GLYCOBIOLOGY

Organizers: Ernest Jaworski and Joseph Welply
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Plenary Sessions Page

Poster Sessions

Glycoprotein Synthesis and Routing

B 001 STRUCTURE, SYNTHESIS, AND FUNCTION OF THE PITUITARY GLYCOPROTEIN HORMONE OLIGOSACCHARIDES. Jacques U. Baenziger, Washington University School of Medicine, St. Louis, MO. 63110.

Lutropin (LH) and follitropin (FSH) are dimeric glycoproteins which contain a common α-subunit and a hormone-specific β-subunit. Both hormones are synthesized in the same cell in the anterior pituitary, the gonadotroph. Despite having common α-subunits and highly homologous β-subunits LH and FSH bear oligosaccharides which differ in structure. LH bears oligosaccharides which terminate with the sequence SO_4-4GalNAcβ1,4GlcNAcβ1,2Manα whereas FSH bears oligosaccharides which terminate with the sequence Siaα-Galβ1,4GlcNAcβ1,2Manα (Sia = sialic acid). The synthesis of sulfated oligosaccharides involves the sequential action of a GalNAc-transferase and a sulfotransferase. A common intermediate, $GlcNAc_2Man_3GlcNAc_2$, acts as the acceptor for either Gal addition by the Gal-transferase and GalNAc addition by the GalNAc-transferase. We have compared human hormone α-subunit, transferrin, α_1-anti-trypsin, J-chain, and a glycopeptide bearing the $GlcNAc_2Man_3GlcNAc_2$ structure as acceptors for Gal and GalNAc addition. ^{3}H-Gal was transferred to each of these substrates by crude pituitary membranes with a similar catalytic efficiency (Vmax/Km) range of 0.7 to 1.7 if transferrin is set = 1. In contrast, the catalytic efficiency for addition of GalNAc to hormone α-subunit was 166 fold greater than for addition to the same oligosaccharide on transferrin, α_1-anti-trypsin, or J-chain. The apparent Km for GalNAc addition to the hormone α-subunit was 25 uM, among the lowest described for glycosyltransferases. The hormone specific GalNAc-transferase was detected in pituitary membranes but not in placental membranes indicating the transferase has a restricted tissue distribution. Unlike the GalNAc-transferase, the sulfotransferase is not peptide specific and requires only the trisaccharide sequence GalNAcβ1,4GlcNAcβ1,2Manα for addition with an apparent Km of 70 uM. The sulfotransferase is also detected in pituitary membranes but not in placental membranes. The absence of GalNAc on FSH indicates that access to the recognition marker on the α-subunit is limited when it is associated with FSHβ but not when it is associated with LHβ. The spectrum of structures present on LH and FSH indicates that the synthesis of sulfated and sialylated oligosaccharides on the glycoprotein hormones is tightly regulated and may be functionally significant. Sulfated and sialylated oligosaccharides may play a role in directing LH and FSH to separate granules within the gonadotroph and/or may modulate hormone biologic potency.

B 002 NUCLEAR PORE GLYCOPROTEINS: STRUCTURE AND FUNCTION, John A. Hanover, Mara D'Onofrio, Christopher M. Starr, Tracy Olson, Barbara Wolff and Min Kyun Park, Laboratory of Biochemistry and Metabolism, NIDDK, National Inst. of Health, Bethesda, MD 20892

The uptake of proteins into the nucleus and the efflux of RNA from the nucleus to the cytoplasm are highly specific, time-, and energy-dependent transport processes. Sorting signals have been identified which direct the import of nuclear proteins through the nuclear envelope (NE). Peptides corresponding to the nuclear localization sequence motif of SV40 large T antigen were chemically coupled to large proteins normally excluded from the nucleus. Transport of such conjugates across the NE was observed after microinjection or, in vitro, using isolated nuclei and cytoplasmic extracts. A similar nuclear localization signal has been found in the human glucocorticoid receptor; a peptide composed of this sequence confers nuclear localization. To determine the structural characteristics of the nuclear localization signal of the SV40 T antigen, a number of monoclonal antibodies have been raised to this sequence. These antibodies interact with intact T antigen, suggesting the localization sequence is exposed on the surface of the molecule, consistent with its recognition by an import "receptor". These antibodies are being used in attempt to interfere with the nuclear uptake process. Attempts are also being made to define the receptor involved in import of proteins into the nucleus.

Nuclear pores mediate transport across the NE. We have identified glycoprotein components of the pores which bear cytoplasmically disposed, O-glycosidically linked GlcNAc. The lectin wheat germ agglutinin binds to these glycoproteins and inhibits both nuclear protein import and RNA efflux from the nucleus. The action of the lectin on these transport processes can be reversed by the addition of a competing saccharide (GlcNAc polymer). To biochemically characterize the nuclear pore proteins, NE from rat liver and CHO cells were used to prepare monoclonal antibodies in mice. These antibodies have allowed immunoaffinity purification the major nuclear pore proteins which range in molecular mass from 45-220 kDa. Sequence from the major nuclear pore protein, a 62 kDa species, was used to devise an oligonucleotide probe. This probe was used to screen a cDNA library, and a clone was isolated which encodes the glycoprotein. The cDNA hybridizes to a mRNA species 2.4 kilobases in length. Sequence from the open reading frame of this clone allowed us to precisely define one of the sites of O-linked GlcNAc addition. Additional molecular characteristics of this protein will be discussed in terms of the structure and function of the nuclear pore.

B 003 GLYCOSYLATION SITE BINDING PROTEIN, A COMPONENT OF OLIGOSACCHARYL TRANSFERASE, IS HIGHLY SIMILAR TO THREE OTHER 57 kDa LUMINAL PROTEINS OF THE ER, William J. Lennarz, M. Geetha-Habib, Howard A. Kaplan and Robert Noiva, Department of Biochemistry and Molecular Biology, UT M.D. Anderson Cancer Center, Houston, TX 77030.

A 57 kDa component of oligosaccharyl transferase, termed glycosylation site binding protein (GSBP), specifically recognizes a photoaffinity probe containing the N-glycosylation site sequence Asn-Lys-Thr. GSBP is present in the lumen of the ER (endoplasmic reticulum) and its release from this compartment results in a loss of N-glycosylation. Antibodies against GSBP were used to identify cDNA clones from a λgt11 expression library. Analysis of the cDNA sequence of GSBP reveals high sequence similarity to three other 57 kDa luminal endoplasmic reticulum proteins: protein disulfide isomerase, the β-subunit of prolyl hydroxylase, and thyroid hormone binding protein. This finding suggests that the capacity to recognize multiple polypeptide domains may reside in a single luminal protein that participates in co- and/or posttranslational modifications of newly synthesized proteins. Several lines of biochemical evidence are consistent with this possibility: a) antibody to thyroid hormone binding protein precipitates photolabeled GSBP, b) protein disulfide isomerase activity is precipitated by antibody to GSBP, and c) purified protein disulfide isomerase can be specifically labeled using the site-directed photoaffinity probe. Studies are underway on the analogous enzyme system involved in protein glycosylation in yeast. Several lines of evidence suggest that in this organism the glycosylation site recognition domain is part of oligosaccharyl transferase proper, rather than a separate subunit. (This work was supported by a National Institutes of Health grant (GM33185) to W.J.L.)

B 004 ENZYMOLOGY OF INTRACELLULAR PROTEIN TRANSPORT, James E. Rothman, Molecular Biology, Princeton University, Princeton, NJ

Recent progress in dissecting the enzymology of vesicular transport through the Golgi stack will be discussed.

B 005 THE PROCESSING OF ASPARAGINE-LINKED OLIGOSACCHARIDE IS AN EARLY MARKER OF THE ENTEROCYTIC DIFFERENTIATION OF HT-29 CELLS. Germain Trugnan*, Eric Ogier-Denis+, Patrice Codogno+, Chantal Bauvy+, Michèle Aubéry+, Isabelle Chantret*, Catherine Sapin* and Alain Zweibaum*. *: INSERM U178, 16 avenue Paul Vaillant Couturier 94807 Villejuif Cedex, France ; +: INSERM U180, UER des Sts Pères, 75006 Paris, France.
Studies on the regulation of the enterocytic differentiation of the human colon cancer cell line HT-29 have recently shown that the posttranslational processing of sucrase isomaltase, a differentiation marker, vary as a function of cell differentiation. Other studies indicate that undifferentiated HT-29 cells accumulate UDP-N-acetylhexosamine which is involved in the glycosylation process. We have now demonstrate that an overall alteration of N-glycan processing is associated with the inability of HT-29 cells to differentiate. Three alterations have been described in undifferentiated cells : (i) a quantitative reduction of the incorporation of labeled mannose into glycoproteins ; (ii) an accumulation of high mannose versus complex glycopeptides and (iii) a partial blockade of the conversion of Man_{9-8}-$GlcNac_2$ species during the trimming of glycoproteins. In contrast, differentiated HT-29 cells display a normal pattern of N-glycan processing. Other experiments have shown that the above described alterations are already present in exponentially growing cells that will not differentiate, whereas they are absent from exponentially growing cells that will differentiate when they reach confluency. From these results we conclude that the N-glycan processing (i) is correlated with the state of enterocytic differentiation of HT-29 cells and (ii) can be used as an early marker of the ability of cells to differentiate.

Lectins and Oligosaccharide Receptors

B 006 MOLECULAR DISSECTION OF RECEPTORS WHICH MEDIATE GLYCOPROTEIN ENDOCYTOSIS. Kurt Drickamer, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, New York 10032

Analysis of the mammalian liver asialoglycoprotein receptor has led to the definition of a COOH-terminal segment of approximately 130 amino acids which constitutes the binding domain for oligosaccharides bearing terminal galactose residues. Comparison of this sequence with carbohydrate-binding segments of other calcium ion-dependent (C-type) animal lectins reveals a distributed pattern of conserved amino acid residues in all of these carbohydrate-recognition domains (CRDs). Two areas will be covered in the presentation. First, ongoing structure-function studies of the known CRDs will be described; this will include the use of genetic and physical methods aimed at determining the way in which specific sugar recognition is achieved by C-type CRDs. In addition, use of the pattern of conserved amino acid residues to identify potential CRDs based on primary structures will be discussed along with experiments in which predicted carbohydrate-binding activity has been demonstrated. Second, attempts to identify other functional domains within the endocytic receptors, such as those critical for proper movement through the endocytic pathway, will be presented; the results suggest that regions beyond the cytoplasmic domain are important for the proper movement of this receptor between the cell surface and intracellular compartments.

B 007 SOLID PHASE OLIGOSACCHARIDE PROBES (NEOGLYCOLIPIDS) IN STUDIES OF OLIGOSACCHARIDE RECOGNITION, Ten Feizi, Section of Glycoconjugate Research, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ, UK.
The development, organization and growth of complex organisms, as well as their interactions with the environment, involve an intricate array of molecular recognition events. There is an increased awareness of the involvement of oligosaccharides of glycoproteins, proteoglycans and glycolipids in many of these processes. Progress in studies of oligosaccharides as recognition structures depends on new detection systems and ways of coping with the heterogeneities of these structures. This communication will be concerned with a new approach, using solid phase oligosaccharide probes (neoglycolipids), for studies of oligosaccharide recognition by mammalian carbohydrate-binding proteins and attachment factors (adhesins) of microbial agents.

B 008 ENDOSOMAL PROCESSING OF PROTEINS FOLLOWING RECEPTOR-MEDIATED ENDOCYTOSIS, P.D. Stahl, J. Blum, R. Diaz, S. Diment, M. Fiani, L. Mayorga and J.S. Rodman, Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110
Early processing of proteins following receptor-mediated endocytosis (RME) may be important for antigen-presentation, hormone activation and the generation of peptide mediators. Recent work suggests that following RME by macrophages, internalized proteins enter a protease containing endosomal compartment where proteolysis occurs. This is the case for at least 4 ligands, ^{125}I parathyroid hormone, ^{125}I ricin A-chain, ^{125}I mannosylated-BSA and ^{125}I dinitrophenol-derivatized-bovine serum albumin (DNP-BSA)-IgG complex. Immunocytochemical studies have demonstrated the presence of cathepsin D in early endosomes. To confirm this, reconstitution studies were carried out using DNP-BSA complexed with anti-DNP monoclonal antibodies and the fusogenic assay conditions described by Diaz, Mayorga & Stahl [J. Biol. Chem. 263: 6093 (1988)]. Following 2 minutes of immune complex internalization at 37° in J774 macrophages, DNP-BSA-IgG was largely present in protease negative vesicles. The presence of protease was detected by incubating vesicles prepared on a post-nuclear supernatant at pH 5 and recording the release of acid soluble ^{125}I. When ligand containing vesicles were allowed to incubate with endosomal fractions under fusogenic conditions prior to pH 5 incubation, ligand degradation was observed. Incubation under non-fusogenic conditions resulted in no proteolysis at pH 5.0. These findings indicate that acid protease is present in a fusogenic compartment whose origin may be endosomal or Golgi membranes.

New Approaches in Glycobiology

B 009 A BIOCHEMICAL AND GENETIC ANALYSIS OF PROTEIN TRANSPORT FROM THE ENDOPLASMIC RETICULUM TO THE GOLGI COMPLEX IN YEAST, S. Ferro-Novick, H. Ruohola, A. Newman, R. Bacon, J. Shim, M. Clague and A. K. Kabcenell, Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Transported proteins are thought to be carried from the endoplasmic reticulum (ER) to the Golgi complex via vesicular carriers. We are using a combined biochemical and genetic approach in yeast to identify components involved in this process. To address this question we have developed a two stage in vitro assay that reconstitutes transport from the ER to the Golgi complex.[1] In the first stage of this reaction prepro-α-factor, synthesized in a yeast translation lysate, is translocated into the ER of permeabilized yeast cells or directly into microsomes. In the second stage, the ER form of α-factor is converted to the Golgi form in the presence of ATP, soluble factors and an acceptor membrane fraction. The nonhydrolyzable GTP analogue, GTPγS, inhibits the second stage but not the first stage of this two stage reaction. Recently, we have identified a putative vesicular intermediate in transit to the Golgi complex. *This intermediate is produced* during the lag of the reaction and its formation is dependent upon the addition of the donor compartment, ATP and soluble components. When this putative intermediate is incubated in the presence of ATP and the acceptor compartment the Golgi form of α-factor is observed. Donor, acceptor and soluble fractions can be separated in this assay. This has enabled us to determine the defective fraction in mutants that block ER to Golgi transport in vivo. YPT1 is a GTP-binding protein that has been implicated in ER to Golgi transport in vivo.[2] We have shown that a ypt1 mutant that fails to bind GTP also blocks transport in vitro. The donor compartment is fully functional in this mutant but the soluble and acceptor fractions are defective. Recently, we have demonstrated a genetic interaction between YPT1 and BET2, a gene whose product is also involved in ER to Golgi transport in vivo.[3] We are also characterizing a genetic interaction among three other genes whose products appear to play a role in ER to Golgi transport in vivo.

1 H. *Ruohola, A. K. Kabcenell and S. Ferro-Novick*, JCB, 107, 1988.
2 N. Segev, J. Mulholland and D. Botstein, Cell, 52, 1988.
3 A. Newman and S. Ferro-Novick, JCB, 105, 1987.

B 010 ANTI-3-FUCOSYLLACTOSAMINE (3-FL) ANTIBODIES: STRUCTURE-FUNCTION RELATIONSHIPS, Donald M. Marcus and Hirohisa Kimura. Departments of Medicine, Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

We are studying the structure and regulation of murine and human antibodies (Abs) against the 3-FL antigenic determinant (also known as Le^x or X). On the basis of Southern filter hybridization and partial sequences of 3 monoclonal antibodies (mAbs) against 3-FL, we suggested previously that there was restricted gene usage by these Abs. We have recently obtained complete sequences of the heavy and light chains of five anti-3-FL Abs and extensive data on three other Abs. The heavy chains of all eight Abs are encoded by V_H441 of the X24 V_H family, 7 of the mAbs use the J_H4 segment and 1 uses J_H3. All of the light chains are encoded by V_K24B and J_K1. The restricted use of gene segments by these Abs is in contrast to the use of multiple V_H and V_L segments by murine Abs against (Á1-6) dextran and group A streptococcal carbohydrate. The V_H441 gene segment is also used to encode Abs against levan and galactan, and the amino acid sequences of the V_H regions of these three families of Abs may be virtually identical. As illustrated in the Table below, however, the CDR3 regions of the Abs are quite different and apparently play a major role in determining the specificity and affinity of the Abs. We are currently undertaking site-directed mutagenesis to test this hypothesis.

	CDR3								
	95	96	97	98	99	100	100A	101	102
Anti-3-FL									
PM81	Q	L	G	E	N	A	M	D	Y
Anti-Galactan									
X44	L	H	Y	Y	G	Y	A	A	-
Anti-Levan									
UPC 10	N	W	D	V	G		F	-	-

B 011 MURINE β-1,4-GALACTOSYLTRANSFERASE: EVIDENCE FOR TWO FORMS OF THE PROTEIN BASED ON CLONING STUDIES, Nancy L. Shaper, Greg F. Hollis† and Joel H. Shaper*. The Oncology Center and *Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and †Monsanto Co., St. Louis, MO 63198.

We have isolated overlapping cDNA clones representing the full-length transcript for murine β-1,4-galactosyltransferase. The full length cDNA is approximately 4.1 kb and contains an unusually long 3'-untranslated region (2582 bp), a coding region of about 1200 bp and a heterogeneous 5'-untranslated region. Inspection of the coding region indicates three distinct structural features: 1) a large, potentially glycosylated, COOH-terminal domain (355 amino acids), which is positioned within the Golgi lumen and contains both the catalytic site and the α-lactalbumin-binding site, 2) a single transmembrane domain of 20 amino acids, and 3) a short NH_2-terminal domain.

Primer extension analysis, S1 protection analysis and RNA blotting demonstrate the presence of two sets of mRNA transcripts which differ in length by about 200 bp. The 5' boundary of the long transcripts maps upstream of two in-frame ATGs. The 5' boundary of the short transcripts maps between these two ATGs.

These results demonstrate that two related forms of β-1,4-galactosyltransferase of 399 and 386 amino acids are synthesized as a consequence of alternative translation initiation. Both forms of the enzyme are identical in primary structure with the exception that the long form (399 amino acids) has a NH_2-terminal extension of 13 amino acids which, in part, potentially encodes a cleavable signal sequence. The structural implications, topological distribution and potential biological significance of the two forms of the enzyme will be discussed.

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Future Applications of Glycosylation in Biotechnology

B 012 SELECTION OF AN EXPRESSION HOST FOR HUMAN GLUCOCEREBROSIDASE; IMPORTANCE OF HOST CELL GLYCOSYLATION, M.L.E. Bergh, C. Naranjo, A.F. Mentzer, G.R. Ostroff and G.D. Barsomian, Genzyme Corp. Boston, MA 02111

Enzyme replacement therapy using glucocerebrosidase (GCR) has been suggested as a possible treatment for Gaucher's disease. Carbohydate-mediated targeting of the enzyme to the site of action is an important aspect of such a treatment. Therefore, the choice of an expression system for the production of recombinant GCR is not only based on the criterium of yield, but also on the glycosylation pattern of the host cell. Insect cells exhibit a glycosylation pattern that is potentially useful for targeting GCR to its site of action. Moreover, a high-yield expression system has been developed recently for this cell type. Therefore, we investigated the usefulness of insect cells for the production of therapeutic GCR. The cDNA encoding human GCR was cloned into the polyhedrin gene of the nuclear polyhedrosis virus *A.californica*.The recombinant virus was used to infect SF9 cells. The infected cells produced high levels of glucocerebrosidase, which was enzymatically active with [^{14}C]Glc-Cer. In addition, the protein was glycosylated and slowly, but completely secreted ($t^1/_2$ appr. 8 h). Secretion into the medium was preceded by processing of the intracellular material from 62 kD (endo H-sensitive) to 58 kD.(endo H-resistant). The difference in MW is due to glycan processing, since processing (but not secretion) was inhibited by the Golgi mannosidase inhibitor DMM. Furthermore, N-Glycanase treatment of both forms yielded the same MW of 54 kD.

B 013 GLYCOSYLATION OF TISSUE PLASMINOGEN ACTIVATOR VARIANTS. Joseph K. Welply, Lyle E. Pegg, Beverly A. Reitz and Thomas G. Warren, Department of Biological Sciences, Monsanto Corporation, St. Louis, Mo., 63198. The synthesis and function of the oligosaccharide at amino acid 117 of tissue plasminogen activator has been studied using a variety of genetically engineered variants of the protein. The normal protein, when expressed in several lines including CHO and C127 cells, contains a high mannose type oligosaccharide at amino acid position 117 within the kringle 1 domain and primarily complex type chains at positions 184 and 448. Of the numerous variants which were constructed, only those containing a deletion of or in one case a single amino acid change within the growth factor domain of the protein were found to have a complex rather than a high mannose sugar at 117. All proteins with complex sugars at 117 exhibited extended circulating serum half-lives upon intravenous injection in rats and rabbits. To determine whether the micro-environment around amino acid 117 affects oligosaccharide structure, 5 variants with single amino acid substitutions at position 118 were constructed. With the exception of a proline substitution at 118, which resulted in the loss of sugar addition to amino acid 117, the other 118 variants contained high mannose at 117. Together, these results suggest that the structure of the growth factor domain and not amino acids near 117 is an important factor in determining the type of oligosaccharide at this site within kringle 1. The growth factor alone does not appear to be sufficient because glycosylation sites of Factor VII, IX and X which are not within kringles but closely follow growth factor domains do not contain high mannose. To determine whether growth factor- kringle combinations generally result in high mannose structures, we are currently constructing a variant of urokinase, a growth factor-kringle protein, which now contains a potential glycosylation site at an amino acid position analogous to 117.

The function of the high mannose structure at 117 is being addressed in binding studies to the mannose receptor of macrophages. Preliminary results indicate that this receptor recognizes normal tissue plasminogen activator whereas the variants that do not contain high mannose sugars are poor substrates.

Glycoproteins in Cell-Cell Recognition

B 014 LYMPHOCYTE HOMING RECEPTORS, Steven D. Rosen, Ted A. Yednock, Joyce S. Geoffroy, Mark S. Singer, and *Jeffrey L. Curtis, Department of Anatomy, University of California, *Respiratory Care Section, VA Medical Center, San Francisco, CA 94143

The migration of blood-borne lymphocytes into secondary lymphoid organs is initiated by a highly specific cell adhesion event between the lymphocytes and and the endothelium of a specialized blood vessels known as high endothelial venules (HEV). Two lymphocyte cell adhesion receptors, known as homing receptors, have thus far been functionally defined: controlling lymphocyte binding to HEV in peripheral lymph nodes (PN) and binding to HEV in gut-associated lymphocyte organs (Peyer's patches [PP]). We have shown (1,2) that the PN homing receptor is inextricably associated with a carbohydrate-binding activity with specificity for sugars structurally related to mannose-6-phosphate (M6P). The PP homing receptor is not specifically inhibited by these sugars. In addition, the PN and PP homing receptors can be discriminated on the basis of their requirements for divalent cations and their sensitivity to proteases. Using selective protease treatments and adhesion-blocking antibodies (directed at lymphocyte surface molecules and HEV-associated molecules), we have been able to establish that lymphocyte migration to lung-associated lymphoid tissue involves a distinct specificity. Although the PN specificity and the lung specificity are different, M6P-like sugars inhibit both. These results will be discussed in terms of the possible role that carbohydrates may play as cell adhesion recognition determinants in this system. (Supported by NIH Grant and Northern California Arthritis Foundation Grant to SDR).

1. Yednock, T.A., L. M. Stoolman, and S.D.Rosen (1987). J. Cell Biol. 104, 713
2. Yednock, T.A., E.C. Butcher, L. M. Stoolman, and S.D.Rosen (1987). J. Cell Biol. 104, 725.

B 015 CELL SURFACE GALACTOSYLTRANSFERASE AS A RECOGNITION MOLECULE DURING FERTILIZATION AND DEVELOPMENT, Barry D. Shur, Department of Biochemistry and Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Galactosyltransferase (GalTase) has at least two well defined subcellular distributions-on the cell surface and within the Golgi apparatus. GalTase in the trans Golgi network participates in the biosynthesis of secretory and membrane-bound glycoproteins. Cell surface GalTase mediates a variety of cell-cell and cell-matrix interactions by binding to its appropriate glycoconjugate substrate on adjacent cell surfaces or in the extracellular matrix. The best studied examples include mouse sperm-egg binding, cell adhesions during preimplantation embryonic development, and cell migration on laminin matrices. In each instance, GalTase is expressed on specific plasma membrane domains where it functions during cell interactions in a spatially and temporally specific manner.

Cell surface GalTase is an integral membrane protein as defined by its resistance to high salt, and by the ability of protease and detergent to release GalTase activity from intact cells. GalTase is recruited to the cell surface from intracellular pools in response to specific extracellular factors. However, molecular, biochemical and cell biological studies show that the expression of GalTase in the Golgi apparatus and on the cell surface are under separate controls, since the enzyme specific activity in each of the two membrane populations can change independent of one another.

GalTase has been purified from sperm plasma membranes and embryonal carcinoma cells and characterized as a β1,4 N-acetylglucosamine:GalTase, which was subsequently cloned from lambda gt10 embryonal carcinoma cell and lactating mammary gland libraries. GalTase cDNA is currently being used to examine GalTase expression during normal and mutant spermatogenesis and during embryonal carcinoma cell differentiation, as well as to explore the function of surface GalTase in vitro.

B 016 SPERM RECEPTOR OLIGOSACCHARIDES AS MEDIATORS OF SPERM-EGG INTERACTIONS IN MICE, Paul M. Wassarman and Jeffrey D. Bleil, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

The mouse sperm receptor, called ZP3, is a glycoprotein (83,000 apparent M_r) that consists of a 44,000 M_r polypeptide chain (402 amino acids), 3 or 4 asparagine-linked (N-linked) oligosaccharides, and an undetermined number of serine/threonine-linked (O-linked) oligosaccharides *(1)*. There are more than a billion copies of ZP3 present throughout the mouse egg extracellular coat, or zona pellucida. As a prelude to fertilization, each acrosome-intact sperm binds in a relatively species-specific manner to tens-of-thousands of copies of ZP3 at the surface of the zona pellucida. Binding to ZP3 induces sperm to undergo the acrosome reaction (membrane fusion) and, consequently, enables them to penetrate through the zona pellucida and to reach, and then fuse with, egg plasma membrane (fertilization) *(2)*. Purified egg ZP3, as well as a specific class of ZP3-derived O-linked oligosaccharides (3,900 apparent M_r), exhibit sperm receptor activity *in vitro (3-5)*. The oligosaccharides, which represent a relatively low percentage of total O-linked oligosaccharide associated with ZP3, fully account for the glycoprotein's sperm receptor activity *in vitro* (i.e., recognition and binding). Furthermore, either enzymatic removal or modification of certain sugars that constitute these oligosaccharides results in destruction of sperm receptor activity *in vitro (6)*. For example, treatment of purified ZP3 and ZP3-derived O-linked oligosaccharides with α-galactosidase results in release of galactose from both substrates and in loss of sperm receptor activity. In addition, galactose oxidase-catalyzed modification of terminal galactose on both substrates results in loss of sperm receptor activity; however, activity is restored by sodium borohydride reduction. Thus, galactose, located in α-linkage at the nonreducing terminus of O-linked oligosaccharides, is at least one of the sugar determinants on ZP3 responsible for binding of sperm to the zona pellucida of ovulated eggs. These and other findings strongly support the conclusion that, during mammalian fertilization, carbohydrates play a fundamental role in species-specific, sperm-egg recognition and binding.

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(2) Wassarman, P.M., *Annu. Rev. Cell Biol. 3:* 109 (1987).
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(5) Bleil, J.D. & Wassarman, P.M., *J. Cell Biol. 102:* 1363 (1986).
(6) Bleil, J.D. & Wassarman, P.M., *Proc. Natl. Acad. Sci., USA 85:* 6778 (1988).

Proteoglycans and Glycoproteins in Cellular Adhesion

B 017 PROTEOGLYCANS IN CELL ADHESION AND CELL AGGREGATION. Jeffrey D. Esko, Richard G. LeBaron, Kathy S. Rostand, and Magnus Höök. Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, Alabama 35294.

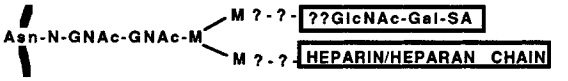
Wild-type Chinese hamster ovary cells synthesize about twice as much heparan sulfate proteoglycans as chondroitin sulfate proteoglycans when grown in monolayer culture. Mutants were obtained that no longer produce heparan sulfate proteoglycans and they were found to be deficient in attachment to defined matrices composed of Type V collagen or a heparin-binding fragment of fibronectin. Although the mutant and wild-type cells attached to matrices of intact fibronectin similarly, mutant cells did not form focal adhesion plaques, suggesting that heparan sulfate proteoglycans are important mediators of cell adhesion. When wild-type Chinese hamster ovary cells were grown as cell aggregates in suspension culture, they synthesized about twice as much chondroitin sulfate proteoglycans as heparan sulfate proteoglycans. Interestingly, heparan sulfate-deficient mutants aggregated into cell spheroids like wild-type cells, but mutants defective in both heparan sulfate and chondroitin sulfate synthesis did not aggregate. These findings suggest that chondroitin sulfate proteoglycans facilitate cell aggregation. Thus, the relative abundance of heparan sulfate and chondroitin sulfate proteoglycans may determine whether cells adhere to each other or to the substratum.

B 018 FAMILIES OF NEURAL ADHESION MOLECULES, Brigitte Schmitz and Melitta Schachner, Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, 69 Heidelberg, Federal Republic of Germany.

Based on the observation that out of two functionally identified cell surface glycoproteins carrying the L2/HNK-1 carbohydrate structure two were recognized as the neural cell adhesion molecules L1 and N-CAM, we formulated the hypothesis that the other glycoproteins of yet unidentified number expressing this carbohydrate epitope are also involved in adhesion. In agreement with this hypothesis we have characterized two other glycoproteins as adhesion molecules, the extracullular matrix molecule J1 and the myelin-associated glycoprotein MAG. The L2/HNK-1 epitope was also detected on integrin, the cell surface receptor for fibronectin and laminin and on P_o, the main glycoprotein from peripheral nervous system myelin which is thought to play a role during the compaction of myelin. Two further carbohydrate epitopes detected by monoclonal antibodies designated L3 and L4 are found to be co-expressed by the above mentioned glycoproteins except for J1 and N-CAM which do not express the L3 and L4 epitopes. A prominent member of the family of glycoproteins carrying these two carbohydrate epitopes is the novel cell adhesion molecule on glia (AMOG), which does not express the L2/HNK-1 epitope. The L2, L3 and L4 epitopes show similar features in that they are expressed by subpopulations of each adhesion molecule, developmentally regulated independently of the protein backbone, and phylogenetically conserved. The three carbohydrate epitopes seem to exert different effects on cell adhesion and neurite outgrowth in vitro. These observations indicate that different cell interactions mediated by a single adhesion molecule may be specified by individual carbohydrate epitopes.

B 019 N-LINKED GLYCOSAMINOGLYCANS AND POLYSIALYLATED INTEGRINS: VARIATIONS ON EXISTING THEMES Ajit Varki, Goran Sundblad, Linda Roux, Shama Kajiji*, Richard Tamura**, Vito Quranta**, and Hudson H. Freeze, Hematology-Oncology Division, and Cancer Center, UC San Diego, Pfizer Research Laboratories*, and Research Institute of Scripps Clinic**, La Jolla, CA 92093

In all systems previously studied, heparin/heparan sulfate chains are attached to core proteins through a xylose residue in O-glycosidic linkage to Serine. We recently described an exception to this rule in bovine pulmonary artery endothelial cells (J. Biol Chem 263: 8879, 8890 &8897, 1988). Peptide:N-Glycosidase F (PNGaseF) was used to specifically release $^{35}SO_4$-labelled molecules from these cells, revealing a novel class of heparan- and heparin-sulfate chains attached to as yet unidentified proteins by N-glycosidic linkage:



Such molecules represented less than 10% of the $^{35}SO_4$-labelled molecules and less than 1% of the total N-linked oligosaccharides from these cells. Since lung tissue is rich in endothelial cells, we have searched for such structures in bovine lung acetone powder. Of the PNGaseF-released N-linked oligosaccharides labelled by reduction with [^{3}H]$NaBH_4$, >10% eluted from a QAE column with 1M NaCl and a portion of this label had properties very similar to those of the $^{35}SO_4$-labelled molecules from endothelial cells. 22 other cell lines examined did not express these novel chains at a detectable level. However, a glycoprotein of 140kDa which is recognized by a specific monoclonal antibody (S3-53) in pancreatic carcinoma cells was found to have N-linked chains substituted with a heterogeneous mixture of sulfated sequences, some of which share properties with the N-linked glycosaminoglycans.

Another murine monoclonal antibody (S3-41) derived by immunization with the same pancreatic carcinoma cell line was found to react with a heterodimer of two non-covalently-linked glycopolypeptides, gp205 and gp125. Upon treatment with exo- or endo-neuraminidases, gp205 undergoes a substantial reduction in molecular weight, suggesting the presence of polysialic acid chains. However, rather large amounts of the enzymes are required to achieve the effects, and similar molecular weight shifts are not seen with PNGaseF. Amino terminal sequencing reveals close homology to the integrin family of cell-surface receptors, with a unique ß-chain amino terminal sequence. A restricted baso-lateral expression of this molecule on epithelial cell surfaces also suggests a role in adhesion. The conclusive structural identification of the polysialic acid chains has not yet been completed. If confirmed, this protein would share features in common with two distinct families of adhesion molecules, the N-CAM family (polysialic acid) and the integrin family (sequence and structural homology).

Glycolipid Modification of Proteins

B 020 TRYPANOSOME GLYCOLIPID ANCHORS: STRUCTURE, SYNTHESIS AND PROCESSING, George A.M. Cross, Anant K. Menon, Satyajit Mayor and Ralph. T. Schwarz
The Rockefeller University, New York, NY 10021.

An increasing number of eukaryotic surface proteins are being found or predicted to be anchored to the cell membrane via glycosyl-phosphatidylinositol, covalently coupled to the carboxyterminal amino acid, after removal of a short hydrophobic peptide present on the preprotein. It was in a variant surface glycoprotein (VSG) of the parasitic pathogenic protozoan *Trypanosoma brucei* that the precise molecular nature of this posttranslational modification was first defined[1]. It seems likely that the glycolipid anchors attached to other proteins share a common core structure with the VSG anchor[2]. The semantic contraction 'glypiation', for glycosylphosphatidylinositolation, has been coined for this process[3], which has sometimes also been referred to as PIG-tailing. No specific function has been ascribed to glypiation, but it could play a role in intracellular sorting, membrane mobility and developmental or temporal regulation of surface molecules, as well as simply acting as the membrane anchor for molecules for which it may either be unnecessary or undesirable to use a more traditional transmembrane polypeptide sequence. Each trypanosome is covered by about 10 million VSG molecules, identical in their polypeptide sequence but generally heterogeneous in the extent of glycosylation, both in the glycolipid anchor and in Asn-linked glycans. The VSG represents about 10% of the total cellular protein of *T.brucei* so, despite its evolutionary distance from higher eukaryotes, this organism can be a useful model for studying the enzymology and cell biology of glypiation. In *T.brucei*, glypiation appears to be an immediate posttranslational event, suggesting the addition, *en bloc*, of a preformed glycolipid precursor. We have chemically characterized a putative glycolipid precursor, which contains the common core structure but lacks galactose branches. We are studying the galactosylation of the glycolipid, which appears to occur after its addition to the protein. To study glycolipid synthesis, we have incorporated radioactive precursors in cell-free extracts, and characterized putative biosynthetic intermediates. The experiments have been extended using inhibitors of glycosylation, and evidence has been obtained for the role of Dolichol-P-mannose as donor of at least one of the three mannose residues.

1 Ferguson, Homans, Dwek & Rademacher (1988) Science 239, 356-363.
2 Homans, Ferguson, Dwek, Rademacher, Anand & Williams (1988) Nature 333, 269-272.
3 Cross (1987) Cell 48, 179-181.

B 021 THE GLYCOINOSITOL PHOSPHOLIPID ANCHOR OF HUMAN ERYTHROCYTE ACETYLCHOLINESTERASE. PALMITOYLATION OF INOSITOL RESULTS IN RESISTANCE TO PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C. W. L. Roberts, S. Santikarn, V. N. Reinhold, J.-P. Toutant, and T. L. Rosenberry, Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106, and Division of Biological Sciences, Harvard School of Public Health, Boston MA 02115.

Several proteins, including acetylcholinesterases (AChEs) from mammalian erythrocytes, recently have been shown to be anchored on the extracellular face of plasma membranes exclusively by a covalently linked glycoinositol phospholipid. Chemical analyses have revealed that anchor components include an inositol phospholipid, glucosamine, mannose, and ethanolamine in amide linkage to the polypeptide C-terminus. Many of these anchored proteins are selectively released from cell membranes by purified bacterial phosphatidylinositol-specific phospholipase C (PIPLC). However, in some cells, particularly human erythrocytes, this release is only partial. For example, PIPLC releases only about 5% of the AChE from human erythrocytes. To investigate this PIPLC resistance, we cleaved the inositol phospholipid from the human erythrocyte AChE anchor with nitrous acid and examined its structure by fast atom bombardment mass spectrometry. This phospholipid contained 1-alkyl-2-acylglycerol in which the alkyl group was 18:0 and the predominant acyl group was 22:4 + 22:5. In addition, a novel palmitoyl group (16:0) was observed in direct acyl linkage to an inositol hydroxyl. This group was directly responsible for PIPLC resistance, because selective palmitate deacylation by ammonia methanolysis generated an inositol phospholipid that was cleaved by PIPLC to yield alkylacylglycerol and inositol-1-phosphate. Polyacrylamide gel electrophoresis in nondenaturing detergents resolves small amounts of AChE histochemical activity and permits rapid discrimination of detergent-binding AChE that retains anchor lipids from hydrophilic AChE that has lost anchor lipids. With this technique it was shown that deacylation of human erythrocyte AChE with 1M hydroxylamine at pH 11 yields a residual alkyllysoplasmanylinositol anchor that binds detergent micelles and is susceptible to PIPLC cleavage. Supported by the NIH (NS16577, DK38181, and RR01494), the Muscular Dystrophy Association of America, and the North Atlantic Treaty Organization.

B 022 STRUCTURE AND FUNCTION OF THE NOVEL LIPOPHOSPHOGLYCAN OF *LEISHMANIA* PARASITES, S. J. Turco and T. B. McNeely, Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40536.

Lipophosphoglycan (LPG) is the major cell surface glycoconjugate of *Leishmania* parasites. Structurally, LPG is a polymer of repeating [PO_4→6Gal(β1,4)Manα1] units attached via a phosphosaccharide core to a novel *lyso*-alkylphosphatidylinositol anchor. 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. The consequence of this inhibition is that induction of the microbicidal oxidative burst by protein kinase C is precluded following infection. Consistent with this hypothesis are the results of three sets of experiments: (*i*) purified LPG from *L. donovani* was found to inhibit protein kinase C isolated from rat brain. LPG was a competitive inhibitor with respect to diolein, a noncompetitive inhibitor with respect to phosphatidylserine, and had no appreciable effect on protein kinase M and protein kinase A. Furthermore, the 1-O-alkylglycerol portion of LPG exhibited the most inhibitory activity toward the enzyme whereas the carbohydrate portion was not quite as effective. (*ii*) addition of purified LPG to human peripheral monocytes attenuated the oxidative burst upon stimulation of the monocytes with either phorbal mystric acid or with opsinized zymosan. (*iii*) incubation of monocytes with a variant of *L. donovani* which lacks LPG resulted in the entry of the variant into the monocytes and its subsequent destruction, which was in contrast to wildtype *L. donovani*. Taken together, these results suggest that one important function of LPG is to play an active role in protecting the parasite within phagolysosomes of host phagocytic cells.

B 023 SIGNALS IN DECAY ACCELERATING FACTOR mRNA WHICH DIRECT GLYCOSYL-INOSITOLPHOSPHOLIPID ANCHORING, M. Edward Medof, Anne T. Litrizza, Christopher A. Hauer and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106

Decay-accelerating factor (DAF) mRNA predicts a 347 amino acid long polypeptide which lacks a cytoplasmic domain as found in conventionally anchored proteins and terminates in 23 C-terminal hydrophobic residues. Although transfection studies using hybrid gene constructs to express chimeric proteins (e.g., CD8·DAF) have established that the signals directing GPL anchor incorporation into DAF are localized to these C-terminal residues, comparisons of mRNAs encoding DAF and other GPL-anchored proteins have failed to reveal consensus sequences that could serve as signals. To determine whether the absence of cytoplasmic extensions, a feature common to DAF and other GPL-anchored proteins, is an essential element in GPL anchor processing, we assembled two gene constructs using oligonucleotide cassettes in our Epstein-Barr virus-based REP2 episomal expression vector: 1) CD8·DAF·CD8/REP2, in which the gene segment encoding CD8's membrane-proximal intracytoplasmic charged amino acids (RNRRR) were appended to DAF's 3' end sequence; and 2) CD8·cyt$^-$/REP2, in which the gene segment encoding CD8's intracytoplasmic region was deleted. Stable CD8·DAF·CD8/REP2 K562 transfectants expressed high levels of phosphatidylinositol-specific phospholipase C (PI-PLC)-resistant (4-12% release) CD8 antigen, which contrasted with the PI-PLC-sensitive (93-95% release) CD8 antigen on control CD8·DF2/REP2 K562 transfectants. As opposed to the CD8·DF2/REP2 K562 transfectants which expressed high levels of PI-PLC-sensitive CD8 antigen, stable CD8·cyt$^-$/REP2 K562 transfectants demonstrated little or no surface CD8. These findings suggest that the addition of charged residues to the hydrophobic 3' end sequence of DAF mRNA is sufficient to abolish GPL-anchor processing. However, removal of intracytoplasmic charged residues from the 3' end sequence of a conventionally-anchored protein such as CD8 is not sufficient to direct GPL-anchoring, and thus, additional information in the 3' end sequence is required.

Banquet Address

B 024 **USE OF GLYCOSYLTRANSFERASES AS PROBES FOR OLIGOSACCHARIDE STRUCTURE AND FUNCTION** *Gerald W. Hart, Sidney W. Whiteheart, Antonino Passaniti, Jonathan S. Reichner, Robert S. Haltiwanger, Gordon D. Holt, and William G. Kelly* Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The recent development of facile methods for the affinity purification of glycosyltransferases has made it feasible to use them as highly-specific probes of saccharide structure/functions **(1)**. Purified sialyltransferases, in conjunction with specific sialidases, have been used to compare the numbers and sialylation states of terminal or penultimate oligosaccharides on the surfaces of B16 melanoma metastatic tumor cell variants **(2)**. While not detectable by more conventional approaches, the sialyltransferase probes found that highly-metastatic cells contain several-fold less penultimate Galß1-4GlcNAc and Galß1-3GalNAc residues on their surfaces than their non-metastatic counterparts. Sialyltransferases have also been used to study the intracellular trafficking of cell surface glycoproteins, indicating that most glycoproteins do not re-cycle back to the Golgi **(3)**. Similar studies with GlcNAc transferase I and a CHO mutant cell line lacking this transferase, indicate a rapid internalization of many cell surface glycoproteins to a mannosidase II-containing compartment of the cell. Bovine milk galactosyltransferase has been used to describe a new form of glycosylation, O-GlcNAc, that is predominantly intracellular, occuring at the cytoplasmic and nucleoplasmic faces of nuclear pores, on important cytoskeletal proteins, and on important non-histone chromosomal proteins **(4)**. These and other studies exemplify the high-selectivity and sensitivity of purified glycosyltransferases as probes of saccharides. As more of these enzymes become available, they are likely to become a major tool in glycoconjugate biochemistry. (Supported by NIH HD13563 and CA 42486)

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Glycoconjugates in Disease

B 025 BRONCHIAL MUCIN CARBOHYDRATES ASSOCIATED WITH CYSTIC FIBROSIS. Herman van Halbeek. Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30613.

Patients with cystic fibrosis (CF), a genetically inherited disease, suffer from hypersecretion of abnormally thick mucus that obstructs the air passages of the bronchial tubes. This blockage leads to the development of infections, thereby increasing the severity of the disease. Both the visco-elastic properties of mucus and the ability of bacteria to adhere to it are dependent, at least in part, on the carbohydrate moieties of bronchial mucin glycoproteins. We are investigating whether the structures of the carbohydrate side chains of bronchial mucins of patients with CF are aberrant when compared to those of healthy individuals. To specifically identify the differences associated with CF, the carbohydrate structures of the mucins of CF patients are also being compared to those of patients with non-genetic chronic diseases of air passages associated with hypersecretion of mucus, namely, chronic bronchitis, bronchiectasis, and lung cancer. The studies include patients with the same disease but different blood groups. The data obtained to date indicate that the blood group of the patient is indeed the dominant factor reflected in the structures of the mucin carbohydrates. These extensive studies are still in their early stages, but preliminary evidence exists that there are unique features in the carbohydrate moieties of bronchial mucins from CF patients. Eventually, detailed knowledge of the structures of these mucin carbohydrates and their putative aberrance in CF may lead to the elucidation of the molecular basis of this disease.

B 026 ALTERED GLYCOSYLATION OF HUMAN CHORIONIC GONADOTROPIN IN TROPHOBLASTIC DISEASE AND ITS USE FOR THE DIAGNOSIS OF CHORIOCARCINOMA,
Kobata A., Amano J., Mizuochi T., and Endo T., Department of Biochemistry, Institute of Medical Science, the University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan

High level of hCG is detected not only in the urine of pregnant women, but in those of patients with trophoblastic diseases including tumors. Accordingly, urinary and serum hCG levels have been measured as a useful markers for the diagnosis and prognosis of trophoblastic diseases as well as pregnancy. We found that the oligosaccharide patterns of hCGs purified from the urine of pregnant women, invasive mole, and choriocarcinoma are different. Only quantitative differences were found in the mucin-type sugar chains. In contrast, the differences detected in the Asn-linked sugar chains were qualitative. The altered glycosylation detected in invasive mole hCG could be induced by the ectopic expression of N-acetylglucosaminyltransferase IV together with the enhanced expression of a fucosyltransferase that forms the Fucα1→6GlcNAc linkage. The alteration in choriocarcinoma could be induced by further modification of the former enzyme to have a wider substrate specificity. Therefore, invasive mole might well be considered as a precancerous state leading to choriocarcinoma. We found that oligosaccharides, which contain the Galβ1→4GlcNAcβ1→4(Galβ1→4GlcNAc β1→2) Manα1→ group, are retarded in a Datura stramonium agglutinin (DSA)-Sepharose column. Because the pentasaccharide structure were detected in the sugar chains of invasive mole hCG and choriocarcinoma hCG, but not in those of hydatidiform mole hCG, the behavior of urinary hCGs from trophoblastic diseases in the lectin column was investigated. All of the hCGs from pregnant women and hydatidiform mole passed through the column irrespective of sialidase treatment. In contrast, high proportion of the hCG from invasive mole and choriocarcinoma were retained in the column after desialylation. Some of the choriocarcinoma samples were retained even before sialidase treatment. Therefore, DSA column can be used as an effective tool to discriminate the malignant diseases from non-malignant trophoblastic diseases. Comparative study of the sugar chains of α- and β-subunits of choriocarcinoma hCG revealed that altered glycosylation is detected in both subunits. The four Asn-linked sugar chains of normal hCG are site specifically synthesized, and each sugar chain is consequently expected to work as a specific signal to express the hormonal activity. Therefore, study of the hormonal activity of invasive mole and choriocarcinoma hCGs may afford a useful information as to the functional role of the sugar moiety of this hormone.

B 027 MODIFICATION OF SCRAPIE AND CELLULAR PRION PROTEINS BY Asn-LINKED GLYCOSYLATION AND GLYCOSYL-PHOSPHATIDYLINOSITOL ANCHORS, Stanley B. Prusiner, Department of Neurology, University of California, San Francisco, CA 94143-0518

Scrapie prions cause a degenerative neurologic disease that can be transmitted to laboratory rodents. The scrapie prion protein (PrP^{Sc}) is the only component of the infectious scrapie prion identified, to date. Although many biochemical and genetic lines of evidence argue that PrP^{Sc} is a major component of the infectious particle, the most convincing data was derived from immunoaffinity purification studies. The mouse PrP gene is on chromosome 2 and is linked to a gene controlling the scrapie incubation time (*Prn-i*). Prion proteins from inbred mice with short and long incubation times differ by two amino acids and prions isolated from these two inbred mice gave different incubation times in specific hosts. Post-translational modification probably accounts for the unusual features of PrP^{Sc}. Molecular cloning of a PrP cDNA identified two potential Asn-linked glycosylation sites. Both the scrapie (PrP^{Sc}) and cellular (PrP^{C}) isoforms were susceptible to digestion by peptide *N*-glycosidase F (PNGase F) but resistant to endoglycosidase H as measured by migration in SDS-PAGE. The extent of Asn-linked glycosylation was the same for the two PrP isoforms as well as PrP 27-30, which is produced from PrP^{Sc} by limited proteolysis of the *N*-terminus. The M_r of PrP 27-30 was reduced from 27-30 kDa by PNGase F digestion to 20-22 kDa while anhydrous hydrogen fluoride (HF) or trifluoromethane sulfonic acid treatment reduced the M_r to 19-21 kDa and 20-22 kDa, respectively. HF not only hydrolyzes the Asn-linked oligosaccharides but also hydrolyzes the *C*-terminal glycosylphosphatidylinositol (GPI) anchor. Structural studies demonstrated that PrP 27-30 possesses Asn-linked, complex oligosaccharides with terminal sialic acids, penultimate galactoses and fucose residues attached to the innermost *N*-acetylglucosamine. PrP^{C} was found almost exclusively on the external surface of cells and can be released by phosphatidylinositol-specific phospholipase C (PIPLC). PrP^{Sc} was not released by PIPLC from scrapie-infected isolated hamster brain cells and cultured murine neuroblastoma (N2a) cells. The majority of PrP^{Sc} in N2a cells appears to be confined to an interior compartment. Whether differences in Asn-linked oligosaccharide or GPI anchor structure distinguish PrP^{C} from PrP^{Sc} and are responsible for the distinct properties displayed by the two PrP isoforms remains to be established. PrP^{Sc} stimulation of a post-translational process which converts PrP^{C} or its precursor into PrP^{Sc} is one possible mechanism for prion replication. This is consistent with observations showing that human prion diseases are manifest as infectious, sporadic and genetic disorders.

Late Addition

B 028 APPLICATIONS OF CARBOHYDRATE CHEMISTRY IN THE DESIGN AND PRODUCTION OF PHARMACEUTICAL PRODUCTS, Russell Greig and George Poste, Smith Kline & French Laboratories, King of Prussia, PA 19406 Advances in carbohydrate chemistry coupled with an increasing appreciation of the importance of glycoconjugates in cell homeostasis and disease, have established this discipline as an emerging area for drug discovery. These can be considered under two headings: carbohydrate structures as targets for pharmacological intervention, and as therapeutic entities in their own right. Cell surface glycoconjugates are involved in several fundamental biological properties including cell recognition, adhesion and motility, and thus aberrations in their display may underlie a spectrum of pathologies ranging from neoplasia to autoimmunity. Pharmacological efforts aimed at restoring normal patterns of glycosylation may have significant therapeutic utility in disease states for which treatment is currently lacking. In addition oligosaccaride analogs themselves may have clinical utility as receptor antagonists, since several ligands and infectious agents interact with target cells through glycosylated receptors. Thus it should be possible to block these interactions by using carbohydrate analogs that mimic the glycosylated region of the target receptor, or by employing compounds that inhibit specific glycosidases and/or glycosyltranferases. Identification of unique carbohydrate antigens on infectious agents, neoplastic cells and specific autoimmune T-cell clones raises the possibility that synthetic oligosaccarides may also be used as vaccines to eradicate, through host-mediated mechanisms, unwanted cell types. These examples illustrate the potential impact of carbohydrate chemistry on drug discovery and treatment of disease.

Glycoconjugate Synthesis, Lectins and Oligosaccaride Receptors

B 100 **PURIFICATION AND CHARACTERISATION OF A MITOGENIC GALACTOSE-BINDING LECTIN FROM TETRACARPIDIUM CONOPHORUM SEEDS**, Theresa Animashaun+, R. Adetoro Togun*, and John E. Kay**, +National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. *Department of Biochemistry, University of Obafemi-Awolowo, Ile-Ife, Oyo State, Nigeria. **School of Biological Sciences, University of Sussex, Brighton BN1 9QG, U.K.
A galactose-specific lectin, mitogenic for T-lymphocytes, has been discovered in the edible seeds of Tetracarpidium conophorum (Mull.Arg) Hurtz & Dalz, a West African plant. Saline extracts of ground, defatted T.conophorum seeds contain a non-specific haemagglutinin which is inhibited by lactose, 1-O-methyl-β-galactose, mellbiose, 1-O-methylα-galactose and galactose, listed in descending order of potency. The lectin was purified using lactose-Sepharose affinity chromatography. Molecular weight studies indicate the lectin is a dimer composed of a single polypeptide chain, approx. M_r 34,000. The N-terminal sequence of the lectin is ELKHIVGPNGL-MDD. The lectin binds to asialo-fetuin. Saline extracts as well as pure lectin from T.conophorum were strongly mitogenic for human, pig and mouse lymphocytes. The stimulation of lymphocytes by T.conophorum lectin as measured by [^{3}H]thymidine incorporation into DNA and [^{35}S]methionine incorporation into protein was comparable with that obtained with the mitogens, concanavalin A (Con A) and phytohaemagglutinin (PHA). The mitogen specifically stimulated murine T cells and did not show any appreciable activation of T-cell depleted mouse lymphocytes. Experiments with cyclosporin A suggest that the mechanism of activation of lymphocytes by T.conophorum lectin differs from that of Con A. T.conophorum lectin is an unusual lectin since it is a T-cell mitogen with specificity for galactose.

B 101 EXAMINATION OF THE WHEAT GERM AGGLUTININ-LIKE PROPERTIES OF PERTUSSIS TOXIN, Gregory J. Tyrrell, Mark S. Peppler, Robert A. Bonnah, Pele Chong, and Glen D. Armstrong, Dept. of Medical Microbiology & Infectious Diseases, University of Alberta, Edmonton, Albetra T6G 2H7, and Connaught Research Institute, Willowdale, Ontario M2R 3T4.
We have determined binding inhibition constants for pertussis toxin and wheat germ agglutinin using fetuin, haptoglobin, transferrin, glycophorin, and α1 acid glycoprotein. Data obtained from these experiments revealed that, although it was reported that pertussis toxin and wheat germ agglutinin apparently recognize similar sugars on glycoproteins, the binding activities of pertussis toxin and wheat germ agglutinin are not identical. In addition, an affinity isolation procedure has been used to identify receptors for pertussis toxin and wheat germ agglutinin in goose erythrocyte membranes. Autoradiography of SDS polyacrylamide gels revealed that surface iodinated erythrocyte receptors were qualitatively similar. However, a solution containing GlcNAc caused the release of erythrocyte receptors from wheat germ agglutinin-agarose but not from pertussis toxin-agarose. This was consistent with the observation that GlcNAc did not inhibit pertussis toxin-mediated hemagglutination. The major goose erythrocyte receptor for wheat germ agglutinin and pertussis toxin had an apparent molecular weight of 139,000. Less prominent receptors with apparent molecular weights of 226,000; 218,000; 191,000; 159,000; 127,000; and 120,000 were also detected.

B 102 A MUTANT FORM OF COLLAGEN IS GLYCOSYLATED IN THE RER. Jeffrey Bonadio, Howard Hughes Medical Institute and The Department of Pathology, The University of Michigan, Ann Arbor, MI 48109. The subcellular location of the glycosyltransferases which accept type I collagen as a substrate is controversial. There is abundant circumstantial evidence from work with connective tissue which argues for a RER localization. However, this evidence was questioned recently by studies [J Biol Chem (1987) 262:3415,10290, and 10296] which localized these enzymes to the Golgi of cultured hepatocytes. We have taken advantage of a mutant fibroblast cell line to address this question. These cells produce a structurally abnormal form of collagen that is unstable and overmodified, and whose transport is blocked inside of cells. We have shown by morphological, biochemical and subcellular fractionation techniques that the abnormal collagen is stuck in the RER. Both glucose and galactose were directly identified on the abnormal collagen following isolation from RER-enriched subcellular fractions. This provides strong evidence that the collagen glycosyltransferases are in fact localized to the RER of connective tissue cells. In the course of these studies, we localized a second gal transferase activity commonly found in the Golgi (UDP-galactose:N-acetlyglucosamine galactosyltransferase) to the RER of human, chick, and bovine fibroblasts. These results suggest that the subcellular location of enzymes which modify collagen and other secreted proteins may have cell and tissue specific subcellular locations.

B 103 **A NEW PROCESSING MANNOSIDASE IN RAT LIVER**, Pedro Bonay and R. Colin Hughes, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.
At least four neutral mannosidase activities have been reported to be involved in processing reactions occurring during biosynthetic assembly of Asn-linked oligosaccharides as the glycoproteins move from the rough ER and through the stacked cisternae of the Golgi apparatus in the rat liver. We report the presence in rat liver of a novel α-mannosidase activity capable of removing α1,3 and α1,6-linked mannose residues from $Man_5GlcNAc$ which is distinct from any previously described α-mannosidase in rat liver. By subcellular fractionation, the $Man_5GlcNAc$ hydrolysing activity has been located in Golgi (heavy-Cis) fraction and also in endosomal fractions. This activity is not inhibited by 5 μM Swainsonine which completely inhibits Golgi II, but it is partially inhibited by 100 μM deoxymannojirimycin. It is inhibited by 1 mM Zn^{+2} and 1 mM EDTA but not by 1 mM Co^{2+}. The product of the purified Golgi (Cis) fraction on $Man_5GlcNAc$ has been identified as Manα1,3 (Man α1,6) Manβ1,4 GlcNAc. The α-mannosidase activity has been purified by conventional chromatographic procedures; the subunit molecular weight is 105 KD and gel filtration under non-dissociating conditions indicates a dimeric structure. In Triton X-114 fraction experiments the activity partitions into the aqueous phase suggesting that it is not an integral membrane protein.

B 104 CHARACTERIZATION OF DE NOVO SYNTHESIZED OVIDUCTAL SECRETORY GLYCOPROTEINS BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS AND LECTIN BINDING, William C. Buhi and Idania H. Alvarez, Department of Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL 32610.

The objective of this study was to characterize de novo synthesized porcine oviductal secretory glycoproteins in explant culture medium by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and lectin binding to assess the surface carbohydrate moieties and provide information for purification. Porcine oviductal tissue obtained throughtout the estrous cycle (Days 0-21), cultured in serum-free medium in the presence of labelled precursors, and analyzed by 1D-PAGE and fluorography revealed the presence of two major bands (115,000 and 85,000 M_r) during estrus which were undetectable at other stages of the estrous cycle. Both proteins, which label with glucosamine and leucine, were further resolved by 2D-PAGE and indicate a pI≤4. The carbohydrate content of the 115K and 85K glycoproteins was evaluated by testing the binding of both iodinated and biotinylated lectins (peanut agglutinin, ricin I, ulex europeaus I, concanavalin A, wheat germ agglutinin and limulus polyphemus) to second dimension slab gels and Western blots, respectively. These studies suggest the 115K and 85K glycoproteins contain fucose, galactose, N-acetyl galactosamine, and N-acetyl glucosamine. Wheat germ agglutinin-agarose affinity columns bind and release retained material as a single peak with N-acetyl glucosamine gradient elution.

B 105 ENDOGENOUS LECTINS: ASSOCIATION WITH SECRETED COMPLEX CARBOHYDRATES, Howard Ceri, Rixun Fang, Wei Sek Hwang and Celia Meade, Departments of Biological Sciences and Pathology, University of Calgary, Calgary, Alberta, Canada.
The specificity of lectins for carbohydrate ligands has prompted their consideration as cell-cell or cell-substrate adhesion molecules. These same properties, however, make lectins important molecules to consider in terms of the secretion, attachment, and organization of secreted complex carbohydrates on mucosal surfaces. Secretion has been studied in two systems, the human lung and quail intestine. Endogenous lectins have been purified from both systems using the secretion as the affinity ligand for purification, and specific antibody raised against the lectins used to localize lectin in each system. In quail the lectin was localized to goblet cells, and found in secretions covering the intestinal mucosa. In human lung the lectin was localized to the ciliated surface of the upper airways, to the ciliated cells themselves and to their underlying secretory glands. In the lower lung the lectin was found in secretory type II pneumocytes. The excess secretion seen in cystic fibrosis was marked by an increase in lectin found at all sites of the lung. This data suggests that lectins co-localize with secretions in secretory cells or glands, and are distributed extracellularly with these complex carbohydrate secretions.

B 106 PRIMARY STRUCTURE OF BOVINE β-1,4-GALACTOSYLTRANSFERASE
Giacomo A.F. D'Agostaro*, Brad K. Bendiak°,and Richard J. Simpson$,*Division of Biophysics and Biomedical Sciences, ENEA Casaccia, 00060 Roma, Italy, °The Biomembrane Institute, Seattle, WA 98119,USA, and$Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research, Melbourne, Australia.
UDP-galactose:N-acetyl-D-glucosamine 4-β-D-galactosyltransferase (GalT) is a Golgi membrane-bound enzyme that participates in the biosynthesis of the oligosaccharide structures of glycoproteins and glycolipids. GalT was purified from bovine milk by sequential affinity chromatography on UDP-hexanolamine-agarose and α–lactalbumin-agarose. SDS-PAGE analysis of the purified enzyme revealed two major polypeptides with M_r of 51 and 42 kDa. N-terminal sequence analysis positively identified the first 15 and 18 amino acid residues of the 51 and 42 kDa GalT polypeptides, respectively. Synthetic oligonucleotides were used to isolate from bovine liver cDNA libraries overlapping cDNA clones that span 1728 nucleotides and code for the entire polypeptide chain of bovine GalT. The sequence of the GalT cDNA reveals a 1206-nucleotide open reading frame that codes for 402 amino acids, including a presumptive N-terminal membrane anchoring domain of 20 hydrophobic amino acids. The colinearity between the cDNA sequence and the N-terminal amino acid sequences of the two bovine milk GalT polypeptides was consistent with the translation frame, indicating that the 51 and 42 kDa polypeptides represent overlapping segments of the C-terminal region of the enzyme , and confirmed the authenticity of the cDNA clones. The primary sequence of bovine GalT predicts that the enzyme is synthesized without a cleaved signal sequence. The N-terminal hydrophobic anchor may function as a stop transfer signal during membrane insertion, to orient the C-terminal region of the GalT polypeptide inside the lumen of the endoplasmic reticulum. This conclusion is in agreement with biochemical studies which indicated that the soluble forms of the enzyme which encompass the C-terminal 324 or 297 amino acid residues of the membrane-bound GalT polypeptide include the catalytic site. These results suggest that the soluble form of GalT can be generated from the membrane-bound form by a proteolytic cleavage between the N-terminal signal-anchor and the catalytic domain.

B 107 STRUCTURE OF O-GLYCOSIDICALLY LINKED OLIGOSACCHARIDES PRODUCED BY INSECT CELLS, Åke P. Elhammer†, Darrell R. Thomsen‡ and Leonard E. Post‡, †Biopolymer Chemistry and ‡Molecular Biology, The Upjohn Company, Kalamazoo, Michigan 49001.

Pseudorabies virus (PRV) gp 50 is a glycoprotein lacking N-glycosidically linked oligosaccharides. This protein was expressed, both in intact form (gp50) and as a truncated dervative lacking the transmembrane and cytoplasmic domains (gp50T), in three different cell types. The cells were labeled *in vivo* with ^{3}H-glucosamine and the immunoprecipitated, radioactive gp50 and gp50T produced, was subjeced to oligosaccharide analysis. Gp50 synthesized by PRV infected Vero cells and by CHO cells transfected with a plasmid containing the gp50T gene, both contained the same three major core oligosaccharides, in approximately the same relative amounts. The smallest of these structures co-migrated with Galβ1-3GalNAc on paper chromatography. All three structures contained between one and three sialic acid residues. By contrast, gp50 expressed in Sf9 cells contained only two types of oligosaccharides. 89% of the structures were composed of only one monosaccharide, GalNAc; the remaining 11% were a disaccharide composed of two GalNAc residues. This structure was resistant to Jack bean β-N-acetylgalactosaminidase. None of the insect cell produced structures contained sialic acid. Gp50T expressed in Sf9 cells contained the same structures as gp50 but in different proportions; 30% of the structures were the disaccharide. Gp50T is secreted at a slower rate than gp50.

B 108 *IN VITRO* N-GLYCOSYLATION IN DICTYOSTELIUM DISCOIDEUM: REDUCED TRANSFER OF TRUNCATED LIPID-LINKED OLIGOSACCHARIDES IN TWO MUTANT STRAINS, Hudson H. Freeze, Petra Koza-Taylor, Jeffrey A. Jones, Univ. of Calif., San Diego Cancer Center and Dept. of Medicine, La Jolla, CA 92093
N-linked oligosaccharides of wild-type Dictyostelium discoideum are derived from the usual lipid-linked precursor (LLO), $Glc_3Man_9GlcNAc_2$-P-P-dolichol. Mutant strains HL241 and HL243 synthesize a truncated precursor, $Man_6GlcNAc_2$, due to the apparent lack of mannosyl transferase(s) needed to form the $Man_9GlcNAc_2$ structure. *In vivo*, N-glycosylation is decreased 3-4 fold in HL243 compared to either HL241 or the wild-type strains, but when cell homogenates of each strain are assayed *in vitro* using an exogenous tripeptide acceptor (octanoyl-Asn-^{125}I-Tyr-Thr-NH_2), both mutant strains are reduced 5-6 fold in N-glycosylation. This decrease is not explained by different rates of synthesis or turnover of the LLO *in vivo*, instability or breakdown of the glycosylating system, by destruction of the tripeptide acceptor, or by an altered Km of the acceptor. Instead, it is likely that the mutants have a limited amount of transferable LLO available for the oligosaccharyl transferase (OT), or that the Km for the truncated LLO is several fold higher than that of the wild-type LLO. OT polypeptide has not been identified in Dictyostelium, but it is unlikely that both strains have mutations in OT. Western blot analysis shows that wild-type cells do not contain the Glycosylation Site Recognition Protein (GSRP) found in many organisms. Additional studies of the oligosaccharyl transferase are in progress. (Supported by NIGMS 32485, and American Heart Association Established Investigator Award).

B 109 OLIGOSACCHARIDES AT INDIVIDUAL N-GLYCOSYLATION SITES OF GLYCOPROTEIN 71 FROM FRIEND MURINE LEUKEMIA VIRUS, Rudolf Geyer*, Janusz Dabrowski#, Ursula Dabrowski#, Dietmar Linder*, Michael Schlüter*, Hans-Henning Schott* and Stephan Stirm*,* Biochemisches Institut, Universität Giessen, D-6300 Giessen and # Max-Planck-Institut für Medizinische Forschung, D-6900 Heidelberg, FRG

Glycoprotein 71 from Friend murine leukemia virus was digested with proteases, and the glycopeptides obtained were assigned to the eight N-glycosylation sites of the molecule by amino acid sequenation. Oligosaccharides liberated from each glycopeptide by endo H or PNGase F were fractionated and subjected to structural analysis by one- and two-dimensional ^{1}H-NMR as well as methylation-GLC/MF. Oligomannosidic oligosaccharides were detected at Asn-168, -334/341 (not separated) and -410. Hybrid species were found at Asn-12 and -334/341. N-acetyllactosaminic glycans - partially intersected and fucosylated Siaα2-3 or Galα1-3Galß1-4GlcNAcß1-2Manα1-6(Siaα2-6 or Siaα2-3Galß1-4GlcNAcß1-2Manα1-3)Manß1-4GlcNAcß1-4GlcNAcß1-(with some branching at -6Manα1-6 and/or -4Manα1-3) - were obtained from Asn-266, -302, -334/341, -374, and -410. In addition, Thr-268, -277, -279, -304/309, as well as Ser -273 and -275 were found to be O-glycosidically substituted by Galß1-3GalNAcα1-, mono- or disialylated at position 3 or/and 6 of Gal and GalNAc, respectively.

Our results demonstrate a selective distribution of oligomannosidic, mixed and N-acetyllactosaminic N-glycans as well as of O-linked oligosaccharides. Only Asn-residues 334/341 and 410 carry different types of N-glycans. The structures of the oligosaccharides found support the concept of a general branch specificity of α1-3-galactosyltransferase and α2-6-sialyltransferase, irrespective of their origin.

B 110 EXAMINATION OF A FUCOSYLATION MUTANT IN THE CELLULAR SLIME MOLD DICTYOSTELIUM DISCOIDEUM, B. Gonzalez-Yanes, M. Gritzali*, R.D. Brown, Jr.* & C.M. West, Dept. of Anatomy & Cell Biology, Coll. of Medicine, and *Food Science and Human Nutrition Dept., IFAS, Univ. of Florida, Gainesville, FL 32610.

Strain HL250 of the cellular slime mold Dictyostelium discoideum was isolated on the basis of its inability to express a common carbohydrate antigen recognized by mouse monoclonal antibody 83.5. Binding of Ab 83.5 can be competed by excess L-fucose, suggesting that L-fucose forms part of the antigenic structure. Total mutant cell L-fucose and protein-associated fucose was found to be <4% that of the parental strain at all stages of development. To explore the basis of the failure to fucosylate, mutant cells were grown on a medium supplemented with 1mM L-fucose, which resulted in a partial restoration of the fucose-containing epitope. This suggested that the mutant lesion was in the conversion of GDP-mannose to GDP-fucose, which could be bypassed in the presence of L-fucose by a salvage pathway. Accordingly, mutant cell-free extracts were able to transfer fucose from GDP-fucose to endogenous acceptor 3X more efficiently than parental extracts, as if fucosyltransferase activity was normal and endogenous acceptor level was elevated. Furthermore, mutant extracts could transport GDP-fucose into vesicles normally. Direct measurement of GDP-mannose to GDP-fucose conversion showed <10% activity in mutant cell extracts compared to parental cell extracts. Current studies are attempting to resolve whether the lesion is associated directly with the enzyme pathway or its regulation.

1mM L-fucose (but not D-fucose) slightly decreased doubling time in the mutant and slightly increased saturation density, but did not have this effect on the parental strain. Since the fucose-containing epitope is eventually expressed exclusively in the outer layer of the spore coat, spore function was examined. Mutant spores were normally viable but this viability diminished rapidly with age in contrast to parental spores. Germination of mutant spores was associated with increased proteolysis of proteins which carry the fucose-containing epitope. We are currently asking whether these changes are associated with the fucosylation defect by determining their reversal in cells rescued with L-fucose.

B 111 **PHOSPHORYLATION OF A VARICELLA-ZOSTER VIRUS GLYCOPROTEIN BY CASEIN KINASE II,** Charles Grose, Larry Keenan, Wallen Jackson, [1]Jolinda Traugh, University of Iowa College of Medicine, Iowa City, IA 52442, [1]University of California, Riverside, CA 92521

Varicella-zoster virus (VZV) is one of the human herpesviruses which is closely cell associated during its infectious cycle. In this study we distinguished a previously described virus-induced protein kinase activity from that of cellular kinases. In particular, we have evaluated the phosphorylation of the major VZV glycoprotein gpI. This M_r 98,000 glycoprotein includes both N-linked complex and O-linked glycans on a M_r 73,000 polypeptide backbone. In a standard protein kinase assay, the purified glycoprotein was phosphorylated in the presence of [^{32}P]ATP as the phosphate donor and uninfected cell lysate as the source of phosphotransferase activity. Because the phosphorylation event was inhibited by heparin, we suspected that the cellular kinase was casein kinase II. Therefore, we repeated the above experiment with VZV glycoprotein gpI and observed that purified casein kinase II catalyzed the phosphorylation of the viral glycoprotein. Two other major VZV structural glycoproteins, gpII and gpIII, were examined in protein kinase assays. In neither instance was there evidence of phosphorylation. In summary, we have demonstrated that one of the major VZV virion glycoproteins is phosphorylated by the cellular enzyme casein kinase II. We suggest that phosphorylation may play a role in the regulation of viral glycoprotein trafficking and/or virion envelopment.

B 112 GLYCOPROTEIN PRIMERS IN CRUSTACEAN CHITIN SYNTHESIS. Michael N. Horst, Biochemistry/Basic Science, School of Medicine, Mercer University, Macon GA 31207 Chitin synthesis in crustaceans such as Artemia involves an initial lipid linked glycosylation of polypeptides yielding a chitoprotein which contains short chitin oligosaccharides (N=2 to 8). This chitoprotein serves as a primer for chitin synthetase in the initial polymerization of macromolecular chitin. Artemia chitoproteins have been purified by affinity chromatography on WGA-agarose and analyzed by SDS-PAGE and immunobloting using a polyclonal antibody to chitin; their molecular weights range from 22 to 50 kD. The chitoproteins appear to serve as acceptors for purified chitin synthetase as demonstrated by altered mobility on SDS-PAGE. The affinity purified chitin synthetase appears to contain bound chitin oligosaccharides as revealed by immunoblotting after SDS-PAGE. These results suggest that crustacean chitin synthetase may serve as a self-primer for chitin synthesis, analogous to the results in fungal systems. Further characterization of the carbohydrate moiety of Artemia chitin synthetase by enzymatic and immunochemical methods is now underway. Supported by NIH Grant GM-30952.

B 113 SULFATION OF THE TUMOR CELL SURFACE SIALOMUCIN OF THE 13762 RAT MAMMARY ADENOCARCINOMA, Steven Hull and Kermit Carraway, Dept. of Anatomy and Cell Biology, University of Miami School of Medicine, Miami, Fl. 33101. ASGP-1, the major cell surface sialomucin of the 13762 rat mammary adenocarcinoma,comprises at least 0.5% of the total cell protein and has sulfate on 20% of its O-linked oligosaccharide. We have used this system to investigate the O-glycosylation pathway in these cells and to determine the temporal relationship between sulfation and sialylation. The two major sulfated oligosaccharides (S-1 and S-2) were isolated by sequential anion exchange and ion supression HPLC. From structural analyses S-1 is proposed to be a branched, sulfated trisaccharide and S-2 its sialylated derivative. Pulse labeling with sulfate indicated that sulfation occurred on a form of ASGP-1 intermediate in size between immature and mature sialomucin. Pulse-chase analyses showed that the intermediate could be chased into mature ASGP-1. The concomitant conversion of S-1 into S-2 had a T1/2 of 5 min. Monensin treatment of the tumor cells led to a 95% inhibition of sulfation with the accumulation of unsulfated trisaccharide and sialylated trisaccharide. These data suggest that sulfation of ASGP-1 is an intermediate synthetic step, which competes with β1,4-galactosylation for the trisaccharide intermediate and thus occurs in the same compartment as β1,4-galactosylation. Moreover, sulfation precedes sialylation, but the two are rapidly successive kinetic events in the oligosaccharide assembly of ASGP-1.

B 114 THE ROLE OF GLYCOSYLATION IN THE SECRETION OF FOREIGN PROTEINS FROM LEPIDOPTERAN INSECT CELLS. Donald L. Jarvis, Christian Oker-Blom, and Max D. Summers. Department of Entomology, Texas A&M University, College Station, Texas, 77843.

Upon infection of Lepidopteran insect cells (eg. Sf9), a recombinant baculovirus vector encoding human tissue plasminogen activator (t-PA) expresses an authentic t-PA product which is both N-glycosylated and secreted. Treatment of the cells with tunicamycin (TM), but not with methyldeoxynojirimycin or castanospermine, inhibits secretion of t-PA. Thus, N-glycosylation per se, but not processing of the oligosaccharide side-chains, is required for the secretion of t-PA from baculovirus-infected Sf9 cells. Experiments examining the role of N-glycosylation in the transport of several other foreign glycoproteins through the Sf9 cell secretory pathway are in progress. Models include both secretory (eg. human ß-interferon) and membrane-bound (eg. Sindbis virus E1 and E2, Rubella virus E1 and E2, and baculovirus gP64) glycoproteins. Secretion of ß-interferon, like t-PA, appears to require N-glycosylation, but not oligosaccharide processing; results on the membrane-bound glycoproteins are forthcoming. Related experiments have revealed a possible explanation for the inability of Sf9 cells to secrete the nonglycosylated forms of some foreign glycoproteins. At least three unrelated proteins (80K, 53K, and 33K) coimmunoprecipitate with the nonglycosylated forms of t-PA, Sindbis E1 and E2, and baculovirus gP64. None of these proteins coprecipitate with any of the recombinant proteins from non-TM-treated cell lysates or when heterologous antisera are used for immunoprecipitation. Furthermore, none of these proteins reacts with antisera against any of the recombinant antigens by western analysis. Together, these results suggest that nonglycosylated recombinant proteins in TM-treated Sf9 cells form heterooligomers with at least three unrelated cellular or viral proteins, which might prevent their progression along the cellular secretory pathway. Further experiments will be undertaken to verify directly the presence of these putative physical complexes.

B 115 COMPLEMENT ACTIVATION BY SERUM MANNAN-BINDING PROTEIN

Toshisuke Kawasaki, Masayuki Ohta, and Ikuo Yamashina, Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, JAPAN

We showed previously that serum mannan-binding protein (MBP), a lectin specific for mannose and N-acetylglucosamine, has an ability to activate complement through the classical pathway (1). In this study, we investigated the molecular mechanism of the complement activation by serum MBP. The activation was monitored first by passive hemolysis test. When SRBC, which had been coated with yeast mannan (ME) and sensitized with serum MBP (ME-MBP), were incubated with human complement, the cells were readily lysed. The lysis of the cells was observed also when C1q-depleted complememnt was used in place of intact complement. This suggests the possibility that $C1r_2s_2$ binds directly to serum MBP just as it does to C1q on immune-complexes, leading to the activation of $C1r_2s_2$. Lines of evidence supporting this hypothesis have been obtained. When ^{125}I-C1s and C1r, or ^{125}I-$C1r_2s_2$ were incubated with ME-MBP, the labeled compounds bound specifically to the cells with high affinities. The radioactivity bound to the cells were analyzed by SDS-PAGE followed by fluorography. The conversion of C1s from its precursor form (single-polypeptide form) to the active form (two-polypeptide form) was observed only when ^{125}I-C1s was incubated with C1r and ME-MBP. 1) K. Ikeda et al., J. Biol. Chem., 262, 7451-7454 (1987)

B 116 CONCERNING THE MICROSOMAL TOPOGRAPHY OF THE SYNTHESIS OF GLcNAc-P-P-DOLICHOL AND $(GLcNAc)_2$-P-P-DOLICHOL, Edward L. Kean, Case Western Reserve University, Cleveland, Ohio 44106.

The orientation in microsomal membranes from the liver of the 15 day embryonic chick of the glucosaminyltransferases that catalyze the biosynthesis of GlcNAc-P-P-dolichol (Gm), and $(GlcNAc)_2$-P-P-dolichol(Gm_2) was examined by following the effect of proteolysis on these activities. The intactness and native orientation of the microsomes was evaluated by measuring the latency of mannose-6-phosphatase which was similar in controls (93% ± 1.4 (7)) (mean ± S.D. (# of experiments)), and after proteolysis (89% ± 5% (7)). The effect of proteolysis was measured on the basal level of GlcNAc-lipid biosynthesis and after stimulation by the allosteric activators, dolichol-P-mannose and phosphatidylglycerol, as described previously (Kean, (1985) J.B.C. *260,* 12561). The GlcNAc-lipids were isolated, partially purified, and the saccharides identified by paper chromatography after mild acid hydrolysis. Two preparations each of Pronase and trypsin were used. Under all conditions, both the basal and stimulatory synthesis of $(Gm)_2$ was extensively inhibited (93% ± 8% (9)). Unlike previous reports, an inhibition of the synthesis of Gm was also observed. However, the latter effect varied with different lots of trypsin and Pronase, ranging from no inhibition to 50-60% inhibition under basal conditions, and from no inhibition to 80% inhibition under stimulatory conditions. These results suggest the sequestering of the GlcNAc-transferase within the microsomal bilayer which some lots of proteolytic enzymes can penetrate while still not disrupting the integrity of the vesicle. Furthermore, these results suggest the presence of cytoplasmically accessible loci on the N-acetylglucosaminyl transferases which influence both the catalytic and allosteric sites concerned with the synthesis of Gm and Gm_2.

B 117 THE *SACCHAROMYCES CEREVISIAE SEC53* GENE ENCODES PHOSPHOMANNOMUTASE. THE *sec59* MUTATION AFFECTS MANNOSE TRANSFER TO DOLICHOL-LINKED OLIGOSACCHARIDE, François Kepes and Randy Schekman,
Department of Biochemistry, Barker Hall, University of California, Berkeley, CA 94720.

The *sec53* and *sec59* conditionally lethal mutations have been isolated on the basis of conferring to yeast cells a defect in secretion of active cell surface enzymes (Ferro-Novick *et al.,* 1984).

Several lines of evidence demonstrate that the *SEC53* gene encodes phosphomannomutase, *i.e.* the enzyme that catalyzes the interconversion of mannose-6-phosphate and mannose-1-phosphate. A defect in glycosylation of α-factor precursor has been reproduced *in vitro.* Glycosylation is restored by addition of GDP-mannose or mannose-1-phosphate, but not by mannose-6-phosphate. Phosphomannomutase activity is dramatically decreased and thermolabile in *sec53* cytosol. Introduction of the *SEC53* gene on a multicopy plasmid into yeast or enterobacteria results in an increase in phosphomannomutase activity. Finally, the Sec53 protein and phosphomannomutase cofractionate upon gel filtration and DEAE chromatography.

sec59 Cells accumulate oligosaccharides lacking mannoses (Runge, Ph.D. thesis, 1985). Given in addition the following data, it appears that the *sec59* mutation affects at least one mannose transfer to dolichol-linked oligosaccharide. Membranes isolated from *sec59* cells show a defect in glycosylation of α-factor precursor; cytosol shows no such defect. Residual glycosylation activity of *sec59* membranes is thermolabile compared to the activity of wild-type membranes. Partial restoration of glycosylation is obtained in reactions supplemented with GDP-mannose or any of its precursors.

B 118 POSTNATAL EXPRESSION OF INTESTINAL GLYCOCONJUGATES IN PIGS, Timothy P. King and Denise Kelly, Rowett Research Institute, Aberdeen, Scotland, AB2 9SB, UK

High resolution in situ cytochemistry using carbohydrate-specific lectins and antibodies was used to investigate the postnatal expression of blood group-related structures in the small intestine of unweaned pigs. The animals were suckled for up to 8 weeks, during which period changes in the terminal glycosylation of both microvillar and mucus glycoconjugates were found to occur. In the first and second postnatal weeks labelled moities consisted predominantly of galactosyl [β-1,3] N-acetyl-galactosamine and galactosyl [β-1,4] N-acetylglucosamine residues; both components of the core regions of blood group substances. Increased fucosylation of intestinal glycoconjugates was evident from the third week and by the seventh and eighth week, blood-group A-like determinants were present in mucus and on enterocyte membranes of several of the animals. There was considerable variability in the lectin and antibody labelling patterns between animals of the same age. Labelling hetergeneity was also evident within individuals; subtle differences in carbohydrate complexion were often observed between adjacent villus epithelial cells. These animal and cellular differences may reflect variations in the expression of the epistatic 'S' system which controls the expression of porcine blood groups A and O. Similar variations may also occur in activation of other epistatic systems, such as those regulating the expression of specific Escherichia coli receptors on intestinal membranes (Bijlsma and Bouw, Vet. Res. Communications, 11, 509-518, 1987).

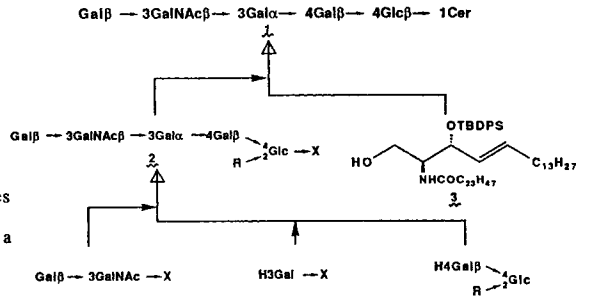
B 119 **BINDING AND DEGRADATION OF SPERM SPECIFIC SULFOGLYCOLIPIDS BY MYCOPLASMA WHICH INDUCE INFERTILITY** by C. A. Lingwood, S. Schraymer, P. Quinn, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8

Sulfogalactosylglycerolipid (SGG) is the major mammalian male germ cell specific glycolipid. *Mycoplasma pulmonis* has been shown to induce male infertility in rodents. We have shown that detergent lysates of this mycoplasma can desulfate and deacylate purified samples of SGG. Moreover, using specific antisera we have found that intact *M. pulmonis* specifically bind to immobilized SGG, thus SGG is a germ cell membrane receptor for these mycoplasma. Rat sperm incubated with *M. pulmonis*, shows a specific reduction in SGG content and indirect immunofluorescent visualization of bound mycoplasma shows a localization coincident with the previously established localization of SGG, that is, on the principle piece and middle piece of the tale, and on the concave surface of the sperm head.

We have previously demonstrated an endogenous SGG binding protein on male germ cells and on the oocyte and have postulated that the interaction between this ligand and its receptor is important in the events culminating in fertilization. The digestion of SGG by *M. pulmonis* may therefore provide the molecular basis for the induction of infertility observed following infection with this organism.

B 120 **A TOTAL SYNTHESIS OF A STAGE SPECIFIC EMBRYONIC ANTIGEN-3 (SSEA-3), GLOBOPENTAOSYL CERAMIDE, IV^{3}GalGb$_4$Cer. USE OF 2,4,6-TRIMETHYLBENZOYL GROUP AS A STEREOCONTROLLING AUXILIARY**, Tomoya Ogawa, and Shigeki Nunomura, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01 Japan

Globopentaosyl ceramide 1 has been isolated from green monkey kidney, human teratocarcinoma as well as from human kidney, and was chemically characterized. 1 showed a strongest reactivity to a monoclonal antibody directed to 4- to 8-cell stage of murine embryos and has been known as the stage-specific embryonic antigen 3 (SSEA-3). Cell surface glycolipids such as 1 are claimed to play significant roles as differentiation antigens during the course of embryogenesis. We achieved a first total synthesis of 1 in an efficient way by using a key glycopentaosyl glycosyl donor 2 that carried a stereocontrolling auxiliary at O-2a as shown in the scheme.



B 121 CORE-SPECIFIC RECEPTOR FOR LIPOPOLYSACCHARIDE ON HEPATOCYTES, James B. Parent, Metabolic Research Division, Naval Medical Research Institute, Bethesda, MD 20814. Endotoxic lipopolysaccharide (LPS), a common structural component of all Gram negative bacteria, is strongly implicated as causative agent of septic shock. The liver is the major organ mediating clearance of LPS from the bloodstream. To determine if hepatocytes have receptors for LPS we have studied the binding of R-form LPS isolated from Salmonella rough mutants deficient in the biosynthesis of their complete (smooth) LPS polysaccharide. LPS was extensively purified and labeled with ^{125}I to 5 μCi/μg using Wood's reagent. Three of the R-form LPS tested (Ra, Rb1 and Rb2) bind to hepatocytes via specific receptors since binding is: (1) competable with excess homologous cold LPS, (2) saturable (max binding 90 ng LPS/10,000 cells), (3) high affinity (1/2 max binding at 1-2 μg LPS/ml), and (4) inhibited by prior mild trypsinization of intact hepatocytes. There was no evidence of specific binding of seven additional R-form LPS tested (Rb3, Rc, Rcp$^-$, Rd1, Rd1p$^-$, Rd2 and Re). These results suggest that hepatocyte LPS receptors recognize the LPS core oligosaccharide and that high affinity binding requires LPS maturation beyond the incomplete core found in LPS isolated from Rb3 mutants. We tested eight S-form (smooth) LPS isolated from E. coli and Salmonella strains and all bound to hepatocytes via specific LPS receptors. Using a photoactivatable radioiodinated LPS probe (Rb2-ASD) and SDS-PAGE analysis, we have identified a 47 KDa LPS receptor on hepatocytes. Underivatized Rb2 LPS inhibits the photoaffinity labelling of the 47 KDa protein with the same dose dependence as it inhibits binding of ^{125}I-Rb2 to intact hepatocytes. The 47 KDa LPS receptor fractionates with cell membranes on both neutral and alkaline sucrose gradients indicating that it is an integral membrane protein.

B 122 FUNGISTATIC PROPERTIES OF THE URTICA DIOICA (STINGING NETTLE) LECTIN, Willy J. Peumans, Willem F. Broekaert, Jan Van Parijs and Els J.M. Van Damme, Catholic University of Leuven, Plant biochemistry Lab, Willem De Croylaan 42, B-3030 Leuven, Belgium. To explain the physiological role of some plant lectins, more precisely these with an high affinity for N-acetylglucosamine oligomers, it has been proposed by several occasions that they may act as antifungal agents. Recently, however, the fungistatic properties of the same lectins have been questioned after it became evident that the observed inhibitory effects of lectins on fungal growth were due solely to contaminating chitinases. To reopen this controversial debate we now report on the fungistatic properties of a lectin found in roots and rhizomes of the stinging nettle (Urtica dioica).

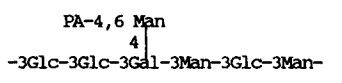
Roots and rhizomes of stinging nettle contain large amounts of a lectin which specifically recognizes N-acetylglucosamine oligomers. This lectin was purified to homogeneity and used in hyphal growth inhibition assays set up with Trichoderma hamatum, Phycomyces blakesleeanus and Botrytis cinerea. In contrast to other chitin binding lectins, which have no inhibitory effect at all, the nettle lectin caused a pronounced growth inhibition with all three fungi at concentrations as low as 50 ug/ml.

Since lectin concentrations in the roots and rhizomes vary between 0.5 and 3.0 mg/g tissue the in vivo concentrations are sufficiently high to inhibit fungal growth, which is an argument in favor of the biological relevance of the nettle lectin as an antimicrobial agent.

B 123 IgE-BINDING PROTEIN: AN ENDOGENOUS IMMUNE SYSTEM LECTIN, Michael W. Robertson, Christine A. Gritzmacher and Fu-Tong Liu, Department of Molecular Biology, Medical Biology Institute, La Jolla, CA 92037 IgE-binding protein (εBP) is a M_r~31K protein isolated from rat basophilic leukemia (RBL) cells. The amino acid sequence, deduced from the cDNA sequence, indicates a unique two-domain structure consisting of a repetitive (9X) sequence (YPGXXXPGA) in the amino terminus portion and predicts a globular carboxy terminus domain. Sequence comparison of εBP to Carbohydrate-Binding Protein 35 (CBP35), a galactose-specific lectin found in many murine cells, shows that 85% of the amino acid residues are identical. In the present study, we show that εBP binding to IgE is readily reversed by the addition of lactose but not glucose or mannose. Moreover, εBP derived from in vitro transcription and translation of cDNA is shown to bind to both IgE-Sepharose and asialofetuin-Sepharose (ASF-Sepharose). Additional studies, utilizing sequence-specific anti-peptide antibodies, indicate that εBP is primarily a cytoplasmic protein and, to a limited extent, is expressed on the surface of RBL cells. Furthermore, Northern blot analysis shows that the εBP transcript is ubiquitously distributed in a variety of murine cells and rat tissues. Although the biological function of εBP is currently unknown, the capacity of εBP to bind specifically to IgE [likely via carbohydrate determinant(s)] provides an interesting example of an endogenous lectin in the immune system.

B 124 EXTENSIVE CO-PURIFICATION OF SECRETORY COMPONENT (SC: AN IgA-BINDING MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY) AND A UDP-GALACTOSE:IgA GALACTOSYL-TRANSFERASE. Stephen Roth, Roy Kerlin, and Edward J. McGuire. Dept. of Biology, University of Pennsylvania, Philadelphia, PA 19104.
SC is a soluble protein found in the milk of many mammals. In its membrane-bound form, on glandular epithelia, the protein is called polymeric immunoglobulin receptor. On the serosal plasma membrane of epithelia, the protein binds plasma IgA, and escorts this immunoglobulin through the epithelium into the glandular secretion. Because of many apparent similarities between SC and some galactosyltransferases, we purified SC according to two methods that each yield seemingly homogeneous preparations of SC. One method (Underdown et al., 1977 Immunochem. 14, 111) utilizes affinity chromatography; the other (Klingmuller and Hilschmann, 1979 Hoppe-Seyler's Z. Physio. Chem. 360, 1895) relies on conventional separation techniques and yields a single protein that has been sequenced. Both preparations also contain a very active galactosyltransferase activity toward human milk IgA. This activity synthesizes a beta-linked galactoside on the alpha chain of the IgA, and the activity is stimulated by alpha-lactalbumin. The enzyme transfers galactose to GlcNAc also, but at one-thousandth the IgA rate. The purified preparations show one silver-stained band with an Mr of 68 kD on SDS-PAGE. The purified preparations also react with poylclonal and monoclonal antibodies against human SC. These data show only that SC and a galactosyltransferase co-purify extensively from human milk. Additionally, however, the binding of IgA to SC-bearing cells is stimulable by alpha-lactalbumin, and cells transfected with the rabbit gene for SC (Mostov and Deitcher, 1986 Cell 46, 613) show an IgA galactosyltransferase activity that is absent in the untransfected, parental strain. Taken together, the results indicate that SC may be a galactosyltransferase that, while on the plasma membrane, acts as a receptor for oligosaccharides synthesized by other enzymes in other cells.

B 125 A NEW LECTIN ACTIVITY IN PEANUT, Morey E. Slodki, Biljana Vucelić-Radović, Martin C. Cadmus and James J. Nicholson, Northern Regional Research Center, ARS, USDA, Peoria, IL 61604
A major group of bradyrhizobial microsymbionts of peanut produces an exocellular heteropolysaccharide (EPS) having the repeat unit structure:



The sugar residues are α-linked. Both side branch D-mannosyl and branch point D-galactosyl residues in EPS from 11-day cultures are 2-O-acetylated. Main chain D-mannosyl residues are also acetylated. The mannosyl side residue bears a 4,6-pyruvic acetal (PA) substituent. Although 10-day EPS is not precipitated by an aqueous extract of defatted peanut meal, a reaction is given by the deacetylated form and by native EPS from a 4-day culture. EPS that is both depyruvated and deacetylated is inactive. Washed 4-day cells are aggregated by peanut extract. These findings support the concept that rhizobial EPS and legume lectin participate in specific nodulation.

B 126 PLANT LECTINS IN BULBS, Els J. M. Van Damme and Willy J. Peumans, Catholic University of Leuven, Plant Biochemistry Lab, Willem De Croylaan 42, B-3030 Leuven, Belgium.
During the last decade evidence has accumulated that plant lectins are not confined to seeds but are also present in a whole variety of vegetative tissues. Recently several lectins have been isolated from bulbs of different monocot plant species e.g. Tulipa sp., Colchicum autumnale, Galanthus nivalis and Narcissus pseudonarcissus. All these species contain reasonable amounts of lectin with some unique properties. Especially the Amaryllidaceae lectins are quite interesting since they exhibit an exclusive specificity towards mannose which makes them very useful tools for glycoconjugate research.
Despite the overwhelming information on the biochemical properties and carbohydrate specificity of lectins in vegetative plant tissues their physiological role is still far from understood. However, recent studies concerning a detailed analysis of the accumulation, disappearance and distribution of the lectin over different tissues throughout the life cycle of Tulipa cv. Attila, Narcissus pseudonarcissus and Galanthus nivalis have indicated that these lectins behave like typical storage proteins. High lectin concentrations were found during the resting period of the bulb whereas after planting the lectin content gradually decreased. However, at the end of the growing season lectins accumulated in the new bulb units.
Most probably these lectins must be considered as a rather special class of storage proteins which in addition to their storage role have carbohydrate binding activity. At present we cannot exclude that these lectins might have an additional function besides their storage role.

B 127 INTRACELLULAR DOLICHOL TRANSPORT, G. Van Dessel, M. De Wolf, A. Lagrou, H.J. Hilderson and W. Dierick, UIA-Laboratory for Pathological Biochemistry and RUCA-Laboratory for Human Biochemistry, University of Antwerp, B2020 Antwerp, Belgium.

Dolichols are a family of long chain 2,3-dihydropolycisprenols which are found in all eukaryotic cells. The subcellular distribution of dolichol, dolichol kinase and dolichylmonophosphate phosphatase has previously been reported. As it is now generally accepted that 'de novo' synthesis of dolichol is localized in the ER-membranes, the omnipresence of dolichol in all cellular membranes points to the presence of an extensive carrier system. This is further suggested by the presence of a relative high amount of this isoprenoid in the cytosol. This dolichol may represent a transport form involving carrier proteins analogous to these described for other lipids.

Dolichol binding capacity of bovine liver pH 5.1 supernatant amounted to $\pm$ 5 pmol/mg protein. The binding showed no linearity with respect to protein concentration, was optimal at pH 7.4 and was abolished after trypsin treatment. Retinol, cholesterol, triglycerides and free fatty acids did not interfere. On Sephacryl S-300 column chromatography the dolichol, cholesterol and fatty acids binding proteins showed a different elution pattern.

The non-specific lipid transport protein from bovine liver is also capable to mediate the transfer of dolichol from single unilamellar vesicles to mitochondria. Phospholipids and cholesterol are transferred to a greater extent than dolichol (ratio 2.1/4.2/1). With respect to dolichol transfer activity a 4,000-fold purification was obtained with a protein yield of 12 %. The transfer activity was influenced by negatively charged phospholipids and by N-ethylmaleimide, but not by salts (1M) or mono- and divalent cations (0.1M).

B 128 PUTATIVE GLYCOSYLATION OF THE RAT LIVER GLUCOCORTICOID RECEPTOR. Ann-Charlotte Wikström, Marc Denis and Göran Andersson. Department of Medical Nutrition and Department of Pathology, Karolinska Institutet, Huddinge University Hospital, S-141 86 Huddinge, Sweden. The glucocorticoid receptor (GR) is an intracellular protein which belongs to the steroid-thyroid hormone receptor family. The majority of these proteins are most likely nuclear proteins. Based mainly upon imunocytochemical studies, GR is considered to be localized also in the cytoplasm. However, GR exerts its function in the nucleus where it acts as a modulator of specific gene function. Deduced from the amino sequence, rat GR contains four putative N-glycosylation sites. Considering the existing models of intracellular GR localization, it is intriguing if cytosolic GR is N-glycosylated. We have studied the putative glycosylation of rat GR.

Rat GR was purified by immunoaffinity- or DNA-cellulose chromatography. After SDS-PAGE and electroblotting to nitrocellulose, the filters were probed with biotinylated lectins and horse radish peroxidase conjugated avidin. A specific reaction pattern indicating both high mannose structures as well as WGA reactive carbohydrate chains was obtained. These reactions were possible to inhibit with specific monosaccharides at a concentration of 0.5 M. A limited protease digestion of GR with either trypsin or α-chymotrypsin gave rise to fragments with a specific lectin reactivity pattern, indicating that the GR steroid-binding domain lacks WGA reactivity.

B 129 DIFFERENTIAL BINDING PROPERTIES OF Gal AND/OR GAlNAc SPECIFIC LECTINS. Albert M. Wu[1] and Shunji Sugii[2]. [1]Department of Veterinary Pathology, College of Veterinary Medicine, Texas A&M University, College Station, Texas, U.S.A., [2]Kitasato University, School of Medical Technology, Department of Serology Immunology, Kitasato Sahamihara-shi, Kanagawa, Japan.

Binding properties of dozens of Gal and/or GalNAc specific lectins, studied by quantitative precipitation and precipitin-inhibition, competitive-binding, and hemagglutinin inhibition assays, were grouped as the following: Galβ1→3 GalNAc specific (*Arachis hypogaea, Bauhinia pupurea alba, Maclura pomifera, Sophora japonica, Artocarpus integrifolia* and *Artocarpin* lectins and ricin; Galβl→4GlcNAc specific (*Ricinus communis, Datura stramonium* (thorn apple), *Erythrina cristagali* and *Geodia cydonium* lectins): GalNAcα 1-3GalNAc (Forssman specific), *Dolichos biflorus, Helix pomatia, Amphicarpaea bracteata,* and *Wistaria floribunda*; GalNAcα 1→3Gal(Human blood group A) specific lectins, *Griffornia simplicifolia* A_4, *Phaseolus lunatus* agglutinin (lima bean), *Glycine max* agglutinin (soybean), *Vicia villosa* (a mixture of two isolectins, B_4 and A_4) and *Wistaria floribunda* , and GalNAcα 1-ser or thr of protein core (*Vicia villosa* B_4, and *Maclura pomifera*). Many of them demonstrate dual specificities such as *Maclura pomifera* is specific for Galβ1→3 GalNAc and GalNAcα 1→linked residues and *Bauhinia purpurea alba* for both Galβ1→3 GalNAc and N-acetyl lactosamine. Grouping of lectins will aid the selection of lectins for carbohydrate residue studied as well as for the interpretation of the distribution and the properties of carbohydrate chain residues on the cell surface.

References: (1) Wu, A.M. and Sugii, S.: Differential Binding Properties of GalNAc and/or Gal Specific Lectins in Molecular Immunology of Complex Carbohydrate. Adv. Exp. Med. & Biol. 228. 205-263. Plenum Press. New York and London. 1988. (2) Wu, A.M. et al.: A Guide for Carbohydrate Specificities of Lectins in Molecular Immunology of Complex Carbohydrate. Adv. Exp. Med. & Biol. 228. 819-847. Plenum Press. New York and London. 1988.

This work aided by grants from the Texas Agricultural Experiment Station (TAES H6194), USDA/SEA/ARS Coop. Agreement No. 58-61125-5-4 USDA/Sea Formula Animal Health Funds (Project 6648) and the Kleberg Foundation.

B 130 MULTIPLE GLYCOSYLATED FORMS OF INTERLEUKIN-3. Hermann J. Ziltener, John W. Schrader, The Biomedical Research Centre, UBC, Vancouver, BC, V6T 1W5.

T cell derived interleukin-3 was biosynthetically labelled with [^{35}S] methionine and affinity-purified using monoclonal or polyclonal anti-IL-3 antibodies. SDS-Page analysis of the purified IL-3 preparations revealed the presence of three bands with apparent Mr of 32,000-36,000, 27,000-31,000 and 21,500-22,500 respectively. Elution of gel slices as well as high performance gel permeation chromatography demonstrated that each of the three [^{35}S] labelled species was active in the standard IL-3 bioassay. Biosynthetic labelling in the presence of tunicamycin, or N-glycanase treatment, revealed only one band, comigrating on SDS-PAGE with synthetic IL-3 at an apparent Mr of 16,000, thus indicating that the heterogeneity of Mr forms is due to different patterns of N-linked glycosylation. IL-3 derived from the myeloid leukemia WEHI-3B showed a significantly different Mr pattern with a broad band between Mr 29,000 and 45,000 and a major peak around Mr 35,000. IL-3 produced by transient expression in Cos 7 cells was found to be different again with major bands at Mr 26,000 and 30,000-31,000. N-glycanase treatment revealed that at least part of the Mr heterogeneity is due to N-linked glycosylation.

Glycoconjugate Techniques and New Experimental Approaches; Future Applications of Carbohydrates in Biotechnology

B 200 CHARACTERIZATION OF THE CARBOHYDRATE CHAINS OF RECOMBINANT HUMAN MACROPHAGE COLONY STIMULATING FACTOR-1 (rhMCSF), Dale Cumming, Ray Camphausen, Tom Salati and Hubert Scoble. Genetics Institute, Inc., One Burtt Road, Andover, MA 01810 Recombinant human MCSF (rhMCSF), purified from Chinese hamster ovary cells, is a disulfide-linked homodimer. Each 223 residue subunit exhibits two sites of Asn-glycosylation and multiple sites of Ser/Thr-glycosylation. As a prerequisite to understanding their contribution to the physiological function of rhMCSF in vivo, detailed structural analysis of the carbohydrate chains is required. Glycopeptides containing Asn-linked oligosaccharides were isolated from a tryptic digest and partially characterized by lectin affinity chromatography. Peptides containing Asn^{122}-linked oligosaccharides bound to ConA-Sepharose, whereas peptides containing Asn^{140}-linked oligosaccharides bound to both immobilized ConA and lentil lectin. The primary structures, established by 500 MHz ^{1}H-NMR, indicate that both Asn^{122} and Asn^{140} are predominantly glycosylated with complex, biantennary oligosaccharides terminated by α2,3-linked sialic acid residues. Asn^{140}-linked oligosaccharides contain an α-fucosyl residue linked to C-6 of the reducing terminus GlcNAc. Identification of the attachment sites and primary structures associated with the Ser/Thr-linked oligosaccharides will also be presented.

B 201 GENE TRANSFER AND MOLECULAR CLONING OF DNA SEQUENCES THAT DETERMINE EXPRESSION OF BLOOD GROUP H STRUCTURES AND AN (α1,2)-l-FUCOSYLTRANSFERASE L.K. Ernst[1], R.D. Larsen[1], V.P. Rajan[2], and J.B.Lowe[1,2], [1]Howard Hughes Medical Institute and [2]The Department of Pathology, University of Michigan, Ann Arbor, MI 48109. Human (α1,2) fucosyltransferases (α1,2-FT) and their cognate cell surface glycoconjugate linkages exhibit tissue-specific expression, are developmentally regulated, and their expression is altered in association with malignant transformation. To understand the molecular basis for these processes, we have established a genetic approach to isolate gene(s) encoding human α1,2-FT's. H-antigen negative mouse L cells were transfected with human genomic DNA. Seven independent mouse L cell transfectant cell lines were isolated that contain 25 to 55 kb of human genomic DNA, express cell surface H structures, and also express α1,2-FT. The structure of these H determinants was confirmed by enzymatic conversion to blood group A determinants with human blood group A α1,3GalNAc transferase, and by hapten inhibition studies. pH-activity profiles and Kms for the substrates GDP-L-fucose and phenyl-β-D-galactoside were determined for the α1,2-FT in human donor cells, in human serum, and in transfectants. The enzymes were indistinguishable by these analyses. Southern blot analysis revealed the presence of two characteristic human DNA restriction fragments in the genome of each mouse transfectant. These fragments were isolated from a phage minilibrary prepared from a representative transfectant and were subcloned into a mammalian expression vector. These plasmids were separately transfected into COS-1 cells; one plasmid directs high level expression of α1,2-FT in COS-1 cells. These cloned sequences may represent a human α1,2-FT gene.

B 202 LOW LEVELS OF GLYCOSYLATION OF A MOUSE/HUMAN CHIMERIC ANTIBODY, Lana Rittmann-Grauer, Mark Guido, Tom Condon, Rodney Jue, Richard Ludwig, Hybritech Incorporated, San Diego, CA 92121

A mouse/human chimeric antibody was constructed by combining the variable light and variable heavy regions from a murine hybridoma specific for human carcinoembryonic antigen (CEM231.6.7) with the kappa and gamma 1 constant region genes cloned from human lymphocytes. The gamma 1 constant region was sequenced. The base sequence AACAGCACG which codes for the potential glycosylation site Asn-Ser-Thr was found 303 based into the CH2 domain of the gamma 1 region. Electrophoresis of the chimeric antibody by SDS/PAGE under reducing conditions followed by periodate oxidation and staining with alkaline phosphatase hydrazide/fast red confirmed the presence of carbohydrate on the human heavy chains and absence on the light chains. The staining of the heavy chain was considerably less than that seen with other mouse and human IgG monoclonal antibodies. Analysis of the sialic acid content of the chimeric antibody indicated a low level of sialylation (0.13 moles of sialic acid/mole of immunoglobulin). This level of sialylation was 5-20 fold less than that seen with other monoclonal antibodies. The neutral sugar content of the XCEM449.08 was also low (5.5 moles of neutral sugar/mole immunoglobulin). The host cell does not appear to be directly responsible for the reduced glycosylation of the antibody because expression of the chimeric construct in three different cell types, SP2/0, P3.653, and MOPC315.36.1 resulted in similar low levels of glycosylation of the antibody. The effects of reduced sialylation and glycosylation on biodistribution and immunogenicity of the chimeric antibody *in vivo* remain to be determined.

B 203 STRUCTURAL CHARACTERIZATION OF OLIGOSACCHARIDES BY LECTIN AFFINITY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, Eric D. Green, Robbin M. Brodbeck, and Jacques U. Baenziger, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Glycoproteins frequently bear highly heterogeneous oligosaccharides, which may be difficult to purify and characterize when only available in small quantities. We have combined the structure-specific nature of lectin affinity chromatography with the efficiency and convenience of high-performance liquid chromatography (HPLC) to develop a lectin affinity HPLC system for the characterization of oligosaccharides. Lectin affinity HPLC columns were prepared by conjugation of L-PHA, RCA_I, RCA_{II}, ConA, *Datura stramonium* agglutinin, and *Vicia villosa* agglutinin to periodate oxidized diol-silica. Analysis of a library of N-glycanase-released, $NaB[^3H]_4$-reduced oligosaccharides of known structure by lectin affinity HPLC revealed that the elution positions of individual oligosaccharides are highly reproducible and correlate with specific structural features. The results of multiple affinity HPLC analyses with different lectin columns can be used to construct a characteristic "fingerprint" for each unique oligosaccharide structure. These "fingerprints" can, in turn, be directly compared with those of known oligosaccharide structures. The utility of lectin affinity HPLC for characterizing heterogeneous oligosaccharide mixtures is significantly increased when combined with other fractionation techniques and selective exoglycosidase degradations. The method is rapid and convenient, providing an important structure-specific dimension to oligosaccharide characterization. Analysis of homogeneous oligosaccharides by lectin affinity HPLC also provides information about the detailed specificity of the conjugated lectins.

B 204 NEW TECHNIQUES FOR THE IDENTIFICATION AND CHARACTERIZATION OF GLYCOPROTEINS ON GELS AND BLOTS, Anton Haselbeck, Sabine Möller, Axel Schmidt, Herbert v.d.Eltz and Wolfgang Hösel, Boehringer Mannheim GmbH, Biochemica Research Center, 8132 Tutzing/Obb, FRG

The analysis of carbohydrate chains on glycoproteins is becoming increasingly important as more therapeutically relevant proteins are produced by genetically engineered organisms with different glycosylation capabilities. For analyzing N-and O-linked oligosaccharides the digestion of glycoproteins with various glycosidases (endo and exo), combined with SDS-PAGE, Western blotting and carbohydrate detection on blots is a fast and easy way to obtain information about the nature of oligosaccharide chains. Along these lines a new staining system suitable for the detection of glycoconjugates on solid supports has been developed. It is both sensitive and selective enough to allow e.g. the detection of glycoproteins on blots at the nanogram level. It has been used in combination with other techniques (e.g. lectin staining) to characterize various glycoproteins which will be presented.

B 205 EFFECT OF N-GLYCOSYLATION ON TISSUE-TYPE PLASMINOGEN ACTIVATOR FIBRIN AFFINITY AND SPECIFIC ACTIVITY, Susan C. Howard, Arthur J. Wittwer, Linda S. Carr, Nikos K. Harakas and Joseph Feder, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167, and Raj B. Parekh, Pauline M. Rudd, Raymond A. Dwek and Thomas A. Rademacher, University of Oxford, Oxford, U.K. Tissue-type plasminogen activator (tPA) isolated from human colon fibroblast (hcf-) conditioned medium is N-glycosylated differently than tPA isolated from the Bowes melanoma (m-) cell line. Both m-tPA and hcf-tPA separate into type I (oligosaccharide at asn-117, -184, -448) and type II (oligosaccharide at asn-117 and -448 only) when chromatographed over lysine-Sepharose. Glycoform assignment was confirmed by reversed phase HPLC of tryptic peptides generated from each. Oligosaccharide analysis of each of these types of t-PA indicates that they have no glycoforms in common, despite having the same primary amino acid sequence (Parekh, et al., submitted). The in vitro activities of type I and type II hcf- and m-tPA were compared with those of unglycosylated tPA isolated from tunicamycin-treated cells. In the presence of fibrinogen fragment stimulator, the kcat of type II tPA was approximately 2.5-fold that of type I tPA from the same cell line, while the Km values were similar. The stimulated activity of aglycosyl tPA was similar to that of type II tPA. In the absence of stimulator, there was little activity difference between type I and type II tPAs, but the activity of aglycosyl tPA was 2-4 fold higher. In a clot lysis assay, type II tPA was 23-26% more effective than type I tPA from the same cell strain, whereas type I and type II m-tPA were about 30% more active, respectively, than type I and type II hcf-tPA. The fibrin binding ability of each tPA correlated very well with clot lysis activity, but not with the stimulated indirect amidolytic activity. Hcf-tPA isolated from a shoulder which eluted from lysine-Sepharose prior to the major type I species was found to have diminished activity.

B 206 MOLECULAR CLONING OF A cDNA THAT DIRECTS EXPRESSION OF SSEA-1 DETERMINANTS AND AN (α1,3)-L-FUCOSYLTRANSFERASE
J. Kukowska-Latallo[1], R.D. Larsen[1], V.P. Rajan[2], and J.B. Lowe[1,2], [1]Howard Hughes Medical Institute and [2]Department of Pathology, University of Michigan, Ann Arbor, MI 48109. Mammalian glycoconjugates may contain L-fucose linked in α anomeric configuration at a number of locations. Terminal fucose residues in α1,3 linkage on cell surface glycoconjugates have been implicated in fundamental events during mammalian embryogenesis. These structures include the stage-specific mouse embryonic antigen (SSEA-1). Terminal α1,3 fucose linkages also represent portions of the human Lewis blood group antigen; these molecules exhibit tissue-specific and developmentally regulated expression patterns. These structures are constructed by α1,3- and α1,4-fucosyltransferase (FT) enzymes. To begin to understand the regulation of expression of fucosylated mammalian glycoconjugates, we have implemented a genetic selection scheme for isolating cDNAs that determine expression of the cognate FTs. Immunologic selection procedures using an antibody directed against the *product* of the enzyme were used to screen a human cDNA library constructed in a mammalian cDNA expression vector. This procedure identified a plasmid containing a cDNA that, when transfected in COS-1 cells, directs expression of cell-surface SSEA-1 structures. This cDNA also directs high level expression of a FT that catalyzes a transglycosylation reaction between the substrate GDP-L-fucose and the acceptor 2'-fucosyllactose. These DNA sequences may encode a human (α1,3)-FT similar or identical to the product of the human Lewis blood group locus.

B 207 ISOLATION OF CLONED cDNAS THAT DIRECT EXPRESSION OF OLIGOSACCHARIDES RECOGNIZED BY GRIFFONIA SIMPLICIFOLIA ISOLECTIN B4
J.B. Lowe[1], R.D. Larsen[1], M.M. Ruff[1], and R.D. Cummings[2]. [1]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109 and [2]Department of Biochemistry, University of Georgia, Athens, GA. Cell surface glycoconjugate structures of mammalian embryos undergo profound spatial and temporal changes during development and differentiation. This includes modulation of the expression of terminally-linked monosaccharides on the polylactosamine chains of some membrane glycoproteins. We have investigated mouse F9 teratocarcinoma cells as *in vitro* model to understand these processes. One of us (R. Cummings) has previously shown that expression of terminal α1,3 galactosyl residues and a cognate α1,3galactosyltransferase (α1,3-GT) is induced in F9 cells differentiated with retinoic acid. To begin to understand the molecular basis for this regulation, we have implemented a genetic scheme to isolate DNA sequences that determine these events. We have constructed a cDNA library in a mammalian expression vector, using mRNA prepared from differentiated F9 cells. A procedure that detects the *product* of the α1,3-GT expressed in differentiated F9 cells was used to screen this library. This method was used to isolate cloned cDNAs that, when transfected into COS-1 cells, direct *de novo* surface expression of molecules recognized by the lectin Griffonia Simplicifolia Isolectin B4 (GSIB4). This lectin recognizes terminal α1,3 linked galactose residues on polylactosamine chains of oligosaccharides; our preliminary enzyme analyses suggest that these cDNAs determine expression of a cognate (α1,3)-GT in transfected, GSIB4-binding cells.

B 208 A HISTOCHEMICAL ASSAY FOR GALACTOSYLTRANSFERASES, Edward J. McGuire, Dan Benau, Whitney Schumacher, Bayard Storey, and S. Roth, Departments of Biology and Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

This abstract describes a histochemical assay for the galactosyltransferases based on a coupled-enzyme assay described earlier (Pierce *et al.*, 1980. Anal. Biochem. 102,441) that yields two moles of NADH for each mole of UDP released from UDP- galactose by the transferase reaction. We have modified this procedure by adding 0.1 U/ml diaphorase and 0.05 mM thiocarbamyl nitroblue tetrazolium to the incubation mixture. The reduction of the tetrazolium by NADH and diaphorase causes intensely colored diformazans to precipitate at sites of galactosyltransferase activity. When applied to suspensions of mouse spermatozoa, using GlcNAc as an acceptor, diformazan appears over the acrosome. This localization agrees with the indirect immunofluorescence localization of sperm galactosyltransferases (Lopez et al., 1985. J. Cell Biol. 101, 1501). This method has also been applied to MDCK epithelial cells that have been transfected with the gene for rabbit secretory component (Mostov and Deitcher, 1986. Cell 46, 613). Secretory component is an IgA-binding member of the immunoglobulin superfamily of proteins that co-purifies extensively with a human milk galactosyltransferase that transfers galactose to IgA. The transfected cells show heavy diformazan deposits when incubated with the complete histochemical mixture and IgA. Under identical conditions, the untransfected, parental cells show no labeling. Both cell types have diformazan deposits when the incubation mixture contains GlcNAc as a galactose acceptor instead of IgA. In all cases, diformazan deposition depends on time, temperature, galactose acceptor and galactose donor. These data suggest, first, that the histochemical assay visualizes galactosyltransferase activities. Second, the results are consistent with the possibility that human secretory component is a galactosyltransferase.

B 209 LOCALIZATION OF GLYCOCONJUGATES AND LECTINS BY TISSUE BLOTTING ON SOLID SUPPORTS, Rafael Pont Lezica and Joseph E. Varner, Department of Biology, Washington University, St. Louis, MO 63130.

The freshly cut surface of a plant or organ pressed on nitrocellulose paper transfers proteins, nucleic acids and metabolites leaving a clear impression of the anatomy on the paper (Cassab & Varner, J. Cell.Biol. 105:2581, 1987). This technique has been shown to be very useful for the rapid localization of specific proteins. We have adapted the technique for the localization of glycoconjugates, complex carbohydrates containing specific sugars and lectins. Glycoconjugates are localized by the reaction with dansyl hydrazide after periodate oxidation of glycoprotein carbohydrates. The resulting hydrazones are reduced with $NaCNBH_3$. This procedure is very sensitive but unspecific since most of the carbohydrates give a positive reaction. The detection of glycoconjugates containig specific sugars is achieved by the use of fluorescent-labeled or peroxidase-conjugated lectins. Excellent patterns were obtained with FITC-lectins at 1 µg/ml (concentrations 100-1000 times lower than that regularly used for classical histochemistry). Lectin activity is probed with sugar-dansyl hydrazones or sugar-BSA-FITC complexes and visualized by their fluorescent emission.

B 210 SEPARATION AND CHARACTERIZATION OF GLYCONJUGATES USING SUPERCRITICAL FLUID CHROMATOGRAPHY-MASS SPECTROMETRY, Vernon N. Reinhold, Douglas M. Sheeley, and Richard S. Johnson, Harvard University School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

Supercritical fluid chromatography (SFC) is a separations technique that employs as its mobile phase a condensed gas (CO_2) above its critical temperature and pressure. These fluids posses favorable physical properties that are intermediate between those of the gas and liquid and provides high molecular weight separations in fused silica capillary columns. With major interests in glycoconjugate characterization we have focused our attention on interfacing SFC with a mass spectrometer (MS). Three major advantages of SFC-MS are: (i) the ease of solvent elimination; (ii) opportunity of utilizing a "soft" ionization MS; and, (iii) the considerable improvements in sensitivity. The SFC column tip was introduced through a closed ion source and heated at the very tip with a resistance wire connected to, and controlled by, the DCI unit. The SFC column restrictor was a laser drilled end cap machined from stainless steel and annealed to the end of the column. With the exception of restrictor tip heating (approx. 1 mm), all other temperatures were maintained equal to that of the SFC oven, 120 °C. To maintain analyte miscibility with the mobile phase all samples were derivatized; several were investigated. Under the CI conditions chosen, only individual adduct ions $(M + NH_4)^+$ were detected for all glyconjugate samples analyzed.

B 211 DNA-MEDIATED CO-TRANSFORMATION OF N-ACETYLGLUCOSAMINYLTRANSFERASE I ACTIVITY, J. Ripka, M. Pierce and N. Fregien, Dept. Anatomy and Cell Biology, Univ. Miami Medical School, Miami, FL 33101

In animal cells, the enzyme $\alpha(1,3)$ mannoside $\beta(1,2)$ N-acetylglucosaminyltransferase I (GlcNAc-TI, EC.4.1.101), catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to the ASN-linked $Man_5GlcNAc_2$ oligosaccharide, which is necessary for complex oligosaccharide biosynthesis. The Chinese hamster ovary (CHO) mutant cell line, Lec1, is deficient in this enzyme, and therefore, synthesizes only mannose-terminating cell surface ASN-linked oligosaccharides. Consequently, Lec1 cells exhibit increased sensitivity to the cytotoxic mannose-binding lectin Concanavalin A (Con A) relative to parental CHO cells. In order to obtain a genomic clone of the GlcNAc-TI gene, Lec1 cells were co-transformed with human A431 cell DNA and the plasmid pBACT-neo7 by $CaPO_4$/DNA-mediated transformation. A single transformant arose from co-transformed Lec1 cells plated in the presence of 10μg/ml Con A and 400μg/ml G418. DNA from the primary transformant was purified and used to transform Lec1 cells, resulting in secondary transformants that proliferated in the presence of both Con A and G418. Both primary and secondary transformants expressed in vitro GlcNAc-TI specific activity. Lec1 cells were also co-transformed with human A431 cell DNA and the plasmid pIBI-30. Transformants were selected in 10μg/ml Con A and demonstrated in vitro GlcNAc-TI specific activity. This work was supported by NIH CA35377 and AR38872.

B 212 SPECIFICITY AND REGIONAL DISTRIBUTION OF GANGLIOSIDE RECEPTORS ON RAT BRAIN MEMBRANES. Ronald L. Schnaar and Michael Tiemeyer, Depts. of Pharmacology and Neuroscience, Johns Hopkins Sch. of Med., Baltimore, MD 21205 Ganglioside-specific binding to rat brain membranes was found using gangliosides covalently derivatized to bovine serum albumin (BSA) as probes. ^{125}I-$(G_{T1b})_4$BSA, (4 G_{T1b} molecules attached per BSA) bound with high affinity (K_D = 2-4 nM) to membranes from rat brain but not liver. Membranes from different brain regions supported different amounts of binding. Membranes from brainstem had 2-fold greater specific binding of ^{125}I-$(G_{T1b})_4$BSA than striatum and hippocampus, while neocortex and cerebellum bound less (8.9, 4.5, 4.0, 2.5 and 1.6 pmol/mg protein, respectively). Spinal cord membrane binding was similar to whole brain (thoracic, 4.9; lumbar 5.8; and whole brain 5.3 pmol bound/mg protein). Inhibition of ^{125}I-$(G_{T1b})_4$BSA binding by gangliosides revealed oligosaccharide structural specificity. Gangliosides having a gangliotetraose core and a $II^3(NeuAc)_2$ group were the most potent inhibitors (IC_{50}'s: G_{D1b}, 73 nM; G_{Q1b}, 77 nM; and G_{T1b}, 107 nM). Other gangliosides were 5 to 20-fold less potent. Kinetic analyses demonstrated that gangliosides are reversible and competitive inhibitors. Most other lipids (e.g. neutral and charged phospholipids and glycolipids) did not inhibit binding. Although phosphatidylglycerol (PG) and phosphatidylinositol (PI) inhibited ^{125}I-$(G_{T1b})_4$BSA binding (IC_{50}'s $\approx$500 nM), their inhibition was non-competitive and irreversible. Thus, $(G_{T1b})_4$BSA is a valuable probe to study ganglioside-specific receptors in brain. Supp. by NIH grants HD14010 and MH18030 (to M.T.).

B 213 SEPARATIONS OF GLYCOPROTEIN-DERIVED OLIGOSACCHARIDES USING HIGH-pH ANION EXCHANGE CHROMATOGRAPHY. Michael W. Spellman and Louisette J. Basa, Medicinal and Analytical Chemistry Dept., Genentech, 460 Point San Bruno Blvd. South San Francisco, CA 94080.

We are investigating the application of high-pH anion exchange chromatography to the separation of Asn-linked oligosaccharides. A nonmetallic HPLC system (Dionex BioLC) equipped with a polymeric pellicular anion exchange column (Dionex HPIC-AS6) and a pulsed amperometric detector was used for the separations. Elution was carried out at a constant base concentration (0.1 N NaOH) using a linear gradient from 0-0.1 M NaOAc and a flow rate of 1 ml/min. Mixtures of high-mannose oligosaccharides (ranging from $Man_5GlcNAc$ to $Man_9GlcNAc$) were baseline resolved in a 40-min gradient. Three closely related biantennary oligosaccharides were also separated under these conditions. Separations of sialylated oligosaccharides were carried out under similar conditions but with a steeper gradient (0-0.2 M NaOAc in 45 min). Although these separations were dominated by the number of residues of sialic acid on each oligosaccharide, significant resolution could be obtained among different structures with the same net charge. The use of pulsed amperometry permits routine detection of underivatized oligosaccharides in the range of 0.1 -1 nmol. Because of the sensitivity of this technique, it can be used in conjunction with reversed-phase HPLC tryptic mapping to characterize the distribution of oligosaccharides at individual glycosylation sites.

For semipreparative applications we have used on-line desalting with an anionic micromembrane suppressor (AMMS) cartridge. The AMMS cartridge can effectively desalt column effluents containing up to 0.2 M sodium ions at a flow rate of 1 ml/min. Salt-free oligosaccharides are then recovered after lyophilization of column fractions. The low dead volume of the cartridge causes minimal peak broadening.

B 214 OLIGOSACCHARIDE PROFILING USING HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (HPAE/PAD) R. Reid Townsend and Mark R. Hardy, Department of Biology, Johns Hopkins University, Baltimore, MD 21218.
The method of HPAE/PAD was applied to the analysis of oligosaccharides released from glycoproteins. Separation of the sialylated isomers of bovine fetuin was used as a model study. Chromatography at pH 6.0 gave only peaks corresponding to di-, tri-, tetra-, and penta-sialylated oligosaccharides. However, elution at pH 13 resulted in the resolution of ≈ 20 peaks. Complete separation of trisialylated oligosaccharides containing a Galβ(1,3)GlcNAc sequence from their Galβ(1,4)GlcNAc- sialylated counterparts was obtained. Additional resolution, based on the combination and branch location of α(2,3) and α(2,6) sialic acids, was also observed at alkaline pH. Oligosaccharides could be detected in the pmol range after direct injection of an N-glycanase digest of bovine fetuin into the chromatograph. The electrochemical response factors for tri-, tetra-, and penta-sialylated structures (provided by Drs. B. Bendiak, D. A. Cumming, and J. P. Carver) were found to be 5.1 +/-20% relative to glucose. Therefore, the relative amounts of the various sialylated oligosaccharides could be surmised directly from elution position and electrochemical response. Detection in the pmol range, without derivatization, and unique chromatographic selectivity make HPAE/PAD an attractive method for the analysis of the oligosaccharides of glycoproteins.

Glycoconjugates in Cell-Cell Recognition and Cell Adhesion

B 300 ROLE OF GALAPTIN IN OVARIAN CARCINOMA ADHESION TO EXTRACELLULAR MATRIX IN VITRO, Howard J. Allen, Daniel Sucato, Barbara Woynarowska and Ralph Bernacki, Roswell Park Memorial Institute, Buffalo, NY 14263

Galaptin is an evolutionarily conserved β-galactoside-binding lectin present in a wide variety of tissues and cells of vertebrate origin. The function of galaptin is unknown; however, data has been presented which suggest that this lectin may play a role in cellular adhesion phenomena. Immunochistochemical studies indicate that galaptin is a major protein of ovarian carcinoma cells present in patient effusions although it is not restricted to the cell surface. ELISA and immunoprecipitation demonstrated that galaptin is a major protein of the A121 ovarian carcinoma cell line, constituting ≤1% of extractable protein bound by DEAE Sephacel. ELISA also demonstrated that galaptin is present in confluent bovine corneal endothelial cells (BCEC) and their extracellular matrix (ECM) synthesized in vitro, at a ratio of 1:10 (ECM:BCEC). Lactose-inhibitable binding of radiolabelled galaptin to BCEC-free ECM demonstrated the presence of a high density of galaptin receptors (>350 ng galaptin bound/CM^2 of ECM). Prior treatment of ECM with lactose increased galaptin binding by 22-48%. Pretreatment of A121 cells with galaptin appeared to inhibit adhesion to ECM while the continuous presence of soluble galaptin appeared to enhance adhesion. The data reported here and other immunohistochemical observations support the hypothesis that galaptin may have a functional role via interaction with the ECM.

B 301 CHANGES IN CARBOHYDRATES IN THE UTERUS ASSOCIATED WITH IMPLANTATION, Thomas Andersson and Arne Lundblad, BioCarb AB, Susan J. Kimber, University of Manchester, Svend Lindenberg, Copenhagen University Hospital. The initial phase of implantation of the mammalian blastocyst consists of close apposition between the trophectoderm and luminal epithelium followed by firm attachment of the trophectoderm to the epithelium. Immunohistochemical techniques in combination with an in vitro model for implantation in the mouse have demonstrated that the interaction between cell surface oligosaccharides on the endometrium and "lectin" molecules on the trophectoderm is essential for blastocyst attachment. A number of monoclonal antibodies (MAB) specific for blood group related oligosaccharides bind to the endometrial epithelium. One of these, 667/9E9 which recognizes the H-type I structure Lacto-N-fucopentaose I (LNF I) exhibits a change in staining pattern during early pregnancy becoming restricted to stretches of cells by the day of implantation.
When blastocysts are cultured with monolayers of endometrial epithelium they attach and show the initial characteristics of implantation in utero. LNF I and the MAB 667/9E9 significantly inhibits attachment. Furthermore, neo-glycoproteins containing LNF I conjugated to albumin and labelled with FITC bind to the abembryonic trophectoderm on day 5 to 6 of development. Recent observations suggest that changes in carbohydrate determinants on the human uterine epithelium occur between the proliferative and the secretory phase in the menstrual cycle. Specifically there is an increase in certain epitopes in the secretory phase which may be associated with preparation of the uterus for receiving the blastocyst.

B 302 BIOCHEMICAL CHARACTERIZATION OF E2, AN ADDITIONAL T-CELL SURFACE MOLECULE INVOLVED IN ROSETTES. Françoise Aubrit, Catherine Gelin, Brigitte Raynal, Danièle Pham, and Alain Bernard, Laboratoire d'Immunologie des Tumeurs de l'Enfant, Institut G. Roussy, 94805 Villejuif Cedex, France.
Using four mAb we demonstrated that, on T-cell surface, a molecule other than CD2 is involved in the rosette phenomenon. These four mAb reacting with human T-cells were able to block rosettes with erythrocytes from many species. They reacted with a 32 kDa molecule detected on all T-cells, monocytes and also non hematological cells. Biochemical studies show striking patterns of the glycosylation states of E2. It is a heavily sialilated and glycosylated molecule. It carries only O-linked oligosaccharides taking account for almost half of its Mr. No N-linked sugar residues were detected. Removal of sialic acid reduced by approximately 4 kDa its molecular mass. Finally, immunopurification of E2 molecule allowed us sequencing 14 amino acid residues of the N-terminal side : -, -, Leu, Phe, Asp, Leu, Ser, Asp, Ala, Leu, Pro, (Pro), Asn, Glu, Asn, -, Lys. This sequence revealed no homology with known proteins.

B 303 TISSUE-SPECIFIC N-GLYCOSYLATION OF A HEPATOCYTE CELL SURFACE PROTEIN INVOLVED IN CELL AGGREGATION. A. Becker, Ch. Hoffmann and W. Reutter, Inst. f. Molekularbiol. u. Biochem. d. Freien Universität, Berlin (West), FRG.

We have isolated a glycoprotein (gp110) from rat hepatocyte plasma membranes that is characterized by a marked heterogeneity im molecular mass (95-115 k) and an exceptionally high content of carbohydrate (>50%). In liver several variants with different molecular mass could be identified which, on deglycosylation with peptide-N-glycanase F, were all converted into a single polypeptide with Mr 48 k. These glycoforms were not due to differential sialylation, since their pI was very similar. By desialylation, however, the Mr could be reduced by 15 k, while the heterogeneity of Mr persisted. Fab-fragments isolated from polyclonal antibodies inhibit the reaggregation of isolated hepatocytes.

A comparison of the expression and glycosylation of gp110 in other organs showed it to be ubiqitously expressed, although expression was strongest in lung, small intestine and salivary gland. Gp110 isolated from these tissues exhibits organ-specific differences in Mr. The different forms of the molecule are converted into a uniform polypeptide of 48 kDa by deglycosylation. However, these glycoforms not only differ in the extent of glycosylation, but also in their isoelectric points, reflecting differences in sialylation. Thus it is evident that gp110 isolated from liver, lung and kidney is sialylated, while gp110 isolated from small intestine and salivary gland is not sialylated. These results demonstrate that the glycosylation of a protein is not solely determined by its polypeptide structure but may be regulated in a tissue-specific manner rendering this glycoprotein a suitable model for such investigations.

B 304 SIALOGLYCOPROTEIN OF VESICULAR STOMATITIS VIRUS: ROLE OF NEURAMINIC ACID IN VIRUS-CELL FUSION, Robert Blumenthal, Settimio Grimaldi and Anu Puri, Section on Membrane Structure and Function, LTB, NCI, NIH, Bethesda, Md. 20892

Treatment of Vesicular Stomatitis Virus (VSV) with Neuraminidase free of proteolytic activity did not affect binding to Vero cells, but significantly enhanced the rate of fusion of the viral membrane with the plasma membrane of the cells. The fusion was measured using an assay for lipid mixing based on the relief of self-quenching of octadecylrhodamine (R18) fluorescence. The pH-dependence of fusion of neuraminidase-treated virus was similar to that seen with untreated virus. Incubation of untreated or neuraminidase-treated VSV with mixed gangliosides resulted in a reduction of the fusion activity. Measurement of fluorescence energy transfer in dansyl-labeled virus indicated that treatment with neuraminidase alters the conformation of the sialaglycoprotein to a state which might more readily mediate pH-dependent fusion.

B 305 CARROT SOMATIC EMBRYOGENESIS DEPENDS ON THE PRESENCE OF CORRECTLY GLYCOSYLATED EXTRACELLULAR PROTEINS, J.H.G. Cordewener, H. Booij and S.C. de Vries, Department of Molecular Biology, Agricultural University Wageningen, De Dreijen 11, 6703 BC Wageningen, the Netherlands.

Very little is known at the molecular level about the functions controlling regeneration of plant cells. We have shown recently that carrot somatic embryogenesis depends on the phytohormone-controlled presence in conditioned medium of several correctly glycosylated extracellular proteins (De Vries et al. 1988, Genes and Development 2, 462-476). This result was based on the observation that addition of tunicamycin, a glycosylation inhibitor, alters the extracellular protein pattern and inhibits somatic embryogenesis at the same time. This inhibition can be reversed by adding, within 24 h after transfer of proembryogenic masses to hormone-free medium, a mixture of extracellular proteins as present in the medium of a normally developing embryo culture. With these extracellular protein preparations it was also possible to complement several non-embryogenic variant carrot cell lines. Since these results suggest that one or more of these extracellular proteins are responsible for proper somatic embryogenesis to occur our attention was focussed on their purification and identification. Several basic peroxidase isoenzymes were isolated from the medium of an embryogenic culture. These isoenzymes were (partially) purified by means of CM-cellulose CL-6B column chromatography. It was shown that some of these peroxidase preparations were able to restore to some extent or even completely embryogenesis in tunicamycin-inhibited wild-type cultures. Data about the physico-chemical properties of these glycosylated proteins will be presented. Furthermore, the physiological aspects and the possible function of these peroxidases during the development of somatic embryos from proembryogenic masses will be discussed.

B 306 CONTRIBUTION OF FINE STRUCTURE OF THE N-LINKED OLIGOSACCHARIDE GROUP OF A RAT IgG2b MONOCLONAL ANTIBODY TO EXPRESSION OF EFFECTOR FUNCTIONS, William Cushley and Anna M. Jaques, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

There is considerable interest in the role of N-linked oligosaccharide groups in regulation of the function of glycoprotein molecules. In the case of antibody (Ab) molecules, much of the work addressing this question has employed tunicamycin to generate non-N-glycosylated Abs for analysis of structure-function relationships. Such studies have indicated that absence of N-linked glycans severely impairs the ability of IgG molecules to fix complement and bind to Fc receptors.

We have employed inhibitors of the enzymes of the pathway responsible for biosynthetic processing of protein-bound N-linked oligosaccharides to generate rat IgG2b monoclonal antibodies bearing carbohydrate structures different from those normally present on the molecule. These antibodies are secreted normally from hybridomas exposed to the inhibitors. Using hybridoma surpernatants and purified IgG2b molecules, we find that the ability of IgG2b molecules bearing manipulated oligosaccharide structures to participate in complement fixation reactions is not reduced relative to control IgG2b molecules. Studies with other IgG subclasses and analysis of Fc receptor interactions will also be presented.

B 307 ENZYMATIC BASIS OF WHEAT GERM AGGLUTININ RESISTANCE IN EL4 VARIANT CELL LINES, Nancy L. Devino and Penny J. Gilmer, Department of Chemistry, Florida State University, Tallahassee, FL 32306-3006.

EL4 murine lymphocytic leukemia cells deficient in cell surface N-acetylneuraminic acid were selected with wheat germ agglutinin (WGA). Cells thus selected were maintained in WGA-free tissue culture medium. WB6, a variant clone of EL4 that survived in 3.2 μg/ml WGA, no longer grows in the peritoneal cavity of the syngeneic mouse. The biochemical basis of the altered carbohydrates is currently under investigation. WB6 has 2-3 fold less total neuraminidase activity than EL4 toward the synthetic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4-MU-NANA). WB6 also has 2-3 fold less total β-galactosidase and α-mannosidase activities toward 4-MU-Galactoside and 4-MU-Mannoside, respectively. In addition, EL4 and WB6 exhibit different sensitivities to sodium chloride concentration during the preparation of these lysosomal enzymes. The fraction of original activity that pellets at 4000 x *g* is independent of [NaCl] in EL4, while WB6 shows a strong positive correlation between pelleted activity and [NaCl]. Differences in the activity of these key oligosaccharide processing enzymes may result in altered pathways of antigen recycling, affecting the relative immunogenicity of the parental and variant cell lines.

B 308 MEMBRANE GLYCOPEPTIDES OF MONENSIN-TREATED BL/VL3 LYMPHOMA CELLS

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Following i.v. injection, arrest in the spleen of 111-In labeled lymphoma cells was clearly reduced if cells had been cultivated for 24 h in the presence of inhibitors of processing. This was also the case after treatment with monensin (MON) 0.1-1.0 μg/ml, 24 h. We have characterized glycopeptides from BL/VL3 murine T lymphoma cells. Following labeling with tritiated precursors (fucose,mannose) surface glycopeptides were released by trypsin and separated by gel filtration on Bio-Gel P6 and by affinity chromatography on immobilized Con-A and LcL. After treatment with MON, a class of high molecular weight glycopeptides was no longer found. There were less complex and more high mannose peptides as judged from affinity for Con-A. The same applied to immunoprecipitated Thy-1 antigen. In addition, by FACS analysis, Thy-1 was not reduced in MON-treated cells. Taken together, our results suggest that cell surface oligosaccharides are dramatically modified, but that certain cell surface antigens at least are present in normal amount. It is tempting to speculate that changes in glycosylation account for the abnormal homing properties of MON-treated cells.

B 309 SIALYL GLYCOCONJUGATES INVOLVED IN THE CAPACITATION PROCESS IN HUMAN SPERMATOZOA

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Before fertilizing mature eggs human spermatozoa must undergo a period of maturation known as capacitation.Capacitation comprehends a series of biochemical and biophysical changes whose nature and sequence remain unclear.It has been suggested by many authors that loss of specific components is one of the key steps in this process.Capacitation,which naturally occurs in the female genital tract,can be acquired in vitro by incubating the spermatozoa in a culture medium containing human serum albumin.In this study,surface sialic acids of washed ejaculated spermatozoa were radiolabeled with $NaJO_4$-NaB^3H_4 and the time course of the release of the sialyl-glycoconjugates over six hours in the capacitation medium was analyzed.Radiolabeled components were progressively released from the sperm during the first four hours,after which a plateau was reached.Gel filtration analysis of the released material revealed two distinct classes of radiolabeled components whose molecular weights judged from the elution profile on Sephadex G-25 were 2-2.5 kD for the first and 4-4.5 kD for the second.Other labeled components eluted as a peak at void volume on Sephadex G-25,G-50 and G-100 were further analyzed by SDS-PAGE.Surprisingly the gels only revealed peaks of radioactivity for macromolecules of molecular weights between 38 and 14 kD.

B 310 PURIFICATION AND CHARACTERIZATION OF AVIAN β1,4 GlcNAC:GALACTOSYLTRANSFERASE

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Cell surface galactosyltransferase (GalTase) participates during embryonic development in a number of cell-cell and cell-matrix interactions. In avian embryogenesis, surface GalTase is localized to areas of inductive interactions, and to surfaces of actively migrating mesenchyme cells. In order to study surface GalTase function during avian embryogenesis, the enzyme from chicken serum was purified to apparent homogeneity by alpha-lactalbumin (α-LA) affinity chromatography followed by preparative SDS-PAGE. The bands containing GalTase activity were identified by extracting and renaturing active enzyme from SDS-PAGE. GalTase activity is associated with both a 30 and a 46 kD band. GalTase from α-LA affinity columns was used to biochemically characterize the enzyme. The K_m's for UDPGalactose and GlcNAc were determined to be 30 μM and 10 mM, respectively. Substrate specificity analysis indicated that the enzyme is a β1,4 GlcNAc:GalTase, since the enzyme is active toward GlcNAc residues on O-linked and N-linked chains, but not toward terminal GalNAc resides, glycolipid substrates, and the α-GalTase substrate N-acetyllactosamine. The enzyme is inhibited with high GlcNAc concentrations, binds to α-LA, and the galactosylated product is sensitive to E. coli β-galactosidase. The gel purified GalTase was used as immunogen to produce anti-GalTase antibody. The antibody labels the 39 and 46 kD bands on Western blots, immunoprecipitates GalTase activity, and inhibits GalTase activity toward ovalbumin and GlcNAc as acceptor substrates. This antibody is currently being used in in vivo studies to assay the role of surface GalTase during early embryonic events. Supported by NIH DE07120.

B 311 THE EXPRESSION OF SYNDECAN, A CELL SURFACE PROTEOGLYCAN, CHANGES WITH STRATIFICATION OF MOUSE EPIDERMAL KERATINOCYTES. Michael Hinkes, Ralph Sanderson and Merton Bernfield. Stanford University School of Medicine, Stanford, CA 94305.

In epidermis, keratinocytes stratify by freeing themselves from the underlying matrix, forming new adhesions with their neighbors, and then dissolving these adhesions prior to desquamation. Thus, this differentiation involves modifications in cell-matrix and cell-cell adhesions. Syndecan, a cell surface proteoglycan known to bind cells to interstitial matrix, surrounds all surfaces of mouse epidermal keratinocytes; modest in the basal layer, heavy in the suprabasal layers, but absent from the most superficial, terminally differentiated layers. This layer-specific difference suggests that syndecan expression changes with keratinocyte differentiation. To assess this hypothesis, syndecan was studied using monoclonal antibody 281-2 before and after calcium-induced stratification and differentiation of newborn mouse keratinocytes in culture. By immunohistology, syndecan is on the lateral and apical surfaces of unstratified keratinocytes. In stratified keratinocytes, it surrounds the entire surfaces of the basal and suprabasal layers but is absent from the uncornified superficial layer, closely duplicating the distribution *in vivo*. Radioimmunoassay of trypsin-released syndecan shows that stratified keratinocytes have 2.7 times more syndecan per cell on their surfaces than unstratified keratinocytes, a difference also seen in syndecan shed into the culture medium. Syndecan extracted from either unstratified or stratified keratinocytes has a modal size of 92 Kd. Hence, calcium-induced stratification of keratinocytes causes: (*i*) syndecan to distribute in a fashion that mimics the *in vivo* situation and (*ii*) increased expression of cell surface syndecan, indicating that the amount of syndecan at the cell surface changes in association with keratinocyte differentiation. Syndecan may be an adhesibe molecule involved in keratinocyte stratification that is lost prior to desquamation. (Supported by NIH HD 06763 and CA 28735).

B 312 A FAMILY OF CELL SURFACE GLYCOPROTEINS DEFINED BY A PUTATIVE ANTI-ENDOTHELIAL CELL RECEPTOR ANTIBODY IN MAN. Geoffrey S. Kansas, Gary S. Wood and Morris O. Dailey, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa 52242 and Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305 and Veterans Administration Medical Center, Palo Alto, California 94304.

An efficient immune response depends directly on the ability of lymphocytes and other blood borne leukocytes to leave the blood and enter lymphoid organs and other sites of immune response or inflammation. A new murine monoclonal antibody termed 515 recognizes a 85-90 kd structure involved in lymphocyte binding to specialized endothelium in postcapillary venules of lymphoid organs (high endothelial venules or HEV). In crossed immunoprecipitations followed by SDS-PAGE analysis, 515 appears to be identical to the anti-lymphocyte homing receptor mAb, Hermes-1. We show here that 515 recognizes peripheral blood leukocytes of all classes examined, but, like Hermes-1, fails to recognize germinal center cells or nearly all cortical (but not medullary) thymocytes. In addition, we demonstrate that 515 recognizes both fibroblasts and epidermal keratinocytes (EK), both *in situ* and in cultured cell lines. Immunoprecipitation and SDS-PAGE analysis reveals that 515-defined antigens from T lymphocytes, neutrophils and the EK cell line HaCaT are clearly distinct, both before and after treatment with peptide N-glycosidase F, which removes all asparagine-linked oligosaccharides. In contrast, the M_r 515-defined antigens from T cells and fibroblasts appear indistinguishable. Thus, the 515 mAb recognizes a broadly distributed family of glycoproteins which may be involved in a variety of adhesive functions.

B 313 NAC, A GLYCOSYLATION MUTANT IN DROSOPHILA WHICH PERTURBS A NEURON - SPECIFIC GYCOCONJUGATE, Flora Katz, Zaid Smith, Wanda Moats, Zelah Pek, and Mark Lehrman[1], Department of Biochemistry and [1]Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX 75235 - 9050.

Antibodies against horseradish peroxidase recognize specifically all neural tissue in Drosophila and other insects. The relevant epitope is a carbohydrate found on a developmentally regulated array of cell-surface proteins. The carbohydrate contains features of a common asn-linked plant glycoconjugate structure exemplified by pineapple stem bromelain and Sophora japonica lectin. A mutation, nac (neurally altered carbohydrate), has been identified in Drosophila that alters this epitope in the adult nervous system. A combination of biochemical analyses have been used to elucidate the structural features of the neuron-specific epitope and the nature of the glycosylation defect in nac. Mutant animals raised at 25°C or 29°C show few behavioral or developmental abnormalities, despite their glycosylation defect. Homozygous mutant flies produced by heterozygous parents and raised at 18°C, however, display several morphological perturbations, including aberrant assembly of the eye facets and abnormal formation of the wing epithelia and venation. In addition, the mutation has a maternal effect, which eliminates the epitope in the embryonic nervous system and produces embryonic lethality at 18° C. Temperature shift experiments suggest the cold-sensitive period for both effects occurs very early in embryogenesis. The mutation has been mapped to 84F4-11,12 on chromosome 3 and has been characterized against deficiencies in this region. It behaves like a null allele.

B 314 NOVEL POLYVALENT CARBOHYDRATES AS THE BASIS FOR CELL RECOGNITION IN MARINE SPONGES, Gradimir N. Misevic and Max M. Burger, F.M.I., CH-4002 Basel, Switzerland.

Cell recognition in the marine sponge Microciona prolifera is mediated by a mucopolysaccharide aggregation factor of $M_r=2x10^7$. The molecular mechanism operating during such a species-specific reaggregation of dissociated cells is based on the highly polyvalent interactions of a cell-binding and a self-association domain. After pronase digestion two major types of repetitive glycans were isolated from MAF by gel electrophoresis. The cross-linking of individual polysaccharides, reconstituting their natural polyvalence, showed that the smaller glycan of $M_r=6000$ (G-6) represents the cell-binding site and the large one of $M_r=2x10^5$ (G-200) is the self-association site. Biochemical and chemical analyses revealed that both of this glycans have high amounts of fucose and glucuronic acid together with mannose, galactose and N-acetyl-glucosamine present on the same polysaccharide chain which is linked to asparagine. This novel glycoconjugates, operating via highly multiple low affinity interactions during cellular recognition in sponges, may exist and function together with polyvalent glycosaminoglycans in cell-cell and cell-matrix associations of evolutionary higher organisms.

B 315 POLYLACTOSAMINE GLYCOSYLATION OF CYTOTROPHOBLAST INTEGRINS IS SPECIFIC FOR THE EARLY PHASE OF HUMAN PLACENTAL DEVELOPMENT, Lenny Moss, Susan J. Fisher, Caroline H. Damsky, Departments of Anatomy and Stomatology, University of California, San Francisco, 94143.

An important factor in formation of the human placenta is the ability of the fetal cytotrophoblast cells to transiently exhibit tumor-like behavior in the establishment of placental access to maternal blood. Invasion of the spiral arteries of the endometrium peaks at 12 weeks of gestation after which the invasive phenotype is rapidly lost. To investigate the adhesive and invasive mechanisms involved, cytotrophoblasts were isolated from human placental chorionic villi at all stages of gestation and grown in primary culture. We found that cytotrophoblasts from both first trimester and term placentas expressed several members of the β1 family of integrins, including the fibronectin receptor (VLA 5). However, when these integrins were resolved by SDS PAGE both the α and β chains of the early, but not the term cytotrophoblast integrins ran as extremely wide bands. Glycopeptides were prepared from pronase digests of the β1 family integrin immune precipitates. Gel filtration (G-50 Sephadex) of the glycopeptides suggested that the adhesion receptors from first trimester, but not term cells, carried very large oligosaccharides. The total glycopeptide fraction from both the early and the late samples was digested with endo-β-galactosidase. The glycopeptides from the first trimester cytotrophoblats, but not from the term cytotrophoblasts, were found to be sensitive to endo-β-galactosidase digestion. After enzyme treatment, the β1 integrin glycopeptides from the first trimester cytotrophoblasts were significantly reduced in size and eluted at a position almost identical to that of the full term cytotrophoblast integrin glycopeptides. Cytotrophoblasts derived from 2nd trimester placenta expressed β1 family integrins containing both the large size endo-β-galactosidase sensitive oligosaccharides, and the smaller size, endo-β-galactosidase resistant oligosaccharides. We suspect that the transient expression of polylactosamine structure on the β1 family integrins during the early stages of gestation, may play a role in the developmentally regulated adhesive and invasive behavior of the human cytotrophoblast.

B 316 CHANGES IN THE CARBOHYDRATE PORTION OF THE LIPOPOLYSACCHARIDE OF MYXOCOCCUS XANTHUS DURING DEVELOPMENT, Sharon M. Panasenko, Barbara Jann, Klaus Jann, Department of Chemistry, Pomona College, Claremont, CA 91711. Myxocossus xanthus, a prokaryotic organism, exhibits a multicellular developmental cycle in which cell-to-cell signalling, aggregation, fruiting, and sporulation occur. We have observed alterations in the composition of the LPS of M. xanthus during the aggregation phase of this developmetal program. The most dramatic change was an increase in galactosamine levels during development on agar but not during vegetative growth or sporulation induced in liquid by glycerol. Furthermore, we have identified a novel methylated amino sugar, 6-O-methyl-galactosamine which was also enriched only in cells developing on agar. The requirement of a solid medium for these changes may reflect the organisms need of a solid surface for aggregation and, indeed, for motility. Since the changes in the composition of the LPS occur during the early aggregation phase of development, we hypothesized that the LPS might play a role in cell-to-cell signalling and/or motility. We have therefore examined mutants which lack the major epitope of the LPS and which are defective in the A type motility of M. xanthus. Unlike the wild type, these mutants fail to accumulate galactosamine and 6-O-methylgalactosamine in their LPS.

B 317 SPECIFIC LOCALIZATION OF FUCOSYL-GLYCOPROTEINS IN THE POLARIZED SITE OF SPERM RECOGNITION AND BINDING IN THE EGG OF UNIO ELONGATULUS,Floriana Rosati and Riccardo Focarelli,Department of Evolutionary Biology,University of Siena,Via Mattioli 4,Siena,Italy. The egg of Unio elongatulus (Mollusca,Bivalvia) is markedly polarized:at the vegetal pole the vitelline coat forms a crater which is encircled by a wrinkled area.This region coincides with the only site of the vitelline coat endowed with the property of recognizing and binding the spermatozoa of its own species and is the only area of the vitelline coat which specifically binds the lectin from Lotus tetragonolobus.LTA treatment of histological sections of oocytes in different developmental stages showed that the glycoproteins containing fucosyl residues are synthesized in the cytoplasm of the young oocyte and then transferred to the periphery.In the final stages of oogenesis they only appear in the wrinkled area of the vegetal region.Three main fucosyl-containing glycopeptides were revealed in the electrophoretic pattern of the isolated vitelline coats after treatment with LTA conjugated with peroxidase.

B 318 SYNDECAN, A CELL SURFACE PROTEOGLYCAN, IS ON B LYMPHOCYTES; DEVELOPMENTAL EXPRESSION CORRELATES WITH CHANGES IN CELL-MATRIX ADHESION. Ralph Sanderson, Paul Lalor and Merton Bernfield, Stanford University School of Medicine, Stanford, CA, 94305

Normal development of B lymphocytes is dependent on cell adhesion to the matrices of stromal cells and interstitial matrices. Early B cell development begins in the bone marrow where cells adhere to stromal cell matrices. Mature B cells leave the marrow and circulate in the blood, lymph and secondary lymphoid organs (lymph nodes, spleen, Peyer's patches). Terminal B cell differentiation to immunoglobulin-secreting plasma cells occurs in interstitial tissue matrices. Therefore, we asked whether syndecan, a cell surface proteoglycan known to be a receptor for interstitial matrix, is expressed on the surface of B lineage cells and whether this expression changes during B cell development. We used fluorescence activated cell sorting and immunohistological staining using lineage specific markers and a monoclonal antibody (281-2) against the core protein of syndecan. Syndecan expression is restricted to cells of the B lineage in bone marrow. Western blotting with 281-2 of detergent extracts from bone marrow-derived leukocytes reveals a characteristic proteoglycan smear with a modal size of 92 kDa. Following heparitinase treatment the smear is reduced to a broad band at approximately 74 kDa, indicating that the antigen is a low molecular weight, predominantly heparan sulfate, form of syndecan. Syndecan expression correlates with specific stages of B cell development: it is (i) expressed on pre-B cells, (ii) progressively lost as the cells mature, (iii) absent on peripheral blood lymphocytes, and (iv) re-expressed on plasma cells. Thus, syndecan is expressed at stages of B cell development when the cells adhere to matrix and is lacking when the cells are released from the marrow and circulate. Syndecan may mediate the adhesion to matrix and thus be involved in the regulation of B cell development. (Arthritis Foundation and NIH HD-06763)

B 319 Effect of mannosidase inhibitors dMM and swainsonine on a class I restricted Mixed Lymphocyte Reaction.

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Bm6 mice are mutants that differ from the parental B6 mice in only two aminoacids located in the H-2 K^b molecule. This difference is not detected in a Mixed Lymphocyte Reaction (MLR), proliferation of the effector cells is not observed. However, when the mannosidase I inhibitor dMM, or the mannosidase II inhibitor swainsonine are added during the B6 anti-bm6 culture, stimulation of proliferation as well as the generation of CTL´s is observed.
Swainsonine as well as dMM remain active during the 5 day culture period. Removal of the T helper cell population in the MLR culture made the system dependent on dMM for stimulation. However, swainsonine does not stimulate the MLR when T helper cells are removed.
This result suggests that the presence or absence of one carbohydrate branch may determine CTL stimulation.

B 320 **O-GLYCOSYLATION OF A CELL-TYPE SPECIFIC GLYCOPROTEIN WITH A GPI ANCHOR,** Elizabeth Smith, Andrew A. Gooley and Keith L. Williams, School of Biological Sciences, Macquarie University, Sydney, N.S.W., 2109, Australia

In the multicellular stage of its development the cellular slime mould Dictyostelium discoideum has two major cell types, prestalk and prespore cells. A developmentally regulated prespore specific antigen, PsA, was purified and sequenced revealing that the amino acid sequence corresponds to that predicted from the cDNA sequence of the D19 gene. While there are no sites for N-glycosylation, several threonine residues predicted from the cDNA sequence cannot be detected in the wild-type, indicating O-linked glycosylation at these sites. PsA is a cell-surface glycoprotein but there is no evidence for a transmembrane domain and the C-terminal sequence is consistent with a GPI anchor, so it seems that the glycoprotein is attached at the membrane via a glycosyl-phosphatidylinositol linkage.

Carbohydrate epitopes on PsA are also present on a number of other developmentally regulated glycoproteins, and are absent in glycosylation defective mutants carrying the modB mutation. This mutation results in a decrease of ~8 kD in the apparent molecular weight of PsA and allows rapid loss of this glycoprotein from the cell surface on papain treatment of cells. The glycosylated region of PsA contains ProThrValThr repeats with different numbers of repeats in different strains, as has been found in human mucins which also have ProThr rich sequences carrying O-linked oligosaccharides. Functional studies suggest that the carbohydrates may have a role in movement of the multicellular organism.

B 321 GLYCOSYLATION VARIANT OF THE FIBRONECTIN RECEPTOR: CORRELATION OF STRUCTURE AND FUNCTION, Tien-wen Tao, Division of Nuclear Medicine, Stanford University Medical School, Stanford, CA 94305

Resistance to wheat-germ-agglutinin (WGA-R) was transferred from a WGA-R mouse melanoma mutant to a Chinese hamster ovary cell line following DNA-mediated transfer and selection. The WGA-R phenotype was accompanied by altered glycosylation as manifested in an increase in electrophoretic mobility of a number of membrane glycoproteins. One of the glycoproteins studied was the fibronectin receptor (FNR). FNR was immuno-affinity purified from extracts of cells surface labeled with ^{125}I and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. The FNR complex of the WGA-R transfectant was structurally altered as compared to that of the WGA-sensitive transfectant control: (1) It was smaller in size with subunits of ≈130kD and ≈95kD while the subunits of the control FNR were ≈150kD and ≈120kD under non-reducing conditions. (2) It migrated as distinct subunits of ≈120kD and ≈105kD under reducing conditions, while the control FNR migrated as one band of ≈140kD. Deglycosylation of the FNRs using N-glycanase abolished the mobility difference, suggesting that the variant FNR is a glycosylation variant with the structural alteration residing in the glycan portion. Meanwhile, the WGA-R transfectant adhered and spread poorly on the cell-binding domain of FN; such interactions are presumably mediated by the FNR. The implication of these observations is that the oligossacharide structure of the FNR is of great importance in the functional expression of the FNR suggesting a potential modulatory role for the carbohydrate moiety of the receptor.

B 322 A GLYCOSYLATION MUTATION AFFECTS CELL FATE IN CHIMERAS OF THE CELLULAR SLIME MOLD DICTYOSTELIUM DISCOIDEUM, J. Houle & C.M. West, Dept. of Anatomy & Cell Biology, Coll. of Medicine, Univ. of Florida, Gainesville, FL 32610

Prestalk and prespore cells form a simple pattern in the pseudoplasmodium of the cellular slime mold Dictyostelium discoideum. Prestalk cells are distinguished from prespore cells by their relatively low expression of a glycoantigen on their surfaces and by reduced intercellular cohesion. We examined the possible significance of these differences using the modB mutation, which eliminates this glycoantigen leading to a reduction intercellular cohesion. modB mutant cells were codeveloped together with normal cells to form interstrain chimeric slugs. Mutant cells labeled by feeding with fluorescent bacteria were highly enriched in the prestalk cell zone, at the anterior region of the slug. In contrast, fluorescently labeled normal cells were concentrated in the rearmost part of the prespore cell zone. Western blot analysis and cell-by-cell double label immunofluorescence of these comixtures showed that mutant cells accordingly underproduced several prespore cell markers. Mutant cells tended not to form spores in chimeras unless they exceeded a threshold proportion of ca. 30%. However, mutant cells showed no tendency to produce excess prestalk cells when cultured alone. These findings are most simply explained by postulating that reduced glycoantigen expression and intercellular adhesion encourage a more anterior cell localization, which in turn causes differentiation into a prestalk cell. Since normal prestalk cells also show reduced glycoantigen expression and intercellular adhesion, this suggests that a similar mechanism may contribute to pattern formation during normal development.

Glycoconjugates and Disease; Glycolipid Modification of Proteins

B 400 TISSUE AND SERUM GLYCOCONJUGATES COMPOSITION IN HUMAN MENINGIOMAS, Bruno Berra, Fausta Omodeo-Salè and Silvana Rapelli, Institute of General Physiology and Biochemistry, School of Pharmacy, University of Milano, Italy.

Data on glycoconjugate content in human meningiomas are scarce and conflicting. Two recent reports agree in the lack of correlation between ganglioside distribution and histological classification; recently it was reported that glycoprotein pattern is characterized by the presence of two fractions (70 and 50 KDa) which however, are present also in other brain tumors. No indications are reported on the serum ganglioside content which might be a useful marker for the follow up of treated patients. We have studied about 40 samples of different histological types and the main results are: 1) solid tumor have a higher ganglioside content as compared with control tissues (leptomeninges); 2) pratically each meningiomas shows a different ganglioside pattern, avoiding a classification on this basis; 3) the only similarity was observed in two cases classified as malignant; 4) meningiomas are characterized by the presence of a glycoprotein (37 KDa) which is absent in the other brain tumors so far examined; 5) a possible correlation was seen between the ganglioside pattern of solid tumors and the karyotype of the patient; 6) in all the observed serum samples with a method worked out in our laboratory there was an increase of ganglioside-associated sialic acid, which might be a good marker for the follow up of the patients.

B 401 A SOLUBLE FORM OF THE CELLULAR PRION PROTEIN IS PRODUCED BY CULTURED CELLS, David R. Borchelt and Stanley B. Prusiner, Department of Neurology, University of California, San Francisco, CA 94143-0518.

Protein molecules which react with antisera raised against the hamster scrapie prion protein (PrP 27-30) have been detected on the surface of primary and immortalized cell lines. PrP immunoreactive molecules in these cell lines possessed properties similar to those observed for cellular PrP (PrP^C): protease sensitivity, and attachment to cell surface via a glycosyl-phosphatidylinositol (GPI) anchor. Exposure of intact cells to *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C (PIPLC) released PrP^C into the medium and enhanced its detection. Biosynthesis studies indicate that PrP^C is post-translationally modified by *N*-linked glycosylation and glycolipidation prior to transport to the cell surface. After reaching the cell surface, 1-10% of PrP^C is released into the surrounding medium during the next 6-12 hr. The PrP^C in the medium was found in the aqueous phase after Triton X-114 partitioning; in contrast, PrP^C bound to cells partitioned into the detergent phase. PrP^C released from the cell surface by PIPLC digestion partitioned into the aqueous phase like the soluble PrP^C found in the medium. Thus, soluble PrP^C appears to lack at least the diacylglycerol moiety of its GPI anchor. 1-10% of PrP^C in hamster brain also partitioned into the aqueous phase of a Triton X-114 extract raising the possibility that PrP^C is released in response to some metabolic signal. Studies on the mechanism of PrP^C release may help elucidate the mode of prion spread and the pathogenesis of scrapie.

B 402 TERMINAL N-ACETYLGLUCOSAMINE (GlcNAc) CLUSTER ANTIGEN (TGCA): COMPOSITION OF IMMUNOREACTIVE EPITOPES, ONTOGENY, PHYLOGENY AND RELATIONSHIP TO NEOPLASTIC TRANSFORMATION, Boris Chechik[1], Bernard Fernandes[1], Tony Pawson[1], and Inka Brockhausen[2], Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5[1], and Research Institute, Hospital for Sick Children, Toronto, Ontario M5G 1X8[2].

TGCA is an epitope(s) of highly branched N-linked oligosaccharides terminating in GlcNAc residues. The anti-TGCA antibody interacts strongly with "truncated" oligosaccharides containing five or six GlcNAc residues at the non-reducing end, and much weaker with epitopes composed of three or four GlcNAc residues linked to mannose. TGCA is found in chicken bursal and thymic lymphocytes, macrophages, capillary endothelial cells, and epithelial cells of the intestine and bronchioles. The expression of TGCA in lymphocytes and epithelial cells of endodermal origin is developmentally regulated. Besides the chicken, the antigen is found in several other avian species, in an amphibian (frog), a reptile (chameleon), and a fish (Rainbow trout), but, among seven mammalian species studied, including humans it is detectable only in mice. The antigen is not found in normal chicken fibroblasts, but appears in chicken embryo fibroblasts after transformation with avian sarcoma viruses.

B 403 STRUCTURE OF UNUSUAL LIPID-LINKED OLIGOSACCHARIDES ISOLATED FROM BRAINS OF PATIENTS WITH NEURONAL CEROID LIPOFUSCINOSIS, Peter F. Daniel, Derrick L. Sauls and Rose-Mary Boustany, Department of Biochemistry, Shriver Center, Waltham, MA 02254. We have shown that abnormal levels of dolichol-PP-oligosaccharides are stored in brain from patients with late infantile and juvenile neuronal ceroid lipofuscinosis (LINCL and JNCL). Normal controls (N=14) had a mean of 2.1 ± 1.9 S.D. nmol/g wet weight; comparable values for controls with lysosomal storage diseases (2 Hurler, a Tay-Sachs and a Fabry), LINCL (N=4) and JNCL (N=7) patients were 4.2 ± 3.9, 12.3 ± 19.9 and 34.9 ± 13.8, respectively. Oligosaccharides containing 2 glucosamine residues and from 3 to 9 mannose residues and, in some instances, glucose residues were liberated by mild acid hydrolysis. Susceptibility of the major accumulated oligosaccharide, $Man_5GlcNAc_2$, to Endo H digestion and subsequent permethylation analysis clearly indicated that it is not an intermediate in the biosynthesis of $Glc_3Man_9GlcNAc_2$-PP-Dolichol but has undergone catabolism, probably either in the ER or in the Golgi. This accumulation may be due to a specific lesion in an enzyme regulating the biosynthesis and/or utilization of lipid-linked oligosaccharide, or it may represent a general membrane dysfunction secondary to the primary defect. Supported by NIH grants NS 24279 and HD 16942.

B 404 THE ROLE OF LIPOPHOSPHOGLYCAN FROM **LEISHMANIA MAJOR** IN CUTANEOUS LEISHMANIASIS: CHARACTERIZATION OF GLYCOSYLATION MUTANTS. Martin J. Elhay, Malcolm J. McConville, Graham F. Mitchell and Emanuela Handman. The Walter and Eliza Hall Institute of Medical Research, Melbourne Victoria 3050, Australia.
Infection of macrophages by the intracellular protozoan parasite **Leishmania major** involves specific attachment to the host cell membrane followed by phagocytosis and intracellular replication. Two promastigote glycoconjugates have been implicated in the initial attachment event: a glycoprotein (gp63) and a lipophosphoglycan (LPG). To investigate more directly the function of LPG, glycosylation mutants of **L.major** were generated. Virulent parasites were mutagenized with N-methyl-N-nitroso-N-nitroguanidine, and variants selected with the toxic galactose binding lectin, Ricin. Twenty mutants were selected by this procedure, their glycolipid profiles analysed and resulting biological and immunochemical behaviour compared. Expression of LPG in mutant clones was examined either by Western blotting using monoclonal and polyclonal antibodies, or by biosynthetic and surface radiolabelling followed by hydrophobic interaction-chromatography. LPG expression varied among the clones, ranging from almost not detectable through to levels similar to that of the parent strain. Changes in the expression of the second major class of glycolipids, the glycoinositolphospholipids (GIPL) were also observed. Clones possessing significant quantities of LPG infected mice normally, whereas those with lower amounts of LPG gave lesions at a very much slower rate. These findings were paralleld by results obtained in vitro using a macrophage cell line J774. Passive transfer of purified LPG onto LPG low, or negative clones prolonged survival in macrophages. These results confirm the essential role of LPG in successful infection.

B 405 COLON CARCINOMA SIALOMUCIN PRODUCTION STIMULATED BY MUCOMODULIN AND CHANGES IN TUMOR CELL-HOST CELL INTERACTIONS. Tatsuro Irimura, Masato Yagita, Elizabeth A. Grimm, David G. Menter, David M. Ota, Debora A. Carlson, Departments of Tumor Biology and General Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Human colon carcinoma HT-29 cells growing *in vitro* produce at least three sialomucins; M_T 900,000, 740,000 and 450,000, and a greater amount of the M_T 900,000 component was associated with a metastatic subline. The amount of M_T 900,000 sialomucins produced by HT-29 cells growing *in vivo* appeared to be greater than the same cells *in vitro*. The synthesis of all three sialomucins by HT-29 cells growing *in vitro* was enhanced by supplementing a conditioned medium from fresh human colon organ culture. These changes were detected by polyacrylamide gel electrophoresis of [^{3}H]-glucosamine-labeled or non-labeled cell lysates on 3% gels, followed by fluorography or staining with ^{125}I-labeled WGA. Incorporated [^{3}H]-glucosamine was sensitive to alkaline reduction. There were no changes detected in the protein profiles, growth rates and morphology of the cells after treatment with the conditioned medium. Dissection of normal colon tissues into different portions showed that the connective tissues produce this soluble factor. This factor apparently is a protein stable at 56°C for 60 min but inactivated by heating at 80°C. We tentatively named the factor as mucomodulin. The mucomodulin-treated human colon carcinoma cells, expressing a greater amount of sialomucins, were less sensitive to the cytolytic effects of recombinant interleukin-2 activated human peripheral blood lymphocytes (LAK-cells). The difference in the killing appeared to result from decreased binding of LAK cells to tumor cells as measured by the conjugation method. Also, the ability of the treated cells to induce platelet aggregation increased. Cell surface sialomucins induced by host tissue factors probably potentiate metastasis of colon carcinoma cells by influencing their interactions with lymphocytes and platelets. (Ref.: Irimura et al., Cancer Res., 48: 2353, 1988)

B 406 ANALYSIS OF OPTICAL ISOMERS OF CHIRO-INOSITOL IN BIOLOGICAL SAMPLES. Kennington, A.S., Frederick, F.C., Larner, J., Kinter, M. and Shen, T.Y., Department of Chemistry, Department of Pharmacology, and Department of Pathology, University of Virginia, Charlottesville, Va. 22901.

Inositol is an essential component of the phosphatidyl-inositol glycan (PIG) anchors of membrane proteins. The presence of either myo-inositol or chiro-inositol has been demonstrated in different membrane anchors. We have developed an HPLC-GC/MS method to analyze the level and the ratio of the D- and L-isomers of chiro-inositol in various biological samples with a sensitivity in the nanomolar range. Exclusively D-chiro-inositol was found in one putative insulin mediator from rat liver, which is structurally analogous to the PIG anchors. An assortment of animal tissue membrane phospholipids, human urine and erythrocyte specimens were also examined. A distinct difference in the amount and ratios of the D- and L- chiro-inositol in these samples was noted.

B 407 PROCESSING OF THE MEMBRANE ANCHOR OF *T.BRUCEI* VARIANT SURFACE GLYCOPROTEINS. S. Mayor , A. K. Menon, M. A. J. Ferguson, R.T. Schwarz and G.A.M. Cross. The Rockefeller University, New York, & University of Dundee, Dundee, Scotland.
The variant surface glycoproteins (VSGs) of *Trypanosoma brucei* are anchored to the cell surface by a complex glycosyl-phosphatidylinositol (core: ethanolamine-P-Man_3GlcN-PI) membrane anchor [Ferguson and Williams, *Ann. Rev. Biochem.* **57**, 285 (1988)]. VSGs from different *T.brucei* variants can be classified into three sub-classes based on carboxyl-terminal peptide sequence homology and the terminal amino acid of the mature protein. The glycolipid membrane anchors of the VSGs from each sub-class have been shown to have a common backbone structure that is variably galactosylated, possessing 0, 4-5, or 8 moles galactose per mole of glycolipid on the average, depending on the sub-class to which they belong. Microheterogeneity in the number of galactose residues within a sub-class can be traced to the presence or absence of terminal residues on a branched structure attached to the mannose residue adjacent to GlcN-PI. Candidate precursor glycolipids have been identified and characterised in *T.brucei*. The glycolipid precursors from trypanosome variants representative of each sub-class were analysed, and the glycan was found to be Man α1-2Manα1-6Manα1-?GlcN, analogous to the Man_3GlcN_1 core of the VSG glycolipid anchors. No evidence for galactosylated forms of the precursor was seen. This variant-independent structure for the candidate precursor strongly supports the hypothesis that the membrane anchor is prefabricated as a non-galactosylated glycolipid which is attached to the protein in the ER and then becomes galactosylated along the secretory pathway. Methods are being developed to study the spectrum and kinetics of appearance of galactosylated forms of the anchor. Preliminary data show that some galactosylation of the anchor occurs in less than 10 min after completion of protein synthesis.

B 408 A NOVEL FAMILY OF GLYCOLIPIDS FROM **LEISHMANIA MAJOR** WHICH ARE SIMILAR TO THE GLYCOSYL-PHOSPHATIDYLINOSITOL MEMBRANE ANCHORS OF EUKARYOTIC PROTEINS, Malcolm J. McConville[1], Emanuela Handman[1] and Antony Bacic[2]. [1]The Walter and Eliza Hall Institute of Medical Research, Victoria 3050 Australia, [2]Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Victoria 3050 Australia.
The cell surface of the parasitic protozoan **Leishmania major** is coated by two main antigens, the glycoprotein gp63 and a heterogeneous lipophosphoglycan (LPG), which are both anchored to the plasma membrane via glycosyl-inositolphospholipids. We have purified and characterized a novel family of glycosyl-inositolphospholipids (GIPLs) from **L.major** promastigotes which are not covalently linked to macromolecules. The glycan moieties of the GIPLs have between 4-10 saccharide residues and contain mannose, galactose and a single non-N acetylated glucosamine residue. Some of the GIPLs contain, in addition, glucose, galactose-6-phosphate and mannose-6-phosphate residues. The lipid moieties of the GIPLs can be released with nitrous acid and specific phospholipase Cs and consist of heterogeneous alkylacyl phosphatidylinositol or lyso-alkylglycerolphosphoinositol moieties. The lipid moiety of one of the GIPLs, a decaglycosylinositolphospholipid is identical to the LPG lipid moiety suggesting that it is the immediate precursor of LPG. Radiolabelling of living promastigotes with NaB^3H_4 after treatment with either galactose oxidase or periodate demonstrated that some of the GIPLs are preferentially exposed on the cell surface. Furthermore three monoclonal antibodies that bind specifically to the surface glycolipids on high performance TLC sheets also bind to the cell surface of **L.major** promastigotes and amastigotes and other **Leishmania** species.

B 409 BIOSYNTHESIS OF THE GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR OF *TRYPANOSOMA BRUCEI* VARIANT SURFACE GLYCOPROTEINS : INCORPORATION OF RADIOLABELLED MANNOSE, GLUCOSAMINE, AND MYRISTIC ACID INTO A PRECURSOR GLYCOLIPID IN A CELL-FREE SYSTEM. A. K. Menon, R. T. Schwarz, S. Mayor, and G. A. M. Cross. The Rockefeller University, New York, NY 10021. USA.
The variant surface glycoproteins (VSGs) of the parasitic protozoan *Trypanosoma brucei*, are attached to the plasma membrane via a glycolipid covalently linked to the carboxyl-terminus of the protein. The glycolipid anchor appears to be acquired immediately upon completion of protein synthesis [Cross, *Cell* **48**, 179 (1987)] suggesting a preformed glycolipid precursor. Candidates for a precursor to the VSG anchor have been identified *in vivo* [Krakow *et.al., J. Biol. Chem.* **261**, 12147 (1986); Menon *et.al., J. Biol. Chem.* **263**, 1970 (1988)]. The precursor has an ethanolamine-Man_3GlcN-PI structure apparently identical to that of the backbone of the VSG glycolipid. The mechanism of biosynthesis of glycolipid anchors is unknown and is of current interest. We have succeeded in incorporating radioactivity into the precursor glycolipid and a related glycolipid upon addition of radiolabelled sugars and fatty acids to a trypanosome membrane preparation [Menon *et.al., Biochem. Soc. Trans.* **16**, 996 (1988)]. Putative biosynthetic intermediates were also labelled. Under our conditions, typical incorporation of radioactivity into the precursor glycolipid (per 10^9 trypanosome equivalents, per μCi input radiolabel) was 2500 cpm using ^{3}H-myristic acid, 850cpm with GDP-^{3}H-mannose, and 200 cpm with UDP-^{3}H-GlcNAc. Incorporation of ^{3}H-mannose was stimulated by the addition of Dol-P and reduced in the presence of amphomycin, an inhibitor of Dol-P-Man synthesis, implying that one, if not all, of the mannose residues of the precursor glycolipid are donated by Dol-P-Man.

B 410 DEMONSTRATED OF FUCOSE-DEPENDENT EPITOPES IN A VIRAL GLYCOPROTEIN, Sigvard Olofsson and Inger Sjöblom, Department of Virology, University of Göteborg, 413 46 Göteborg.
We have previously shown that the herpes simplex virus type 1 (HSV-1) specified glycoprotein C (gC-1) contains carbohydrate-dependent antigenic epitopes. Although these epitopes are of peptide nature, they are dependent on galactose on adjacent oligosaccharides for their expression. In the present investigation was investigated if these epitopes where expressed in a mouse neuroblastoma cell line (C1300) which is almost devoid of galactosyl transferases. The assay consisted of an enzyme-linked solid phase assay in which the effect of sequential removal of peripheral monosaccharides by chemical treatment or glycosidase digestion on antigenic activity was measured. It was found that the carbohydrate-dependent epitopes were expressed in gC-1 synthesized in C1300 despite lack of terminal galactose residues. The glycoprotein from C1300 cells contained many oligosaccharides with peripheral fucose units, which seemed to be more or less absent in gC-1, produced in epitheloidal cells. In addition, fucosidase treatment abolished the antigenic activity of the carbohydrate-dependent epitopes. Altogether, the results indicated that the carbohydrate-dependent epitopes of gC-1 from C1300 cells were (i) stabilized by N-linked oligosaccharides rather than O-linked ones and that (ii) fucose in a peripheral position, associated with the (1-6) branch of N-linked oligosaccharides could compensate for absence of terminal galactose.

B 411 DISSECTION OF THE BIOSYNTHESIS OF THE GLYCOLIPID MEMBRANE ANCHOR OF TRYPANOSOME VARIANT SURFACE GLYCOPROTEINS USING INHIBITORS OF GLYCOSYLATION. Ralph T. Schwarz, Satyajit Mayor, Anant K. Menon and George A. M. Cross. The Rockefeller University, New York, NY 10021, USA.
Several eukaryotic cell surface proteins, including the variant surface glycoproteins (VSGs) of *Trypanosoma brucei*, are anchored to the membrane by glycosyl-phosphatidylinositol. The glycolipid anchor is covalently linked to the carboxyl-terminus of the protein and appears to be acquired immediately upon completion of protein synthesis. A putative glycolipid precursor to the VSG anchor has been identified *in vivo* [Krakow *et.al., J.Biol.Chem.* **261**, 12147 (1986); Menon *et.al., J.Biol.Chem.* **263**, 1970 (1988)] and synthesised *in vitro* [Menon *et.al., Biochem. Soc. Trans.* **16**, 996 (1988)]. The precursor has a Man_3GlcN-PI backbone structure identical to that of the VSG glycolipid anchor but lacks a variable galactose side-chain. Inhibitors of glycosylation pathways have been useful in studying the biosynthesis and function of N-linked oligosaccharides [Schwarz and Datema, *Adv. Carbohyd. Chem.* **40**, 287 (1982)] and are potential tools in the dissection of the biosynthesis of the glycolipid precursor to the VSG glycolipid anchor. Several glycosylation inhibitors were tested *in vivo* and *in vitro*. One of the inhibitors reduced incorporation of radiolabels (^{3}H-glucosamine, ^{3}H-myristic acid) into the precursor glycolipid *in vivo* leading to the accumulation of a putative biosynthetic lipid intermediate. The data obtained support the hypothesis that Dol-P-Man is involved in the formation of the mannose core of the precursor to the VSG glycolipid anchor.

B 412 DIFFERENTIAL RELEASE OF CELLULAR AND SCRAPIE PRION PROTEINS FROM SCRAPIE-INFECTED NEUROBLASTOMA CELLS AND DISSOCIATED HAMSTER BRAIN CELLS BY PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C, Neil Stahl, David R. Borchelt, Michael P. McKinley and Stanley B. Prusiner, Univ. of California, San Francisco, CA 94143-0518

The only identified component of the infectious scrapie prion is a host-encoded protein called PrP^{Sc}, which appears to differ posttranslationally from a normal cellular protein isoform (PrP^{C}) derived from the same gene. While both isoforms contain glycosylphosphatidylinositol modifications, it is not known if their GPI anchor structures differ. We have compared some properties of the prion proteins in scrapie-infected mouse neuroblastoma (N2a) clones versus cells prepared by collagenase dissociation of normal and scrapie-infected hamster brain. In both cell preparations, we find that the normal isoform PrP^{C} is released by Bacillus thuringiensis phosphatidylinositol-specific phospholipase C (PIPLC), while PrP^{Sc} is resistant to release. We then asked whether PrP^{Sc} is exposed on the cell surface by measuring its accessibility to proteases or biotinylation with a membrane impermeant reagent. Cells were treated with 1 mg/mL NHS-SS-biotin for 10 minutes before quenching with Tris and protein extracts from these cells were analyzed. Most of the PrP^{Sc} in the scrapie-infected neuroblastoma cells is inaccessible to biotinylation. In contrast, the majority of PrP^{Sc} in the dissociated brain cells is accessible to proteinase K, suggesting that it is exposed on the cell surface. Almost all of the PrP^{C} was localized to the cell surface on both types of cells. Thus the PIPLC resistance of PrP^{Sc} in neuroblastoma cells, but not the dissociated hamster brain cells, apparently results from aberrant subcellular localization.

B 413 THE MEMBRANE ANCHORAGE AND THE FUNCTIONAL CAPACITY OF CD16 EXPRESSED ON GRANULOCYTES AND NK CELLS. Periasamy Selvaraj, Olli Carpen, Margaret L. Hibbs and Timothy A. Springer. Center for Blood Research, Department of Pathology, Harvard Medical School, Boston MA 02115

The cell surface receptors for the Fc region of IgG are involved in binding and clearance of immune complexes from the circulation and antibody dependent cellular cytotoxicity. The occupancy of Fc receptor by its ligand also modulates a variety of immune responses. CD16 is a low affinity FcγR (FcγR III) expressed on granulocytes, NK cells and tissue macrophages. We found CD16 is anchored to the membrane of granulocytes via phosphatidylinositol glycan -moiety and can be released from the cell membrane by phosphatidylinositol-specific phospholipase C (PIPLC) whereas the CD16 expressed on NK cells, cultured monocytes and lung macrophages are resistant to PIPLC treatment. SDS PAGE analysis of N-glycanase treated CD16 from NK cells, cultured monocytes and granulocytes showed the protein backbone of NK cell CD16 and macrophage CD16 is ~5 kd higher than granulocyte CD16. Redirected killing of hybridoma targets expressing anti-CD16 showed that NK cell CD16 is able to trigger killing whereas granulocyte CD16 is unable to trigger killing. These results show that CD16 found on granulocytes and NK cells have different membrane anchors and differ in their funtional capacity to trigger killing.

Late Additions

B 500 STIMULATION OF NK AND LAK CELL-MEDIATED LYSIS BY A HIGH MANNOSE-TYPE GLYCOPEPTIDE, Patricia B. Ahrens and Helmut Ankel, Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226
The carbohydrate on target cells effects their sensitivity to lysis by either human natural killer (NK) cells or peripheral blood cells grown in the presence of IL-2, lymphokine activated killer (LAK) cells. Parent CHO cells are poorly lysed by NK and LAK cells, but CHO cells deficient in GlcNAc transferase I, an enzyme necessary for processing of the complex-type saccharide chains on N-linked glycoproteins, are good targets. These mutant CHO cells have predominantly high mannose-type oligosaccharides. We tested the effect of exogenous soluble saccharides on effector cell function. Lytic activity of both NK and LAK cells against either CHO or K-562 targets was enhanced by a high mannose-type glycopeptide (10-20 μM) with at least 7 mannosyl residues. None of the shorter high mannose structures containing between 3 and 5 mannosyl residues were stimulatory at comparable concentrations. Likewise, di-, tri- and tetra-antennary complex-type oligosaccharides did not change the extent of lysis. Our data indicate that carbohydrate boosting of NK and LAK lytic activity requires a high mannose-type oligosaccharide containing α-1,2 linked mannosyl residues. We conclude that high mannose structures on target cells trigger the lytic response of NK and LAK cells and that this is mimicked when such structures are present in solution. Supported by NIH grant CA46956.

B 501 SPECIFICITY DRIFT OF INFLUENZA N2 NEURAMINIDASES FOR α2,3 AND α2,6 LINKED SIALIC ACIDS Linda G. Baum and James C. Paulson, Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.
The neuraminidase of influenza A cleaves sialic acid residues from cell surface glycoconjugates, presumably to facilitate release of budding virus from host cells. There are few studies which have systematically examined the substrate specificity of any of the nine known influenza serotypes (N1-N9) during antigenic drift. A number of influenza A viruses of the N2 serotype, isolated from 1957 to 1977, were assayed for their ability to release ^{14}C-neuraminic acid from a soluble glycoprotein, α1-acid glycoprotein, enzymatically modified to contain neuraminic acid in either the α2,3 or α2,6 linkage. Viral strains isolated in 1957 preferentially cleave sialic acid in the α2,3 linkage, with minimal ability to release sialic acid in the α2,6 linkage. Strains isolated in 1968 can cleave α2,6 linked sialic acid to a limited degree, but still demonstrate greater activity towards α2,3 linked sialic acid. Isolates from 1972 to 1977 have approximately equal activity towards both the α2,3 and α2,6 linkages. The broadening of neuraminidase specificity may facilitate viral elution from infected cells. Preliminary evidence indicates that human viruses with dual specificity elute more rapidly from erythrocytes than do earlier viruses which cleave primarily the α2,3 linkage. It will be of interest to correlate the temporal changes in neuraminidase specificity with amino acid sequence changes among the various N2 strains, and to relate these changes to the known crystal structure of the enzyme (Colman et al, Nature 303:41, 1983). Supported by NIH grant AI-16165.

B 502 STRUCTURAL FINGERPRINTING OF ASN-LINKED CARBOHYDRATES AT SPECIFIC ATTACHMENT SITES IN GLYCOPROTEINS BY MASS SPECTROMETRY, Steven A. Carr, Mark F. Bean, Gerry D. Roberts, Mark E. Hemling and Michael Huddleston, Smith Kline and French Laboratories, King of Prussia, PA 19406 USA
A new mass spectrometry-based strategy has been developed that 1) defines the sites of attachment of Asn-linked oligosaccharides in glycoproteins, 2) determines the extent of glycosylation at each site, and 3) provides composition, molecular heterogeneity and linkage information for oligosaccharide chains at the specific attachment sites. In this carbohydrate "fingerprinting" strategy potential glycopeptides are identified by comparing the HPLC chromatograms of proteolytic digests of a glycoprotein obtained before and after digestion with a glycosidase, usually peptide:N-glycosidase F (PNGase F). The glycopeptide-containing HPLC fractions are analyzed by fast atom bombardment mass spectrometry (FABMS) prior to and after digestion with PNGase F to identify the former glycosylation site peptide and its sequence location. Carbohydrates extracted from these fractions as the peracetates are analyzed by FABMS, and again following permethylation. Molecular weight-related ions for each of the parent oligosaccharides present in the fraction reveal information about the composition in terms of hexose, deoxyhexose, N-acetylhexosamine and sialic acid, while their relative ratios reflect the relative abundances of the oligosaccharides. Furthermore, the derivatives formed are directly amenable to methylation analysis for determination of linkage. This strategy enables the structures of carbohydrates at specific attachment sites to be determined using only a few nanomoles of glycoprotein. Application of the methodology to tissue plasminogen activator, soluble CD_4, and other glycoproteins will be discussed.

B 503 PROGRESS IN THE PICOMOLE LEVEL CHARACTERIZATION OF INTACT CARBOHYDRATES BY MASS SPECTROMETRY. A.L. Burlingame, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

In order to optimize the structural information obtainable on picomole quantities of intact carbohydrates using liquid matrix secondary ion based mass spectrometric strategies, a knowledge of how to manipulate the parameters required to produce high quality mass spectra is required. In the case of the oligosaccharides, these factors include the hydrophobicity/hydrophilicity ratio, control of the site of charge localization, control of vibronic energy content, and minimization or elimination of matrix-associated chemical noise. Instrumental factors include control of the sputtering process, minimization or elimination of the chemical noise, control of vibronic energy, and optimization of overall sensitivity including the use of electro optical multichannel array detection on both double focusing instruments and tandem double focusing instruments.

In this laboratory, considerable effort is being directed toward development of mass spectrometric methods for structural characterization of intact N-linked oligosaccharides obtained from glycoproteins by enzymic cleavage using PNGaseF. We have demonstrated the utility of reductive coupling of p-aminobenzoic acid ethyl ester (ABEE) with the PNGaseF liberated free oligosaccharide reducing terminus for HPLC separation, charge localization and induction of an abundant fragmentation pattern in the negative ion mode (Webb, 1988). More recently, we have shown that the sensitivity of these analyses may be increased significantly by increasing the hydrophobicity of this derivative.

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Webb, J.W.; Jiang, K.; Gillece-Castro, B.L.; Tarentino, A.L.; Plummer, T.H.; Byrd, J.C.; Fisher, S.J.; Burlingame, A.L. Anal. Biochem., 1988, *169*, 337-349.

B 504 **AN INTEGRATED, MODULAR, HPLC APPROACH TO GLYOPROTEIN MAPPING**, Steven R. Carter, Dennis G. Gillen, Joseph D. Olechno,Dionex Corp., 501 Mercury Drive, Sunnyvale, CA 94086. A number of liquid chromatographic methods have been developed in recent years for carbohydrate/glycoprotein mapping (fingerprinting). However few, if any of the techniques have been able to demonstrate sufficient selectivity or sensitivity to allow routine application in research or quality control environments. The development of high selectivity microbread™ pellicular anion exchange packing materials, high sensitivity Pulsed Amperometric Detection and high purity oligosaccharide standards now makes this possible. An integrated system will be described that allows mapping of glycoprotein isoforms, multiple column selectivity for glycopeptide/peptide mapping, analysis of microheterogeneity at individual glycosylation sites and compositional analysis.

B 505 RAPID UP AND DOWN REGULATION OF THE HUMAN LYMPHOCYTE-NODAL HEV INTERACTION BY PHORBOL ESTERS. L.M, Stoolman and H. Ebling. Pathology Department, University of Michigan, Ann Arbor, MI 48109-0602. A variety of lymphocyte activation signals act, in part, through rapid modulation of the phorbol-sensitive, protein kinase C. We have found that short-term phorbol treatment alters the expression of adhesion receptors for nodal HEV in a time and concentration dependent fashion. Gradient-purified human peripheral blood mononuclear cells (PBMC) were treated with neuraminidase (0.005U, 30', 37°C) to enhance detection of binding sites, incubated with varying concentrations of phorbol esters (37°C), chilled to 4°C and then examined for binding to PNHEV and expression of the phosphomannosyl-binding lectin implicated in the adhesive process. 15-30' incubations at sub-nanomolar concentrations of phorbol 12-myristate, 13-acetate (PMA) resulted in a 25-50% increase in binding activity. Greater than 1-5 nM resulted in marked inhibition of the binding to PNHEV (>75%). The phosphomannosyl-binding lectin is similarly down-regulated at high phorbol concentrations. The rank order of inhibitory potencies for several phorbols were identical in the HEV and lectin assays. In particular, 4 alpha phorbol, an inactive derivative, neither up-regulated nor down-regulated activity. Phorbol treatment also increases binding of PBMC to unidentified cells in the sinusoids and germinal centers. Time course studies conducted at 5nM PMA show enhanced binding to germinal centers and sinusoids before maximal inhibition of binding to PNHEV is evident (15-30' incubation). The adhesion receptor(s) responsible for the upregulation of binding to the HEV, germinal centers and sinusoids have not been identified. These data strengthen the linkage between lectin activity and binding to nodal HEV and suggest that rapid modulation of adhesion receptors occurs during lymphocyte activation.