6]HHMI and Dept. of Pathology, Univ. of Michigan, Ann Arbor, MI 48109.

Sialyl Lewis x (SLex, NeuAc-α-2,3-Gal-ß-1,4-[Fuc-α-1,3]-GlcNAc) has been identified as a ligand for ELAM-1, the endothelial leucocyte adhesion molecule. Using a combination of enzymatic and chemical synthetic approaches, we have synthesized the sialyl-Lewis x tetrasaccharide as well as a related Lewis x allyl glycoside. In two separate approaches, SLex was synthesized either from N-acetyllactosamine using a human placental α-2,3-sialyltransferase followed by a cloned human α-1,3-fucosyl- transferase, or directly from human milk-derived NeuAc-α-2,3-Gal- ß-1,4-GlcNAc using the fucosyltransferase alone. The O-allyl- glycoside of Lewis x (Gal-ß-1,4-[Fuc-α-1,3]-GlcNAc-O- allyl) was produced by chemical synthesis of N-acetyl-lactosamine-O-allyl followed by fucose addition using the fucosyltransferase. 500 MHz ^{1}H and ^{13}C NMR spectra were obtained for both carbohydrate structures using COSY, TOCSY, homonuclear J-resolved, HMQC, HMBC and dept-135 techniques. ROESY and NOESY experiments revealed NOE's indicating a folded conformation for both structures, with fucose and galactose interacting. Especially strong were interactions between fucose H5 and H6 with galactose H2. The presence or absence of the sialic acid residue did not appear to strongly effect interactions between other residues within the molecule. Understanding the solution conformation of these molecules may be helpful in designing drugs to block ELAM-1.

P 220 STEREOCHEMICAL ASSIGNMENT OF THE 1-CARBOXYETHYL AND 1-CARBOXYETHYLIDENE ANTIGENIC DETERMINANTS OF RHODOCOCCUS EQUI CAPSULAR POLYSACCHARIDES BY NMR SPECTROSCOPY. James C. Richards and Wayne B. Severn, Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6

Rhodococus equi is a Gram-positive encapsulated bacterium which is found widely in the environment. This organism is primarily a horse pathogen, causing serious and often fatal respiratory disease in 4-12 week old foals. Seven serotypes of *R. equi* are currently recognized which differ in the nature of their capsular polysaccharide antigens. Our structural studies have indicated these polysaccharides to be high-molecular-weight heteroglycans composed of tri- or tetrasaccharide repeating units. In addition, specific sugar residues carry acidic non-carbohydrate components that can be related to the immunological properties of the polysaccharide antigens. Thus, the capsular polysaccharides of serotypes 1,3 and 6 contain 1-carboxyethylidene (pyruvic acid) acetal groups, while the serotypes 2 and 3 antigens carry the biosynthetically related 1-carboxyethyl (lactic acid) ether substituents. It is interesting to note that the serotype 3 polysaccharide antigen contains both pyruvic acid and lactic acid moieties. NOE based strategies were investigated for determination of the stereochemistry of these chiral antigenic determinants. *NOE measurements on the intact serotype* 3 polysaccharide lead to the stereochemical assignment of the pyruvate acetal carbon. The chirality of the lactate α-carbon was related to that of the attached sugar moiety in a conformationally rigid lactone derivative of the glycose. This latter approach has been successfully applied to the stereochemical analysis of the 3-0-(1-carboxyethyl)-α-L-rhamnopyranosyl residues present in the serotype 2 polysaccharide, as well as muramic acid, a component of bacterial cell-wall peptidoglycan.

P 221 CARBOHYDRATE PROFILES OF PRIMARY BREAST CARCINOMAS AND THEIR METASTASES. Rye P.D., Dearing S., and Walker R.A., University of Leicester, Breast Cancer Research Unit, Clinical Sciences, Glenfield General Hospital, Groby Road, Leicester LE39QP, U.K.

The ability of tumour cells to metastasize is the critical and often lethal event in the course of human neoplastic disease. It is well documented[(1)] that glycoconjugates of the tumour cell have a role to play in the complex metastatic process, yet a specific function remains elusive. We have studied the carbohydrate profiles of 38 primary breast tumours and their corresponding axillary lymph node metastases with six lectins (WGA, PNA, CONA, LTA, UEAI, and HPA) and two antisera reactive with glycoconjugates (HMFG2 and P5252), using an immunohistochemical approach. Our findings as expected showed considerable heterogeneity between individual tumours. However unlike previous studies, where specific differences in metastatic glycoconjugate expression have been observed[(2)], we have shown that there is almost complete homology in the carbohydrate phenotypic profiles of individual primary tumours and their corresponding lymph node metastases. The staining patterns of WGA and LTA showed some heterogeneity but only in six and five cases respectively, and this was limited to small quantitative differences (>20%) in numbers of cells staining.
There is considerable evidence that tumour subpopulation interactions exist both within individual tumours and between primary tumours and their metastases, to regulate growth[(3)]. In light of this we suggest that such an interaction exists between primary breast tumours and their lymph node metastases to influence phenotypic glycoconjugate expression and thence metastatic cell behaviour.

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P 222 CHARACTERIZATION OF A HUMAN TUMOR-ASSOCIATED MUCIN CARRYING THOMSEN-FRIEDENREICH EPITOPES, John Samuel and B. Michael Longenecker, Dept. of Immunology, University of Alberta, Edmonton, AB T6G 2H7, Canada.

The Thomsen-Friedenreich (TF) epitope (Gal β 1-3-GalNAc) has been long recognized as an important cancer-asssociated antigen. Its expression in human carcinomas has been shown to be of significance in relation to cancer progression, metastasis and prognosis. We have recently isolated a high molecular weight glycoprotein carrying multiple TF epitopes from human carcinoma cell lines and pleural effusion fluids of carcinoma patients (1). The high molecular weight (>10^6 D), sensitivity to alkali (0.1 M NaOH), extractability with perchloric acid (0.6 M) and buoyant density (1.35 g/ml) of this antigen indicated that it is a mucin-like molecule. The amino acid composition analysis of TF antigen isolated from a colon carcinoma cell line (LS174T) showed that it was rich in serine, threonine and proline and low in aromatic amino acids and methionine, typical of mucins. The amino acid composition of this antigen was significantly different from that reported for the recently characterized intestinal mucins MUC2 and MUC3. The native and deglycosylated TF mucin samples did not show any reactivity with a panel of monoclonal antibodies (including MAbs HMFG-1, HMFG-2 and SM-3) specific for the peptide epitopes of the epithelial mucin MUC1. The studies on the reactivity of the native mucin with a panel of MAb specific for various carbohydrate structures indicated the presence of sialyl Le^x, Le^a, and Tn epitopes. Sialyl Le^x determinant has been recently shown to be involved in cell adhesion and may have a potential role in cancer metastasis. These results suggest that the human tumor-associated TF antigen is a novel carcinoma mucin having a potential role in metastatic spread.

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P 223 **OVER-EXPRESSION OF LAMP-1 MOLECULES ON THE CELL SURFACE RESULTS IN STRONGER ADHESION TO ELAM-1 PRESENTING CELLS**, Ritsuko Sawada and Minoru Fukuda, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

We have recently compared the surface expression of lamp molecules among different tumor sublines derived from a human colon cancer and found that highly metastatic sublines express more lamp on the cell surface than poorly metastatic sublines. In addition, the lamp-1 and lamp-2 from the highly metastatic sublines contain more polylactosaminoglycans than those from the poorly metastatic ones, with the concommitant increase in sialyl Le^x structure with high affinity in antibody binding assay (Saitoh et al., J. Biol. Chem., in press). Since lamp molecules are the major carriers for polylactosaminoglycans, the above results suggest that highly metastatic tumor cells express more ligands for ELAM-1 and GMP-140, which might lead into a stronger adherence to endothelial cells at metastatic sites.

In order to test this hypothesis, the poorly metastatic subline was transfected with cDNA encoding lamp-1, of which the cytoplasmic tail is mutated from tyrosine to histidine. As shown previously, this mutation renders the lamp-1 molecule to be transported to the plasma membrane instead of being transported to the lysosomes (Williams and Fukuda, J. Cell Biol. 111, 955-966, 1990). Different cell lines expressing different amounts of surface lamp-1 were isolated and tested for adhesive property. The results revealed that cells expressing more lamp-1 on the cell surface can adhere more strongly to endothelial cells activated by interleukin 1. This was also true between those cell lines and CHO cells expressing human ELAM-1.

These results strongly suggest that lamp molecules on the cell surface provide ligands for ELAM-1 and GMP-140, and the amount of cell surface lamp may determine the ability of metastasis of tumor cells. (Supported in part by grant CA48737 from the National Cancer Institute).

P 224 THE EFFECT OF LECTINS ON THE HEPATIC KILLING AND CLEARANCE CANDIDA ALBICANS BY THE ISOLATED PERFUSED MOUSE LIVER, Richard T. Sawyer, Ronald E. Garner, John A. Hudson, and Michael N. Horst, Department of Biomedical Science, Mercer University School of Medicine, Macon, GA 31207

The isolated perfused mouse liver model was used to study the effect of various lectins on hepatic trapping and killing of Candida albicans. After exhaustive washing 10^6 C. albicans were infused into mouse livers. At the time of recovery, 63 ± 2% [mean ± SEM] of the infused C. albicans were recovered from the liver and 14 ± 1% were recovered from the effluent for a total recovery of 77 ± 2%. This indicates that 86 ± 9% of the original inoculum was trapped by the liver and that 23 ± 2% was killed within the liver. When included in both pre- and post- perfusion buffers (0.2mg lectin/ml), Ulex europeaus lectin (UEL; binding specificity for fucose) decreased hepatic trapping of C. albicans by 37% and eluted trapped C. albicans from the liver when included only in post-perfusion buffer. By comparison, UEL had no effect on the trapping of killing of yeasts when C. albicans was treated with UEL prior to infusion. When Lens culinaris lectin (LCL; mannose) was included in the perfusion buffers hepatic killing of C. albicans increased by 16% with no significant effect on hepatic killing when yeasts were treated with LCL prior to infusion. Forty to 54% of the infused C. albicans were killed when yeasts were treated prior to infusion with Con-A (mannose), Glycine max lectin (galactose, N-acetyl-galactosamine), or Arachis hypogea lectin (galactose), or when these lectins were included in the perfusion buffers. The increased hepatic killing C. albicans was independent of whether Con-A was included only in the perfusion buffer or if yeasts were treated with Con-A prior to infusion. Increase killing of Con-A treated C. albicans was dose-dependent. The data suggest that a fucose-containing receptor on the surface of either sinusoidal endothelial cells or Kupffer cells may be involved in the trapping of C. albicans by the perfused mouse liver. Lectins with binding specificity for mannose, galactose and N-acetyl-galactosamine increased hepatic killing of C. albicans.

Supported by an NIH RCDA HL01819, NHLBI and Grant 16121-50 from the Medical Center of Central Georgia.

P 225 IS DIABETIC NEPHROPATHY AN ACQUIRED SPHINGOLIPIDOSIS? James A. Shayman, Gayatri D. Deshmukh, Ivan Zador, and Norman S. Radin, Nephrology Division, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, 48109-0676.

Nephropathy is a major complication of diabetes mellitus occurring in ~50% of affected individuals. Early manifestations include hypertrophy and hyperfiltration. We have recently reported associations between renal epithelial cell growth, hormonal signaling, and glucosylceramide metabolism (J. Biol. Chem. (1990) 265, 12135 and (1991) 266, in press). Because UDP-glucose is a precursor for glucosylceramide formation, we studied the levels of glycolipids and the activities of related enzymes in early diabetic hypertrophy. Glucosylceramide (145 ± 1.8 v 200 ± 7.4 μg/mg kidney) and ganglioside GM3 (231 ± 14 v 373 ± 31 μg/mg kidney) levels were increased in rats two weeks following streptozotocin treatment. Corresponding changes in UDP-glucose and UDP-galactose were observed. Renal glycolipid levels were no different in control animals compared to diabetic animals concurrently treated with insulin. Glucosylceramide synthase and β-glucosidase activities were no different in diabetic versus control kidneys. These data indicate that the difference in glycolipid concentrations were due to substrate availability and not altered metabolism. To assess the functional significance of increased glycolipid levels, male rats were treated with the glucosylceramide synthase inhibitor D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Treatment with inhibitor for three days was sufficient to reverse the renal hypertrophy. We suggest that glycosphingolipid synthesis respresents an alternative metabolic pathway for glucose in diabetic nephropathy and may play an important role in the associated functional complications.

P 226 SIALYLATION AND MANNOSE PHOSPHORYLATION AS A FUNCTION OF CULTURE CONDITIONS FOR RECOMBINANT HUMAN DEOXYRIBONUCLEASE PRODUCED IN CHO CELLS. Mary B. Sliwkowski, Jane V. Gunson and Thomas G. Warner‡, Cell Culture & Fermentation R & D Dept. and ‡Medicinal & Analytical Chemistry Dept., Genentech, Inc., 460 Pt. San Bruno Blvd., S. San Francisco, CA 94080.

Recombinant human deoxyribonuclease (rhDNase) is under study as a potential therapeutic for aerosol treatment of the DNA-rich secretions which clog the lungs of patients with cystic fibrosis and chronic bronchitis. rhDNase is a glycoprotein with two N-linked carbohydrate sites and when produced in Chinese hamster ovary (CHO) cells, a mixture of high mannose and complex oligosaccharide side chains were obtained. The acidic pI (3-4) of this rhDNase allowed characterization of the carbohydrate charge heterogeneity by direct analysis of whole culture fluid on isoelectric focusing (IEF) gels. Under standard growth conditions in batch cultures, a reproducible mixture of rhDNase charge forms was obtained. IEF in combination with enzymatic digestion showed that the charge heterogeneity was due to production of glycoprotein species differing in both sialic acid content of complex chains and phosphorylation of high mannose chains. CHO-derived rhDNase was then used as a model system to study the effects of culture conditions on glycoprotein charge heterogeneity of recombinant products. Significant changes were observed in both sialic acid and mannose phosphate content of rhDNase during the time course of cultures grown under various conditions. Metabolic labeling and enzyme inhibitor studies were used to investigate possible sources of these charge differences. The results of these studies will be discussed. The asialoglycoprotein receptor is known to contribute to glycoprotein clearance (1) and receptors for mannose phosphate have also been shown to occur on the surface of various cells (2). While these clearance mechanisms are not particularly relevant for a therapeutic agent delivered by aerosol to the bronchial airways, variation in sialylation and phosphorylation could be important issues for production of many pharmaceutical proteins.

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2. Dahms, N. M., Lobel, P., and Kornfeld, S. (1989) *J. Biol. Chem.* **264,** 12115-12118.

P 227 SELECTIN AND INTEGRIN DEPENDENT MONOCYTE ATTACHMENT TO VENULES IN RHEUMATOID ARTHRITIS, James S. Grober, Brian L. Bowen, Hazel Ebling, Judy Shih, Craig Thompson, Praveen Reddy, David A. Fox and Lloyd M. Stoolman, Departments of Pathology and Medicine, University of Michigan, Ann Arbor, MI 48109

Blood monocytes are the principal reservoir for tissue macrophages in rheumatoid synovitis. Receptor-mediated adhesive interactions between circulating cells and the synovial venules initiate recruitment. These interactions have been studied primarily with cultured endothelial cells. Thus the relative contributions of specific adhesion receptors, such as the endothelial selectins (LEC-CAMs) and the leukocytic integrins, have not been evaluated directly in diseased tissues. We, therefore, examined monocyte-microvascular interactions in rheumatoid synovitis by modifying the Stamper-Woodruff frozen section binding assay initially developed to study lymphocyte homing. Specific binding of monocytes to venules lined by low or high endothelium occured at concentrations as low as 5 X 10^5 cells/ml. Monoclonal antibodies (Mabs) specific for P-selectin (CD62, GMP-140/PADGEM) blocked adhesion by >90% in all synovitis specimens examined. Mabs specific for E-selectin (ELAM-1) blocked 20-50% of monocyte attachment in several RA synovial specimens but had no effect in others. LFA-1, Mo-1/MAC-1, and β2-chain-specific MAbs individually inhibited 30-50% of adhesion. P-selectin mediated adhesion to the microvasculature was also detected in sections of acutely inflamed tonsil. In contrast, the P-selectin positive venules of minimally inflamed foreskin showed 5-20 fold lower levels of adhesion and similar venules in placenta showed no attachment. We conclude that P-selectin associated with the synovial-microvasculature initiates shear-resistent adhesion of monocytes in the Stamper-Woodruff assay and stabilizes bonds formed by E-selectin and the β2-integrins. These findings implicate P-selectin in monocyte recruitment during rheumatoid synovitis and demonstrate that the frozen section assay permits direct evaluation of leukocyte-microvascular adhesive interactions in inflamed tissues.

P 228 THE ROLE OF β1-3-N-ACETYLGLUCOSAMINYL-TRANSFERASE IN REGULATING THE EXPRESSION OF NEOLACTO COMPOUNDS IN HUMAN LEUKOCYTES, Cheryl L.M. Stults and Bruce A. Macher, San Francisco State University, Department of Chemistry and Biochemistry, San Francisco, CA 94132.

Previous studies have shown that leukocytes express different families of glycosphingolipids on their cell surfaces depending on their lineage. For example, myeloid cells express neolacto neutral glycosphingolipids, whereas lymphoid cells express only the globo series. The initial step in the synthesis of type 2 glycosphingolipids involves an initiating form of the enzyme, β1-3-N-acetylglucosaminyltransferase (β1-3GlcNAcT), which catalyzes the following reaction: Galβ1-4Glcβ1-1Cer (lactosylceramide) + UDP-GlcNAc → GlcNAcβ1-3Galβ1-4Glcβ1-1Cer (lactotriglycosylceramide). This reaction may be a key step in the synthetic pathway of neolacto structures in human leukocytes. Therefore, extracts from several cell lines representative of both myeloid and lymphoid lineages were assayed for the presence of β1-3GlcNAcT. Our results (ELISA and TLC immunostain) indicate that myeloid cells contain the initiating form of β1-3GlcNAcT and lymphoid cells do not. This is consistent with our TLC immunostain results which show that myeloid cells express neutral neolacto glycosphingolipids and lymphoid cells do not.

Another step in the synthesis of type 2 compounds involves the elongation of the polylactosamine chain. The following reaction is also catalyzed by a β1-3GlcNAcT (elongating form of the enzyme): Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer (neolactotetraosylceramide) + UDP-GlcNAc → GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer. We tested both myeloid and lymphoid cell lines for the presence of an elongating form of β1-3GlcNAcT by using neolactotetraosylceramide as a substrate. Our data show that an elongating form of the enzyme is expressed in all myeloid and lymphoid cell lines tested. Therefore, our results indicate the presence of two forms of β1-3GlcNAcT in human leukocytes: one that is involved in the initial reaction and one that is involved in the elongating reaction.

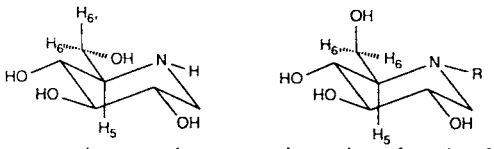
P 229 DIFFERENTIAL IMMUNE RESPONSE TO THE CARBOHYDRATE AND PEPTIDE PARTS OF NOVEL SYNTHETIC TUMOR GLYCOPEPTIDE IMMUNOGEN, Jan Thurin, Charles Hackett, Laszlo Otvos, Hans Loibner, The Wistar Institute, 3601 Spruce street, Philadelphia, PA 19104-4268, USA.

Several highly specific tumor-associated glycosphingolipids have been identified that could be used as human tumor vaccines capable of inducing carbohydrate-specific immunoglobulin responses. To better understand how carbohydrate antigens are recognized, in order to induce more effective immune responses, we constructed an experimental immunogen by coupling a gastrointestinal tumor-associated carbohydrate hapten, the Y-antigen (Fucα1→2Galβ1→4GlcNAc(3←1αFuc)β1→), to a peptide containing sequences of viral proteins know to be targets of murine T helper cells and antibodies. Immunization with the glycopeptide yielded Y-hapten specific IgM responses together with a strong T helper cell reactivity to the viral epitope in the glycopeptide vaccine. Neither Y-hapten alone, T helper cell epitopic peptide alone, nor a mixture of hapten and peptide induced an immune response. Covalent coupling of the Y-hapten and the T helper cell epitopic peptide was therefore necessary for inducing the immune response. The striking observation was that strong IgG responses were obtained to the peptide parts of the immunogen, but that a rather weak IgM response was observed to the saccharide part. This suggests that T cell help is directed differentially to B cells recognizing carbohydrate *versus* peptide determinants of a molecule.

P 230 DRUG RESEARCH ON AZASUGARS, INHIBITORS OF N-GLYCOPROTEIN PROCESSING GLYCOSIDASES,

Leon van den Broek, Agnes Tan, Stan van Boeckel, Hidde Ploegh and Jan Bolscher, *Scientific Development Group, Medicinal Chemistry III RDL, Organon International BV, P.O. Box 20, 5340 BH Oss, The Netherlands, and the Netherlands Cancer Institute, Amsterdam.*

The ability of the glucosidase inhibitor 1-deoxynojirimycin (dNM) and a series of *N*-alkylated dNM derivatives to interfere with biosynthesis, transport and maturation of the glycoprotein α_1-antitrypsin in intact HepG2 cells was investigated. Inhibition of endoplasmic reticulum glucosidase I and II by dNM and its derivatives resulted in an intracellular accumulation of α_1-antitrypsin with glucose-containing high-mannose type oligosaccharides (precursor). *N*-Alkylation of dNM increased its potency in inhibiting endoplasmic reticulum glucosidases, as determined from the concentration required for half maximal inhibition. *N*-Alkylated derivatives of dNM were better able to inhibit glucosidase I than glucosidase II (deduced from the number of glucose residues retained in Endo H-released oligosaccharides). The inhibition of glucosidase activity imposed by alkylated dNM derivatives was less easily reversed than that by dNM, an effect most pronounced for *N*-methyl-dNM. Branching of the alkyl group of dNM derivatives decreased the inhibitory potency; the effect was much stronger for the *iso*-butyl- than for the *iso*-pentyl analogue. Although dNM and its derivatives interfered strongly with intracellular oligosaccharide processing, they did not completely block *N*-glycan maturation of α_1-antitrypsin even at the highest concentrations tested. Upon *N*-alkylation of dNM the conformation (as judged from ^{1}H NMR studies) of the molecule changed from *gauche-trans* (dNM) to *gauche-gauche*, bringing the C_6-OH group in a position perpendicular to the pyranose ring; a position close to that of the C_1-OH group of another potent glucosidase I inhibitor castanospermine.



gauche-trans and *gauche-gauche conformation of dNM*

A. Tan et al. *J. Biol. Chem.* 266 (1991) 14504.

P 231 STRUCTURAL CHARACTERIZATION OF AN ADJUVANT-ACTIVE, TRITERPENE GLYCOSIDE.

Katherine Vogele, Catherine Costello+, Karen Lövgren#, Bror Morein#, Jan Thurin. The Wistar Institue, Philadelphia, PA 19104; +Dept. of Chemistry, MIT, Cambridge, MA 02139; #National Veterinary Institute, Department of Virology, Uppsala, Sweden.

A commercially available extract from the bark of *Quillaja-Saponaria Molina* has been shown to generate prolonged humoral and cellular immune responses, the most notable of these in chimpanzees against gp120 of HIV-1. This bark extract is comprised of over twenty different compounds which are currently incompletely characterized. We have structurally characterized the major adjuvant-active compound using analytical experiments and bioassays at each stage of our purification scheme.

A multi-step purification scheme was derived to separate the twenty or so, structurally similar compounds found in the commercially available bark extract. The separations were achieved by reverse phase, silicic acid, and DEAE chromatography. Structural characterization of this compound required analytical experiments at each stage of the purification scheme. These experiments included high-performance thin layer chromatography; tandem, negative FAB, and CID-MS; glycosyl analysis using TMS methylglycoside and alditol acetate; elemental analysis; ^{1}H and ^{13}C 1D-NMR, and homonuclear, heteronuclear, multiple quantum, and nuclear Overhauser 2D-NMR experiments.

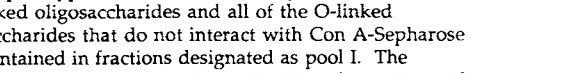
The structure of the major adjuvant-active *Quillaja-Saponaria Molina* compound differs from previously reported structures in both the number and type of sugars and in the triterpene ring system. The previously reported Higuchi structure comigrates with ours in silicic acid, yet is inactive as an adjuvant.

P 232 CHARACTERIZATION OF TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS IN HUMAN CARCINOMAS. Wei-Chun Wang, Hans Marquardt, Karl Erik Hellstrom, and Ingegerd Hellstrom. Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA98121.

Two monoclonal antibodies, designated BR64 (IgG1) and BR96(IgG3), bind selectively to carcinomas of the colon, breast, ovary and lung, but not to normal cells (Hellstrom et al 1990, Cancer Research 50, 2183). In order to characterize the corresponding antigens, we labeled human breast carcinoma 3396 cells with radioactive sugar precursors. The labeled glycoproteins were digested with pronase and the glycopeptides fractionated by concanavalin A-Sepharose chromatography. The complex-type tri- and tetraantennary, bisected biantennary Asn-linked oligosaccharides and all of the O-linked oligosaccharides that do not interact with Con A-Sepharose were contained in fractions designated as pool I. The glycopeptides in pool I were digested with endo-β-galactosidase and subsequently fractionated on Sephadex G-50. The resulting oligosaccharides with the smallest molecular weight were purified by immunoaffinity chromatography using monoclonal antibodies covalently linked to Sepharose. The antigen(s) was eluted with 0.2 M glycine/HCL buffer, pH3.0, and further analyzed by high-performance liquid chromatography on an AX-10 column, compositional analysis, lectin chromatography, chemical defucosylation and methylation analysis. Our results show that a tetrasaccharide with the structure Fuc α1-2Gal β1-4(Fuc α1-3)GlcNAc is the minimal antigenic determinant defined by antibody BR96 and Fuc α1-2Gal β1-4GlcNAc β1-3Gal is the minimal antigenic determinant defined by antibody BR64. These findings suggest that certain carcinoma cells express high levels of α1-3 fucosyltransferases which lead to the expression of oncodevelopmental antigens that provide potentially useful markers for the study of these tumor cells.

P 233 PROBING THE ADHESIVE SPECIFICITY OF THE ENDOTHELIAL-LEUKOCYTE-ADHESION-MOLECULE ELAM-1 WITH NEOGLYCOLIPIDS, Chun-Ting Yuen[1], Wengang Chai[2], Margot Larkin[1], Mark S. Stoll[1], Jaqui O'Brien[1], André Lubineau[3], Tim J. Ahern[4], Alex M. Lawson[2] and Ten Feizi[1], [1]Glycoconjugates and [2]Clinical Mass Spectrometry Sections, MRC Clinical Research Centre Harrow, UK, [3]Laboratoire de Chimie Organique, Universite' de Paris-Sud, France, [4]Genetics Institute Inc, Cambridge, Massachusetts.

Solid-phase binding studies[1] using (a) suspensions of Chinese hamster ovary cells transfected to express cell surface ELAM-1, and (b) immobilized neoglycolipids derived from a series of structurally defined oligosaccharides have shown that ELAM-1 binds to fuco-oligosaccharides with the terminal non-reducing sequences:



This communication will be concerned with an investigation of the minimum backbone chain length among oligosaccharides of the lacto/neolacto series that can support ELAM-1 binding.

[1]Larkin M, Ahern TJ, Larsen GR and Feizi T submitted for publication.

The Biological Function of Glycosaminoglycans, Glycosylphosphatidylinositols and Other Complex Carbohydrates

P 300 THE STRUCTURE OF THE LIPOPHOSPHOGLYCAN FROM *LEISHMANIA MAJOR* AMASTIGOTES.
Antony Bacic**, Susan F Moody, Emanuela Handman and Malcolm J McConville* The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, *The University of Dundee, Medical Sciences Institute, Dundee, Scotland, **The University of Melbourne, Parkville, Victoria.
The etiological agent of human cutaneous leishmaniasis, *L.major* is a protozoan parasite that cycles between an intracellular, non-motile amastigote form within the phagolysosomal compartment of the mammalian host macrophage and an extracellular motile promastigote form in the alimentary canal of the sandfly vector (*Phlebotomine*). The major cell surface glycoconjugate of *L.major* promastigotes, a lipophosphoglycan (LPG) plays a key role in both parasite uptake and survival within the macrophage. A biochemically and immunogenically distinct form of LPG is present on the amastigote surface. Like promastigote LPG, this amastigote specific form of LPG is also likely to play an important role in attachment, uptake and survival.

LPG from amastigotes and promastigotes share the expected tripartite structure of a GPI-anchor, a glycan core and a phosphorylated disaccharide repeat backbone, however, these molecules are chemically distinct. Amastigote LPG is larger in molecular weight (100-6kDa) than promastigote LPG (40-5kDa). Galactose, glucose, mannose, glucosamine and inositol are common to amastigote and promastigote LPG, but arabinose is absent from the amastigote LPG. The lipid anchor of promastigote and amastigote LPG are both composed of alkylglycerols with slight compositional variation.

The structures of the phosphorylated repeats and the hexasaccharide core were determined by monosaccharide analysis, methylation analysis and exoglycosidase digestions.

P 301 A NEW METHOD FOR THE ANALYSIS OF AMIDE LINKED HYDROXY FATTY ACIDS IN LIPID A FROM *RHIZOBIACEAE*. U. Ramadas Bhat and Russell W. Carlson, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602 (U.S.A.)
Lipid A represents the ubiquitous, covalently bound hydrophobic component of bacterial lipopolysaccharides (endotoxins). Lipid As isolated and characterized so far from rhizobial species, indicate dramatic variation in the backbone structure as well as substitution by hydroxy fatty acids. The sugar backbone either consists of glucosamine (GlcN) and galacturonic acid or GlcN and 2,3-diaminoglucose (DAG). The published procedures for amide-linked fatty acids do not release all the fatty acids. Hence, we have developed a method to characterize hydroxy fatty acids still linked to the amino groups of GlcN or DAG. The method involves a mild methanolysis procedure to release GlcN or DAG methyl glycosides along with the amide-bound hydroxy fatty acids and analysis by FAB-MS. The mild methanolyzates after trimethylsilylation are analyzed by the E.I.-M.S. and C.I.-M.S. The method is very useful in analyzing lipid A types with both GlcN and DAG in the backbone and also to determine the heterogeniety in samples.

P 302 POLYMORPHIC EXPRESSION OF GANGLIOSIDES IN THE RAT LIVER, Danièle Bouhours and Jean-François Bouhours, Institut National de la Santé et de la Recherche Médicale, Unité 76, 75739 Paris Cedex 15, France
Gangliosides are synthesized sequentially from lactosylceramide by 3 types of enzymes: GalNActransferase, Galtransferase, and several sialyltransferases. It is therefore likely that the ganglioside composition of an organ is altered when a mutation impairs the expression of one of these enzymes. As the rat liver is an experimental model in many fields of biology and the reference organ for glycosyltransferase purification from the Golgi apparatus, we have undertaken the study of liver ganglioside composition of inbred rats of 8 strains. Several phenotypes were found upon HPTLC of gangliosides from male and female rats. They could be classified into 2 types, the SHR-type displayed a high percentage of GD1b, GT1b, and GQ1b (**b** pathway of synthesis), whereas the DA-type was characterized by a high percentage of GM1 and GD1a (**a** pathway of synthesis). Analysis of the ganglioside profiles of F1 and F2 hybrids indicated that the SHR-type of ganglioside composition was controled by a single autosomal dominant gene. Assays of glycosyltransferases on Golgi fractions showed a defect of GD3 synthase in DA rats. Therefore the ganglioside pattern of the rat liver is controlled by a gene which probably determines the expression of SAT-2 activity for the synthesis of GD3 from GM3.

P 303 POLYMORPHISMS OF BLOOD GROUP A/H-ACTIVE GLYCOSPHINGOLIPIDS IN THE RAT SMALL INTESTINE. Jean-François Bouhours, Danièle Bouhours, Gunnar C. Hansson, Jonas Angström, and Per-Ake Jovall. Institut National de la Santé et de la Recherche Médicale U 76, 75739 Paris Cedex 15, France, and Department of Medical Biochemistry, University of Göteborg, Göteborg, Sweden.
The polymorphism of blood group active neutral glycosphingolipids that was discovered in the rat intestine (1) has been reinvestigated using 15 commercially available strains of inbred rats. 12 strains were found to express only H-active glycolipids and 3 strains to express in addition A-active glycolipids. However, each group could be divided into two phenotypes, indicating the presence of a new polymorphism. One H and one A phenotypes were identical to those already described. The new H and A phenotypes were characterized by the presence of either a H-active hepta- (H-7) or A-active octaglycosylceramide (A-8). A-8 was isolated and characterized as a glycolipid with a linear chain containing a Galβ1-3GlcNAc repeat (2). A genetic analysis after mating the 4 types of strains demonstrated that the capacity of synthesizing blood group A-active glycolipids is inherited as a dominant trait and that the expression of H-7 or A-8 is inherited as a single recessive trait.

1. Breimer, M. E., Hansson, G. C., Karlsson, K.-A., and Leffler, H. (1980) FEBS Lett. 114, 51-56
2. Bouhours, D., Angström J., Jovall, P.-A., Hansson, G. C., and Bouhours, J.-F. (1991) J. Biol. Chem. , in press

P 304 STRUCTURES OF THE OLIGOSACCHARIDES FROM THE LIPOPOLYSACCCHARIDE CORE REGION OF *BRADYRHIZOBIUM JAPONICUM* 61A101C AND ITS SYMBIOTICALLY DEFECTIVE LIPOPOLYSACCHARIDE MUTANT, JS314. Russell W. Carlson* and Bhagyalakshmi S. Krishnaiah, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602 (U.S.A.).

This presentation describes the structure of the lipopolysaccharide (LPS) core oligosaccharides from *Bradyrhizobium japonicum* 61A101c and its symbiotic and LPS defective mutant, JS314. Previous work has shown that the mutant JS314 LPS lacks the O-chain polysaccharide [Stacey et al. 1991. *MPMI* 4:332-340]. The only core oligosaccharide released from the parent LPS by prolonged (5 h) mild acid hydrolysis, and the major core oligosaccharide from the mutant LPS, is a trisaccharide consisting of a terminal mannosyl residue α-linked to C4 of a glucosyl residue which, in turn, is α-linked to C4 of Kdo. An unusual aspect of this trisaccharide is that the Kdo residue is present as α-2,7-anhydro-3-deoxy-D-*manno*-2-octulofuranosonic acid. This anhydro Kdo residue is almost certainly formed during prolonged mild acid hydrolysis as reported previously for Kdo [McNicholas et al. 1987. *Carbohydr. Res.* **165**:17-22]. A disaccharide core component was also released by mild acid hydrolysis of the mutant LPS. This component consists of terminal 4-O-methylmannose α-linked to C5 of Kdo. The Kdo residue in this disaccharide is present in two forms: as a normal reducing Kdo pyranose residue and as an anhydro residue, possibly 4,8-anhydro-3-deoxy-D-*manno*-2-octulosonic acid. It is hypothesized that this anhydro Kdo residue is also formed during the prolonged mild acid hydrolysis. The parent strain does not produce this disaccharide on mild acid hydrolysis; however, 4-O-methylmannose is found exclusively in the O-chain fraction released from the parent LPS. Additionally, a small amount of O-chain is found in the mutant LPS. The results imply that the O-chain is attached to the remainder of the LPS through the 4-O-methylmannose-Kdo disaccharide component of the core region.

P 305 ANTICOAGULANT HEPARAN SULFATE PROTEOGLYCANS DISTRIBUTION BETWEEN CELL SURFACES AND CULTURE MEDIA VARIES WITH DIFFERENT CELL TYPES.
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Anticoagulantly active heparan sulfate proteoglycans (aHSPG) bind and activate antithrombin III (AT). aHSPG accumulate on the extracellular matrix of the endothelial cell line RFPEC and on the subendothelial basement membrane of rat aorta (de Agostini et al. (1990) J.Cell.Biol. 111:1293). LTA, a cell line derived from mouse fibroblasts, also synthesizes aHSPG (de Agostini et al. (1990) Proc.Natl.Acad.Sci. 87:9784). To study the localization of aHSPG produced by RFPEC and LTA cells, we have developed a ligand binding assay that allows to detect aHSPG released by cells in culture media by testing ^{125}I-AT binding to nitrocellulose immobilized soluble aHSPG. aHSPG can be quantitated using purified aHSPG from RFPEC cells (Kojima et al. (1991) J.Biol.Chem., submitted) as standard. Using this assay in conjunction with ^{125}I-AT cell binding assay we detected aHSPG both on the cell layers and in the culture media of RFPEC and LTA cells. The overall production of aHSPG by these two cell lines is comparable and is by and large released into the culture media. Indeed after 8 days in culture the amounts of aHSPG present on the cell layers only represented respectively 62% and 23% of the total aHSPG produced in 24h by RFPEC and LTA cells. Furthermore, we have studied the evolution of the production of aHSPG by RFPEC and LTA cells over time in culture. After confluence RFPEC constantly increased their levels of aHSPG both on the cell layers and into the media. In contrast LTA cells kept constant amounts of aHSPG on their cell layers and released constant amounts of soluble aHSPG in the media. For instance between day 8 and day 14 in culture RFPEC increased their aHSPG from 0.52 to 0.67 pmol/10^6cells on the cell layers and from 0.81 to 1.21 pmol/10^6cells/24h into the media whereas LTA cells kept their aHSPG constant at 0.4 pmol/10^6cells on the cells and at 1.79 pmol/10^6cells/24h in the media. The accumulation of aHSPG on RFPEC monolayers after confluence may be linked to differentiation and production of basement membrane by these cells. LTA cells are originating from fibroblasts which do not make basement membrane, and these cells do not modify their aHSPG production after confluence. These data demonstrate that cells are able to differentially target aHSPG.

P 306 GD2 GANGLIOSIDE ON HTLV-I INFECTED T CELLS: A POSSIBLE ACTIVATION OF β1,4 N-ACETYLGALACTOSAMINYL-TRANSFERASE GENE BY p40^{tax}. K. Furukawa[1], K.S. Furukawa[1], T. Akagi[2], Y. Nagata[1], Y. Yamada[3], K. Shimotohno[2], N-K.V. Cheung[4], K.O. Lloyd[4], H. Shiku[1], [1]Department of Oncology, Nagasaki University School of Medicine, Nagasaki 852, Japan, [2]Department of Virology, National Cancer Center, Tokyo 104, Japan, [3]Nagasaki Citizen Hospital, Nagasaki 852, Japan, [4]Sloan Kettering Cancer Center, NY 10021.

Characteristic profiles of ganglioside expression in adult T-cell leukemia (ATL) and HTLV-I$^+$ cells were demonstrated by using monoclonal antibodies, and the involvement of HTLV-I p40^{tax} protein in the expression of gangliosides in these cells was investigated. Among various leukemia cell lines, ATL lines and HTLV-I$^+$ cells were shown to specifically express GD2. Leukemia cells from ATL patients expressed low level of GD2 but the ratio of GD2 positive cells increased by 40-70% during in vitro culture in the presence of interleukin-2. The appearance of GD2 in the cultured cells corresponded with the expression of the HTLV-I genome, especially with p40^{tax} expression. Cultured lymphocytes expressing p40^{tax} (induced by transfection with a retroviral vector) showed high expression of GD2 in comparison with control lymphocytes. The presence of high level of β1,4 N-acetylgalactosaminyltransferase mRNA in these cells was demonstrated in Northern blot hybridization by probing with cDNA for this enzyme (GM2/GD2 synthase). These results indicate that high GD2 expression in these cells was due to neosynthesis from precursor GD3 by activation of this enzyme.

P 307 VARIABLE SUBCELLULAR LOCALIZATION OF GLYCOSPHINGOLIPIDS (GSLs), Baiba K. Gillard, Lisa T. Thurmon and Donald M. Marcus, Departments of Medicine, and Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

Although most GSLs are thought to be located in the outer leaflet of the plasma membrane, recent evidence indicates that GSLs are also associated with intracellular organelles. We now report that distribution of GSLs between the plasma membrane and intracellular organelles, and among different intracellular organelles, depends on both GSL and cell type. GSL localization was determined by indirect immunofluorescence microscopy of fixed permeabilized cells on coverslips. The same GSL showed variable subcellular localization in different cells. For example, antibody to GalCer localized primarily to the plasma membrane of HaCaT II-3 keratinocytes and SW13 adenocarcinoma cells, but to intracellular organelles in other epithelial cells. GalCer localized to small vesicles and tubulovesicular structures in MDCK cells, and to the surface of phase-dense lipid droplets in HepG2 hepatoma cells. Furthermore, within a single cell type, individual GSLs were found to have different patterns of subcellular localization. In HepG2 cells LacCer was associated with small vesicles, which differed from the phase-dense vesicles stained by anti-GalCer, and globoside was associated with the intermediate filaments of the cell cytoskeleton. Together with observations from other laboratories on the enrichment of GSLs in the apical plasma membrane of polarized cells, these findings indicate the existence of specific sorting mechanisms that regulate the intracellular transport and localization of GSLs.

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P 308 CARBOHYDRATE SPECIFICITY OF THE MAMMALIAN C-TYPE LECTIN, PULMONARY SURFACTANT-ASSOCIATED PROTEIN A (SP-A). *John Haurum, Steffen Thiel, Jens Chr. Jensenius. Department of Immunology, Institute of Medical Microbiology, University of Aarhus, 8000 Aarhus C, Denmark.*

Pulmonary surfactant is a complex mixture of phospholipids and proteins lining the inner surface of the alveoli. Four surfactant associated proteins (SP-A, SP-B, SP-C and SP-D) have been described. SP-A, the most abundant surfactant-associated protein, is a glycoprotein synthesized by the type II alveolar epithelial cells. The C-terminal sequence of SP-A shows a marked homology with the carbohydrate recognition domain (CRD) of mammalian C-type lectins. Accordingly, SP-A was shown to bind to immobilized carbohydrates in the presence of calcium. Furthermore, the SP-A molecule contains an N-terminal region with a collagenlike sequence. Thus, the overall structure of SP-A is similar to the structure reported for mannan-binding protein, another C-type lectin. Several biological functions have been ascribed to SP-A such as regulation of surfactant secretion and reuptake, modulation of surfactant function, interaction with the C1q-receptor found on phagocytic cells, and enhancement of phagocytosis of *Staphylococcus* aureus and herpes simplex virus by alveolar macrophages.

In this study we examined the carbohydrate specificity of SP-A in an Enzyme-Linked Lectin-Binding Assay (ELLBA). The SP-A used was purified from normal human lavage fluid by DEAE ionexchange chromatography. Microtiter wells were coated with mannan from bakers yeast. The wells were blocked with HSA and incubated with SP-A diluted in calcium containing buffer. Bound SP-A was detected with biotinylated chicken anti-rSP-A immunoglobulin followed by AP-Avidin. Nonspecific binding was estimated by replacing calcium with 10 mM EDTA in the buffer during incubation with SP-A. The ligand binding characteristics of human SP-A were assessed by inhibition studies where various carbohydrates were included in serial dilutions in the calcium containing buffer during the incubation with SP-A.

ManNAc resulted in the most efficient inhibition (CI_{50}≈ 4 mM), followed by Mal, Glc, Man, and ManN (CI_{50}=9, 11, 12, and 18, respectively). Moderate inhibition of binding was exerted by GlcN, GalN, GlcNAc, and Gal (CI_{50}=30, 39, and 39). At 50 mM GalNAc and Fuc did not inhibit binding of SP-A to mannan. The results indicated that SP-A binds with high affinity to ManNAc. This was further verified by carbohydrate affinity chromatography using TSK-beads activated with divinylsulphone and coupled with ManNAc.

P 309 HEAT AND HYPOXIA-REOXYGENATION STRESS: PARALLEL INDUCTION OF STRESS PROTEIN SYNTHESIS AND SPECIFIC PROTEIN GLYCOSYLATION. Kurt J. Henle, and William A. Nagle, Depts. of Medicine, Radiology, and Physiology/Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205-5484.

The cellular heat stress response is well documented in terms of elevated synthesis of heat shock proteins and the expression of thermotolerance. We have recently identified a glycosylation phenomenon that occurs in parallel with the above events; this includes elevated activity of several glycosyltransferases (J Cell Physiol 142:372-78, 1990) and selective labeling of specific glycoproteins, e.g., GP50 (J Cell Sci 95:555-61, 1990, Exp Cell Res 196:184-91, 1991). General features of the glycosylation stress response were qualitatively similar in a series of five mammalian cell lines, with GP50 as the major induced glycosylation product. In contrast to typical heat shock proteins, GP50 appeared as a strongly basic protein, when analyzed by NEPHGE, and was found at lower constitutive levels in control cells. GP50 shared characteristics with HSP47 (affinity to collagen and heparin). Stress-induced glycosylation of GP50 was demonstrated with tritiated D-Man, D-GlcN, and more weakly with L-Fuc; the former labels, but not L-Fuc were removable by digestion with endoglycosidase F. Incorporation of D-Man was reduced, but not eliminated by 0.5 μg/ml tunicamycin or by 10 μg/ml cycloheximide. Similarly, tunicamycin was effective in potentiating cell killing at 45°C, both with and without prior thermotolerance induction.

A second, prompt glycosylation phenomenon was observed when D-Man label was present during heat stress. At temperature above 43°C preferential labeling of a M_r 60,000 (GP60) was observed, whereas lower hyperthermic temperatures caused relatively more labeling of GP50.

Specific protein glycosylation was not restricted to heat stress, but was also induced by some forms of oxidative damage. For instance, hypoxia/reoxygenation, but not H_2O_2 (100 μM, 30 min 37°C) induced significantly increased glycosylation of GP50. Our data support the hypothesis that GP50 and GP60 are essential components of an integrated cellular stress response that mediates the expression of stress tolerance and enhanced adaptation to environmental stress (Supported by CA-33405).

P 310 ISONEOLACTO-SERIES MONOSIALOGANGLIOSIDES OF BOVINE ERYTHROCYTES, E.L. Hogan, S. Dasgupta, *J. Glushka and *H. van Halbeek, Department of Neurology, Medical University of SC, Charleston, SC 29425 and *Complex Carbohydrate Research Center, University of GA, Athens, GA 30602 Three monosialogangliosides of the isoneolacto-series have been purified to homogeneity by DEAE-sephadex and Biosil A column chromatography. They are designated as Ggs-1, 2 and 3 in order of decreasing Rf values on tlc. The neuraminidase (in the absence of detergent) digested product of Gg-1 migrates at a higher Rf than asialo Gg-2 and 3 (that comigrate indicating close structural similarity). The structures of the individual gangliosides have been established by GC-MS, stepwise specific exoglycosidase hydrolysis and 500-MHz ^{1}H-NMR spectroscopy of both the native gangliosides and their free oligosaccharides. The molar composition of Gg-1 is NeuGc:Gal:GlcNAc:Glc 0.8:3.9:2.7:1. Both Gg-2 and Gg-3 contain an additional mole of galactose and the sialic acid composition of Gg-2 and 3 differs. 2D-NMR spectroscopy of Gg-3 oligosaccharide has established its *biantennary structure as* Galα1→3Galβ1→4GlcNAcβ1→6 and NeuGcα2→3Galβ1→4GlcNAcβ1→3 linked to βGal of a neolactotetraosylceramide core. 600-MHz ^{1}H-NMR spectroscopy of the exoglycosidase (α- and β-galactosidase and β-hexosaminidase) digested oligosaccharides of Gg-1 and Gg-2 confirms structural identity with Gg-3 with the exceptions that **Gg-1** lacks the terminal αGal and **Gg-2** contains **NeuAc** instead of **NeuGc** as described in **Gg-3**. Hence the structures of Ggs-1, 2, and 3 have been established as follows:

Gg-1: NeuGc-Iso-nLcOse$_6$Cer
Gg-2: NeuAc-Iso-nLcOse$_6$Cer
Gg-3: NeuGc-Iso-nLcOse$_6$Cer

Gg-2 and Gg-3 contain the *same structures as* described by Watanabe et al. (1979) in a mixture but, **Gg-1** is a novel ganglioside of the isoneolacto-series.
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P 311 Fractionation of Heparin-Derived Oligosaccharides on Affinity Columns of Immobilized Basic Fibroblast Growth Factor. Masayuki Ishihara[1], David J. Tyrrell[1], Michael C. Kiefer[2], Philip J. Barr[2], and Robert J. Stack[1]. [1]Glycomed Inc., 860 Atlantic Ave., Alameda, CA 94501 and [2]Chiron Corp., 4560 Horton St., Emeryville, CA 94608.

Partial depolymerization of heparin with nitrous acid at controlled pH yielded a complex mixture of oligosaccharides with terminal anhydromannose residues. The reduced products were fractionated by gel permeation chromatography into pools of discretely-sized, chemically heterogenous structures. The tetra-, hexa-, and octasaccharide pools were sequentially applied to a basic fibroblast growth factor (bFGF) affinity column and eluted with buffers of increasing ionic strength. Hexasaccharides fractionated in this manner were divided into six subpools, which were then analyzed for charge heterogeneity by ion-exchange HPLC. In general, the degree of charge correlated with the degree of retention by the bFGF affinity column. Panning experiments with syndecan-expressing human lymphoblastoid cells (UC 729-6) on bFGF-coated culture plates (Kiefer et al, 1990 PNAS 87: 6985) showed clear differences in the ability of these sub-pools of hexasaccharides to inhibit cell binding in vitro.

P 312 IDENTIFICATION OF A MACROPHAGE-BINDING DETERMINANT ON LIPOPHOSPHOGLYCAN (LPG) FROM *Leishmania major* PROMASTIGOTES. Michelle Kelleher*, Antony Bacic+& Emanuela Handman*. The Walter & Eliza Hall Institute of Medical Research, Melbourne, 3050; and + Plant Cell Biology Research Centre, School of Botany, University of Melbourne, 3050. *Leishmania* are obligatory intracellular parasites in mammalian macrophages that gain entry by receptor-mediated phagocytosis. The major cell surface glycoconjugate lipophosphoglycan (LPG) and the glycoprotein gp63 have been implicated in this process. The monoclonal antibody WIC 79.3 specific for *Leishmania major* LPG, that had been shown to block attachment of promastiogtes of *L. major* to macrophages, was used to identify a macrophage-binding determinant of LPG. LPG has a tripartite structure consisting of a series of phosphorylated oligosaccharide repeats and a phosphorylated saccharide core attached to a conserved, unusual lysoalkyl phosphatidylinositol (PI) lipid anchor. WIC 79.3 bound exclusively to the phosphorylated repeats of LPG, and not to the saccharide core or lipid anchor. Furthermore, the epitope recognised by WIC 79.3 mapped to the phosphorylated oligosaccharides P5b, PO_4-6[Gal(β1-3)Gal(β1-3)Gal(β1-3)Gal(β1-4)Manα1-, which is unique to LPG of promastigotes of *L. major*. Two other phosphorylated oligosaccharides P3, PO_4-6[Gal(β1-3)Gal(β1-4)Manα1- and P4b, PO_4-6[Gal(β1-3)Gal(β1-3)Gal(β1-4)Manα1- were recognised by WIC 79.3 but with considerably lower (approx. 100 fold) affinities. The phosphorylated oligosaccharide P5b inhibited attachment of promastigotes of *L. major* to the macrophage cell line J774 to the same degree as phosphoglycan (derived from LPG), suggesting that P5b is a site of *L. major* LPG that is recognised by macrophage receptors(s) and is an important determinant in the attachment of promastigotes to host macrophages and initiation of infection. A number of monoclonal antibodies have been raised to other epitopes of the PORs of *L. major* promastigote LPG. The specificity of the monoclonal antibody is being established in order to study developmental and functional roles of the various epitopes of LPG.

P 313 TLC-FAB MASS SPECTROMETRY IN COMBINATION WITH TLC OVERLAY AS A METHOD TO SCREEN FOR RECEPTOR-ACTIVE GLYCOLIPIDS AFTER CHEMICAL MODIFICATION. Boel Lanne, K.-A. Karlsson, M.H. Nori-Sorkhabi, G. Stenhagen, S. Teneberg, L. Uggla
Glycobiology Group, Dept. of Medical Biochemistry, University of Göteborg, Box 33 031, S-400 33 Göteborg, Sweden, Fax 46-31-413190

When studying the detailed binding characteristics of lectins, eg microbial adhesins, chemical modifications of carbohydrate receptors are of value. However, such chemical derivatizations often lead to mixtures of reaction products, among which binding activity can be found. A convenient and fast method suitable for this analysis is separation by thin-layer chromatography followed by overlay with radiolabeled ligand and autoradiography. Structural information of modified glycolipids can then be obtained by fast-atom bombardment mass spectrometry directly of the reaction products separated on the thin-layer plate. The whole lane with chromatographed glycolipids is cut out and continously moved within the mass spectrometer for atom bombardment, allowing several spectra to be collected per mm lane.

Results will be presented where the technique was applied to the receptor glycolipid of enterotoxigenic *E. coli* K99, that causes severe diarrhoea in calves and piglets. *N*-glycolyl-GM3 ganglioside was modified in several ways, including exchange of the *N*-glycolyl group with *N*-trifluoroacetyl, which perserved the binding capacity, and coupling of the carboxyl group to several amides. Aliphatic amides with up to three carbons still bound the bacteria.

P 314 STRUCTURAL AND IMMUNOLOGICAL CHARACTERIZATION OF O-ACETYLATED-G_{D2}, Adriana E. Manzi[1], Eric R. Sjoberg[1], Anne Dell[2], Kay-Hooi Khoo[2] and Ajit Varki[1,]*Department of Medicine, Cancer Center, University of California, San Diego, La Jolla, California and* [1]*Imperial College of Science, Technology and Medicine, Department of Biochemistry, London.* Gangliosides are a structurally heterogenous group of glycosphingolipids with one or more sialic acid residues, that are synthesized in the lumen of the Golgi apparatus and transported to the cell surface. O-acetylation on sialic acids of gangliosides has been detected at C4,7(8),and 9 hydroxyl groups. Ganglioside O-acetylation is intricately regulated in a spatial and temporal manner throughout development of tissues derived from the neuroectoderm. This suggests that O-acetylated gangliosides may mediate intercellular adhesion events giving rise to appropriate tissue development. We have recently used Golgi enriched membrane preparations isolated from a human melanoma cell line to study the biosynthesis of O-acetylG_{D3}. Using this *in vitro* assay to study ganglioside O-acetylation in the human melanoma cell line M21, we discovered the formation of a new base labile species whose chromatographic behavior is consistent with that of O-acetylG_{D2} .HPLC analysis of the sialic acids from the putative [*acetyl*-^{3}H]O-acetylG_{D2} obtained in this assay indicated that both C7 and C9 hydroxyls can be esterified. Analyses of total lipid extracts from metabolically labeled M21 cells showed a species co-migrating with G_{D3} that upon alkali treatment co-migrates with G_{D2}. We purified this base labile species from M21 cells grown in culture, and analyzed it by HPTLC immuno-overlay, FAB-MS in both positive and negative ion modes, permethylation followed by GC-MS, and 500 MHz ^{1}H-NMR. This analysis is consistent with the following structure:

{Neu5,9Ac2α(2-8)Neu5Acα(2-3)}[GalNAcβ(1-4)]Galβ(1-4)Glcα(1-1)Cer

Comparison of negative FAB-MS spectra of G_{D2} and O-acetyl G_{D2} allowed us to localize the O-acetyl ester to the terminal sialic acid.

P 315 NOVEL *O*-GLYCOSIDIC LINKAGES IN EUBACTERIAL SURFACE LAYER GLYCOPROTEINS, Paul Messner[1], Judith Kolbe[1], Rudolf Christian[2], Günther Allmaier[3], Gerhard Schulz[4], and Uwe B. Sleytr[1], [1]Zentrum für Ultrastrukturforschung, Universität für Bodenkultur, A-1180 Wien, [2]Scientific Software Company, A-1140 Wien, [3]Institut für Analytische Chemie, Universität Wien, A-1090 Wien, and [4]Sandoz Forschungsinstitut, A-1235 Wien, Austria

Glycosylated proteins are a common feature of eukaryotic organisms. They fulfill there a broad spectrum of functions. In prokaryotic organisms glycoproteins have been identified mainly as costituents of crystalline cell surface layers (S-layers). Glycosylated S-layers are more frequently found in archaebacteria than in eubacteria (1).
Structural studies on eubacterial S-layer glycoproteins have shown that both linear and branched oligosaccharides can occur. Their constituents comprise of a wide range of hexoses, deoxy sugars, amino sugars and uronic acids. Also, new types of *N*- and *O*-glycosidic carbohydrate-protein linkages were observed in S-layers (1,2).
We have analyzed the S-layer glycoproteins of several *Clostridium thermohydrosulfuricum* strains in greater detail. A common feature of most of these S-layers is presence of tyrosine as the linkage amino acid to the glycan chain. In *C. thermohydrosulfuricum* S102-70 a non-repititive hexasaccharide unit is linked via β-D-glucose to tyrosine. This forms a novel type of *O*-glycosidic linkage (2). Presently we are investigating several other S-layer glycoproteins of this group and would like to determine how common those linkages are among eubacteria.

References: (1) Messner, P. & Sleytr, U.B. (1991) *Glycobiology* 1, (in press); (2) Messner, P., Christian, R., Kolbe, J., Schulz, G. & Sleytr, U.B., *Proc. Natl. Acad. Sci. USA*, (submitted).

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P 316 Studies on the GPI-anchored Folate Binding Protein identified on ovarian carcinomas by two MAbs

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Two MAbs, MOV18 and MOV19, produced in our laboratory against ovary carcinoma associated antigens, identify a 38kDa GPI-protein (gp38) which is overexpressed on ovarian tumors. Recently, gene cloning from IGROV1 cDNA demonstrated that gp38 is a folate binding protein (L.R. Coney et al. Cancer Research 1991, in press). Since gp38 can be detected as a membrane protein or as a soluble molecule released in the culture medium of different ovary carcinoma cell lines, we investigated the biochemical characteristics of both forms and the possible mechanism involved in the generation of the soluble protein. As detected by HPLC analysis, the soluble protein is smaller than the membrane PI-PLC released gp38 and doesn't express the cross-reactive determinant (CRD) unmasked on several GPI proteins after bacterial PI-PLC cleavage. This suggests that mechanisms other than the endogenous PI-PLC induced release could normally be involved in the generation of the soluble protein. A model for a better understanding of these phenomena is represented by the gp38 release which follows, 5 days after a PI-PLC treatment, the resynthesis of the protein. The precursor-product relationship between the two forms of FBP are at present being investigated as well as the folate binding capacity and the internalizing property of the receptor on ovarian carcinomas.

P 317 CHARACTERIZATION AND SUBSTRATE SPECIFICITY OF AN α-L-FUCOSIDASE FROM *HALIOTIS RUFESCENS*. Allen K. Murray, Glycozyme, Inc., 3873 S. Main Street, Santa Ana, CA 92707-5710.

The characteristics and substrate specificity of an α-L-fucosidase from the marine mollusk, *Haliotis rufescens*, have been investigated to determine the usefulness of this enzyme in glycoconjugate structural determinations. The enzyme is active between pH 2.5 and 4.5 with an optimum at pH 3.5. Activity decreases markedly above pH 5.0 and is reduced to <10% at pH 7.0. The the pH stability has been investigated with 50% activity remaining after exposure to pH 7.0 at 25°C or pH 8.0 at 4°C. Temperature stability studies indicate the enzyme retained 68% activity after exposure to 60°C for ten minutes. The α-L-fucosidase is active against the synthetic substrates *p*-nitrophenyl-α-L-fucopyranoside and 4-methylumbelliferyl-α-L-fucopyranoside. The substrate specificity of the enzyme has been investigated using a number of naturally occuring substrates. The enzyme has both α-1,2 and α-1,3 activity using 2'-fucosyllactose and 3-fucosyllactose as substrates.

P 318 STRUCTURE AND MICROHETEROGENEITY OF THE N-LINKED GLYCAN CHAINS FROM A STYLAR GLYCOPROTEIN ASSOCIATED WITH THE EXPRESSION OF SELF-INCOMPATIBILITY IN *NICOTIANA ALATA*, David Oxley[1], James R Woodward[1], David Craik[2], Anne Dell[3], Kay-Hooi Khoo[3], Sharon L A Munro[2], Adrienne E Clarke[1] and A Bacic[1]; [1]Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville 3052, Australia; [2]The Victorian College of Pharmacy, Parkville 3052, Australia; [3]Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2A2, United Kingdom.

The interacting partners during fertilization in higher plants are pollen grains and the female pistil. If mating is compatible, pollen produces a tube which grows through the pistil to the embryo sac. In many plant families, the products of the self-incompatibility genes (*S*-genes) operate to prevent inbreeding by inhibiting the growth of self pollen tubes. We have used *Nicotiana alata* (Solanaceae), an ornamental tobacco, to study the molecular basis of self-incompatibility. The products of the *S*-gene are glycoproteins with ribonuclease activity which we have purified from the stylar tissue and we have cloned genes encoding several *S*-alleles.

As part of our broad program directed towards understanding the molecular mechanism by which the *S*-glycoproteins inhibit pollen tube growth, we have characterised their glycosyl residues. We have investigated the number of N-linked glycan chains by enzymic cleavage with peptide:N glycosidase F (N-glycanase) and endo-β-N-acetylglucosamidase H. *S*-glycoproteins from different alleles contain different numbers of N-linked chains; S_1, S_2, S_3, S_6 and S_7 have 1, 3, 4, 3 and 3 chains respectively. We have isolated the N-linked glycan chains from the S_1- and S_2-glycoproteins by anion-exchange HPLC using the Dionex Corp (Sunnyvale, CA) Carbopac PA1 column. Both the S_1- and S_2-N-linked glycan chains can be fractionated into three peaks (a, b, c). They are in the approximate ratio of 5:93:2 and 7:57:36 for S_1 and S_2 respectively. The major N-linked oligosaccharides are xylosylated hybrid structures. Fucose is absent. This contrasts with the structures of the N-linked glycan chains of the *S*-glycoproteins from *Brassica campestris* which displays sporophytic self-incompatibility. No O-linked glycans have been detected on the S_2-glycoprotein. We are in the process of chemical (cyanogen bromide) and proteolytic cleavage of the S_2-glycoprotein to determine site-specific microheterogeneity of N-linked glycan chains.

These studies will allow us to establish if the N-glycan chains play a role in biological function analagous to that demonstrated for sperm-egg interactions in mammalian systems.

P 319 THE KINETICS OF GLYCOLIPID BIOSYNTHESIS IN *THE PROTOZOAN PARASITE LEISHMANIA MAJOR*, Lorna Proudfoot, Michael A.J. Ferguson and Malcom J. McConville, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

In addition to utilizing glycosyl-phosphatidylinositol (GPI) glycolipids as membrane anchors for the major surface proteins, *Leishmania* parasites synthesize two additional classes of GPI, the low molecular weight glycoinositolphospholipids (GIPLs)[1] and the heterogeneous lipophosphoglycans (LPG's)[2]. The GIPLs are major cellular glycoconjugates in all stages of the parasite and, in *L. major*, are structurally similar to the membrane anchor of the LPG. To determine the relationship between the GIPLs and LPG, promastigotes in logarithmic growth phase were metabolically labelled with [^{3}H]-glucosamine and [^{3}H]-galactose. In continuous labelling experiments, [^{3}H]-GlcN was incorporated predominantly into the GIPLs, in particular GIPL-2, with lower incorporation into LPG. The kinetics of incorporation of [^{3}H]-GlcN label into GIPLs relative to LPG decreased when promastigotes were placed in glucose-free medium. These results suggest that the GIPLs are major metabolic end-products, as well as being biosynthetic precursors to LPG, consistent with the finding that they are expressed in high copy number at the cell surface. The effect of Brefeldin A and monensin on GIPL and LPG biosynthesis has also been investigated, to identify the site of synthesis of these glycoconjugates.

[1]McConville, M.J., Homans, S.W., Thomas-Oates, J.E., Dell, A., and Bacic, A. (1990) J. Biol. Chem., 265, 7385-7394.

[2]McConville, M.J., Thomas-Oates, J.E., Ferguson, M.A.J. and Homans, S.W. (1990) J. Biol. Chem., 265, 19611-19623.

P 320 STRUCTURAL CHARACTERIZATION OF GPI ANCHORS BY ESI-MS AND CID

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GPI anchors represent a class of amphiphilic molecules that serve to bridge the polar and nonpolar domains of cellular constituents. In this role of basic structural integration, anchor components bear a series of modifications that enhance molecular specificity but exacerbate the problems of structural characterization. GPI anchors are attached at the protein C-terminus and exhibit a conserved glycan core with membrane anchorage dependent on an aliphatic terminus. This complex conjugate, with unique physical and chemical properties has challenged conventional isolation and characterization procedures, where most of the characterization has relied on anchor degradation and identification of selected components.

Electrospray provides an alternative approach. Here molecular ionization is not by energetic particle bombardment, (e.g., FAB-MS), but is through desolvation, where analytes are charged as a consequence of their solution acidic or basic character. A common example is the multiple protonation (and subsequent desolvation by electrospray) of peptides which results in multiple positive charges on the gas phase ion. Since mass measurement is a function of mass-to-charge, this multiple charging brings high molecular weight analysis into the realm of accessible instruments. Additionally, multiply-charged ions generally exhibit greater instability upon collision induced dissociation, (CID). These combined factors allow peptide-attached anchor analysis using ESI-MS-CID-MS.

The multiply-charged parent ion profile of ESI-MS yields a clear understanding of anchor glycoforms and their molecular heterogeneity, while CID contributes to sequence and linkage information of these complex structures. Anchors from AChE and T. brucei VSG have been investigated with these techniques to clarify existing and define new structures.

P 321 GLYCOSYLATION PATHWAYS IN THE BIOSYNTHESIS OF GANGLIOSIDES IN MELANOMA AND NEUROBLASTOMA CELLS LINES.

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In order to understand the factors that control the overall profile of glycolipid (GL) expression in cells we have determined the relationship between ganglioside expression and the levels of glycosyltransferases in a number of melanoma and neuroblastoma cell lines. Cell lines representing tumors with (i) GM3, (ii) GM3 and GM2, (iii) GM3 and GD3, and (iv) GM3, GM2, GD3 and GD2 as their major gangliosides were chosen. Sialyl-transferases were measured in extracts of crude membrane preparations using [14C]NeuAc-CMP as donor and either Lac-Cer (for GM3 synthase) or GM3 (for GD3 synthase) as acceptors. N-Acetyl-galactosamine transferase levels were measured using [14C]GalNAc-UDP as the donor and GM3 (for GM2 synthase) or GD3 (for GD2 synthase) as acceptors. GM2 and GD2 synthesis was shown to be carried out by the same N-acetylgalactosamine transferase, as has been demonstrated for the liver enzyme by other investigators. In general, the levels of activities of the specific transferases correlated with the actual ganglioside content of the cells. However, it was noted that in cells rich in GD2, the levels of GD3 synthase was relatively low. This suggests that the level of GD2 synthase in cells is governed by the level of an enzyme representing an intermediate step in the pathway (GD3 synthase) as well as the enzyme responsible for the final step in the pathway (GD2 synthase). Also, some cells with low GM2 synthase levels had quite high levels of GM2 ganglioside. In conclusion, the overall pattern of gangliosides characteristic of a particular cell type is dependent on the levels of specific enzymes in the biosynthetic pathway, as well as other factors such as the concentration of appropriate GL acceptors in the cell on which the enzymes act and the levels of the next enzyme in the pathway.

P 322 TRYPANOSOMA CRUZI TRANS-SIALIDASE AND NEURAMINIDASE ACTIVITIES CAN BE MEDIATED BY THE SAME ENZYMES.

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When trypomastigotes of T. cruzi emerge from cells of the mammalian host, they contain little or no sialic acids on their surfaces. However, rapidly upon entering the circulation, they utilize a unique cell surface trans-sialidase (TS) to specifically transfer α(2-3)-linked sialic acid from extrinsic host-derived macromolecules to parasite surface molecules. The T. cruzi TS does not utilize cytidine 5' mono-phospho-N-acetyl-neuraminic acid as a donor substrate, but readily transfers sialic acid from exogenously supplied α(2-3)-sialyllactose (Cell 65:1117, 1991). We have now found that the TS can be the same enzyme as a previously described T. cruzi neuraminidase (J. Exp. Med. 174:179, 1991). Both enzymes are recognized by two independently derived monoclonal antibodies, are anchored to the membrane by glycosylphosphatidylinositol, co-purify by ion exchange, molecular sieving and hydrophobic chromatography, have maximal activities between pH 6.5 and 7.5, and are inactivated by heating at 56°C. Furthermore the neuraminidase and trans-sialidase reactions are coupled. An increase of the concentration of acceptors of the transfer reaction decreases the amount of free sialic acid released through the neuraminidase reaction. We conclude that a single enzyme can catalyze the transfer or the hydrolysis of macromolecular-bound sialic acid. The predominant direction of the reaction will depend on the availability of appropriate oligosaccharide acceptors of sialic acid.

P 323 CELL-FREE SYNTHESIS OF LIPID-LINKED SACCHARIDES AND GLYCOSYLPHOSPHATIDYL-INOSITOLS IN LYSATES OF *PLASMODIUM FALCIPARUM*. CHARACTERIZATION OF PUTATIVE PRECURSORS OF THE GPI-MEMBRANE ANCHOR,

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A cell-free system for the study of glycolipids in *P. falciparum*, the causative agent of malaria in man, was established. Isolated and hypotonically lysed parasites are incubated with radioactive precursors. The reaction products were extracted sequentially with chloroform/methanol (2:1;CM) and chloroform/methanol/water (1:1:0.3;CMW). Labeled reaction products in CM were analyzed on Silica G60 plates using chloroform/methanol/acetic acid/water (25:15:4:2) as a solvent. In CM six peaks (R_f 0.06;0.20;0.32;0.5;0.65;0.85; numbered 1 through 6) are seen after labeling with GDP-[^{3}H]mannose. The material under peak 6 bound to DEAE-cellulose and can be cleaved by mild acid hydrolysis, liberating mannose. Its formation was sensitive to Amphomycin, an antibiotic known to inhibit the synthesis of Dol-P-man and Dol-P-glc, and thus, may be Dol(?)-P-man. Peaks 2,4, and 5 are sensitive to PLD as are peaks 1 and 3 upon prolonged incubation. Two peaks (R_f 0.47 and 0.65) were labeled with UDP-[^{3}H]glucosamine and were tentatively identified as PI-glucosamine and PI-N-acetylglucosamine by HPAEC analysis (Dionex, Sunnyvale, CA). Compounds extractable with CMW which upon partition between water and butanol are recovered in the butanol phase were run on SI 50 000 Silica plates (Merck, Darmstadt) and developed with chloroform/methanol/ 25% ammonia/1M ammonium acetate/water (180:140:9: 8:23) as a solvent. Two major peaks (R_f 0.75 and 0.85) which are sensitive against PLD treatment are detectable and their glycan moieties were analyzed on HPAEC.

P 324 OCCURRENCE OF SULFATE ON THE N-LINKED OLIGOSACCHARIDES OF HUMAN ERYTHROPOIETIN, Thomas W. Strickland*, Beverly Adler*, Ken Aoki*, Sheilah Asher*, Patricia Derby*, Eugene Goldwasser** and Gary Rogers*, *Amgen Inc., Amgen Center, Thousand Oaks, CA 91320 and **Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637

In order to investigate sialidase resistant negative charges present on the N-linked oligosaccharides of human erythropoietin (Takeuchi et al., JBC 263, 3657, 1988 and Strickland, T.W., unpublished observations), a series of experiments were undertaken to determine whether sulfate moieties might account for such charges. Chinese hamster ovary cells which had been transfected with the human erythropoietin gene were incubated in the presence of ^{35}S-labeled sulfate. The r-HuEPO purified from the medium was found to contain the ^{35}S label. An N-glycanase digest, followed by SDS-PAGE and fluorography revealed that the label was exclusively on the N-linked carbohydrate of r-HuEPO. When the oligosaccharides released from ^{35}S-labeled r-HuEPO were chromatographed on a Dionex Carbopac PA-1 using a sodium acetate gradient in the presence of 0.1 M sodium hydroxide, the ^{35}S label co-eluted with a group of peaks [detected by the pulsed amperometric detector (PAD)] eluting at approximately 250 mM sodium acetate. In this run the bi, tri, and tetra-sialylated oligosaccharides of r-HuEPO eluted at approximately 90, 120, and 150 mM sodium acetate, respectively. From the PAD profile, the sulfated oligosaccharides account for approximately 3% of the total r-HuEPO oligosaccharides. To compare the level of sulfation of urinary derived human erythropoietin (u-EPO) to r-HuEPO, the N-linked oligosaccharides were removed from each using N-glycanase, reduced with sodium borotritide, and subjected to chromatography on a Carbopac PA-1 as above. The major radioactivity peak in the u-EPO profile co-eluted with the major sulfated r-HuEPO oligosaccharide suggesting that u-EPO also contains sulfated oligosaccharides and that these sulfated species are more prevalent in u-EPO than in CHO cell expressed r-HuEPO.

P 325 COS Cell Mutants Defective in Biosynthesis of Heparan Sulfate Proteoglycan (HSPG).
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COS cell mutants defective in the biosynthesis of heparan sulfate proteoglycan (HSPG) have been isolated by a panning selection procedure using ethyl methanesulfonate-treated COS cells and basic fibroblast growth factor-coated culture dishes. These mutant cell lines (CM-2, CM-8, CM-9, and CM-15) exhibited significantly reduced $^{35}SO_4$-incorporation into heparan sulfate (HS). For one of these cell lines, CM-15, structural analysis revealed that N-sulfated glucosamine residues were present much less frequently in HS than that derived from wild-type cells. Furthermore, CM-15 was 3-fold deficient in HS N-sulfotransferase activity. Transfection of CM-15 cells with a cDNA encoding the core protein of hamster syndecan resulted in the expression of syndecan with a reduced ratio of SO_4-incorporation into HS versus chondroitin sulfate as compared to syndecan expressed by the wild-type COS cells. These results suggest that CM-15 is partially deficient in HS N-sulfotransferase activity and demonstrate the utility of our selection method to identify mutant cell lines with defects in HSPG biosynthesis.

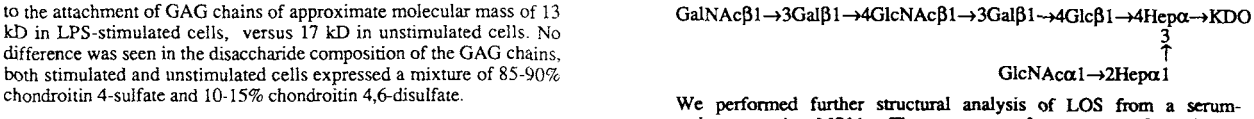
P 326 BIOSYNTHESIS AND TURNOVER OF CHONDROITIN SULFATE PROTEOGLYCAN IN HUMAN MACROPHAGES, Lars Uhlin-Hansen*, Trude Wik and Svein O. Kolset, Institute of Medical Biology, University of Tromsø, 9000 Tromsø, Norway. *Present address: National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892.

Human macrophages cultured *in vitro* were labeled with [^{35}S]sulfate and the metabolic turnover of proteoglycans were studied. The [^{35}S]sulfate was incorporated almost exclusively into chondroitin sulfate proteoglycan (CSPG). After a labeling period of 24 h about 50% of the [^{35}S]macromolecules were recovered from the culture medium, whereas the other 50% were associated with the cells. About 20% of the [^{35}S]macromolecules in the cell fraction were trypsin releasable and therefore considered to be membrane-associated. Pulse-chase kinetics indicated that most, if not all, of the membrane bound CSPG was endocytosed and degraded in the lysosomes by a two step process. In the first step the [^{35}S]CSPG was rapidly cleaved to yield free glycosaminoglycan (GAG) chains ($t_{1/2}$ = 20 min). In the second step, the GAG chains were completely depolymerized ($t_{1/2}$=2.5 h). When the cells were stimulated with lipopolysaccharide (LPS) from *E.coli* about 3 times more CSPG was released to the culture medium, whereas the turnover of the cell-associated CSPG was not significantly affected. The [^{35}S]CSPG molecules released from LPS-stimulated cells were of smaller molecular size than those released from unstimulated cells, due to the attachment of GAG chains of approximate molecular mass of 13 kD in LPS-stimulated cells, versus 17 kD in unstimulated cells. No difference was seen in the disaccharide composition of the GAG chains, both stimulated and unstimulated cells expressed a mixture of 85-90% chondroitin 4-sulfate and 10-15% chondroitin 4,6-disulfate.

P 327 THE STRUCTURES OF LIPO-OLIGOSACCHARIDES OF *NEISSERIA GONORRHOEAE*, Ryohei Yamasaki, Bradely E. Bacon, Deborah E. Kerwood and Kevin Quinn, University of California and Center for Immunochemistry, San Francisco, CA 94121.

Gonococcal lipooligosaccharides (LOSs) are important antigenic and pathogenic components of the outer membrane of *Neisseria gonorrohoeae*. Gonococci produce several different but discrete LOS components. Recent studies of gonococcal LOS revealed that the antigenicity and immunogenicity of the LOS is complex, and this complexity arises from the following factors: (a) antigenic similarity between the gonococcal LOS and host glycosphingolipids; (b) the presence and absence of human antibodies specific for certain glycosphingolipids; (c) the *in vivo* sialylation of gonococcal LOS and the resulting change in the serum sensitivity of the bacteria; (d) the interaction of gonococcal membrane proteins with glycosphingolipids that mimic gonococcal LOS components.

Many gonococcal serum-sensitive strains produce two LOS components that are recognized by MAbs 1-1-M and 3F11. We have determined the structures of OS derived from the above two LOS components by chemical and 2D NMR methods (1); N-acetylgalactosaminylated lactoneotetraose is linked to the diheptose(GlcNAc)-KDO core in the case of the MAb 1-1-M-defined LOS as shown below. The only structural difference of the two LOS components is GalNAc, and the lactoneotetraose is exposed at the non-reducing end of the MAb 3F11-defined LOS component.



We performed further structural analysis of LOS from a serum-resistant strain, MS11. The structure of the MS11 OS will be presented and the antigencity of gonococcal LOS will be discussed.

1. R. Yamasaki, B.E. Bacon, W. Nasholds, H. Schneider and J.M. Griffiss, Biochemistry (1991), *in press.*

P 328 METABOLISM OF GLYCOSYLPHOSPHATIDYLINOSITOL(GPI)-ANCHORED HEPARAN SULFATE PROTEOGLYCANS IN RAT OVARIAN GRANULOSA CELLS, M. Yanagishita, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892

Rat ovarian granulosa cells synthesize two distinct species of plasma membrane-intercalated heparan sulfate (HS) proteoglycans; glycosylphosphatidylinositol (GPI)-anchored and core protein-intercalated HS proteoglycans. Both species of HS proteoglycans are primarily localized on the plasma membrane. Cell surface localization of GPI-anchored and protein-intercalated HS proteoglycans can be determined by their accessibility to exogenously added phosphatidylinositol-specific phospholipase C (PI-PLC) and trypsin, respectively. Kinetic parameters for the processes involving their transfer from the Golgi to the cell surface, endocytosis and secretion, and the modes of intracellular degradation were determined by metabolic labeling experiments using [^{35}S]sulfate and various chase protocols in combination with the use of PI-PLC and trypsin. The data demonstrated that (1) both HS proteoglycan species are transferred from the Golgi to the cell surface with an average transit time of ~12 min, (2) GPI-anchored HS proteoglycans are endocytosed with a $T_{1/2}$ ~3 h without being shed into the medium. After endocytosis, they are degraded rapidly ($T_{1/2}$ ~25 min) without generating recognizable degradation intermediates, and (3) protein-intercalated HS proteoglycans are partly (~30%) shed from the cell surface into medium and the remaining ~70% are endocytosed with a $T_{1/2}$ ~4-6 h. After endocytosis, they undergo slow ($T_{1/2}$ ~4 h), stepwise degradation generating distinct degradation intermediates. These results indicate that two HS proteoglycan species synthesized by rat ovarian granulosa cells have distinct secretory, endocytotic and intracellular degradation pathways probably due to the differences in their membrane anchor structures.

P 329 ATTACHMENT INHIBITION OF *CHLAMYDIA TRACHOMATIS* TO MAMMALIAN HOST CELLS BY CHEMICALLY MODIFIED HEPARIN ANALOGS, Jian Ping Zhang[1] and Richard S. Stephens[2], [1] University of California, Berkeley, [2]University of California, San Francisco, CA 94143-0412, USA

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that is the cause of a wide variety of diseases in humans. Chlamydiae attach to host cells and enter 'nonprofessional phagocytic' cells by receptor-mediated endocytosis. Heparin is known to be a potent inhibitor of attachment of chlamydia to mammalian cells *in vitro*. Previous experiments in our laboratory indicated that a heparin-like glycosaminoglycan mediates attachment of chlamydiae to host cells. We investigated the specificities of heparin inhibition using chemically modified heparin analogs. *O*-sulfated/*N*-desulfated heparin inhibited attachment of chlamydia nearly as effectively as heparin (i.e. > 90%). In contrast, *O*-desulfated/*N*-sulfated heparin, and completely desulfated heparin, had little effect on chlamydial attachment. In the context of host cell biology, heparan sulfate was determined to be as effective as heparin in chlamydial attachment inhibition,whereas other glycosaminoglycans had no competitive effect. Heparan sulfate binding proteins were used to pretreat host cells or chlamydiae prior to attachment assessments. Only pretreatment of organisms reduced attachment by >90%. These findings demonstrate a requirement for *O*-sulfation of heparin inhibitors or chlamydial attachment, and suggest a specific and functional role for chlamydia-associated heparan sulfate in attachment of chlamydiae to host cells.

P 330 Predominant basolateral localization of GPI-anchored proteins in a polarized thyroid epithelial cell line.

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We characterized the polarity of some endogenous membrane proteins in FRT (Fisher rat thyroid) epithelial cell line and compared it with that observed in kidney and intestinal cell lines. Using immunofluorescence and domain selective surface biotinylation combined with immunoprecipitation we found that the distribution of several apical (DPPIV) and basolateral markers (ßNaK-ATPase, uvomorulin, transferrin receptor, 35-40 Kd Ag, ZO1) was identical to that displayed by these antigens in the model epithelial cell lines MDCK and Caco2. Unexpectedly, we observed a different distribution of the endogenous GPI-anchored proteins in the plasma membrane of FRT cells, compared to that one shown by other cell lines. It has been shown in different kidney and intestinal epithelial cells (MDCK, LLCPK, Caco2 and SKCO-15) that endogenous GPI-anchor proteins are apically polarized. Furthermore, recombinant transfer of GPI to basolateral or secretory proteins results in their targeting to the apical domain suggesting a possible role of GPI as an apical targeting signal. We found that endogenous GPI anchored proteins of FRT cells are preferentially localized on the basolateral domain, while some of them are apical and some are not polarized. Exogenous GPI anchored protein introduced by transfection also showed a different distribution to that observed in MDCK cells. Decay accelerating factor (DAF) which is apically localized in MDCK, was found equally distributed on both membrane domains of FRT cells. A chimeric GPI anchored protein (gD1-DAF) formed by the ectodomain of the Herpes glycoprotein gD1 and the DAF signal for GPI-addition, was basolateral in FRT cells, whereas it has been shown to be apically distributed in MDCK cells. We conclude that GPI does not appear to act as an apical targeting signal in FRT.

Glycoconjugates and The Cell: Organelles, Extracellular Matrix, Cell-Cell Recognition, and Development

P 400 LECTIN BINDING ON FRESH AND CAPACITATED HUMAN SPERM, Marie C Béné, Bernard Foliguet, Gilbert C Faure. Laboratoire d'Immunologie & IVF unit, Faculté de Médecine, CHU & Maternité Régionale, 54000 Nancy, France.

The fertilizing ability of sperm has been shown to depend on the presence or exposure of critical membrane molecules on these cells. Several proteic capacitation structures have thus been identified, as well as a number of glycosylated residues. Such studies have mostly been performed on animal sperm (boar, pig, bull...) with selected lectines. We used a large panel of 15 fluorescein-conjugated lectins to identify sugar residues on the surface of fresh or capacitated human sperm obtained for enumeration and vitality assessment prior to in vitro fertilization attempts in the local IVF unit. Capacitation was induced by gradient centrifugation on Percoll. Fresh sperm was used undiluted and capacitated sperm was resuspended in RPMI 1640, at the initial concentration. All lectins (Vector, USA) were used diluted 1:50 in phosphate buffered saline (PBS). After 30 minutes incubation, the samples were washed in PBS supplemented with bovine serum albumin, and fixed in .1% paraformaldehyde. The number of labelled sperm cells, and the fluorescence intensity with each lectin were assessed in flow cytometry (Epics Profile, Coultronics, Hialeah FL). The remaining labelled sperm cells were cytocentrifuged and examined in UV light and confocal microscopy in order to determine the topography of lectin binding. Individual patterns of sperm-lectin reactivity were identified, and correlated with the outcome of IVF. Inter-individual differences were noted in terms of number of positive sperm, lectin binding pattern after capacitation and labelling intensity, both on fresh and capacitated sperm. Direct observation of the labelling allowed to identify the areas (acrosome, ring, intermediate piece or tail) critically affecting sperm fecundability. These data suggest that flow cytometry is a valuable tool to assess the presence or appearance of glycosylated structures important in human fecondation.

P 401 DIVERSITY OF SPECIFICITY OF MONOCLONAL ANTIBODIES OBTAINED BY IMMUNIZATION WITH SYNTHETIC CARBOHYDRATE ANTIGEN CD15 (Le^x) N.V.Bovin, * O.E.Galanina, *E.Yu.Korchagina, S.P.Sidorenko*, S.V.Mikhalap*, Shemyakin Institute of Bioorganic Chemistry, Moscow 117871, and *Kavetsky Institute of Oncology Problems, Kiev 252022, USSR

Trisaccharide Galβ1--4(Fucα1--3)GlcNAc is an antigenic determinant of CD15 (alias Le^x, X, SSEA-1). This trisaccharide as well as its sialylated form (SiaLe^x) mediates number of cell adhesion processes taking part in particular in inflammations as ligands of selectins. At the same time an antigenic determinant of blood group antigen Le^a is a trisaccharide Galβ1--3(Fucα1--4)GlcNAc having a similar topography of Gal and Fuc moieties as trisaccharide Le^x. We studied the possibility of specific anti-Le^a monoclonal antibodies (MA) generation when Le^x antigen was used as an immunogen.

Spacered trisaccharide $Le^xO(CH_2)_3NH_2$ was bound to an activated polymer along with phosphatidylethanolamine (PE). Polymeric neoglycolipid Le^x-pol-PE obtained was incorporated into *S. minnesota* and mice were immunized by the conjugate. The specificity of the MA obtained was studied by ELISA (plates sensibilized by polymeric conjugates of trisaccharides Le^a and Le^x). From 117 anti-Le hybridomas 78 produced MA reacting equally with Le^x and Le^a, 19 reacting considerably better with Le^x and 20 preferring Le^a. Only a few hybridomas produced MA highly specific to Le^x and one hybridoma - MA to Le^a. So, an immunization with Le^x may result in production of specific anti-Le^a MA.

P 402 LAMININ AS A LECTIN-LIKE MOLECULE. Roger Chammas, Silvio S. Veiga, Luiz R. Travassos* and Ricardo R. Brentani. Ludwig Institute for Cancer Research - São Paulo Branch and *Disciplina de Biologia Celular, Escola Paulista de Medicina - São Paulo, Brasil.

Adhesion and migration on basement membranes are keysteps along tumor progression. It is likely that those events are mediated by specific receptors to basement membrane components, as laminins and collagens. We have characterized a murine melanoma glycoprotein (gp120/140) as a specific receptor for EHS-laminin. This receptor is immunologically related to VLA-6 integrin, which is a laminin receptor in several human models. Differently from most extracellular matrix component interactions with *their integrin receptors, laminin interaction* with VLA-6 is not RGD-dependent. We could indeed show that, in the murine model, such an interaction was sensitive to mild oxidation of integrin carbohydrates, as well as to endoglycosidase digestion of the receptor with n-glycanase. Exoglycosidase treatment of the receptor allowed us to identify galactoside residues as recognition elements in laminin interaction with this integrin. Laminin interactions with sulfatides, heparan-sulfate proteoglycan and now with integrin oligosaccharides strenghthen the concept that this extracellular matrix glycoprotein may act as a lectin-like molecule.

Supported by FAPESP.

P 403 CHEMICAL AND BIOCHEMICAL STUDIES ON OLIGOSACCHARYLTRANSFERASE: A KEY ENZYME IN N-LINKED GLYCOPROTEIN BIOSYNTHESIS. James K. Coward, Jung Lee, Richard S. Clark, and Shyamal Banerjee, Interdepartmental Program in Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, The University of Michigan, Ann Arbor, MI 48109.

We have initiated research to elucidate the mechanism of the reaction catalyzed by yeast oligosaccharyltransferase (OST, EC 2.4.1.119). As a first step, we have synthesized small substrates (peptides, lipid disaccharide) and products (glycopeptides) in order to characterize the reaction more completely. Specifically, we have used the tripeptide, Bz-Asn-Leu-Thr-NH_2, the corresponding *iso*Asn-containing tripeptide, and three stereospecifically labeled *d1*Asn-containing tripeptides as substrate probes. In addition, we have used glycopeptides and glyco*iso*peptides containing either one or two GlcNAc residues to establish unambiguously the structure of the product of this *unusual reaction. Using either biosynthetic* ^{3}H-labeled lipid oligosaccharide (LOS) or lipid disaccharide (LDS), we have shown by HPLC that the ^{3}H-labeled glycopeptide product is the commonly accepted regioisomer; i.e., no *iso*peptide-containing product is formed. Using chemically synthesized LDS, we have carried out a large-scale reaction and shown that the biosynthetic glycopeptide is identical by ^{1}H-NMR and HPLC to a synthetic glycopeptide containing chitobiose linked to the tripeptide *via* Asn. Using synthetic LDS and three deuterium labeled tripeptide substrates, we have shown by ^{1}H-NMR that each of the three deuterium atoms are retained in the glycopeptide product, thus excluding two possible nucleophilic activation mechanisms. Recent results which extend these studies will also be presented. (Supported in part by a grant from the NIH, GM30286, and the Program in Protein Structure & Design, The University of Michigan)

P 404 PURIFICATION AND CHARACTERIZATION OF AN α-L-FUCOSIDASE FROM *AMPULLARIA*, Tamao. Endo[1], T. Tsukada[2], M. Hiraiwa[3], Y. Uda[3] and A. Kobata[1], [1]Dept. of Biochemistry, Inst. of Medical Science, University of Tokyo, Tokyo, [2]Research Institute of Tokyo Zouki Chemical Co.,Chiba, and [3]Lab. of Health Chemistry, Niigata College of Pharmacy, Niigata, Japan.

Glycoconjugates containing α-linked fucose residues are widely distributed and their structural features are complicated and diversity. Since content and structures of these fucosylated glycoconjugates are changed during cell differentiation, development and transformation, they are considered to play many important roles on cell recognition and it is therefore very important to determine their structures. α-L-Fucosidase is not only a good tool for structural analysis but also for elucidation of biological function of the fucosylated oligosaccharides. Many α-fucosidases from various sources including mammalian tissues, bacteria, invertebrates, and plants were isolated and characterized. In this paper, we will report an α-fucosidase from *Ampullaria* which shows novel substrate specificity.

The enzyme was purified about 285-fold from the crude enzyme extract by procedures involving ammonium sulfate fractionation, heat treatment and chromatographies on DEAE-Sepharose, hydroxylapatite and L-fucosylamine-CH-Sepharose. The final preparation was sufficiently free from other glycosidase activities and gave a single protein band which corresponded to α-fucosidase activity on disc gel electrophoresis. The enzyme has two optimum pH values, 3.0 and 6.0, and the apparent Km value and the maximum velocity for p-nitrophenyl α-L-fucoside were calculated to be 0.45 mM and 1.46 μmol/min/mg of protein, respectively. Toward natural substrates, the enzyme hydrolyzed Fucα1→2Gal, Fucα1→4GlcNAc, and Fucα1→6GlcNAc linkages, but not Fucα1→3GlcNAc linkage. The substrate specificity revealed was unique and different from any α-fucosidases studied previously, and the enzyme is a useful reagent for the sequencing and characterization of the fucosylated oligosaccharides.

P 405 LECTIN-CARBOHYDRATE INTERACTIONS IN THE REGULATION OF HEMOPOIESIS. Othmar Förster, Walter Krugluger, Martina Allmaier, Alois Gessl, Markus Köller, Georg Boltz-Nitulescu. Inst. of Exptl. Pathology, Univ. of Vienna, Austria.

Carbohydrates have been identified as important recognition structures in the hemopoietic system. Previously we have reported that soy bean agglutinin (SBA) binds weakly to the majority of blast cells in freshly isolated rat bone marrow (BM) but strongly to mature macrophages (MΦ) (W.Krugluger et al., J.Leukocyte Biol. **48**: 451, 1990). At least 2 different SBA-binding structures have been identified: a 26 kDa glycoprotein on freshly isolated BM-cells (BMC) and a 160 kDa glycoprotein on BM-derived MΦ (BMDMΦ). To investigate the functional role of lectin carbohydrate interactions in the hemopoietic system we have cultured rat BMC in the presence or absence of colony stimulating factor (recomb. murine GM-CSF) and/or SBA. It was found that addition of SBA to BMC cultures markedly enhanced the proliferation inducing effect of rmGM-CSF. SBA alone did not induce proliferation but seemed to induce differentiation. SBA did not seem to recruit additional cells into the MΦ precursor pool since the number of methyl cellulose colonies was not increased by SBA. However, pretreatment of BMC with GalNAc lead to an increase of colony number, which may be due to the removal of an endogenous lectin. The presence of such lectin was demonstrated by SDS-PAGE analysis of material eluted from bone marrow by GalNAc. We conclude that lectin-carbohydrate interactions may play a role in the regulation of myelopoiesis *in vivo*.

Supported by the Austrian Science Research Fund and Anton Dreher Gedächtnisschenkung für Medizinische Forschung.

P 406 PHOSPHORYLATED *N*-LINKED OLIGOSACCHARIDE BIOSYNTHESIS IN *DICTYOSTELIUM*, Hudson H. Freeze, *Ole Hindsgaul, Mie Ichikawa, and Melinda Larson, La Jolla Cancer Research Foundation, La Jolla, CA 92037 and * University of Alberta, Edmonton, Alberta, T6G 2G2 Canada.

N-linked oligosaccharides on lysosomal enzymes in *Dictyostelium* contain Man-6-P as a phosphomethyldiester (Man-6-P-OCH_3). Its role in lysosomal enzyme targeting is unproven. We have identified enzymes that are probably involved in the biosynthesis of this unusual molecule. The first two enzymes are similar to those used by mammalian cells, but the third is unique. First, a previously identified GlcNAc-1-P:oligosaccharide transferase donates GlcNAc-1-P from UDP-GlcNAc to selected Man residues to form a GlcNAc-1-P-6-Man diester. We have now identified an "uncovering" enzyme that converts the diester to a monoester (Man6P) and is specifically inhibited by GlcNAc-1-P. This enzyme also resembles its mammalian counterpart. Finally, a novel Man-6-P-specific phosphate methyltransferase completes the pathway. This enzyme prefers model oligosaccharides with terminal α1→2 linked Man-6-P residues on structures that resemble the known phosphorylated branches (Km 0.15mM). All three membrane bound enzymes co-fractionate on sucrose gradients, and are good candidates for the first enzymatic markers of the *Dictyostelium* Golgi. *N*-linked oligosaccharides synthesized later in development are more highly processed than in vegetative cells, and lack the terminal α1→2 linked Man residues required by GlcNAc-1-P:oligosaccharide transferase. We, therefore, expected that the enzymes would all be down-regulated. Surprisingly, GlcNAc-1-P transferase increases about 5 fold during development, and while uncovering enzyme remains about the same, phosphate methyltransferase decreases to almost undetectable levels late in development. Since Man-6-P containing proteins continue to be made, the results suggest that Man6P addition and processing are carefully regulated (NIGMS32485).

P 407 STRUCTURE, FUNCTION, AND TISSUE-SPECIFIC EXPRESSION PATTERNS OF MURINE α(1→3)FUCOSYLTRANSFERASE GENES , Kevin M. Gersten and John B. Lowe, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109

Oligosaccharide structures represent a major component of the antigenic makeup of animal cell surfaces and are thought to function in cellular interactions during development, differentiation, oncogenic transformation, and inflammation. Evidence suggests that α(1→3) fucosylated oligosaccharides mediate morula compaction and leukocyte adhesion to the vascular endothelium. Analysis of the biological effects of modifying the glycan products expressed in an animal, through transgenic and embryonic stem cell technologies, may provide important insights into the significance of fucosylated oligosaccharides. The mouse is an ideal system to further characterize the role played by α(1→3) fucosylated oligosaccharides during development and in the immune response because it is a mammalian species with defined genetics. The murine system will also facilitate the definition of the developmental and tissue-specific expression of fucosyltransferase genes, and the genetic elements which control these patterns. In preparation for these studies, we have begun to isolate murine α(1→3) fucosyltransferase (α(1→3)FT) genes using human α(1→3)FT sequences and cross hybridization techniques. The protein predicted by one murine α(1→3)FT gene shares 75% amino acid identity with the human Fuc-TIV α(1→3)FT and is predicted to have a type II membrane topology. Transfection of the murine gene into cultured cells results in the expression of an α(1→3)FT which efficiently utilizes the type II acceptor *N*-acetyllactosamine to form the Lewis x determinant [Galβ1→4(Fucα1→3)Glc*N*Ac]. The enzyme reacts only 2/5 as efficiently with the substrate Neu*N*Acα2→3Galβ1→4Glc*N*ac to form the sialyl Lewis x determinant, and is incapable of determining cell surface expression of this antigen in COS-7 cells. Biochemical analyses defined the optimal Mn^{2+} concentration and pH for enzyme activity as 15 mM and 7.5, respectively. The α(1→3)FT exhibits an apparent Michaelis constant of 16.6 μM for GDP-Fucose and 660 μM for *N*-acetyllactosamine. Tissue-specific expression studies indicate that the α(1→3)FT gene is expressed in a variety of tissues, with highest transcript levels occurring in bone marrow, colon, and stomach. The characterization of an α(1→3)FT pseudogene and two additional candidate α(1→3)FT genes will also be presented.

P 408 RESOLUTION OF TWO PATHWAYS FOR PROTEIN DEGRADATION IN THE ENDOPLASMIC RETICULUM BY DIFFERENTIAL SENSITIVITY TO OLIGOSACCHARIDE STRUCTURE, Reza Green[1], Su Kui[1], Timothy Stoller[2], and James Rocco[1], [1]Dept. of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029 and [2]Dept. of Developmental Biology and Cancer, Alb. Einstein Coll.of Medicine, Bronx, NY 10461.

Degradation of newly synthesized proteins in the lumen of the endoplasmic reticulum (ER) appears to be part of a quality control mechanism that ensures that only properly folded polypeptides are transported to distal compartments of the secretory pathway. The nature and location of the relevant proteases, and the structural determinants of degradation, are, however, still unclear. Using heterologous expression of yeast preproαfactor in GH3 (rat pituitary) cells, we have resolved two pathways for ER degradation which function sequentially and are differentially sensitive to N-linked oligosaccharide structure. In GH3 cells, glycosylated proαfactor (gpαf) synthesized at the ER is efficiently degraded following transport to a late ER-early Golgi compartment ($t_{1/2}$= 25-30 min). The substrate for constitutive proteolysis is gpαf containing $GlcNac_2$-Man_{5-8} oligosaccharides. Notably, Man_9-gpαf is stable, indicating that oligosaccharide processing beyond Man_9 is required to generate a protease-sensitive conformation.

A second degradation mechanism is evident when ER glucosidases I and II are inhibited by treatment of cells with deonxynojirimycin (DNM). A large proportion of newly synthesized glucose-containing gpαf (Glu-gpαf) is proteolyzed much more rapidly than is control gpαf. Importantly, in contrast to constitutive degradation, this process is unaffected by incubation at 15°C, localizing the second proteolytic apparatus to the proximal cisternae of the ER. Preliminary experiments indicate that only DNM-induced, and not constitutive, degradation can be reconstituted in a cell-free translation system containing canine microsomes; this will considerably facilitate characterization and purification of the relevant protease(s). These data reveal a hierarchy of proofreading mechanisms in the ER that sense secretory protein conformations at different stages of transport. The different substrate selectivities exhibited by the two pathways argue that 1) small changes in carbohydrate structure can alter polypeptide conformation, and 2) protease susceptibility in this system can be used as a sensitive biological monitor of protein folding. (This work was supported by NIH grant GM35801 to R.G.)

P 409 CELL SURFACE β1,4 GALACTOSYLTRANSFERASE MEDIATES NEURAL CREST CELL MIGRATION AND NEURULATION *IN VIVO*, Helen J. Hathaway and Barry D. Shur, Department of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030.

Mesenchymal cell migration and neurite outgrowth are mediated in part by binding of cell surface β1,4 galactosyltransferase (GalTase) to *N*-linked oligosaccharides within the E8 domain of laminin. In this study, we determined whether cell surface GalTase functions during neural crest cell migration and neural development *in vivo* using antibodies raised against affinity purified chicken serum GalTase. The antibodies specifically recognized two embryonic proteins of 67 and 54 kDa, both of which express GalTase activity. The antibodies also inhibited chick embryo GalTase activity and inhibited neural crest cell migration on laminin matrices *in vitro*. Anti-GalTase antibodies were microinjected into the head mesenchyme of stage 7 - 9 chick embryos or cranial to Henson's node of stage 6 embryos. Anti-avian GalTase IgG decreased cranial neural crest cell migration on the injected side but did not cross the embryonic midline and did not affect neural crest cell migration on the uninjected side. Anti-avian GalTase Fab crossed the embryonic midline and perturbed cranial neural crest cell migration throughout the head. Neural fold elevation and neural tube closure were also disrupted by Fab fragments. Cell surface GalTase was localized to migrating neural crest cells and to the neural epithelial basal lamina by indirect immunofluorescence, whereas GalTase was undetectable on neural crest cells prior to migration. These results suggest that during early embryogenesis cell surface GalTase participates in neural crest cell migration, perhaps by interacting with laminin, a major component of the basal lamina. Cell surface GalTase also appears to play a role in neural tube formation, possibly by mediating neural epithelial interaction with its basal lamina. Supported by DE 07120 to BDS.

P 410 CLONING, CHARACTERIZATION AND DISRUPTION OF AN α-1,2-MANNOSYLTRANSFERASE FROM *SACCHAROMYCES CEREVISIAE*, Alex Häusler and Phillips W. Robbins, Center for Cancer research, Massachusetts Institute of Technology, Cambridge, MA 02139

A gene encoding an α-1,2-mannosyltransferase from *Saccharomyces cerevisiae* was cloned and sequenced[1]. The α-1,2-mannosyltransferase activity was purified and could be assigned to a 41 kD protein band on SDS-PAGE. Degenerate oligonucleotide primers corresponding to the amino acid sequences from internal peptides were used for polymerase chain reactions on yeast genomic DNA. A specific reaction product was used to screen a genomic library of *S.cerevisiae*. A fragment of about 5.7 kb was isolated which contained a 1329 base pair open reading frame encoding the peptide sequences of the purified α-1,2-mannosyltransferase. The gene, designated *MNT1*, encodes a 442 amino acid polypeptide with a calculated molecular weigth of 51.4 kD. The corresponding mRNA has a length of approximately 1.6 kb. Overexpression of the *MNT1* gene increased this α-1,2-mannosyltransferase activity about 2.5-fold. The enzyme contains a putative membrane spanning domain near its N-terminus and its topology is thus similar to that of mammalian Golgi glycosyltransferases. Disruption of the *MNT1* gene revealed that the α-1,2-mannosyltransferase is involved in the elongation of *O*-linked oligosaccharide chains.

[1] Häusler and Robbins (1992) Glycobiology in Press

P 411 PROCESSING OF GLUCOSYLCERAMIDES IS REQUIRED FOR MAINTENANCE OF THE EPIDERMAL PERMEABILITY BARRIER Walter M. Holleran, Yutaka Takagi, and Peter M. Elias, Department of Dermatology, University of California, San Francisco and Dermatology Service (190), Veterans Admin Medical Center, 4150 Clement St., San Francisco, CA 94121.

The outer layer of the mammalian epidermis (stratum corneum) contains abundant interstitial lipids which are critical for preventing water loss through the skin. These lipids, which are enriched in ceramides (Cer), cholesterol and fatty acids, form membrane bilayers in the interstices of the stratum corneum (SC). During the terminal stages of epidermal differentiation, the content of glucosylceramide (GlcCer) decreases as the ceramide (Cer) content increases. To determine whether enzymatic hydrolysis of glucosylceramide to ceramide is important for epidermal permeability barrier function, we first characterized ß-glucocerebrosidase (ß-GlcCer'ase) activity in murine epidermis. Next, we assessed whether inhibition of ß-GlcCer'ase effected epidermal barrier function, lipid content, and stratum corneum morphology. We found abundant mRNA for ß-GlcCer'ase and significant enzymatic activity present in whole epidermal extracts. Daily topical applications of bromoconduritol-B-epoxide (BrCBE) to murine skin caused a gradual, reversible perturbation in barrier function (increased transepidermal water loss) that was not reversed by coapplications of Cer. This effect correlated with an 80% inhibition of ß-GlcCer'ase activity while other glycosidases were not affected. Moreover, BrCBE applications increased GlcCer content in both whole epidermis and isolated SC, while Cer content remained largely unchanged. Light microscopic histochemistry showed increased PAS-positive staining in BrCBE-treated SC, consistent with increased GlcCer content. Electron microscopy (ruthenium tetroxide) of these specimens revealed marked changes in intercellular membrane bilayers of the SC, consistent with incomplete GlcCer hydrolysis. No evidence of toxicity or changes in the lamellar body secretory system were observed. These findings suggest that GlcCer-to-Cer processing is critical for epidermal permeability barrier maintenance, and persistence of GlcCer into the stratum corneum induces altered membrane bilayers resulting in abnormal barrier function.

P 412 CELL SURFACE GLYCOCONJUGATES IN THE ADHESION OF PNEUMOCYSTIS CARINII TO MAMMALIAN CELLS, Michael N. Horst, Anna N. Walker and Ronald E. Garner, Departments of Basic Medical Sciences and Pathology, School of Medicine, Mercer University, Macon GA 31207

Pneumocystis carinii is an opportunistic pathogen that is a major cause of morbidity in immunocompromised patients. Using immunochemical methods, we have previously shown that chitin is found in the cell wall of all forms of this organism: trophozoites, cysts and intracystic bodies (Walker, et al., 1990, Infect. Immun. 58, 412). In the present study, we have examined the distribution of chitin in the P. carinii cell wall using colloidal gold labeled lectins and chitinase. Transmission electron microscopy indicated that chitin does not appear to form a continuous layer in the P. carinii cell wall; rather, a punctate pattern of labeling is observed with both tomato lectin-gold (ToL-Au) and chitinase-gold conjugates. We have also found that treatment of P. carinii with Lyticase, a beta 1,4 glucanase, removes chitin from the cell wall, suggesting that chitin may be attached to glucan as it is in Saccharomyces. When P. carinii were grown in tissue culture using mink lung cells as the feeder layer, fluorescence microscopy with fluorescein-ToL (Fl-ToL) showed a similar punctate pattern of labeling in the Pneumocystis cell wall. Fl-ToL labeled the host cell Golgi apparatus and its associated vesicles; such labeling is most likely due to ToL binding to the N-acetylchitobiosyl core of N-linked glycoproteins. In cells infected with P. carinii, fluorescent labeled vesicles were observed in the feeder cells adjacent to the P. carinii attachment site; similar vesicles have been observed by Nielsen and Settnes (APMIS 99:219-225,1991). Based upon our fluorescence and Namarski interference microscopy data, we believe that these vesicles are being recruited to the site of attachment. The effect of certain antibiotics, such as brefeldin, on this recruitment process are now in progress. Possible signals for recruitment include Pneumocystis binding to receptors on the host cell surface or release of signaling molecules by P. carinii. In order to identify putative attachment molecules, both feeder cells and P. carinii infected feeder cells have been labeled with 3H glucosamine and radiolabeled components separated by SDS-PAGE. Autoradiographic and Western blot analysis revealed the presence of an 80 kD chitoprotein only in infected cells. Further studies on the role of chitin, glucan and mannan in the adhesion of P. carinii to the host cells are currently underway. Supported by NIH Grant AI-29638.

P 413 THE GALACTOSYLATION OF BOVINE RHODOPSIN, Edward L. Kean and Jr-Min Ju, Departments of Ophthalmology and Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

While trace amounts of galactose have been observed in bovine rhodopsin, it is unresolved whether or not this sugar is a natural constituent of rhodopsin, even as a transitory component. Most analyses of rhodopsin have not reported its presence. From the structures of the oligosaccharide chains of rhodopsin, the presence of a terminal unsubstituted GlcNAc provides a site for galactosylation, and indeed the capacity for rhodopsin to add this sugar in *in vitro* reactions is clear. This situation implies the functioning of strict regulatory mechanisms in the retina which govern the galactosylation of this key molecule of the visual process. There has been little information published concerning the properties of this reaction. With the possibility that such a study might provide information concerning the capacity of rhodopsin to be modified in this manner, as well as information concerning regulation, many of the kinetic properties of this reaction were examined using preparations from milk, rat liver, and retina from cattle and the embryonic chick as enzyme sources, and rhodopsin, opsin, GlcNAc, and ovalbumin as acceptors. Golgi-enriched fractions of retina showed very limited activity as compared to other tissues; the V_{max}/K_m ratios being from 0.1% -0.2% of that of the other tissues examined. Using the enzymes from rat liver and retina, it was observed that the conformation of the molecule may play a role in galactosylation since opsin (light-bleached rhodopsin) was shown to be a much better acceptor of galactose than rhodopsin. The light-dark effect was not seen with the milk enzyme, nor with the galactosylation of ovalbumin. α-Lactalbumin inhibited the galactosylation by each of the enzymes. As revealed by the use of specific galactosidases, the linkage of galactose in enzymatically galactosylated rhodopsin catalyzed by the retina enzyme was shown to be β-(1-4). Supported in part by EY 00393 and EY 03685, and the Ohio Lions Eye Research Foundation.

P 414 EXPRESSION OF LEWIS X AND SIALYL LEWIS X EPITOPES IN AVIAN B LYMPHOCYTE DEVELOPMENT CORRELATES WITH TISSUE SPECIFIC LOCALIZATION OF IMMUNE CELLS, Robert D. Larsen[1], Louise M. Carlson[1], James Pickel[2], John B. Lowe[1], Craig B. Thompson[1], and Kelvin P. Lee[1], [1]Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109-0650 and [2]NCI-Frederick Cancer Research Program, Frederick, MD 21702-1201.

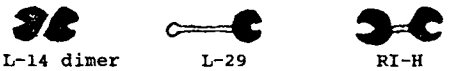
Among avian species, normal B lymphocyte development requires that pre-B cells home to a specialized organ, the Bursa of Fabricius, to undergo further maturation. This homing occurs between day 8 and day 14 of embryogenesis. During this time period/developmental stage, we find that the bursa becomes populated with cells that express sialyl-Lewis x (sLex) epitopes but which remain Lewis x (Lex) negative. Following initial colonization, bursal lymphocytes undergo a critical period of expansion from day 15 to several days post-hatch (day 21). From day 15 to day 18, more than 99% of the bursal lymphocytes switch their cell surface phenotype from high level expression of sLex epitopes to high level expression of Lex epitopes. This high level expression of Lex epitopes is maintained until 1 week post-hatch. From one week post-hatch onward, a progressively larger population of bursal lymphocytes downregulates surface expression of Lex (by nearly 100 fold), to the point where, at 10 weeks post-hatch, these Lex-negative cells constitute nearly all of the bursal population. Only these "low expressors" of Lex epitope are found in peripheral lymphoid organs and most likely represent mature B cells.

To examine this process at the molecular level, we used a cloned human fucosyltransferase gene that is expressed in the human myeloid lineage (Fuc-TIV, ELFT), to isolate a chicken homologue. Using this putative chicken α(1,3)FT as a probe, we have identified a 4 kb message that is expressed in bursa, thymus, gizzard, eye, and brain. Expression of this message correlates with B cell surface expression of Lex and sLex epitopes during the developmental processes alluded to above. Experiments are underway to determine the DNA sequence of the candidate chicken α(1,3)FT gene and to test for functionality of the clone by transfection into COS-7 cells. These data indicate that expression of the Lex and sLex carbohydrate epitopes is associated with specific stages of B cell development, and further suggest that these carbohydrate molecules may participate in processes that determine tissue-specific localization of developing immune cells.

P 415 L-29 and RI-H: two lectins from epithelial linings. Leffler, H., Oda, Y., Lindstedt, L., Herrmann, J., Gitt, M., Apodaca, G., Mostov, K., Barondes, S. Dept. Psychiatry, UC San Francisco, CA 94143

The common defining feature of soluble lactose binding (S-Lac) lectins is that they contain a lactose binding domain (LBD) of about 14 kDa. To understand their biological role we have examined the structure, specificity and localization of two S-Lac lectins, RI-H and L-29, in mucosal tissue.

RI-H has a new structure (shown compared to L-29 and another common S-Lac lectin, L-14 in the scheme below) consisting of two active LBDs (shown filled) within one peptide. The two RI-H LBD sequences and specificities are more related to the L-29 LBD than to L-14.



L-14 dimer L-29 RI-H

L-29 is abundant in columnar epithelial cells of intestine, lung and kidney while RI-H is restricted to intestine.

The interaction between an LBD and a potential ligand requires, besides the appropriate binding specificity, a colocalization of the two. In order to address the latter we have initiated studies of L-29 in polarized epithelial cells (MDCK) grown on filters where it is abundantly expressed (>10^6 molecules per cell). A fraction was externalized by a non-classical pathway, predominantly to the apical medium. This suggests that L-29 may interact with luminal components such as mucins, apical membrane glycoproteins and microorganisms. High temperature and a Ca ionophore increased L-29 secretion suggesting regulation.

Supported by grants from TRDRP, CA and NIH.

P 416 EXPRESSION OF CLONED ß-GALACTOSIDE α2,6-SIALYLTRANSFERASE ALTERS TERMINAL GLYCOSYLATION IN BACULOVIRUS-INFECTED INSECT CELLS IN SPITE OF ENDOGENOUS SIALIDASE ACTIVITY. Peter Licari and James E. Bailey, California Institute of Technology, Pasadena, CA 91125.

A recombinant baculovirus (ST-AcMNPV) containing a rat liver ß-galactoside α2,6-sialyltransferase gene under the control of the polyhedrin promoter was constructed. *Spodoptera frugiperda* cells infected with this virus produced ß-galactoside α2,6-sialyltransferase DNA, appropriate sized RNA transcripts, and the protein while mock-infected and wild-type infected cells did not. Activity of α2,6-sialyltransferase synthesized in ST-AcMNPV infected cultures was demonstrated by the *in vitro* sialylation of asialofetuin. Studies with FITC-labelled lectin specific for the the NeuAcα2,6Gal linkage indicated that a variety of cellular and/or viral proteins associated with the cell surface that are not ordinarily sialylated were sialylated *in vivo* in ST-AcMNPV infected cells but not in controls. This occurred relatively late in infection. Furthermore, intracellular localization of lectin binding was observed. Besides demonstrating *in vivo* activity of the *heterologous transferase*, these studies suggest that the necessary oligosaccharide and CMP-sialic acid precursors exist in these cells. The existence of an endogenous, cellular sialidase was demonstrated. The relative activity levels of endogenous sialidase and recombinant α2,6-sialyltransferase may explain the disparity in time between the beginning of α2,6-sialyltransferase at 24 hours post-infection and lectin binding very late in infection. In spite of the endogenous sialidase activity, these results demonstrate the ability to alter the oligosaccharides of glycoproteins produced in insect cells via the expression of heterologous glycosyltransferases using engineered baculoviruses.

P 417 A GLYCOSYLATED DOMAIN THAT FACILITATES SECRETION IN YEAST, Vivian L. MacKay, Michael Zavortink and Susan Welch, ZymoGenetics, Inc., Seattle, WA 98105

Mating-type **a** cells of *Saccharomyces cerevisiae* produce an extracellular acid protease (called the Barrier or Bar protease) that has three proposed domains. The first two exhibit strong homology to the two catalytic domains of aspartyl proteases. The third domain (191 amino acids) is unique and contains three potential asparagine-linked glycosylation sites and 33% serine + threonine. Electrophoretic mobilities and Concanavalin A binding of Bar protease ± incubation with endoglycosidase H indicate that this domain has substantial amounts of O-linked carbohydrate.

Deletion analysis has shown that the first 36 amino acids of the third domain (with the amino-terminal signal peptide) are necessary and sufficient to promote export of the protease or of heterologous proteins. This segment has one sequence (PWSTNE) that could be either a phosphorylation site or an O-linked carbohydrate site. Mutation of the serine to alanine decreased the steady-state level of extracellular protease by *ca.* 50% and eliminated the microheterogeneity seen on Western blots of the parent polypeptide. These results suggest that O-linked glycosylation of this segment contributes to the protein's secretion/export, but is not the only factor.

P 418 **THE STRUCTURE AND ROLE OF O-GLYCANS ATTACHED TO LYSOSOMAL MEMBRANE GLYCOPROTEINS, LAMP-1 AND LAMP-2,** Kentaro Maemura, *Sven R. Carlsson, Wei-Chun Wang, Ni Lee, and Minoru Fukuda, La Jolla Cancer Research Foundation, La Jolla, CA 92037 and *University of Umeå, Umeå, Sweden.

We have shown previously that both human lamp-1 and lamp-2 molecules contain *N*-acetylgalactosamine, which suggest the presence of *O*-glycans in these molecules (J. Biol. Chem. 263, 18911-18919, 1988). The present studies revealed that these *O*-glycans are attached as a cluster at the hinge-like region and lamp-1 and lamp-2 were found to contain 6 and 7-10 *O*-glycans, respectively. These *O*-glycans apparently protect the hinge-like domain, which is otherwise exposed to proteases in the lysosomal lumen. The structures of *O*-glycans from lamp-1 and lamp-2 were examined after glycopeptides containing *O*-glycans were isolated by a Jacalin-column. In both lamp-1 and lamp-2, about 12-14% of the *O*-glycans contain *N*-acetyllactosaminyl repeats, indicating that lamp-1 and lamp-2 are also the carriers for polylactosaminoglycans present in *O*-glycans. Considering that these poly-*N*-acetyllactosamines can carry sialyl Le^X structures, some O-glycans can be ligands for selectins.

These *O*-glycans also contribute to the stability of lamp molecules. Some of HL-60 sublines that are defective in induced differentiation were found to express much less poly-*N*-acetyllactosamine in *N*-glycans. The half-lives of lamp molecules in most of these cells are shorter than those in wild-type HL-60 cells. However, one of the cell lines compensates such defect by synthesizing more *O*-glycans, and the half-life of lamp molecules are almost the same as that of wild-type.

These results indicate that *O*-glycans in lamp molecules play critical roles in maintaining the stability of lamp molecules and providing ligands for selectins. (Supported by CA48737 and O3X-07886).

P 419 THE 1B2 CARBOHYDRATE EPITOPE AND A CORRESPONDING GALACTOSIDE-BINDING LECTIN CO-LOCALIZE IN THE RAT OLFACTORY SYSTEM: BIOCHEMICAL AND CELL BIOLOGICAL ANALYSES. Nagesh K. Mahanthappa and Gerald A. Schwarting, E.K. Shriver Center for Mental Retardation, Waltham, MA 02254; and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

A monoclonal antibody, 1B2, has been demonstrated to recognize the Galβ1→4GlcNAc→R structure [Young, *et al.*, JBC 256:10967-10972 (1981)]. Our laboratory has demonstrated that in the rat this antibody stains a subset of sensory neurons in the olfactory epithelium (OE). Neurons continuously turnover in the normal OE, and those that are 1B2+ appear to be of intermediate maturity. This 1B2-immunoreactivity is also observed in the olfactory nerve and in a subset of olfactory bulb (OB) glomeruli (the terminal synaptic zones of OE neurons). The 1B2-epitope appears to be expressed both by a glycolipid, as observed by immunostaining of total OE lipid separated by HPTLC, and by a 50 kD glycoprotein, as observed by immunoblot analysis of total OE protein separated by SDS-PAGE. Interestingly, an antiserum directed against a 14.5 kD rat lung galactoside-binding lectin (gift of S.H. Barondes, UCSF) with specificity for Galβ1→4GlcNAc [Leffler, *et al.*, JBC 261:10119-10126 (1986)] stains the rat OB nerve layer and glomeruli in a distribution similar to 1B2. Immunoblot analysis with the anti-lectin antiserum reveals the presence of a ~14 kD immunoreactive protein in the OB. We are currently purifying the lectin activity from both the rat lung and olfactory system to verify their identity by peptide mapping. In order to directly test the ability of the purified lectin to recognize the 1B2 epitope, it will be labeled for binding studies with OE and OB glycoconjugates. The presence of these molecules in OE neuron cultures is also being analyzed with the goal of assaying *in vitro* the function of the carbohydrate-lectin interaction in neurite outgrowth and synaptogenesis.

P 420 COOPERATIVE BINDING OF L29, A SOLUBLE LACTOSE-BINDING LECTIN, TO LAMININ OLIGOSACCHARIDES, Stephen M. Massa, Douglas N.W. Cooper, Hakon Leffler and Samuel H. Barondes, Departments of Psychiatry and Neurology, University of California, San Francisco School of Medicine, San Francisco, CA 94143

The homologous endogenous soluble lactose-binding lectins, L14-I and L29, are secreted and bind to the extracellular matrix glycoprotein laminin. These lectins have distinct fine carbohydrate specificities, and L14-I forms noncovalent homodimers in solution, while L29 is primarily monomeric. Because of these differences, we hypothesized that the characteristics of their binding to laminin might differ. The binding of ^{125}I-labelled recombinant lectins to surface-immobilized laminin was therefore explored. Unexpectedly, the binding of L29 was found to be highly cooperative, with fractional binding rising 4 to 5 fold between 6 pM and 500 pM added lectin. Over a similar concentration range, L14-I exhibited typical isotopic dilution, consistent with noninteractive binding. Digestion of L29 with bacterial collagenase, which removes the N-terminal, proline-glycine rich, non-carbohydrate binding portion of the molecule, eliminated cooperativity with little effect on fractional binding at low lectin concentrations. Cooperativity was unaffected by mercaptoethanol, dithiothreitol and iodoacetamide treatment of the lectin. Binding of L29 to N-acetyllactosamine-conjugated BSA, but not unglycosylated BSA was also found to exhibit cooperativity. Fluorescence and CD measurements and molecular sieve chromatography of L29 over a range of concentrations, in the presence and absence of free disaccharide ligands, revealed no evidence for interaction between L29 molecules in solution. The K_i for lactose inhibition of L29-laminin binding was similar for samples at high and low fractional binding. Taken together, these data suggest that cooperative L29 binding is due to monomer binding to sterically constrained oligosaccharides, which then induces secondary multimer formation. The putative multimerization does not increase the apparent affinity of lactose-inhibitable binding.

P 421 MOLECULAR COMPLEMENTARITY BETWEEN SPERM SURFACE β1,4-GALACTOSYLTRANSFERASE AND EGG COAT GLYCOPROTEIN ZP3 MEDIATES FERTILIZATION IN THE MOUSE, David J. Miller, Mary B. Macek, and Barry D. Shur. Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Despite its fundamental importance, the molecular basis underlying mammalian gamete recognition has remained unclear. In mouse, the best-studied mammlian species, sperm surface β1,4-galactosyltransferase (GalTase) has been suggested to function as a receptor by binding oligosaccharide residues in the egg coat, or zona pellucida. Results from others suggest that the ability of the zona pellucida to bind sperm is conferred by alkali-sensitive serine/threonine-linked oligosaccharides of the ZP3 glycoprotein. However, it has not been determined whether sperm GalTase and ZP3 are, in fact, complementary gamete receptors whose interaction mediates sperm-egg binding. We show that sperm GalTase specifically binds to alkali-sensitive oligosaccharides on ZP3, but not to other zona pellucida glycoproteins. However, all zona pellucida glycoproteins are recognized by nonsperm GalTase, demonstrating a more stringent substrate specificity for the sperm GalTase. Furthermore, the interaction between sperm GalTase and ZP3 is required for sperm-egg binding, since blocking or removing the GalTase-binding site on ZP3 inhibits its ability to bind sperm. After the exocytotic release of the sperm acrosome, sperm GalTase loses its ability to bind ZP3, consistent with the inability of acrosome-reacted sperm to bind ZP3 or to initiate binding to the zona pellucida. Following fertilization, ZP3 is modified by egg cortical granule secretions so that it loses sperm receptor activity and is no longer recognized by sperm GalTase, although it is still recognized by nonsperm GalTase. Therefore, the loss of ZP3's sperm receptor activity following fertilization can be accounted for by a selective loss of the sperm GalTase binding site. These results demonstrate that sperm surface GalTase and the egg coat glycoprotein ZP3 are complementary adhesion molecules that mediate mouse gamete binding. Supported by NIH grants HD23479 to B. D. S. and by National Research Service Award HD07418 to D. J. M.

P 422 ACIDIC GLYCANS AS NOVEL MEDIATORS OF CELL ADHESION AND MIGRATION, Misevic G.N., Department of Research, University Hospital of Basel, Hebelstr 20, CH-4031 Basel, Switzerland

Cell aggregation and migration in sponges is mediated by the new class of acidic glycan adhesion molecules (AGAMs). Immunological, biochemical and chemical analyses revealed that these novel carbohydrate structures have in part some properties of both glycosaminoglycans and neutral N-linked polysaccharides. Similar AGAM structures were shown to be present in sea urchin and mice embryos, and normal and malignant adult human cells by means of two types of monoclonal antibodies (Block 1 and 2) which block sponge cell adhesion and recognize sponge AGAMs. Confocal immunofluorescence microscopy and immunogold electron microscopy of *L. pictus* sea urchin blastula and gastrula embryos with Block 1 and 2 revealed that AGAMs are localized within cell-cell contacts, attachment sites of apical lamina to microvilli and cilia, and blastocoel extracellular matrix. The Fab fragments of the Block 1 and 2 antibodies completely inhibited sea urchin blastula cell reaggregation and also caused dissociation of living embryos. Both blocking effects were abolished by ether the total sea urchin polysaccharides or the purified sponge AGAMs, but not with the mixture of glycosaminoglycans (hyaluronic acid, chondroitin sulfate A, B, and C, heparin, heparan sulfate and keratan sulfate). More direct evidence about the function of AGAMs in sea urchins were provided by strong promotion of blastula cell-cell adhesion through highly polyvalent AGAMs of the sponge proteoglycan molecule. Polyvalent AGAMs also enabled fast cell movement when used as a substratum. Migration of blastula sea urchin cells on AGAMs coated glass was 1-10 μm/min versus no movement on plane glass. Preliminary functional studies with the highly metastatic and motile mouse melanoma cell lines Nr 1 and 4 showed that their cell-cell adhesion and attachment to the substratum is also strongly promoted by AGAMs. Contrary, the low metastatic lines did not respond to AGAMs. In human colon carcinoma tissues expression of AGAMs was in direct correlation with the degree of cell invasiveness and occurrence of metastasis. These findings supported the notion that AGAMs may mediate reversible adhesion necessary for migration of not only the embryonal but also metastatic cells. Since the novel AGAMs are expresses in variety of multicellular organisms they could be considered as phylogenetically common cell adhesion and migration molecules operating during morphogenesis and possibly metastasis.

P 423 ALTERED GLYCOSYLATION AND BASOLATERAL DELIVERY OF AN ENDOGENOUS MDCK LAMP-2, Ivan R. Nabi, Douglas Fambrough[1] and Enrique Rodriguez-Boulan, Department of Cell Biology, Cornell University Medical College, New York, NY and [1]Department of Biology, Johns Hopkins University, Baltimore, MD

Madin-Darby Canine Kidney (MDCK) epithelial cells cultured on porous polycarbonate filters form polarized monolayers with defined apical and basolateral surface domains separated by impermeant tight junctions. Using surface immunoprecipitation at 37°C we have demonstrated that an endogenous MDCK lysosomal membrane glycoprotein, the AC17 antigen, is targeted to the basolateral plasma membrane and/or early endosomes prior to delivery to lysosomes. The AC17 antigen was identified as a homologue of the LAMP-2 family of lysosomal glycoproteins by N-terminal sequencing which revealed 85% sequence identity with human LAMP-2. After plating MDCK cells at confluence for one day the AC17 antigen has an increased molecular size (~110 kD) compared to 3 and 7 days of culture (~100 kD). The AC17 antigen endo F digestion product from both 1 and 3 day cultures has an identical size (40 kD) indicating that the increased size of the glycosylated protein is due to altered N-linked oligosaccharide processing. The AC17 antigen from one day cultures is selectively sensitive to β-endogalactosidase digestion after which it comigrates with AC17 antigen of three day cultures indicating that the increased size is the result of increased polylactosamine glycosylation. The altered glycosylation is not specific for the AC17 antigen as both it and an apical MDCK sialoglycoprotein, Gp114, exhibit increased binding to the polylactosamine specific lectin, L-PHA, in one day cultures. MDCK cells plated for one day exhibit a slower rate of AC17 antigen delivery to the basolateral domain but the rate of acquisition of endo H resistance is identical for both one and three day cultures. This suggests that the delayed delivery might be due to oligosaccharide processing in the Golgi apparatus. Experiments are in progress to establish the relationship between polylactosamine glycosylation and basolateral delivery of an MDCK LAMP-2.

P 424 ROLE OF SIALYL LEWIS X/A OLIGOSACCHARIDES IN CAPILLARY TUBE FORMATION, M. Nguyen[1,2], N.A. Strubel[1], and J. Bischoff[1,3] Department of Surgery, Children's Hospital[1], Brigham and Women's Hospital[2], Departments of Cell and Molecular Physiology and Surgery, Harvard Medical School[3], Boston, MA 02115

Growth of new capillaries (angiogenesis) is a complex and highly regulated process which occurs normally during certain physiologic events. To identify cell surface molecules involved in this process, we have used an in vitro model for capillary morphogenesis in which clonal bovine capillary endothelial (BCE) cells can be induced to form capillary-like structures on fibronectin-coated dishes. Since carbohydrates are known to mediate many cell-cell interactions, we tested the effect of various anti-carbohydrate monoclonal antibodies on capillary tube formation in vitro. Antibodies directed against sialyl Lewis A, sialyl Lewis X and $(Gal\beta1,4GlcNAc)_n$ oligosaccharide structures inhibited capillary tube formation, while antibodies directed against Lewis A, Lewis B, A, B, H, and Vim-2 carbohydrate antigens had no effect. Addition of a soluble oligosaccharide analog of sialyl Lewis A (300μg/ml) to BCE cells blocked tube formation. Analogs to sialyl Lewis X, Lewis X, and Lewis A had no effect on tube formation. Since human endothelial leukocyte adhesion molecule-1 (ELAM-1) is known to bind sialyl Lewis X and Lewis A oligosaccharides, we used a cDNA encoding the lectin domain of human ELAM-1 for Northern blot analysis of RNA isolated from BCE cells. Using this probe we detected a 3 kb RNA species whose signal increased 2-3 fold upon capillary tube induction. We then isolated a 2.85 kb cDNA clone from a BCE tube cDNA library by nucleic acid hybridization using the same probe. This bovine cDNA encodes a protein that is 71% identical to human ELAM-1 with the exception that the fourth and fifth complement regulatory protein repeat domains of human ELAM-1 are absent in the BCE cDNA. We postulate that this alternate form of ELAM-1, expressed in capillary endothelial cells, is essential in capillary morphogenesis.

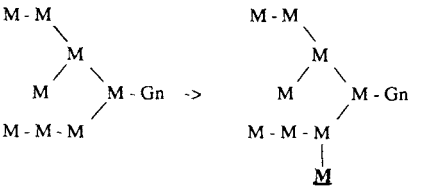
P 425 GANGLIOSIDE MODULATION OF INTEGRIN FUNCTION IN A RECONSTITUTED SYSTEM, Leland D. Powell and Ajit P. Varki, Cancer Center and Department of Medicine, UCSD School of Medicine, La Jolla, CA 92093. Results from several groups suggest a functional interaction between several disialogangliosides (G_{D3}, 9-O-acetylG_{D3} and G_{D2}) and integrin molecules responsible for adhesion to vitronectin and/or fibronectin. Based on these results, we have created a reconstituted system in which to study directly both the structural features of and functional consequences of integrin-ganglioside interaction(s). Highly purified vitronectin receptor (VNr) from human placenta was reconstituted into artificial liposomes created by the detergent dialysis technique from mixed phospholipids and highly purified gangliosides. By measuring the binding of VNr-containing liposomes to vitronectin adsorbed to microtiter wells, the functional consequences of ganglioside(s) on receptor-ligand binding were studied. Inclusion of G_{D2} at a level of one mole percent of total phospholipid increased binding by approximately two fold, whereas G_{M2} was without effect. To measure the direct binding of G_{D2} to the VNr, G_{D2} was labeled to a high specific activity (>10 Ci/mmole) by galactose oxidase/NaB^3H_4. However, when studied in the presence of detergents (necessary for receptor solubility), no direct receptor-ganglioside binding was seen, suggesting that it may be of a low affinity relative to that of the detergent (NP-40 or Lubrol PX). Solid phase methods to study receptor-ganglioside binding are being developed. Once established, they will be used to determine both the stoichiometry of receptor-ganglioside binding and to probe the structural features of gangliosides required for receptor interaction(s).

P 426 FUNCTIONAL ROLE OF THE CARBOHYDRATE COMPONENT OF SOMATOSTATIN (SRIF) RECEPTORS. S. Rens-Domiano and T. Reisine. Dept of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

SRIF is a neurotransmitter in the brain whose physiological actions are mediated by cell surface receptors. Using lectin affinity chromatography, we have established that SRIF receptors are glycoproteins and the carbohydrate groups associated with the receptors are N-linked, are of either hybrid or complex types and contain terminal sialic acid residues. Treatment of solubilized brain SRIF receptors with PNGase F and Endoglycosidases F and H resulted in a decrease in high affinity binding of agonists to SRIF receptors. More specifically, neuraminidase treatment of solubilized SRIF receptors, to remove only the terminal sialic acids, also resulted in a decrease in high affinity agonist binding to the receptor. Further studies have been conducted to understand the novel functional role of sialic acid residues on SRIF receptors. Overnight treatment of intact AtT20 cells, a pituitary tumor cell line containing a high density of SRIF receptors, with neuraminidase resulted in the reduction of high affinity binding of the stable agonist [125I]MK 678 to SRIF receptors. This treatment did not effect the maximal ability of SRIF to inhibit forskolin-stimulated cAMP production in AtT20 cells. Treatment of AtT20 cell membranes with neuraminidase for 5 hours resulted in a 75% decrease in specific, high affinity [125I]MK 678 binding sites. However, when the membranes were pretreated with SRIF agonist, neuraminidase was only able to decrease high affinity binding by 24%. These data would suggest that the desialylated SRIF receptors are functionally active and remain coupled with G-proteins, but exhibit a reduced affinity for agonist. The protection from the effects of neuraminidase on SRIF receptors afforded by SRIF agonist occupancy suggests that the sialic acids may be associated with the ligand bind site of SRIF receptors. Specifically, the negatively charged sialic acid residues may promote high affinity agonist binding to SRIF receptors through electrostatic interactions since SRIF contains a charged lysine residue critical for high affinity binding. Studies are currently underway to further investigate this hypothesis. Supported by NIMH grant 45533, NRSA Post-doctoral Fellowship NS 09002 and a NARSAD Young Investigator Award to SRD.

P 427 PURIFICATION OF YEAST α–1,6-MANNOSYL-TRANSFERASE WHICH INITIATES OUTER CHAIN FORMATION, Pedro A. Romero and Annette Herscovics, McGill Cancer Centre, McGill University, Montreal, Quebec Canada, H3G 1Y6

The α-1,6-ManT which catalyses the first step specific to yeast *N*-linked oligosaccharide biosynthesis has been purified.



The enzyme was solubilized by extracting a 145,000 xg pellet of *Saccharomyces cerevisiae mnn1* mutant with 1% Triton-X 100; it was then adsorbed to Concanavalin A-Sepharose, and eluted with α-methylmannoside. After exhaustive dialysis it was chromatographed on DEAE Trisacryl which removed 90% of the α-1,2-ManT using α-methyl mannoside as substrate. The enzyme was further purified by sequential chromatography on hydroxylapatite, Sephacryl S-200 and CM Trisacryl. SDS-PAGE of the individual fractions eluted from the last two columns followed by silver staining of the gels showed a major band whose intensity corresponded to the peak of enzyme activity. The apparent molecular weight of the enzyme is about 62 kDa. Following blotting onto nitrocellulose, tryptic peptide analysis of the 62kDa band yielded sequences which were used to synthesize denegerate oligonucleotides for PCR reactions on yeast genomic DNA as template. PCR products were obtained with different pairs of primers, and these will be used to isolate the gene from a yeast genomic library.
(Supported by NIH Grant GM-31265).

P 428 EXPRESSION OF A SOLUBLE GALACTOSE-SPECIFIC LECTIN IN HUMAN THYMIC EPITHELIAL CELLS. Jeff Seilhamer[1], Mabel Pang[2], Angelo Delegeane[1], Leanne Vollger[2], Christel Uittenbogaart[2] and Linda Baum[2]. Incyte Pharmaceuticals Inc., Palo Alto, CA 94304[1], UCLA School of Medicine, Los Angeles, CA 90024[2].

Soluble galactose specific lectins, a family of homologous carbohydrate-binding proteins, are expressed in many tissues in a developmentally regulated manner, although their function is unknown in most cases. A member of this family has been identified in neonatal human thymic stroma, and in primary cultures of isolated human thymic epithelial (TE) cells. Direct immunostaining, using an antiserum to IML-1, a human galactose-specific lectin isolated and cloned from placenta, revealed strong reactivity in cultured TE cells, as well as in individual epithelial cells in sections of human thymus; the epithelial nature of these cells was confirmed by counterstaining with antibodies to cytokeratin. In addition, Western blot analysis demonstrated a single 14kD species in extracts of both cultured TE cells and of intact thymic epithelium depleted of thymocytes.

Interaction between thymic epithelial cells and immature cortical thymocytes is crucial for proper selection of immunocompetent T cells; immature cortical thymocytes are known to have increased levels of exposed galactose residues on Galβ1,3GalNAc sequences on cell surface glycoproteins, relative to mature thymocytes and T cells, as shown by binding of peanut agglutinin (PNA). To examine whether lectin-ligand recognition is involved in thymocyte-epithelial interactions, a binding assay has been developed which demonstrates the ability of PNA-positive T cell lines to adhere to monolayers of cultured TE cells. It will be of interest to define the role of the galactose-specific lectin in human thymocyte development.

P 429 DIFFERENTIAL GLYCOSYLATION EFFICIENCY AT SPECIFIC SEQUONS IN RABIES VIRUS GLYCOPROTEIN. SL Spitalnik, AT Remaley, JR Eshleman, WH Wunner, SH Eshleman, Dept. of Pathology and Laboratory Medicine, University of Pennsylvania, and Wistar Institute, Philadelphia, PA 19104.

The sequon Asn-X-Ser/Thr is a necessary but not sufficient signal for N-linked glycosylation. To further define the signals on a protein which regulate its N-linked glycosylation, the glycosylation efficiencies at individual sequons of rabies virus glycoprotein (RGP) were compared. RGP is the only protein on the viral surface, is the major antigenic stimulus during viral infection, and can serve as a vaccine against lethal infection. The vaccination efficacy of RGP is critically dependent on its glycosylation. RGP (ERA strain) is a 505 amino acid transmembrane protein with sequons at Asn residues 37, 247, and 319. Biochemical studies on intact ERA strain rabies virus suggest that glycosylation only occurs at Asn 247 and Asn 319. To further study RGP glycosylation, wild-type RGP cDNA was subcloned into the pSG5 expression vector and 7 mutants were constructed by site-directed mutagenesis. In each mutant one or more sequons was abolished by replacing the Ser/Thr residue with Ala. Glycosylation of RGP was studied in a cell-free transcription/translation/glycosylation system and also by transfection into CHO cells. These studies showed that RGP was completely glycosylated at Asn 247 and Asn 319, but inefficiently glycosylated at Asn 37. The glycosylation efficiency at each individual sequon was not influenced by glycosylation of the other sequons. Glycosylation at either Asn 247 or Asn 319 alone enabled high cell surface expression of RGP; glycosylation at Asn 37 alone allowed only low surface expression of RGP. In contrast, non-glycosylated RGP accumulated intracellularly and was not detected on the surface of CHO cells. These results show the differential glycosylation efficiency at specific sequons can be detected with both cell-free and transfection systems and that this differential glycosylation influences the cell surface expression of RGP.

P 430 MANNAN-BINDING PROTEIN, A SERUM LECTIN, IS AN ACTIVATOR OF THE COMPLEMENT SYSTEM AND IS AN ACUTE PHASE PROTEIN. *S.Thiel, U.Holmskov, S.B.Laursen, J.C.Jensenius. Institute of Medical Microbiology, Aarhus University, DK and Institute of Medical Microbiology, Odense University, DK.*

Human serum mannan-binding protein is a lectin specific for mannose and GlcNAc terminated carbohydrate structures. After binding to the relevant polysaccharides it is an efficient activator of the Clr_2Cls_2 complex via a mechanism independent of antibody and C1q. MBP consist of a COOH-terminal globular carbohydrate recognition domain (CRD) preceded by a 60 a.a. long collagen-like region, stabilized by a short 25 a.a. cysteine rich NH_2-terminal domain. MBP and C1q thus have the same distinct division of collagen-like and globular modules. When the molecules are viewed in the electron microscope the they show a marked similarity. The 5'-flanking region of the MBP gene contains several consensus sequences including a heat-shock promoter sequence and glucocorticoid responsive elements. This could mean that the synthesis of MBP could be induced by acute phase conditions (e.g. infections or surgery) as has prewiously been indicated by Northern blots of liver tissue.

MBP was isolated from human serum by affinity chromatography on mannan-Sepharose followed by size permeation chromatography and ion-exchange chromatography. Antisera were prepared by immunising rabbits. A sandwich-ELISA was developed using anti-MBP antibody from two different rabbits. When normal donors are analyzed a wide range of MBP serum concentration is seen (a range of more than 100 fold).

The serum concentration of MBP was followed in consecutive samples from people undergoing major surgery (the insertion of an artificial hip). Blood samples were collected one day (-1) before the operation, the day after the operation, the following 6 days and at day 14 and day 70. A significant increase in serum MBP concentration was observed following the surgery (an approximately 3-fold increase as compared to the day -1 sample). The maximum MBP concentration were reached at about day 4 and were back to normal level at day 70. Also the serum concentration of C-reactive protein (CRP) was measured and was shown to increase dramatically (between 40 and over 100 fold) at day 1. The data are consistent with MBP being an acute phase protein.

P 431 BIOSYNTHESIS OF ENDO H-RESISTANT INVERTASE $Man_{5\text{-}10}GlcNAc_2$ OLIGOSACCHARIDES IN alg3 MUTANT YEAST: in vivo AND in vitro STUDIES. Robert B. Trimble[+], Paul H. Atkinson[*] and Mary Fran Verostek[+], [+]NYSDOH, Albany, NY 12201, and [*]MAF, Upper Hutt, New Zealand.

Alg3 yeast mutants synthesize endo H-resistant oligosaccharides whose precursor for elongation is Man1α2Man1α2Man1α3-(Man1α6)Man1ß4GlcNAc$_2$ (Verostek, M.F., et. al., J. Biol. Chem. 266, 5547). To characterize alg3 glycan elongation in vivo, oligosaccharides on alg3,sec18 invertase synthesized and secreted at 26°C were released with PNGase F and purified by Bio-Gel P4 chromatography. Large-($Man_{>30}GlcNAc_2$) and intermediate-($Man_{5\text{-}10}GlcNAc_2$) sized oligosaccharides were pooled separately, and the smaller ones were exchanged with ^2H_2O for 1D and 2D DQCOSY ^1H NMR analyses at 500 MHz. Although there was little or no substitution of the terminal α1,6-core-linked mannose, addition of α1,6-, α1,2- and α1,3-mannoses to the α1,3-linked core branch of a majority of the Man_5 precursor was analagous to core-filling reactions seen on wt invertase glycans. In addition, two minor types of oligosaccharides found were: those which retained glucose and those consistent with mannan elongation. Glucose retention appeared to be due to inefficient trimming from minor glucosylated intermediates, while mannan elongation was by extension of a new α1,6-linked branch from the α1,3-core-linked residue as seen in wt processing. $Man_5GlcNAc_x[^3H]ol$ isomers from alg3 and ovalbumin were compared as acceptors for elongation in vitro by solubilized mannosyl transferases using GDP-Man. Bio-Gel P-4 chromatography of products showed that the alg3 isomer was efficiently elongated to $Man_{8,7}GlcNAc_2[^3H]ol$ by either wt or alg3,sec18 membranes, while the ovalbumin form was not. By 1D ^1H NMR, the Man_8 product was the two major endo H-resistant isomers seen in vivo, confirming that an α1,3-linked mannose had not been added to the α1,6-core-linked residue in vitro. Thus, the α1,6-linked branch additions forming $Man_9GlcNAc_2$-PP-Dol from $Man_5GlcNAc_2$-PP-Dol appear dispensible for normal yeast mannan synthesis. Partial support by GM23900 (RBT).

P 432 ROLE OF GLUCOSE IN PROTEIN GLYCOSYLATION BY alg3 YEAST, Mary Fran Verostek and Robert B. Trimble, NYSDOH, Wadsworth Center, Albany, NY 12201

Derepression of the slightly leaky alg3 mutant in the sec18 background at 37°C revealed the oligosaccharides on invertase restricted to the ER to be 20% wt $Man_8GlcNAc$ and 80% Man1α2Man1α2Man1α3(Man1α6)Man1ß4GlcNAc$_2$ (Verostek, M.F., et. al. (1991) J. Biol. Chem. 266, 5547). It was not clear from this work whether the oligosaccharide-lipid (OSL) precursors were glucosylated in alg3 yeast. Therefore, an alg3,sec18,gls1 strain was constructed to delete the GLS1-encoded glucosidase I responsible for trimming the terminal α1,2-linked glucose from newly-transferred $Glc_3Man_xGlcNAc_2$ oligosaccharides. This strain was transformed with a multicopy plasmid (pRB58) carrying the external invertase (SUC2) gene, which resulted in a 5-10 fold increase in overall invertase activity. Preparative amounts of the ER-form of external invertase, derepressed at 37°C, were purified as a source of oligosaccharides. The N-linked glycans, released by sequential treatment with endo H and PNGaseF, were separately sized on Bio-Gel P4. $Glc_3Man_8GlcNAc$, $Glc_3Man_5GlcNAc_2$ and $Man_5GlcNAc_2$ were found in about a 1:1:7 molar ratio, suggesting that transfer of over 80% of the $Man_5GlcNAc_2$ occurred without prior addition of the three glucoses to the OSL precursor. Recovery of $Glc_3Man_8GlcNAc$ and not $Man_8GlcNAc$ implied absence of glucosidase I, which was verified by incubation of [^{3}H]Glc_3[^{14}C]$Man_9GlcNAc$ with solubilized membranes from either alg3,sec18,gls1 or alg3,sec18,GLS1. Bio-Gel P4 chromatography of the products showed that [^{3}H]Glc was removed only in the presence of the GLS1 gene product. It is not clear whether alg3's vigor is due to the leakage of 20% of the oligosaccharides past the alg3 block to become wt $Glc_3Man_9GlcNAc_2$ or to the relaxed specificity of yeast oligosaccharyltransferase in glycosylating nascent peptides with truncated nonglucosylated oligosaccharides. Partial support by GM23900 (RBT).

P 433 CHARACTERIZATION OF A CYTOSOLIC FUCOSYLATION PATHWAY IN DICTYOSTELIUM, C. M. West, B. Gonzalez-Yanes, J. M. Cicero* & R. D. Brown, Jr.†, Dept. of Anatomy & Cell Biology, Coll. of Medicine, *Dept. of Ent. and Nematol., & †Food Sci. & Human Nutrit. Dept., IFAS, Univ. of Fla, Gainesville, FL

FP21 is a 21kD fucoprotein which fractionates with the cytosol after high-speed centrifugation of gently lysed Dictyostelium cells. Less than 0.7% of FP21 is associated with vesicles. In proliferating cells, 4 X 10^5 fucoses/cell are associated with FP21 as O-linked oligosaccharides equal in size to 4.8 glucose units. FP21 is underfucosylated in a mutant strain (HL250) that depends on extracellular fucose for synthesis of GDP-fucose. To determine the site of FP21 fucosylation in the cell, cytosolic and vesicular preparations from strain HL250 were compared for their ability to fucosylate FP21. Cytosolic preparations fucosylate endogenous FP21 in a time-, concentration-, and divalent cation-dependent fashion, with a K_m for GDP-fucose of 1.4 μM. Normal cell cytosol is inactive unless provided with purified mutant FP21, demonstrating that FP21 is fully fucosylated in normal cells. Both mutant and normal cytosols are also able to fucosylate a type 1 (galß1-3glcNAcß-) glycolipid substrate, but not related substrates, with K_ms for the type 1 glycolipid of 1 mM and for GDP-fucose of 1.6 μM. Competitive inhibition between FP21 and the type 1 glycolipid shows that the same enzyme fucosylates both substrates, suggesting that the FP21-fucosyltransferase is an α1-4 fucosyltransferase which lacks α1-3 activity. Intact and permeabilized vesicle preparations from wild-type cells are unable to fucosylate FP21 or the type 1 glycolipid by a divalent cation-dependent mechanism, and thus are devoid of FP21 fucosyltransferase. Since control experiments showed that vesicle leakage is minimal during cytosol preparation, these results indicate that FP21 is synthesized and fucosylated in the cytosolic compartment, by an unusual soluble fucosyltransferase. Fucosylation of FP21 is correlated with rescue of the slow-growth phenotype of strain HL250 by exogenous fucose.

P 434 EFFECTS OF OVER-EXPRESSION OF β1,4-GALACTOSYLTRANSFERASE ON GLYCOPROTEIN BIOSYNTHESIS IN F9 MOUSE TERATOCARCINOMA CELLS, Adel Youakim and Barry D. Shur, Dept. of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

β1,4-Galactosyltransferase (GalTase) is involved in the biosynthesis of *N*-acetyllactosamine-containing oligosaccharides. We have examined the effects of over-expressing GalTase on glycosylation of glycoproteins in F9 cells. F9 cells were transfected with either the short form (encoding a protein of 386 amino acids) or the long form (encoding a protein of 399 amino acids) of the GalTase cDNAs under the control of the constitutive promoter for phosphoglycerate kinase. Cells transfected with either construct possessed about 3-fold greater total GalTase activity than control F9 cells. [6-^{3}H]-Galactose-labeled glycopeptides obtained by pronase digestion of metabolically-labeled cells showed no differences between control and transfected cells as assessed by Bio-Gel P-6 gel filtration chromatography and Tomato lectin affinity chromatography. Analysis of immunoprecipitated ^{3}H-galactose-labeled LAMP-1 glycoproteins showed no differences either in the quantity of labeled glycoprotein or in mobility by SDS-PAGE. Furthermore, analysis of the LAMP-1-derived glycopeptides by Bio-Gel P-6 gel filtration chromatography also indicated no differences between control and transfected cells. These results indicate that GalTase levels in F9 cells can be increased several fold above normal without any detectable change in the glycosylation of glycoproteins, suggesting that GalTase is not rate limiting in the biosynthesis of oligosaccharides in these cells.

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Late Abstracts

RNA POLYMERASE II IS A GLYCOPROTEIN.

William G. Kelly, Michael E. Dahmus, and Gerald W. Hart.* Dept. of Biological Chemistry, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205; *Dept. of Biochemistry and Biophysics, University of California, Davis, CA 95616. N-acetylglucosamine attached to protein via the hydroxyl of a serine or threonine (O-GlcNAc) is a post-translational modification found on the nuclear and cytosolic faces of many intracellular proteins in all eukaryotes (for review, see **Ann. Rev. Biochem.**, 58:841). O-GlcNAc-modified proteins include nuclear pore components, endoplasmic reticular membrane residents, cytoskeletal proteins, viral proteins, and numerous chromatin proteins, including, but not restricted to, transcription factors. Interestingly, this modification is found on RNA polymerase II- specific transcription factors, but it has not been found on the polymerase I or III-specific factors that have been examined.

We have found that the large catalytic subunit of RNA Polymerase II itself is also modified by O-GlcNAc. The O-GlcNAc is restricted to the C-terminal domain (CTD) of the protein, a region distinguished by the presence of a heptapeptide repeat (consensus sequence YSPTSPS) that appears 52 times in mammalian Pol II. The CTD of Pol II is known to be rapidly and extensively phosphorylated during its transition from an initiating to an elongating transcription complex. Only the unphosphorylated form of the large subunit (Pol IIA) contains O-GlcNAc, whereas this modification is not detectable on the phosphorylated form (Pol IIO), suggesting that the glycosylation and phosphorylation of Pol II may be mutually exclusive events. Reverse-phase HPLC peptide mapping studies suggest that the O-GlcNAc is found at a unique site on the CTD, in contrast to the multiple (>30) sites of phosphorylation. These data imply that O-GlcNAc may play a role in regulating the initiation of the rapid phosphorylation cascade that accompanies transcription elongation. Supported by HD13563, GM33300, and The March of Dimes Predoctoral Fellowship Program.

REMOVAL OF TERMINAL SIALYLATED GALACTOSE RESIDUES FROM CYTOTOXIC T LYPHOCYTES ABROGATES CYTOTOXICITY WITHOUT EFFECTING ANTIGEN SPECIFIC CELL PROLIFERATION,

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We have approached the problem of identifying functional roles for the carbohydrate moities of cell surface glycoconjugates by exploiting commercially available glycosidases to sequentially remove specific monosaccarides from the surface of an alloreative cytotoxic T-cell line. Treated cells were characterized by flow cytometery using fluorescencent lectins specific for known carbohydrate structures and monoclonal antibodies to T-cell specific phenotypic markers. Treated cells were further analyzed for their ability to recognize and lyse alloantigen bearing target cells in a four hour chromium release assay and for their ability to proliferate in response to such cells in a three day thymidine incorporation assay.

From the results of such experiments we conclude: 1) The binding of most monoclonal antibodies to T-cell phenotypic markers is unperturbed by such enzymatic treatments, the exceptions being an anti-CD3 antibody which showed enhanced binding after treatment of the cells with sialydase and a Thy-1 specific monoclonal which showed slightly reduced binding after beta-galactosidase treatment. 2) Removal of cell surface sialic acid leads to a slight enhancement of cytotoxicity. 3) Progressive removal of sialylated galactose residues leads to an increasing loss of cytotoxicity with a concomitant increase in cell proliferative capacity.