ALTERED GLYCOSYLATION IN TUMOR CELLS

Sen-Itiroh Hakomori, Donald Marcus and Christopher Reading, Organizers
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Altered Glycosylation in Tumor Cells

Historical Perspective

R 001 INTRODUCTORY REMARKS AND HISTORICAL PRESPECTIVES, Sen-itiroh Hakomori, Biochemical Oncology, Fred Hutchinson Cancer Research Center, and the Departments of Pathobiology, Microbiology & Immunology, University of Washington, Seattle, WA 98104.
While studies on oncogenes have been the most fashionable current trend in cancer research, the mechanism can be applied only to some viral carcinogenesis. With a large number of probes, oncogenes have been found to be expressed only in 10-15% of human cancers. How can we explain the mechanisms of the progression of the majority of human cancers? In striking contrast, essentially all experimental and human cancers are characterized by aberrant glycosylation, many instances of which can be detected by specific carbohydrate binding probes, such as monoclonal and polyclonal antibodies, lectins, and enzymes. Although the physiological and pathological implications of the role of aberrant glycosylation in carcinogenesis are largely unknown, the products per se, defined by various probes, have been increasingly recognized as useful markers for cancer detection (diagnosis) and as targets for therapy. Some structures have been characterized as being closely associated with the metastatic potential of tumor cells, and others are useful to indicate the degree of differentiation (i.e., malignancy of tumors). An important characteristic of aberrant glycosylation is that it is expressed in a large variety of carbohydrate chains carried by various molecular species bound to either lipids, membrane proteins, or pericellular components. We are still at the stage of assembling our knowledge of these structures, yet a great deal of effort is being made to understand the mechanism of aberrant glycosylation on an enzymatic, genetic, or epigenetic basis and its physiologyical significance. This symposium is assembled to summarize our current knowledge and provide perspectives on future trends in research in this area. A brief historical review since 1929, when E. Witebsky, L. Hirszfeld, and their associates opened this area, will also be presented.

Carbohydrate Structure and Antigenicity

R 002 CELL SURFACE PROTEOGLYCANS AND THE NEOPLASTIC PHENOTYPE
Renato V. Iozzo, Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104, USA.
Cell surface proteoglycans are strategically positioned to regulate interactions between cells and their surrounding environment. Such interactions play key roles in several biological processes such as cell recognition, adhesion, migration and growth. These biological functions are in turn necessary for the maintenance of differentiated phenotype and for normal and neoplastic development. There is ample evidence that a special type of proteoglycan bearing heparan sulfate side chains is localized at the cell surface in a variety of epithelial and mesenchymal cells. This molecule exhibits selective patterns of reactivity with various constituents of the extracellular matrix and plasma membrane, and can act as growth modulator or as a receptor. Certainly, during cell division, membrane constituents undergo profound rearrangement and proteoglycans may be intimately involved in such processes. The present work will attempt to describe the recent advances in our understanding of these complex macromolecules and will focus on the structure and metabolism of heparan sulfate proteoglycan synthesized by human colon carcinoma cells. Our recent studies indicate that the synthesis of heparan sulfate proteoglycan in these cells is highly dependent on that of the protein core, since xyloside-mediated initiation of heparan sulfate chains is more evident after inhibition of protein core biosynthesis. Following various post-translational modifications, the heparan sulfate proteoglycan is rapidly transported to the cell surface where it becomes an integral membrane component via a small leucine-rich hydrophobic domain in the protein core. The cell surface proteoglycan is then partially released into the extracellular space by limited cleavage of the protein core, and partially internalized and converted into smaller species through a series of degradative steps, involving the sequential action of proteases and endoglycosidases. Our results indicate that the biosynthesis, cell surface expression and catabolism of heparan sulfate proteoglycan is strictly controlled and provide a working model that may help further our understanding of its functional role in the normal and transformed phenotype.

(Supported in part by NIH grant CA-3981)

R 003 STRUCTURE, FUNCTION AND TRANSFORMATIONAL CHANGE OF THE SUGAR CHAINS OF GLYCOHORMONES, Akira Kobata, Department of Biochemistry, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced in trophoblastic cells. In 1975, Moyle et al. (1) reported for the first time that the sugar chains of hCG play key roles for the expression of biological activity of the hormone. Since then, several research groups have reported supporting data for this notion by demonstrating that hormonal activity of hCG is lost by eliminating its sugar moiety by enzymatic or chemical method. Structures of all asparagine-linked sugar chains included in hCG molecule were reported by us (2) and that of mucin-type sugar chains by Kessler et al (3). Comparative study of the asparagine-linked sugar chains of α- and β-subunits, also performed in our laboratory (4), revealed that α-subunit contains a non-fucosylated monoantennary and a non-fucosylated biantennary complex type sugar chain, while ß-subunit a fucosylated and a non-fucosylated biantennary sugar chain. The location of a specific sugar chain at a definite loci of hCG molecule will be of particular importance in considering the functional role of the sugar chains of this hormone, because Calvo and Ryan (5) recently reported that the glycopeptide obtained from α-subunit but not from β-subunit inhibits dose-relatedly the hormonal action of hCG. In contrast to normal hCG, hCGs purified from urine of choriocarcinoma patients contain both fucosylated and non-fucosylated forms of mono-, bi- and triantennary complex type sugar chains (6). Another unusual feature of the sugar chains of choriocarcinoma hCGs is that they contain abnormal biantennary sugar chains in which two outer chains are linked to Manα1→3 arm of the trimannosyl core (6). The sugar chains of hCGs purified from urine of hydatidiform mole patients were exactly the same as normal hCG. However, hCGs from invasive mole patients contain triantennary sugar chains, although they do not contain the abnormal biantennary sugar chains. The structural change observed in the sugar chains of hCGs from various trophoblastic diseases indicates that the structural change of the sugar chains of hCG induced by malignant transformation of trophoblasts is brough about by two steps. The first step is the ectopic expression of the usual *N*-acetylglucosaminyltransferase IV, and the second step is the deterioration of the enzyme to have looser substrate specificity. These complicated changes in the structure of the sugar chains of hCG molecule will become a useful marker for the diagnosis and prognosis of choriocarcinoma.

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R 004 IDIOTYPES AND REGULATION OF MURINE ANTI-3-FUCOSYLLACTOSAMINE ANTIBODIES, Donald M. Marcus, Masato Umeda, Hirohisa Kimura and Richard Cook, Depts. of Microbiol. and Immunol., Baylor College of Medicine, Houston, TX 77030

Antibodies against carbohydrate determinants play an important role in protection against bacterial pathogens, and also identify many tumor-associated antigens. On the other hand, certain autoantibodies against erythrocyte and neural carbohydrate antigens can be injurious. The role of idiotype networks in regulating antibody synthesis is receiving increasing attention. Many monoclonal antibodies against tumor associated antigens are directed against the 3-fucosyllactosamine (3-FL) antigenic determinant, Gal(β1-4)[Fucα1-3]GlcNAc-R; this determinant is also known as SSEA-1, Le^X or X. We have undertaken an analysis of the regulation of these antibodies and of their idiotypic, structural and genetic diversity. We described previously the preparation of syngeneic monoclonal anti-idiotypic antibodies (6C4 and 6B1) that reacted with 50% of a panel of monoclonal anti-3-FL antibodies (1). We found subsequently that the idiotopes and anti-3-FL antibodies were found in the sera of unimmunized BALB/c mice (2). We have recently obtained the sequence of the N-terminal 30-40 amino acids of the heavy and light chains of 4 BALB/c anti-3-FL monoclonal antibodies. The light chains are virtually identical to each other and to the germline sequence of the V_K24B gene, which encodes V_L regions of Abs against group A Streptococcal carbohydrate (3). The heavy chains are virtually identical to each other and to the germline sequences of the two known V_H genes of group 4, V_H441 and X24 (4,5). The two genes exhibit 98% homology and they encode the V_H regions of antibodies against galactans and levans. These data suggested that antibodies against 3-FL antibodies might arise in part from idiotype network interactions stimulated by immune responses against levan and galactan. An anti-idiotypic column was used to purify antibodies from a pool of sera obtained from unimmunized mice. In addition to antibodies against 3-FL, the eluate from this column also contained specific anti-levan antibodies. These observations provide initial evidence for the existence of idiotypic determinants that are shared by antibodies against two non-crossreactive anti-carbohydrate antibodies. Our current studies are directed toward a more complete characterization of the idiotopes of these antibodies and to an analysis of the functional role of idiotype network interactions in regulating anti-carbohydrate antibodies.

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R 005 STUDIES ON THE BIOSYNTHESIS AND METABOLISM OF G_{M3} GANGLIOSIDE--MODULATION OF CELL SURFACE LEVELS DURING CELL CYCLE AND BY AN EXTRACELLULAR SIALIDASE. C.C. Sweeley and S. Usuki, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

The synthesis and turnover of GM_3 ganglioside may be important in the regulation of growth by extracellular factors. The carbohydrate chains of GM_3 and more complex gangliosides are believed to be assembled in the Golgi apparatus and degraded in secondary lysosomes. However, recent evidence suggests that a portion of cell surface and/or exogenous ganglioside may recycle to cell surface without degradation in the lysosomes.

To test the hypothesis that cell surface GM_3 ganglioside may be degraded by an extracellular sialidase we have studied the turnover of doubly labelled GM_3, prepared *in vivo* by pulse-labelling FS-4 fibroblasts with [1-^{14}C]-N-acetylmannosamine and [3,3-$^{3}H_2$]serine. During a 24 hour chase period there was a 35% decrease in the [^{14}C]-sialic acid content of GM_3 without loss of ^{3}H from the ceramide moiety. We have also studied sialidase activity in the medium as a function of cell density, using GM_3 ganglioside as substrate. This sialidase activity was about 3-fold higher in medium from sparse cultures than it was in medium from confluent cultures. Labelled free sialic acid in the medium increased over a 24 hour period after pulse-labelled fibroblasts were seeded at a pre-confluent cell density (1×10^4 cell/cm^2) and grown in the absence of labelled precursors. These results and the reported inhibition of EGF-mediated cell growth by GM_3 will be summarized in a hypothetical model of cell cycle and density dependent regulation of growth.

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Biosynthesis and Regulation of Carbohydrate Structures

R 006 PROLINE-RICH PROTEINS: PRODUCTS OF TISSUE-SPECIFIC MULTIGENE FAMILIES, David K. Ann, Paul S. Wright, and Don M. Carlson, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616.

Our research has focused on a group of unusual proteins and glycoproteins high in proline, or the so-called proline-rich proteins (PRPs). PRPs are secreted products of the parotid and submandibular glands of various animals, and comprise about 20% of the total protein in human saliva. Treatment of rats, mice, and hamsters with the ß-agonist isoproterenal causes a dramatic induction of the PRP multigene families; after about 10 days of treatment PRP mRNAs make up about 70% of the total mRNAs. PRPs have the following characteristics: (1) amino acid compositions (mol %) are 25-45% Pro, 18-22% Gly and 18-22% Arg; (2) PRPs are acidic or basic, and may be glycosylated and/or phosphorylated; (3) PRPs are composed of four regions, signal peptide, transition region, repeat region and carboxyl-terminal region; (4) PRPs are generally acid-soluble proteins and glycoproteins. The upstream regions of the mouse PRP gene MP2 and of the hamster PRP gene H29 each contain putative regulatory units for cAMP induction, an enhancer element, and the expected TATA and CAAT boxes. We have recently confirmed that deletion of the putative cAMP inducible sequence (-639 to -627 bp in MP2) completely obviates the induction of PRP mRNAs in transfected PC-12 cells. Glycosylation sites in PRP GP66sm are introduced by one base change (C to A) in proline codons (CCA) giving threonine codons (ACA). The major oligosaccharide of GP66sm is a hexasaccharide (Gal NAc(1), Gal(2), Glc NAc(1), Neu Ac(2)). Evidence for an unusual protective role or defense mechanism has been shown for PRPs by their induction in response to tannins in the diet.

Altered Glycosylation in Tumor Cells

R 007 CLONING AND CHARACTERIZATION OF THE GENE ENCODING GALACTOSYLTRANSFERASE. Gregory F. Hollis[1], James R. Douglas[1], Joel H. Shaper[2], Nancy L. Shaper[2], and Ilan R. Kirsch[1], NCI-Navy Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20814[1] and Cell Structure and Function Laboratory, The Oncology Center, Johns Hopkins School of Medicine, Baltimore, MD 21205[2].

We have isolated a cDNA clone coding for bovine galactosyltransferase by immunological screening of a lambda phage expression library. This clone hybridizes to a 4.8 kilobase bovine mRNA and is homologous to sequences found in the human and mouse genomes. Mouse cDNA and genomic clones were isolated using the bovine cDNA clone as a probe. The mouse galactosyltransferase transcript is approximately 4.8 kilobases in size, and is coded for in multiple exons spread out over 50 kilobases of genomic DNA on mouse chromosome 4. Sequence analysis indicates that the mouse and bovine genes encoding galactosyltransferase have been highly conserved, retaining greater than 80% homology at the nucleotide level. The structure of these clones suggest that the C-terminal domain of galactosyltransferase is oriented within the lumen of the golgi.

R 008 BIOSYNTHESIS OF LACTOSAMINOGLYCANS, Dirk H. van den Eijnden, Anky H.L. Koenderman Willem M. Blanken and Wietske E.C.M. Schiphorst, Department of Medical Chemistry, Vrije Universiteit, P.O.Box 7161, NL-1007 MC Amsterdam, The Netherlands.

We have studied the acceptor properties of Novikoff tumor cell ascites fluid β-galactoside β3-N-acetylglucosaminyltransferase (β3-GlcNAcTase), bovine colostrum β-N-acetylglucosaminide β4-galactosyltransferase (β4-GalTase), Novikoff tumor cell β-galactoside β6-N-acetylglucosaminyltransferase (β6-GlcNAcTase) and calf thymus β-galactoside α3-galactosyltransferase (α3-GalTase) towards several glycoproteins and glycolipids as well as a variety of oligosaccharide structures that form part of N-linked glycans and blood group I/i-active structures. These studies have revealed several pathways leading to the formation of cell-surface lactosaminoglycans (LAG).

- The synthesis of linear LAG (blood group i-active) chains occurs by the alternating action of the β3-GlcNAcTase and the β4-GalTase. The β3-GlcNAcTase, which is the key enzyme in this process, not only is involved in the elongation of these glycans but also in the initiation of their synthesis on N-glycoproteins and possibly glycolipids. This enzyme highly prefers acceptor substrates which contain the Galβ1→4GlcNAcβ1→2(Galβ1→4GlcNAcβ1→6)Man pentasaccharide structure occurring on tri'- and tetraantennary N-linked glycans. Such acceptor structures on the other hand are relatively resistent to α2→6-sialylation. The presence of the Galβ1→4GlcNAcβ1→6 to Man branch on these glycans thus promotes LAG formation.
- Initiation of LAG chain synthesis occurs preferentially at the 1→2/1→6 and the 1→6/1→6 branches of tri'- and tetraantennary N-linked glycans, but not at the 1→2/1→3 branch which is preferentially α2→6-sialylated.
- The β3-GlcNAcTase can act on Galβ1→4(Fucα1→3)GlcNAcβ-R. This indicates that the synthesis of oligomeric X-antigenic structures may proceed by the repetitive attachment of Fuc, GlcNAc and Gal residues in this order to Galβ1→4GlcNAc-R acceptors.
- Branched LAG (blood group I-active) may be formed by the action of the β6-GlcNAcTase. This enzyme acts directly after the β3-GlcNAcTase on the acceptor structure R_1β1→3Gal(NAc)-R_2 yielding a R_1β1→3(GlcNAcβ1→6)Gal(NAc)-R_2 branching point. Subsequent β4-galactosylation of the two GlcNAc branches occurs in a highly preferred order in which the 1→6-linked GlcNAc residue is galactosylated first.
- Further chain elongation of branched LAG can occur at both branches; the β3-GlcNAcTase shows no branch specificity. α3-Galactosylation, however, preferentially occurs at the 1→6-branch. As a result α3-galactosylated bloodgroup I-active structures are elongated at the 1→3-branch and contain short 1→6-branches, which are terminated by an α3-linked Gal residue.

Carbohydrates in Cellular Recognition and Adhesion

R 009 ENDOGENOUS LECTINS AND GLYCOCONJUGATES PARTICIPATE IN ADHESIVE INTERACTIONS IN DICTYOSTELIUM, Samuel H. Barondes and Wayne R. Springer, Department of Psychiatry, University of California, San Francisco, CA 94143 and Department of Psychiatry, Veterans Administration Medical Center, San Diego, CA 92161. Formation of aggregates during differentiation cellular slime molds depends on both specific cell-substratum adhesion and cell-cell adhesion. Glycoconjugates have bee implicated in both of these adhesive processes. Specific cell-substratum adhesion that is critical for ordered cell migration into aggregates is dependent on an endogenous developmentally regulated soluble lectin, discoidin I. It is extensively synthesized during the aggregation process, is packaged in multilamellar bodies along with complementary glycoconjugates of bacterial origin, and is secreted around the aggregating cells. Interaction of the lectin with the cell surface is mediated not via its carbohydrate binding site but instead by a domain that contains the tripeptide arg-gly-asp also found in the vertebrate adhesion molecule fibronectin. The carbohydrate binding site of discoidin I interacts with the bacterially derived glycoconjugates as part of the secretory process and possibly also as part of the mechanism of adhesion to substratum. Work in Gerish's laboratory has implicated a cell surface glycoprotein with subunit molecular weight 80,000 (gp-80) in developmentally regulated cell-cell adhesion in Dictyostelium discoideum. We have found a related molecule, p-CAM, in Dictyostelium purpureum with subunit molecular weight of 37,000. A monoclonal antibody directed against a glycoconjugate epitope found on both gp-80 and p-CAM blocks cell-cell adhesion in species. Since purified oligosacchadrides derived from p-CAM block cell-cell adhesion in D. purpureum, they appear to participate directly in the adhesion process. The role of the p-CAM oligosaccharides in this interaction is presently being examined.

(Supported by a grant from NSF and by The Veterans Administration Medical Center.)

R 010 ENDOGENOUS GALACTOSIDE-SPECIFIC LECTINS AS MEDIATORS OF TUMOR CELL ADHESION, Reuben Lotan and Avraham Raz, Department of Tumor Biology, The University of Texas-M.D. Anderson Hospital and Tumor Institute at Houston, TX [R. L.], and Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel [A. R.]. Endogenous carbohydrate-binding proteins have been found in different vertebrate tissues and hemopoietic cells. Although lectins with different sugar specificities have been described, the most prevalent ones are those that bind galactosides. The ability of some cells to bind exogenous carbohydrate-containing ligands (e.g., glycoproteins or neoglycoproteins) suggested that sugar-binding proteins are present on the cell surface and that these might mediate intercellular recognition and adhesion by binding complementary glycoconjugates on adjacent cells or on the extracellular matrix. We have found that extracts of various cultured murine and human tumor cells including melanoma, sarcoma, carcinoma, neuroblastoma and large cell lymphoma exhibit a galactoside-inhibitable hemagglutinating activity. One of the initial indications that lectin activity is present also at the cell surface was the finding that asialofetuin, a glycoprotein possessing several branched oligosaccharide side chains bearing terminal nonreducing galactosyl residues, binds at the cell surface and can induce homotypic aggregation of suspended tumor cells. Asialofetuin glycopeptides failed to induce aggregation and actually inhibited the induction of cell aggregation by the intact asialofetuin molecule suggesting that the "polyvalent" asialofetuin serves as a crosslinking bridge between lectin molecules present on adjacent cells. Monoclonal antibodies (mAbs) were prepared against crude B16 mouse melanoma cell extract and selected for ability to inhibit lectin-induced hemagglutination. Immunoblotting of total cell extracts of melanoma, sarcoma or carcinoma cells revealed that these mAbs bind to two proteins of M_r 14,500 and 34,000, respectively. These proteins comigrated on SDS polyacrylamide gels with lectin molecules purified from cell extracts by affinity chromatography on immobilized asialofetuin columns. An analysis of mAb binding to intact cells by indirect immunofluorescence revealed the presence of the lectins on the surface of melanoma, carcinoma, sarcoma and neuroblastoma cells. The binding of the antilectin mAbs to the surface of several tumor cell types resulted in inhibition of asialofetuin-induced homotypic aggregation, suppression of cell-to-substrate attachment and a non-cytotoxic inhibition of tumor cell colony formation in vitro (in agarose) and in vivo (in the lungs of syngeneic mice). These findings implicate tumor cell surface lectins in the mediation of specific intercellular interactions and regulation of growth. (Supported by grant CA39422 from the National Cancer Institute).

Altered Glycosylation in Tumor Cells

R 011 ALTERATIONS IN SIALYLATION OF HIGH AND LOW METASTATIC TUMOR LINES AND THEIR POSSIBLE ROLE IN METASTASIS, Volker Schirrmacher, Peter Altevogt, Elke Lang, Rosemarie Benke and Eckhard Pflüger, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG.
Plastic adherent variants can be generated under reproducible conditions from the suspension growing highly metastatic ESb cell line. Such variants have a greatly decreased ability to release from the locally growing tumor and have a greatly decreased overall malignancy in vivo. The changes in adhesiveness affect the kinetics, the quantity and the organ distribution of metastases. Associated with the changes in adhesiveness we see a change in the glycosylation of distinct cell surface glycoproteins which could be identified as differentiation antigens and as cell surface receptors with the help of defined monoclonal antibodies. The altered glycosylation patterns were also detectable with plant lectins. The implications of these altered carbohydrates on cellular adhesion and metastasis will be discussed.

R 012 THE RECEPTOR FUNCTION OF GALACTOSYLTRANSFERASE DURING CELLULAR INTERACTIONS, Barry D. Shur, Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Galactosyltransferase (GalTase) is present on the cell surface in addition to its conventional intracellular location in the Golgi apparatus. Through the use of biochemical, genetic, and immunological probes, it has been shown that cell surface GalTase participates in a variety of cellular interactions by recognizing and binding to its appropriate glycoconjugate substrate on adjacent cell surfaces and in the extracellular matrix. The receptor function of surface GalTase in cell interactions has been confirmed by using competitive GalTase substrates, affinity-purified GalTase, GalTase substrate analogues and substrate modifiers, specific glycosidase digestions and monospecific anti-GalTase IgG. With these reagents, GalTase has been shown to mediate mouse sperm binding to the egg zona pellucida, cell interactions in the preimplantation mouse embryo, and cell migration on basal lamina matrices. The glycoconjugate substrates for surface GalTase have been identified as lactosaminoglycans, and laminin, in particular, has been shown to be the substrate for surface GalTase during cell migration on basal lamina matrices.

Recent results show that GalTase is redistributed on the cell surface to new plasma membrane domains where it will participate in subsequent cell interactions, suggesting that GalTase functions repeatedly as a recognition molecule during cell interactions in development. The redistribution of surface GalTase appears to be mediated by the cytoskeleton, possibly involving spectrin-like proteins anchored to actin filaments beneath the plasma membrane. Currently, the function of GalTase during cell interactions is being pursued with the aid of molecular genetics, whereby GalTase expression can be manipulated positively and negatively in transfected cell lines and in transgenic mice. The consequences of altered surface GalTase expression on cellular interactions during fertilization and embryonic development are being examined.

Oncodevelopmental Expression of Specific Carbohydrates

R 013 **DIFFERENTIATION OF HUMAN TERATOCARCINOMA STEM CELLS, Peter W. Andrews, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104.** Human embryogenesis is largely inaccessible to experimentation; yet, there are clearly differences between human embryonic development and that of experimental animals. Cell lines derived from human teratocarcinomas, tumors that present a caricature of embryogenesis, provide a source of experimental information about the properties of human embryonic cells, and differentiation in human teratocarcinomas could, in turn, be used to investigate aspects of human embryogenesis. Teratocarcinomas consist of stem cells (embryonal carcinoma, or EC, cells) and tissues derived from them by differentiation; the EC cells can initiate multiple pathways of differentiation which probably parallel those of the early embryo, and in the mouse, EC cells most closely resemble pluripotent cells of the primitive ectoderm. Substantial differences between human and murine teratocarcinomas exist and findings in one species cannot always be extrapolated to the other. For example, human EC cells express on their surface the globoseries oligosaccharide antigens SSEA-3 and -4, which belong to the P blood group system, but not the lactoseries antigen SSEA-1, whereas murine EC cells lack SSEA-3 and -4, but express SSEA-1. Such differences might indicate that human EC cells represent a different population of embryonic cells, or they might reflect differences in human and mouse embryogenesis.

We have cloned and defined a human EC cell line, NTERA-2 cl.D1, that can be induced to differentiate into a variety of cell types, including neurons, by exposure to retinoic acid in vitro. The approach that we have taken to analyzing its differentiation is first to define the various derivative cell types in terms of their expression of specific cell surface antigens identified by monoclonal antibodies. These antigens can then serve as markers for following changes in the composition of cultures as differentiation progresses, and for manipulating the culture to analyze the developmental relationships of the cells. When NTERA-2 EC cells differentiate the globoseries antigens SSEA-3 and -4 disappear, while cells expressing antigens A2B5 (ganglioside GT_3), ME311 (ganglioside 9-O-acetyl GD_3) and SSEA-1 (Le^x epitope) appear. To some extent these antigens are distributed on different subsets of cells which reflect different cell lineages leading to neuronal and non-neuronal derivatives of the EC cells. In collaboration with Drs. Fenderson and Hakomori, we have analyzed the glycolipids that carry the various antigenic epitopes that we have studied, and proposed that a key event in the differentiation of human EC cells is a switch in synthesis of oligosaccharide core structures from globoseries to lacto- and ganglioseries. This implies developmental regulation of specific galactosyl, glucosaminyl and sialyl transferases and we are currently investigating this.

R 014 HUMAN CELL SURFACE SIALOGLYCOPROTEINS DEFINING DIFFERENTIATION AND MALIGNANCY, Minoru Fukuda, Sven R. Carlsson, Paul D. Siebert, and Juha Viitala, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

It has been increasingly evident that tumor cells often represent cells which may be arrested at various stages of differentiation. We have been, therefore, interested in determining whether carbohydrate markers and their carriers, glycoproteins, can distinguish the pathways of divergence of pluripotent stem cells into terminally differentiated cells (1). To this end, we have been studying two major sialoglycoproteins which are expressed in hematopoietic cells. The first one, glycophorin A, is specifically expressed in erythroid cell lineage. We found that glycophorin A in K562 immature leukemic cells migrates slower than that in mature erythrocytes in SDS-PAGE analysis (2). By isolating cDNA clones for glycophorin A from K562 mRNAs, we discovered that the amino acid sequence of glycophorin A is the same in mature erythrocytes and K562 cells (3). By using isolated cDNAs, we further discovered that glycophorin A and glycophorin B are encoded by separate, single copy genes which are coordinately regulated by tumor-promoting phorbol ester (4). The second sialoglycoprotein, leukosialin, was recently discovered by us to be present in all white cells including immature erythroid nucleated cells but not in erythrocytes (5). The apparent molecular weight of leukosialin varies significantly depending on cell types but share the same polypeptide portion, Mr 52,000, as evidenced by pulse-labeling experiments (5). Furthermore, we discovered that leukosialin is heavily glycosylated by O-linked oligosaccharides (90 oligosaccharides/molecule) and that the structures of O-linked oligosaccharides are characteristic to cell lineage and maturation stages (6,7). More recently, we have isolated a novel sialoglycoprotein which contains polylactosaminoglycans. This glycoprotein is ubiquitously expressed in all cells examined except erythrocytes, and is heavily glycosylated by N-linked oligosaccharides and N-linked oligosaccharides appear to be characteristic to cell types. In particular, it is notable that the expression of this glycoprotein is closely correlated to tumorigenecity; this glycoprotein is minimally present in tumorigenic cells, which are obtained by cell-cell hybrids or oncogene transfection.

Studies on these three distinct sialoglycoproteins will hopefully enable us to understand structure and function of cell surface glycoproteins in differentiation and malignancy.

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R 015 CELL SURFACE CARBOHYDRATES IN HUMAN HEMATOPOIETIC CELLS, Carl G. Gahmberg, Department of Biochemistry, University of Helsinki, 00170 Helsinki, Finland.
We have earlier developed techniques to specifically radioactively label cell surface-exposed carbohydrate residues in glycoproteins and glycolipids (1,2). The labeled glycoproteins have been analyzed by a variety of different techniques. Using human bone marrow cells and different hematoipoietic cell lines we have been able to identify glycoprotein patterns characteristic of cells of defined stages of differentiation (3,4). Using the erythroid line-specific marker glycophorin A as a model system it has become apparent that the degree of glycosylation of individual polypeptides may change during cellular differentiation. This may have profound effects on the interactions with antibodies and other ligands (5,6).
Recently we have been able to introduce new carbohydrate units specifically into hematopoietic cell surface glycoconjugates using hydrazine derivatives (7). This technique makes it possible to study functional aspects of surface carbohydrates, and has been applied to study human lymphocyte interactions. Using phorbol esters cell surface glycoprotein capping (8), and cell aggregation results. The receptor involved has been identified as the LFA-1 molecule (9). The protein has been purified in milligram amounts from human lymphocytes and is being characterized.

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R 016 CELL SURFACE GLYCOCONJUGATES AND CARBOHYDRATE-BINDING PROTEINS: POSSIBLE RECOGNITION SIGNALS IN SENSORY NEURONE DEVELOPMENT, Thomas M. Jessell[†] and Jane Dodd, Center for Neurobiology and Behavior and [†]Howard Hughes Medical Institute, Columbia University, College of Physicians and Surgeons, New York, NY 10032.
Dorsal root ganglion (DRG) neurons transmit cutaneous information from the periphery to the dorsal horn of the spinal cord. Subpopulations of DRG neurons that subserve distinct sensory modalities project to discrete regions in the dorsal horn. Several lines of evidence suggest that the formation of specific sensory connections during development may involve cell-surface interactions between sensory axons and spinal cord cells. We have therefore begun to analyse molecules that are expressed on the surface of functional subpopulations of DRG and dorsal horn neurones. The use of monoclonal antibodies has revealed that distinct subsets of DRG neurones express globo- and lactoseries carbohydrate differentiation antigens. The expression of defined carbohydrate structures correlates with the embryonic lineage, peptide phenotype and the central termination site of sensory neurones. Moreover, glycoconjugates with similar or identical structures have been implicated in cellular interactions that contribute to earlier stages of embryonic development.
Since small-diameter DRG neurones that project to the superficial dorsal horn express N-acetyllactosamine backbone structures, B-galactoside-specific binding proteins (lectins) represent possible receptors for these oligosaccharide. Two lectins have been identified that are expressed early in development in the superficial dorsal horn. These complementary molecules may contribute to the development of sensory afferent projections in the spinal cord.

R 017 ONCODEVELOPMENTAL CHANGES OF GLYCOPROTEIN-BOUND CARBOHYDRATES, Takashi Muramatsu, Department of Biochemistry, Faculty of Medicine, Kagoshima University, 1208-1, Usukicho, Kagoshima 890, Japan.

Carbohydrate moieties of glycoproteins undergo marked alterations during development, and some of the developmentally regulated sequences are expressed abnormally in tumor cells. The over-all picture of the developmental alterations revealed in the mouse will be summarized at first. The carbohydrate alteration is a very complex phenomenon; both the non-reduring ends and the core structure are altered during the process. Monoclonal antibodies and lectins usually detect changes in non-reducing ends. Studies using the teratocarcinoma system as a model indicate that many of the markers are carried by poly-N-acetyllactosamines in early embryos. Among the developmentally regulated markers, the following ones will be covered in detail.

α-Galactosyl residues. ECMA 2 is an oncodevelopmental carbohydrate antigen expressed in preimplantation embryos, visceral endoderm and primordial germ cells, and is defined by an α-galactosyl residue. Receptors for Griffonia simplicifolia agglutinin (GS-I) is a marker of extraembryonic endoderm. Furthermore, patients with germ cell tumors frequently develop an antibody recognizing α-galactosyl residues on poly-N-acetyllactosamine of embryonal carcinoma cells. As an aid to study α-galactosyl cell-surface markers, a new endo-β-galactosidase was isolated from the culture fluid of Clostridium perfringens. The enzyme (endo-β-galactosidase C) catalyzed the following reaction.

Gal(α1-3)Gal(β1-4)GlcNAc-R ⟶ Gal(α1-3)Gal + GlcNAc-R

The susceptible substrates included bovine thyroglobulin glycopeptides and a pentaglycosyl ceramide. Blood group B antigen was not cleaved. Using the enzyme, GS-I binding sites in glycoproteins from teratocarcinoma OTT6050 were proved to be Gal(α1-3)Gal.

Peanut agglutinin (PNA) receptors. The receptors are expressed in many sites in mouse embryos. In the somite, the cells near the neural tube expressed them more intensely: a kind of gradient was observed about the mode of expression. The receptors are also present in many of human cancer cells. The major oligosaccharides in the receptors from human gastric cancer cells were determined to be Gal(β1-3)GalNAc and Gal(β1-3)[Gal(β1-4)GlcNAc(β1-6)]GalNAc. Incomplete sialylation is probably the reason of the expression in cancer cells.

Fuc(α1-3)[Gal(β1-4)]GlcNAc. The structure is recognized by anti-SSEA-1 and also by Lotus tetragonolobus agglutinin, and is probably involved in cell-matrix interaction. In pararell with the disappearance of these markers from differentiating embryonal carcinoma cells, the activity of the fucosyltransferase forming the linkage is lost from the cells. The enzyme has been purified from embryonal carcinoma cells. Further studies on the enzyme may be helpful in understanding the mechanism of the differentiation-dependent cell-surface change.

R 018 DEVELOPMENTALLY REGULATED GLYCOCONJUGATES IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM, Gerald A. Schwarting, Sharon F. Suchy and Miyuki Yamamoto, Department of Biochemistry E.K. Shriver Center, Waltham, MA 02254.

The nervous system develops through a sequence of cellular events, including cell proliferation, migration to particular sites, and various steps of morphological differentiation leading to the establishment of afferent and efferent connections. A general hypothesis is that these events are regulated by the interaction of molecules expressed on cell surfaces, in the extracellular matrix, or by diffusable substances. There is increasing evidence that glycoconjugates are involved in specific cell-cell interactions in the development of the mammalian nervous system. Monoclonal antibodies have been raised in our laboratory against developmentally regulated carbohydrate antigens in the brain which recognize distinct classes of cells in the developing central nervous system. One of these antibodies, 7A, was raised against a homogenate of embryonic rat forebrain, is expressed almost exclusively on germinal layers of the cerebral cortex, beginning as early as day 11 of gestation. Typical staining is seen at embryonic day 15, in which only the ventricular and subventricular zones of the cerebral cortex are intensely labeled. Molecular species possessing the 7A antigen are identified as neutral glycolipids that contain the X-determinant oligosaccharide Galβ1-4[Fucα1-3]-GlcNAc... A second antibody, 4F4, also raised against embryonic rat forebrain defines a different cellular staining pattern from 7A in E15 cerebral cortex. 4F4 immunoreactivity is associated with the molecular layer and the subplate of the cortex. Fiber-like structures in the intermediate zone are also stained. These results suggest that the most differentiated portion of the cortex is labeled by 4F4. Chemical analysis of the 4F4 reactive glycoconjugates reveals the presence of acidic glycolipids in embryonic but not adult CNS. The major antigenic glycolipid which is maximally expressed at P14 in the rat appears to be identical to a SO_3^--GlcUA(β1-3) Gal(β1-4) GlcNAc.... containing glycoconjugate identified from human peripheral nerve. Western blotting analysis also reveals the presence of several high molecular weight proteins which react with 4F4 antibodies. The staining pattern of 4F4 antibody in the cerebral cortex is complementary to that of 7A antibody. 7A antibody recognized X-determinant carbohydrate antigens and is expressed only in proliferating cells of the cortex. This is of potential significance in that 4F4 reactive glycolipids and X-determinant glycolipids are both derived from the same biosynthetic precursor, lactoneotetraosylceramide (LNT) and that sulfated glucuronic acid-LNT (4F4) and fucosyl-LNT (X-determinants) are expressed on mutually exclusive populations of cells in the embryonic cortex. These results indicate that modulation of carbohydrate structures takes place during cellular maturation. In this case fucosylation of LNT decreases while sulfation and glucuronylation of the same precursor increases.

Altered Glycosylation in Tumor Cells

R 019 CELL SURFACE MOLECULES IN MAMMALIAN DEVELOPMENT, Davor Solter, The Wistar Institute, Philadelphia, PA 19104.
Numerous cell surface molecules have been identified in mouse embryos using polyclonal and monoclonal antibodies. Chemically characterized molecules which exhibit stage specificity (stage specific embryonic antigens-SSEA) were exclusively carbohydrates complexed with lipids and/or proteins. Additional glycoprotein molecules have been identified using functional assays but their detailed chemical structures is unknown. Appearance of cell surface molecules in correct temporal and spatial sequence during development is an essential regulating feature in embryogenesis. In mammals, potential immune response to unique embryonic antigen must be subverted, so that it cannot interefere with the development of the fetus. Recent observations, demonstrating differential expression of maternal and paternal genome during development, suggest one of the possible mechanisms by which embryo can evade the immunological recognition.

The Use of Lectins to Analyze Tumor Cells

R 020 **ANALYSIS OF TUMOR CELL OLIGOSACCHARIDES AND METASTATIC POTENTIAL USING SEVERAL GENOTYPIC LECTIN RESISTANT TUMOR CELL MUTANTS.**
James W. DENNIS, Mount Sinai Hospital Research Institute, Division of Cancer and Cell Biology, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5.
Neoplastic transformation has been associated with a variety of structural changes in cell surface carbohydrates, most notably increased sialylation and β1-6 linked branching of complex-type asparagine-(Asn)-linked oligosaccharides (ie. -GlcNAcβ1-6Manα1-6 Manβ1-). However little is known about the relevant glycoproteins or how these transformation-related changes in oligosaccharide biosynthesis may affect the malignant phenotype. We recently identified that a cell surface glycoprotein, gp130, as the major target of increased β1-6 linked branching and the expression of these oligosaccharide structures on gp130 appears to be directly related to the metastatic potential of the cells. We have selected glycosylation mutants of a metastatic tumor cell line which are deficient in both β1-6 GlcNAc transferase V activity and metastatic potential in situ. In addition swainsonine, a potent inhibitor of Golgi α-mannosidase II which blocks processing prior to the addition of the β1-6 antennae, also inhibited metastasis but not tumor growth. Moreover, induction of increased β1-6 branching in clones of a non-metastatic murine mammary carcinoma correlated strongly with acquisition of metastatic potential. The results indicate that increased β1-6 linked branching of complex-type oligosaccharides on gp130 may be an important feature of tumor progression related to increased metastatic potential.

R 021 LECTINS AS PROBES OF GLYCOCONJUGATES STRUCTURE, Irwin J. Goldstein, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.
Lectins of known carbohydrate binding specificity provide valuable tools for the detection, isolation and characterization of glycoconjugates. Of special interest are lectins which exhibit unique carbohydtate binding specificities. Three such plant lectins are present in snow drop bulbs, elderberry bark and Griffonia simplicigolia seeds. The snow drop (Galanthus nivalis) lectin binds solely to mannose and exhibits specificity for Manal, 3Man units; it precipitates yeast mannans, but not glycogen or dextrans. Glycopeptides with terminal Man 1, 3Man units are retarded on immobilized snow drop lectin. The elderberry bark lectin (Sambucus nigra L.) (SNA) recognizes Neu5Ac 2,6GalNAc/Gal sequences. Immobilized SNA separates Neu5Ac 2,6lactose, which binds, from Neu5Ac 2,3lactose, which elutes in the void volume of the column. Immobilized SNA also resolves oligosaccharides and glycopeptides based on the number of NANA 2,6Gal units present in each molecule. Griffonia simplicifolia $I\text{-}B_4$ isolectin binds glycoconjugates containing a-galactosyl end groups, e.g. murine laminin. The use of immobilized lectin columns for resolving complex mixtures of glycoconjugates will be discussed. Support for these studies was provided by NIH Grant GM 29470 and CA 20424.

R 022 USE OF LECTINS IN GLYCOPROTEIN ANALYSIS OF METASTATIC TUMOR CELLS IN HUMANS, Tatsuro Irimura, Department of Tumor Biology, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.
Tumor metastasis is a specific process resulting from tumor cell-host interactions; however, only a few highly metastatic cells in the primary tumor are able to metastsize. Metastatic tumor cells express a wide variety of characteristics necessary for such interactions. We have been studing structural changes and functional significance of cell surface glycoproteins (GPs) in experimental animal tumor cells selected in vivo for high metastatic potentials, as well as human tumor cells from primary tumor sites and metastase. Lectins are useful tools for biochemical and histochemical analyses, and for the purification of tumor cell with different metastatic protentials. In histochemical experiments, we have found that human carcinoma of distal colon or rectum bound Ulex europeus arrlutinin-1 (UEA1) but not Cytisus sessilifolius agglutinin, another anti-H lectin having slightly different carbohydrate specificity. This "H-like" structure was expressed in the tumors on a GP have M_r >300,000. The reactivity of UEA 1 to this GP from primary tumors that did not develop metastases was significantly higher than those that developed metastases. However, there was no significant difference in the GPs between primary tumors and their metastases in lymph nodes or liver. Cellular GPs from human colon carcinoma cell lines established from liver metastases in nude mice were analysed by a combination of polyacrylamide gel electrophoresis, chemical modification of the GP carbohydrate chains and lectin staining. Increased expression of a wheat germ agglutinin (WGA) binding GP of M_r 800,000 was detected in cells derived from metastases. This was a sialoGP, becuase WGA reactivity was eliminated by treatment of the GP with mild acid in the polyacrylamide gels before staining with lectin. The differential WGA reactivity was probably not due to structural differences in the carbohydrate chains, because WGA reactivity was restored similar extent to those cell lines derived from primary sites and metastasis, if the desialyzed GP was further processed for Smith degradation. This GP has been purified by WGA-agarose affinity chromatography followed preparative electrophoresis. References: Irimura, T. and Nicolson, G.L. Carbohyd Res. 115: 209-220, 1983; Irimura, T. and Nicolson, G.L. Carbohyd. Res 120: 187-195, 1983; Irimura, T. and Nicolson, G.L. Cancer Res 44; 791-798, 1984; Irimura, T., Ota, D.M., Cleary, K.R. and Nicolson, G.L. In; Biology and Treatment of Colorectal Cancer Metastasis, A.J. Mastromarino (ed.), pp. 57-72, 1986; Irimura, T., Tressler, R.J. and Nicolson, G.L. Exp. Cell Res. 165; 403-416, 1986. (Supported by USPHS grant RO1-CA39319)

Altered Glycosylation in Tumor Cells

R 023 LEUKEMIA- AND TUMOR-ASSOCIATED GLYCOPROTEINS REVEALED BY LECTINS, Chritopher L. Reading, University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston TX 77030.

We have previously described a procedure for Western-Enzyme Linked Lectin Analysis (WELLA) of total cellular glycoproteins (Glycoconjugate J, 2:293-302, 1985). Additional steps allow examination of cell surface glycoproteins using WELLA. Intact cells are reacted with sulfo-N-hydroxysuccinimidyl-biotin prior to detergent extraction. Biotin-labeled glycoproteins are purified on immobilized avidin or immobilized lectins and released with SDS. Western transfers of these glycoproteins are visualized with a panel of lectins using lectin-peroxidase, and the relative amounts of components can be evaluated using avidin-peroxidase. Using the WELLA with PWM-HRP we detected two glycoproteins of Mr 210K and 140K which were present on K562 cells but not on normal bone marrow mononuclear cells. Glycoproteins bound by PWM were purified by gel filtration in SDS, detection by affinity chromatography, and preparative SDS PAGE. The bands were cut out of the gel, homogenized, and used to immunize mice. Monoclonal antibodies were obtained which reacted with the Mr 210 glycoprotein (monoclonal antibody MDA010) and with the MW 140 glycoprotein (monoclonal antibody MDA011) on Western transfers. These antibodies also bound fresh acute leukemia cells. MDA010 reacts with ALL and some AML samples and MDA001 reacts with AML only. Similar procedures with UEA-HRP detect a MW 180K glycoprotein on human colon carcinoma cells which appears to be a subset of CEA. These techniques represent simple methods for detection, isolation, and production of monoclonal antibodies to tumor-associated glycoconjugates.

Blood Group Tumor-associated Antigens

R 024 GENERAL CHANGES OF BLOOD GROUP ANTIGENS AND INCOMPATIBLE BLOOD GROUP ANTIGENS IN HUMAN CANCER, Sen-itiroh Hakomori, Biochemical Oncology, Fred Hutchinson Cancer Research Center, and the Departments of Pathobiology, Microbiology & Immunology, University of Washington, Seattle, WA 98104. Since the majority of human cancers arise from epithelial cells and blood group antigens are abundant in epithelial cells and their secretions, aberrant glycosylation associated with the majority of human cancers have been in blood group and related antigens. The following changes have been chemically identified, and most of the antigens have been detected by their respective monoclonal antibodies: (i) Deletion of A or B determinants and accumulation of their precursors (H, Ii). (ii) Enhanced subterminal $\alpha 1\rightarrow 3$ fucosylation of the precursor type 2 chain as well as enhanced internal $\alpha 1\rightarrow 3$ fucosylation of type 2 chain leading to the accumulation of Le^x and multimeric Le^x. (iii) Enhanced sialylation followed by subterminal $\alpha 1\rightarrow 3$ or $1\rightarrow 4$ fucosylation of type 1 or type 2 chain leading to formation of sialyl Le^x or sialyl Le^a. (iv) Enhanced fucosylation coupled with subterminal $\alpha 1\rightarrow 3$ or $1\rightarrow 4$ fucosylation leading to imcompatible Le^b or Le^y. (v) Incompatible blood group A or A-cross-reacting antigens that have been identified as (a) real type 1 chain mono- or difucosyl A (ALe^a), ALe^b) in tumors of blood group B or O; (b) Tn antigen; and (c) Forssman antigen. Tn antigen is the major imcompatible A antigen.

R 025 ALTERED BLOOD GROUP EXPRESSION IN HUMAN PREMALIGNANT AND MALIGNANT COLONIC TISSUES
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Recently carbohydrate structures related to blood group substances are being recognized as important cancer-associated antigens in the GI tract. Three main types of changes in carbohydrates have been observed in colorectal cancer using specific monoclonal antibodies. These are: 1) expression of an antigen not usually found in normal mucosa; 2) deletion of an antigen usually expressed by normal mucosa with or without accumulation of precursors; and 3) neosynthesis which occurs by modification of existing structures. When A,B,H antigen was examined, the most frequent finding was the reexpression of blood group antigens (A,B,H & Le^b) in the distal segment (90% of distal colon cancer). Over 60% of colon cancers of both proximal and distal colon expressed blood group antigens (A or B) incompatible with the host's blood type. Deletion of the host's blood group antigen from cancer cells was also observed in nearly half of the colon cancers occurring in the proximal colon. T antigen which is related to M & N blood group antigens and whose epitope is thought to reside on precursor carbohydrate structures is expressed preferentially in colon cancer tissues and in premalignant (adenomatous) polyps. The examples of modification of existing structure$_x$ are Le^x and Le^y antigen expression in colon cancer cells. The respective epitopes for Le^x and Le^y antigens, the positional isomers of Le^a and Le^b antigens, can reside either on short or extended type 2 lactosamine backbones. Only the Le^x and Le^y antigens present on the extended backbone with or without internal fucosylation were preferentially expressed in malignant colonic tissues and rarely expressed in normal mucosa. Furthermore, the expression of extended and polyfucosylated extended Le^x and Le^y antigens was limited exclusively to premalignant (adenomatous) polyps and was absent from nonmalignant (hyperplastic) polyps. These monoclonal antibodies therefore appear to be promising adjunct for the diagnosis and perhaps therapy of colon cancer and may also provide insight into the biochemical event involved in polyp to cancer sequence.

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R 026 BLOOD GROUP ANTIGEN EXPRESSION IN EPITHELIAL TUMORS: INFLUENCE OF SECRETOR STATUS
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Normal distal colonic epithelium is characterized by the presence of $Lewis^a$ and X ($Lewis^x$) antigens and, in general, by the absence of $Lewis^b$ and Y ($Lewis^y$) antigens. Colonic cancers, on the other hand, produce both Le^b and Y antigens in the majority of cases (1). This expression is unrelated to the blood group of the patient and, more significantly, is not dependent on the secretor status, i.e. non-secretors express Le^b and Y antigen in their colonic tumors (1). The specificities of a group of anti-Y antibodies reacting with colonic tumors will be compared and a new anti-type 1 H (2) will be described.

The influence of secretor status on the expression of blood group antigens in normal epithelia and secretions and in carcinomas will be discussed in more detail. In saliva, both type 1 (Le^a and Le^b) and type 2 (X and Y) expression is governed by secretor type (3). In tissues, this control is not as strict. Nevertheless, in non-secretor individuals the expression of Le^b and Y is generally absent or minimal in comparison to secretors. In the urinary tract, blood group expression is maximal in the collecting ducts and urothelium. Differential expression is seen in different cell types. Secretor individuals have low or no expression of Le^b and Y antigen in these tissues (4). The influence of the secretor gene in normal epithelia is important in understanding blood group expression in epithelial tumors.

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R 027 SYNTHETIC THOMSEN-FREIDENREICH (TF) AND Tn ANTIGENS FOR PRODUCTION OF ANTI-CARCINOMA MAbs AND GENERATION OF ANTI-CARCINOMA T CELLS, B. Michael Longenecker, Carina Henningsson, Grant MacLean and Tony Noujaim, Department of Immunology, Faculty of Pharmacy, Department of Medicine, University of Alberta and BIOMIRA Inc., Edmonton, Alberta, Canada T6G 2H7.

Our group has synthesized several well-defined tumor-associated carbohydrate haptens and conjugated them to immunogenic carriers by way of appropriate linker arms (called synthetic tumor-associated antigens=S-TAGs). Monoclonal antibodies generated to our Thomsen Freidenreich (TF) or Tn S-TAGs react with the majority of human adenocarcinomas and detect cancer-associated mucins. Epiglycanin (Epi) is a mucin-like membrane glycoprotein which has multiple copies of TF and Tn determinants produced by the highly lethal murine spontaneous mammary adenocarcinoma cell line, TA3-Ha. Epi protects TA3-Ha cells against rejection by the immune system. We reasoned that an appropriate immune response directed against TF and/or Tn determinants might cause rejection of TA3-Ha cells. Preimmunization with purified Epi or the appropriate S-TAG provided good protection against a TA3-Ha tumor transplant. Several attempts to transfer protection to syngeneic mice using anti-Epi immune sera or TF or Tn MAbs failed. Consequently we investigated whether T cells which mediate a DTH reaction could specifically recognize TF or Tn carbohydrate determinants and mediate an anti-TA3-Ha effect. DTH effector cells were generated using an in vivo - in vitro protocol designed to examine the fine antigen specificity of the DTH effectors. Using this protocol together with our S-TAGs we have now provided the first conclusive evidence that DTH effector T-cells can specifically recognize carbohydrate determinants in an MHC restricted fashion. We have also demonstrated that Epi and S-TAGs can be used for the in vitro generation of carbohydrate specific anti-tumor effector T-cell populations. This provides the rationale for the use of S-TAGs for cancer immunotherapy.

R 028 BLA : A GLYCOLIPID BLOOD GROUP ANTIGEN ASSOCIATED WITH BURKITT'S LYMPHOMA CELLS. Thomas Tursz, Joelle Wiels, Marc Lipinski and Senitiroh Hakomori* Institut Gustave Roussy, 94805 Villejuif, France and Fred Hutchinson Cancer Research Center*, Seattle, USA.

The monoclonal antibody 38.13 is reacting with most Burkitt's lymphoma (BL) derived lines, either containing the Epstein-Barr virus (EBV) or not, but not with EBV-positive non-malignant lymphoblastoid cell lines. The target antigen, BLA, was shown to be a neutral glycolipid, identified as globotriaosylceramide (Gal α1-4 Gal β1-4 Glc β1-1 ceramide). This substance is known as the blood group antigen Pk, a normal intermediate in the P substance synthesis. The enzyme activities involved in the synthesis and degradation of globotriaosylceramide, and the degree of exposure of this glycolipid have been studied with various BL and non-BL and lymphoblastoid cell lines. The activity of UDP-Gal : LacCer α-galactosyl-transferase of BL cells was consistently higher than that of non-BL cell lines, and the enzyme activity can be correlated with the antigen expression at the cell surface. Though not detected on normal B cells from peripheral blood, bone marrow and lymph nodes, BLA was found on a small subset of B cells located within the germinal center of normal tonsils. These normal BLA + cells shared most morphological and antigenic features (CALLA+, C3d-EBV receptor+, SIgM+, SIgD-) with BL cells. It is suggested that this novel B cell population could represent the normal counterpart of Burkitt's cells. BLA could behave as a "homing" antigen, with possible implications in the peculiar clinical localizations of BL.

Tumor-associated Carbohydrate Antigens And Diagnostic Applications

R 029 Cancer-Associated Serum Glycoprotein Antigens Expressing Type 1 and Type 2 Chain Polylactosamines.

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Seven tumor-associated carbohydrate antigens carrying type 1 and type 2 chain polylactosamines present in the sera of patients with various malignant and non-malignant disorders were investigated using monoclonal antibodies. Four types of type 2 chain determinants; Le^x, poly-Le^x, fucosyl-Le^x (Le^y), and sialyl-Le^x-i were measured with the specific monoclonal antibody, FH-2, ACFH-18, AH-6 and FH-6, respectively. Among the four antigens, the incidence of sialyl-Le^x-i was the highest (ca. 30%) in the cancer sera. The incidence of sialyl-Le^x-i antigen was especially high in the sera of patients with lung adenocarcinoma (76%) while that of Le^y antigen was highest in hepatocellular carcinoma (34%). Interestingly, serum sialyl-Le^x-i antigens were highly glycosylated glycoproteins, while Le^y antigens were less glycosylated, as judged from the solubility of the antigens in 0.6 N perchloric acid. Three types of type 1 chain polylactos amine determinants; disialyl-Le^a, disialyl-Lc_4 and sialyl-Le^a were measured by monoclonal antibodies FH-7, FH-9 and N19-9 (Centcor), respectively. Among the three antigens, the incidence of sialyl-Le^a antigen in the cancer sera was the highest, especially in pancreas adenocarcinoma. The serum levels of type 1 chain polylactosamine antigens in non-malignant disorders were generally higher than those of type 2 chain antigens, suggesting that type 2 chain antigens are more specific for malignancy. For instance, the levels of disialyl-Le^a antigens were frequently high in non-malignant disorders such as kidney diseases, autoimmune diseases and liver cirrhosis. The disialyl-Le^a antigens in the sera of non-malignant disorders as well as those in malignant disorders were highly glycosylated, and their behaviors on molecular sieve chromatography and ion-exchange chromatography showed no significant difference. These results collectively indicate that the high-molecular-weight serum glycoprotein defined by anti-carbohydrate monoclonal antibodies are highly heterogenous as to their biochemical nature and have the different degrees of cancer specificity.

R 030 CANCER-ASSOCIATED CARBOHYDRATE ANTIGENS, John L. Magnani, Lab of Structural Biology, NIDDK, National Institutes of Health, Bethesda, Maryland 20892.

Many monoclonal antibodies which detect differentiation or cancer-associated antigens are directed against carbohydrates. Of about 500 monoclonal antibodies that we have obtained from different laboratories, 124 bind carbohydrates. We have determined the carbohydrate structure of the epitope for 93 of these antibodies. Most tumor markers of gastrointestinal and pancreatic cancer are sialylated oligosaccharides found in mucins in patient's sera. Some of these oligosaccharides, such as sialylated Le^a (19-9), are also found in glycolipids. Determining the structure of this particular antigen has been helpful in evaluating its genetic distribution in cancer patients and for predicting other tumor markers for gastrointestinal and pancreatic cancer. Other oligosaccharide epitopes on mucins are not found in glycolipids and must be released intact from glycoproteins to be analyzed. A new method has been developed to analyze these oligosaccharides by chromatography on thin layer plates followed by immunostaining with monoclonal antibodies after covalent attachment of the oligosaccharides to the silica gel. Using this method 10 pmol of carbohydrate antigen released from glycoproteins is detected. Many antibodies that we have analyzed bind the same oligosaccharide sequence but have different cell specificities. In some cases the antibodies bind slightly different epitopes on the same oligosaccharide, while in other cases, the antibodies have different binding affinities for the same epitope. The binding of these monoclonal antibodies to cells probably depends on the density of antigens on the cell surface, each antibody requiring a different density. Thus, cells containing antigen below a certain threshold concentration may not bind low affinity antibodies.

Therapeutic Applications

R 031 THE USE OF MONOCLONAL ANTIBODIES TO CARBOHYDRATE ANTIGENS IN AUTOLOGOUS BONE MARROW TRANSPLANTATION, Edward D. Ball, Departments of Medicine and Microbiology, Dartmouth-Hitchock Medical Center, Hanover, NH 03756. Normal myeloid cells (granulocytes, monocytes, and their precursors) express a large repertoire of surface antigens, many of which have been identified by monoclonal antibodies (MoAbs). Some of the antigens identified by MoAbs have been determined to be functional molecules such as complement receptors and Fc receptors. A large number of MoAbs reactive with normal human granulocytes have been found to react with the carbohydrate lacto-n-fucopentaose III hapten have been produced, all but one of which are III (LNF-III) which is expressed on both lipid and protein cell surface molecules. Many MoAbs to the LNF-III hapten have been produced, all but one of which are IgM MoAbs. Another highly immunogenic molecule on the surface of monocytes is a gp-55 defined by several MoAbs including AML-2-23 and MY-4. Myeloid leukemia cells express the same surface antigens as their normal counterparts and in a similar developmental program. The LNF-III hapten can be found on cells from 95% of patients using PM-81 MoAb. The gp-55 can be found on up to 70% of myeloid leukemia cells using the AML-2-23 MoAb. Studies employing complement ($C^{'}$) dependent lysis have shoen that leukemic progenitor cells from 75% of patients express the LNF-III-hapten and 40% express the gp-55 molecule. Like normal myeloid progenitor cells, leukemic progenitor cells have a slightly less mature phenotype than the circulating blast cell population. Leukemic cells and their progenitors can be lysed with these MoAbs in the presence of rabbit $C^{'}$. Studies using the HL-60 promyelocytic cell line have revealed that a 5 log reduction in leukemic cells admixed with normal marrow can be achieved with two separate one hour treatments with the MoAbs and complement. Recent clinical studies have taken advantage of the ability of MoAbs to eliminate leukemic progenitor cells from marrow samples from patients with acute myelogenous leukemia (AML). A study underway at the Dartmouth-Hitchcock Medical Center (DHMC) and the Scripps Clinic employs the PM-81 and AML-2-23 MoAbs to treat marrow obtained from patients with AML in remission, a time when leukemic cells are present but in low numbers, for the purpose of autologous bone marrow transplantation (ABMT). Nineteen patients have been treated in this manner (14 at DHMC, 5 at Scripps). Regeneration of the MoAb-treated marrow has been satisfactory. From the group of patients transplanted in first - third complete remission (N=14), 9 of 14 are surviving with a median followup of 10 months. The limitations of ABMT are the 1) failure to eradicate leukemic cells in vivo; and 2) the failure of the purging method to eliminate leukemic cells in vitro. Current studies are focused on means of improving both modalities. Since neuraminidase treatment of leukemic cells results in increased exposure of the LNF-III-hapten for MoAb binding, we are studying the safety and efficacy of treating remission bone marrow with neuraminidase prior to MoAb treatment. Other studies are focused on the development of immunotoxins, conjugates of MoAbs and plant or bacterial toxins. A ricin AML-2-23 conjugate has been developed which kills 90-100% of clonogenic leukemic cells and only kills 30% of colony-forming unit granulocyte-monocyte. Approaches to the eradication of leukemic cells in vivo include improved chemoradiotherapeutic modalities as well as exogenous administration of immunotoxins or heteroantibodies. Thus bythese manipulations it may be possible to improve the efficiency of leukemic cell killing and improve upon the results of ABMT in patients with AML.

R 032 *MONOCLONAL ANTIBODY TARGETING OF LYMPHOKINE-ACTIVATED KILLER CELLS FOR THE* TREATMENT OF HUMAN MALIGNANCIES. C. J. Honsik and R. A. Reisfeld, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Monoclonal antibodies (Mab) 14.18 (IgG3) and 11C64 (IgG3) directed against disialogangliosides GD2 and GD3, respectively, when used in conjunction with human peripheral blood mononuclear cells (PBMCs) stimulated with human recombinant IL-2 (rIL-2) lyse human melanoma, small cell lung carcinoma, and neuroblastoma cells by antibody-dependent cellular cytoxicity (ADCC). Such Mab "armed" effector cells are specifically directed to targets expressing the given disialoganglioside without detectable cross-reactivity. In addition, ADCC as well as the natural killing ability of human PBMC's is augmented by a brief co-incubation with recombinant IL-2 (rIL-2). PBMCs augmented by rIL-2 and "armed" with Mab elicited a significant suppression of tumor growth in the xenotransplant nude mouse model. Our results suggest that once a threshold level of activation of PBMC's is achieved, additional rIL-2 does not significantly enhance cytolytic augmentation over a 3 log range of increasing rIL-2 concentrations. Furthermore, anti-GD3 Mab 11C64 together with rIL-2-stimulated PBMCs from melanoma patients with widely differing tumor burden effectively lyse melanoma tumor targets in ADCC. Our results also suggest that GD2 and GD3 represent distinct and relevant immunotherapeutic target structures on melanoma whereas GD2 does the same for neuroblastoma and some small cell lung carcinoma cell lines. Taken together, our data suggest that targeting of activated human effector cells may provide a new and effective cancer immunotherapy protocol.

R 033 GD2 GANGLIOSIDE AS MELANOMA ASSOCIATED ANTIGEN AND MELANOMA PROGRESSION MARKER, Jan Thurin, Magdalena Blaszczyk-Thurin, Meenhard Herlyn, David Elder*, DuPont Guerry III*, Alan E. Lichtin*, Wallace H. Clark Jr*, Dorothee Herlyn, Zenon Steplewski and Hilary Koprowski, The Wistar Institute, Philadelphia, PA 19104 and *The Pigmented Lesion Study Group, University of Pennsylvania, Philadelphia, PA 19104. A monoclonal antibody, ME 361, detecting GD2 and GD3 gangliosides (GD3 to a lesser extent) was produced and characterized for use as a therapeutic reagent in human malignant melanoma. The detailed binding specificity was characterized on purified and structurally characterized gangliosides. The antigens were found in all melanoma cell lines studied so far (=20 lines) and bound to melanoma cells in FACS analysis, correlating with the amount of antigen extractable from the cell lines. The antibody mediates cytoxicity in ADCC and CDC using melanoma cells and human effector cells. The current status of the clinical trials performed with the IgG2a switch variant of the ME 361 will be discussed. The antibody was also used to study the expression of the GD2 and GD3 antigens in melanoma progression. Data will be presented that demonstrates the distinct appearance of the N-acetylgalactosaminyl transferase activity (necessary for the GD2 synthesis from its precursor GD3), which correlates with the onset of competence for metastasis and increased growth rate in melanoma progression and that occur at the transition between the radial growth phase and vertical growth phase of this disease.

Carbohydrate Structure

R 100 MOLECULAR BASIS OF DIFFERENTIAL SBA LECTIN BINDING IN METASTATIC ESB CELLS AND A PLASTIC-ADHESIVE LOW-METASTATIC VARIANT, Peter Altevogt, Elke Lang, Volker Schirrmacher, German Cancer Research Center, D-6900 Heidelberg FRG.
Cell surface carbohydrates have been proposed to play an important role in tumor metastasis. By selection for plastic adhesiveness we have established a variant tumor line (ESb-MP) from the metastatic murine lymphoma ESb. In contrast to the parental line, the variant is i) significantly decreased in malignancy, ii) grows as a primary tumor with late onset of metastasis, iii) is altered in cell surface carbohydrates as indicated by its binding to FITC-conjugated SBA lectin. Here we show that the major SBA-binding glycoprotein is the T200 antigen. In ESb cells, T200 antigens do bind SBA only after Neuraminidase treatment. Enzymatic studies suggested however, that glycans detected by the lectin with or without Neuraminidase treatment are different. Studies on the biosynthesis of T200 antigens showed that the antigens expressed on both type of cells differ in expression of O-linked glycans that can be recognized by SBA. The additional O-linked sugars on ESb-MP T200 molecules seem to be expressed after trimming of a second T200 precursor chain that can be detected by pulse labelling in ESb-MP cells but not in ESb cells. Our results suggest that altered glycosylation may require altered protein forms that could be created post transcriptionally. Recent data on molecular cloning of the T200 antigen support this possibility (1). The role of subtle glycosylation differences for cell behaviour are discussed.
1. Saga et al., PNAS 83,6940, 1986.

R 101 BIOSYNTHESIS OF THE P1 AND P^k BLOOD GROUP ANTIGENS IN HUMAN KIDNEY.
P. Bailly+, F. Piller+, B. K. Gillard*, D M. Marcus* and J.P. Cartron+. + INSERM U76, CNTS-Institut, 75015 Paris, France. * Baylor College of Medecine, Houston TX 77030.
The P^k and the P1 antigens are glycolipids which carry both the Galα(1-4Gal determinant on lactosylceramide (LacCer) and on lactoneotetraosylceramide (PG), respectively. From studies on individuals lacking either one or both of the antigens, two biosynthetic models have been proposed : (A) two different α(1-4 galactosyltransferases are responsible for the synthesis of the two antigens or (B) an unidentified regulatory protein modifies the acceptor substrate specificity of one single enzyme (1). Human kidney is known to contain both the P^k as well as the P1 antigen and has been found to express high levels of α(1-4 galactosyltransferase activity (2). Microsomal preparations from kidneys with P1 phenotype are able to transfer galactose in α(1-4 linkage to LacCer as well as to PG, thus forming the P^k and the P1 antigenic structures. The 1-4 linkage has been established by methylation analysis of the products and the anomeric linkage was determined by digestion with specific galactosidases. Furthermore, the biosynthesis of the P1 antigen was confirmed by immunological characterization of the glycolipid product using a murine monoclonal anti-P1 antibody (3). In addition, the relationship between the α-galactosyltransferase and the P1 blood group system was established. Kidneys typed P2 contain transferase activity capable to transfer galactose in α(1-4 to LacCer only but not to PG. This report is the first evidence for the in vitro biosynthesis of the P1 blood group antigen and work is in progess to determine whether one or two enzymes are responsible for the synthesis of the P^k and the P1 antigenic structures.
1)Marcus, D. et al. (1981) Sem. Hematol. 18, 63-71. 2)Bailly, P. et al. (1986) Biochem. Biophys. Res. Commun.,in press. 3)Bailly, P. et al. (1986) Mol. Immunol.,in press.

R 102 MATURATION OF THE CELL SURFACE SIALOMUCIN (ASGP-1) OF 13762 ASCITES TUMOR CELLS. K.L. Carraway, J. Spielman and S.R. Hull, Univ. of Miami School of Medicine, Miami, FL 33101.
Maturation of ASGP-1 involves O-glycosylation and subsequent sulfation of a polypeptide of approx. 190Kd, to produce a sialomucin whose molecular weight exceeds 500Kd. Previous studies have shown that newly-synthesised ASGP-1 requires a minimum of 70-80 min to reach the cell surface. The half time for appearance at the cell surface is >4hr, suggesting that most of the molecules reside for some time within a subcellular compartment(s) before being transported to the plasma membrane. O-glycosylation occurs throughout this time. To investigate events involved in maturation, cells were pulse-labeled for 5 min with thr and $glcNH_2$, and the ASGP-1 PNA(peanut lectin)-precipitated at intervals during a chase. Chromatography on Sepharose CL 2B indicated that 80% of ASGP-1 approached mature size within 90 min. Comparison of immature ASGP-1 with desialylated ASGP-1 revealed that most of the size change seen during maturation resulted from sialylation. Oligosaccharide analysis confirmed the presence of sialic acid in the mature form, whereas immature ASGP-1 contained predominantly non-sialylated tri- and tetrasaccharides. Monensin blocked maturation of the ASGP-1 polypeptide, and inhibited transport to the cell surface by 70%. Maturation was also inhibited by CCCP, an uncoupler of oxidative phosphorylation.

R 103 HUMAN NATURAL ANTI-α-GALACTOSYL IgG ANTIBODY REDUCES THE METASTATIC POTENTIAL OF MURINE MO4 TUMOUR CELLS. V. Castronovo, J.M. Foidart, M. Li Vecchi, J.B. Foidart, M. Bracke, M. Mareel, & P. Mahieu. Universities of Liege and Ghent (Belgium).
Anti-α-galactosyl antibody (a-Gal Ab), a natural IgG antibody present in high titer in all normal human sera and specifically recognizing Gal α (1→3) structures, has been implicated in the removal from circulation of human red blood cells (RBC) exposing usually cryptic α-D-galactopyranosyl (α-D-Galp) residues. There are several lines of evidence that the metastatic potential of murine tumour cells is strongly correlated with the expression of α-D-Galp residues on their surface. We have therefore conducted a study to determine whether the metastatic potential of MO4 cells, a highly malignant murine fibrosarcoma cell line, is reduced in syngenic C3H-HE mice by a-Gal Ab. The results indicate that : (1)the survival rate of mice is considerably higher (24/30) in the group receiving MO4 cells first incubated with a-Gal Ab than in the group receiving MO4 cells only (6/30); (2)the pulmonary surface occupied by MO4 colonies is also much higher (± 36%) in this latter group than in the former (± 10%); (3)on the contrary, the surface of metastases in the other organs examined (spleen, liver, kidneys) represents only 1% in both groups of mice. The observed protective effect on lung metastasis formation is due to a binding of a-Gal Ab to α-D-Galp end groups of MO4 cells, inducing both an increase in their sequestration into the liver and the spleen and a decrease in their attachment capacity to laminin. Since Gal α (1→3) structures can become prominent on some human adenocarcinoma cells^{R}, as on human senescent, thalassemic and sickle RBC, it is tempting to speculate that natural a-Gal Ab may also have a protective effect on metastasis formation by malignant cells bearing α-D-Galp end groups in human.

R 104 A MAJOR CELL SURFACE GLYCOCONJUGATE OF CULTURED TRACHEAL EPITHELIAL CELLS CARRIES SULFATED GLYCANS OF THE POLY(N-ACETYL) TYPE, L. S. Forsberg, S. Varsano, I. Iwamoto and J. A. Nadel, CVRI, Univ. of California, San Francisco, CA 94143.
Canine tracheal epithelial cells in primary culture display a prominent cell surface glycocalyx, extending 200-300 nm from the bilayer. Glycoconjugate components are shed continuously, suggesting a role in mucus secretion and/or bacterial adherence. A major component is a trypsin-accessible cell surface mucin containing O-glycosidically linked glycans of the poly(N-acetyllactosamine) type. The glycans are novel in that they are partially sulfated. For structural studies, cells were labeled with $[^{35}S]SO_4$ and $[^{3}H]$glucosamine for 20 h. Cell surface glycoconjugates released by trypsin yielded a major fraction at the void volume ($Mr > 10^6$) on Sepharose Cl-4B. Over 70% of both radiolabels can be degraded to low Mr components by endo-β-galactosidase (E. freundii), and to a lesser extent by keratanase. Chromatography of the Vo fraction on DEAE-Sephacel in 8 M urea/0.5% Triton yielded a single peak eluting at 0.26 M NaCl, indicating a charge density less than corneal keratan sulfate (0.43 M NaCl). Digestion of the Vo fraction with Pronase gave a major fraction of 200-300 kDa on Cl-4B, typical of the protease-resistant glycosylated domains of a mucin like glycoprotein. Alkaline-borohydride treatment, endo-β-galactosidase digestion, TLC and Sephacryl S-200 analysis show that the lactosamine glycans have Mr 7,000 and contain the sequence GlcNAc-6-SO4-β1-3Gal as a major constituent. Immunofluorescence studies with FITC-lectins show that pokeweed mitogen and Datura stramonium lectin, both specific for lactosamine sequences, bind intensely to the cell surface. Other components of the epithelial cell glycocalyx include a chondroitin sulfate proteoglycan and hyaluronic acid.

R 105 ISOLATION OF THE CONCANAVALIN-BINDING COMPONENT OF AN ASCITES TUMOR CELL SURFACE SIALOMUCIN COMPLEX. S.R. Hull and K.L. Carraway, Univ. of Miami School of Medicine, Miami, FL 33101.
The 13762 rat mammary adenocarcarcinoma ascites tumor contains a cell surface complex of a sialomucin (ASGP-1) and a Concanavalin A-binding component (ASGP-2) which comprises about 1% of the total cell protein. This complex has been purified by CsCl density gradient centrifugation of Triton X-100 extracts of microvilli isolated from the 13762 cells. Purification of the ASGP-1 and ASGP-2 from isolated complex is achieved by gel filtration in guanidine hydrochloride on Sepharose 2B. ASGP-2 represents at least 0.2% of the ascites cell protein. Carbohydrate analysis shows the presence of mannose, galactose and glucosamine, indicating the presence of N-linked, but not O-linked, oligosaccharides. ASGP-1 has been postulated to mask histocompatibility antigens at the surfaces of metastatic tumor cells. We suggest that ASGP-2 may play a role in the expression of the ASGP-1 at the ascites tumor cell surface.

R 106 USE OF A CHEMICALLY-SYNTHESIZED GLYCOLIPID AS AN IMMUNOGEN TO OBTAIN MONOCLONAL ANTIBODIES DIRECTED TO CANCER-ASSOCIATED CARBOHYDRATE ANTIGENS. Reiji Kannagi, Katsuyoshi Shigeta, Yukishige Ito, Tomoya Ogawa, and Sen-itiroh Hakomori. (Kyoto University, RIKEN, Fred Hutchinson Cancer Research Center)

Many monoclonal antibodies raised against human cancer cells are shown to recognize antigens carried by glycolipids. More recently, purified cancer-associated glycolipids are directly used for immunization of mice, and several useful monoclonal antibodies have been established. This new protocol provides a more effective way to obtain antibodies directed to cancer-associated glycolipids. However, cancer-associated glycolipids are very minor membrane components, and sometimes it is preferrable to obtain the glycolipid antigens through chemical synthesis. We developed a method to obtain the cancer-associated lactoganglioseries glycolipid having the following carbohydrate structure: GalNAcβ1→4(GlcNAcβ1→3)Galβ1→4Glcβ1→1Cer by chemical synthesis using lactose unit, ceramide unit and hexosamine donors as synthons and made the synthetic glycolipid available as an immunogen. The two monoclonal antibodies we obtained (YI328-18 and YI328-51, both IgG_3) specifically recognized the abnormal branching structure. These results indicate that the application of synthetic glycolipids as immunogens enables to raise specific monoclonal antibodies directed to cancer-associated glycolipids which are very minor components.

R 107 SYNTHETIC APPROACHES TO NEOANTIGENS BASED ON CORE OLIGOSACCHARIDE STRUCTURE OF N-LINKED GLYCOPROTEINS, J.J. Krepinsky[1,2,3], H.H. Lee[1], G.R. Perdomo[1] and J.F. Harris[4], [1]Ludwig Institute for Cancer Research, Toronto, Ontario; Departments of [2]Medical Genetics and [3]Medical Biophysics, University of Toronto; and [4]Regional Cancer Centre, London, Ontario. Several oligosaccharide structures appear in malignant tissues while they are absent from that tissue in healthy state. Availability of such a structure in an antigenic form could provide an immunogen giving rise to antibodies recognizing specifically the structure. The antibodies could be used for diagnostic and other purposes. One of such structures is bisected core oligosaccharide of N-lined glycoproteins; it has been synthesized in two parts, and one of them was linked to BSA via a nonpolar linker or through Asn to a polyacrylamide molecule. The polymeric carrier is investigated since BSA apparently interacts with the carbohydrate to produce antibodies not defined exclusively by the carbohydrates.

R 108 MODULATION OF THE INTERACTIONS BETWEEN IgG AND Fc-RECEPTORS OF HUMAN MONOCYTES BY THE CARBOHYDRATES OF Fc DOMAIN, Michel G. Malaise, Paul Franchimont and Philippe R. Mahieu, State University of Liege, Liege, Belgium.

The Fc-receptors (Fc-R) of monocytes (MC) are capable of mediating different events upon interaction with IgG molecules, mainly phagocytosis and ADCC. In order to determine whether the first step of this interaction, i.e. the receptor-ligand binding, is modulated by the carbohydrates of Fc domain, we have first set up a solid phase RIA for direct measurement of the binding capacity of human IgG to peanut agglutinin (PNA), to concanavalin A (Con A) and to pokeweed mitogen (PWM) which mainly bind to β-galactose, alpha-mannose and dimers of N-acetyl-β-glucosamine respectively. The mean specific binding ($\pm$ 1 SD) of the 96 normal IgG tested to PNA (6.7% $\pm$ 7) and toPWM (5.2 $\pm$ 5) was statistically higher ($p < 0.001$) than that to Con A (2.5 $\pm$ 2.4), whereas no significant differences were observed between the mean specific bindings to PNA and to PWM. The ability of those IgG to inhibit the Fc-R was then measured by a classical rosette assay. The inhibitory capacity of IgG was inversely correlated to their binding ratios to PNA and Con A only. By affinity chromatography, three groups of IgG were separated: the IgG purified on Agarose-PNA columns slightly reduced the Fc-R function (40-45% inhibition); the IgG purified on Sepharose-Con A columns exhibited the highest inhibitory properties (85-85% inhibition); the IgG that did not bind to PNA- and Con A-columns possessed intermediate inhibitory properties (65-70% inhibition). Incubation of MC with D-galactose (10mM) significantly improved their capacity ot form IgG-rosettes, whereas their incubation with D-mannose (10 mM) significantly reduced the Fc-R function. Scatchard plots of ^{125}I-IgG1 myeloma protein binding to MC were linear in basal conditions, as well as after a prior incubation of MC with D-galactose or D-mannose. MC bound about 16,000 molecules of IgG1 per cell in each instance. In contrast, the mean association constant (Ka) for IgG1 binding was $2.59 \pm 0.50 \times 10^8$ M^{-1} in basal conditions, $4.4 \pm 0.75 \times 10^8$ M^{-1} after D-galactose incubation, and $1.35 \pm 0.50 \times 10^8$ M^{-1} after D-mannose incubation. These data show that the interaction between human IgG and Fc-R depends, at least in part, on the presence of "accessible" galactosyl or mannosyl residues in the Fc domain, and that the modulation of the Fc-R function induced by these carbohydrates is due to a change in the affinity rather than in the number of a single class of high affinity binding sites.

R 109 A CARBOHYDRATE, EPITOPE ASSOCIATED WITH HUMAN SQUAMOUS LUNG CANCER.
S. Mårtensson[1], C. Due, B. Nilsson, H. Eriksson, L. Olsson[2] and A. Lundblad[1]

1Department of Clinical Chemistry, University Hospital, S-220 07 LUND, Sweden. 2. Cancer Biology Laboratory, State University Hospital, DK-2100 Copenhagen, Denmark.
An epitope identified by a monoclonal antibody (designated 43-9F) and with high specificity for human squamous lung carcinoma (SLC) has previously been described (ref.). The expression of the 43-9F epitope on SLC cells is associated with high proliferative activity and ability of SLC cells to grow as tumours in nude mice. The antibody has also been shown to detect the epitope in serum from patients with SLC, and thus may be useful as a serum marker for this disease. The epitope is present in SLC cells in glycoproteins and is also released into the circulation. In order to characterize the epitope further, glycolipids were extracted from SLC cells. The neutral glycolipids were separated on TLC plates and overlayed with the 43-9F antibody. Detection was carried out with an ELISA technique. The staining revealed a major band in the pentaglycolipid region. The pentaglycolipid was isolated and further characterized by FAB-MS. The structure was established as Galβ1-3(Fuc α1-4)GlcNAc β1-3Galβ1-4Glc-Cer.
Two monoclonal antibodies, Le^a-01 (BioCarb AB, Lund, Sweden) and CO-514 (ATCC, Rockville, Md., USA) with known specificity for the Le^a structure gave the same pattern as the 43-9F antibody when used in the overlay assay described. 43-9F, Le^a-01 and CO-514 were also used in a cell binding assay and for immunoblotting of glycoproteins from SLC cells separated by SDS-PAGE. In these assays the antibodies had different properties indicating that the 43-9F antibody recognized an additional structure, not seen by Le^a-01 and CO-514.
Ref: Pettijohn, D. et al (1986) Cancer Res. in press.

R 110 INHIBITION OF METASTATIC POTENTIAL BY FUCOSIDASE: AN NMR STUDY IDENTIFIES FUCOGANGLIOSIDE AS A CELL SURFACE METASTASIS MARKER
Carolyn E. Mountford, Lesley C. Wright, George L. May, Kerry T. Holmes and Marlen Dyne. Ludwig Institute for Cancer Research (Sydney Branch), Blackburn Building, University of Sydney, N.S.W. 2006, Australia.

NMR spectroscopy is able to detect subtle changes to the surface chemistry of cells. We have previously shown that high resolution ^1H NMR methods can identify tumour cells with the capacity to metastasise, and now report that the long T_2 relaxation value (500 - 800 ms) observed in metastatic rat mammary adenocarcinoma cells is removed by treatment with fucosidase. 2D scalar correlated NMR (COSY) spectra of fucosidase treated cells show that a cross peak, consistent with scalar coupling between the methyl and methine groups on fucose and usually associated with malignancy and metastatic ability, is absent. No metastases were observed in eight out of ten rats injected subcutaneously with enzyme treated cells compared to an eighty percent rate of metastasis with untreated cells. NMR studies on isolated cellular lipids identify the long T_2 relaxation value only in the ganglioside fraction. In addition 60% of ^{14}C labelled fucose is incorporated into cellular lipid, over 85% of which is found in the ganglioside fraction. We propose that fucogangliosides are an indicator of metastatic potential in rats and may be involved in immunosuppression. The observation that a cell surface metastasis marker has an NMR signal with a characteristically long relaxation value has important consequences for the future use of magnetic resonance imaging and spectroscopy in the cancer clinic.

R 111 **IDENTIFICATION OF A GLYCOLIPID ANTIGEN PRESENT ON RAT ACUTE MYELOCYTIC LEUKEMIA CELLS**, Luann M. Pendy, Eric G. Bremer, and Herbert Kaizer, Rush University, Chicago, IL 60612.

An IgM monoclonal antibody (RM124) has been produced that mediates suppression of rat acute myelocytic leukemia (AML) in vivo in the presence of complement. In addition to its reactivity with the AML cells, immunofluorescence studies have shown that RM124 also binds to 5% of the mature granulocytes in normal rat bone marrow. We have chosen to study the nature of the antigen to which RM124 binds to further our understanding of the effector mechanisms operating in tumor suppression. Three RM124-positive bands have been identified in the glycolipid fraction of AML cell lipid extracts by thin-layer chromatography immunostaining techniques. The major RM124-positive band has been isolated by HPLC and co-migrates with an orcinol-positive band similar in R_f to that of the H1 glycolipid from human type O erythrocytes. These data indicate the monoclonal antibody, RM124, binds to a glycolipid with 5-6 sugar residues that is present on rat AML cells. More precise molecular characterization of the antigen and its distribution in normal tissues is currently being investigated.

R 112 COMPARISON OF β-N-ACETYLGLUCOSAMINYLTRANSFERASE V ACTIVITIES IN ROUS SARCOMA VIRUS-TRANSFORMED BABY HAMSTER KIDNEY (RS-BHK) AND BHK CELLS. M. Pierce & J. Arango, Anatomy & Cell Biol, U of Miami Medical School, PO Box 016960, Miami, Fl 33101.

Recent studies have demonstrated that Rous sarcoma virus-transformed Baby Hamster Kidney (RS-BHK) cells express two-fold higher levels of the N-linked oligosaccharides that contain the sequence [GlcNAc β(1,6)Man α(1,6)] than do non-transformed BHK cells (Pierce and Arango, JBC 261, 10772, 1986). Kobata and co-workers have shown that N-acetylglucosaminyltransferase V (GnT V), the enzyme that synthesizes the [GlcNAc β(1,6) α Man] branch, exhibits a two-fold higher specific activity in Py-BHK compared to BHK cells, while other GlcNAc transferases have the same specific activity. We have investigated the activity of GnT V in RS-BHK and BHK cells using both a synthetic oligosaccharide and asialo-agalacto-fetuin triantennary glycopeptide as acceptors and UDP-[^{3}H]-GlcNAc as substrate. The radioactive products were separated by a newly-devised reverse-phase chromatographic technique. Using the synthetic substrate (GlcNAc β(1,2)-αMan(1,6)-βMan-O-$(CH_2)_8COOCH_3$), the enzymatic product was shown by Dr. O. Hindsgaul, Univ. of Alberta, to be identical to the predicted synthetic tetrasaccharide product. Formation of product was linear with time up to 6 hr and with protein concentration up to 0.40 mg per 0.020 ml assay. The specific activity of GnT V was 1.8 times greater in RS-BHK cells than BHK cells when measured under optimal assay conditions and using saturating amounts of UDP-GlcNAc and either oligosaccharide acceptor. Further studies will study the kinetic properties of GnT V from both cell types to attempt to define the mechanism(s) by which this specific increase in activity is produced by tumor virus transformation of BHK cells.

Abstract Withdrawn

R 114 RELEASE, ISOLATION AND CHARACTERIZATION OF SULFATED N-LINKED OLIGOSACCHARIDES FROM MAMMALIAN CELL LINES Linda Roux, Sylvia Holojda, Goran Sundblad, Hudson Freeze and Ajit Varki, University of California, San Diego 92093

Sulfated N-linked oligosaccharides have been considered rare, and specific structures from only a few glycoproteins have been described (including pituitary hormones, ovalbumin, and D.discoideum lysosomal enzymes). We have recently reported that such oligosaccharides are in fact widely distributed among many different types of tissue culture cell lines. We have now developed improved methods for the metabolic labelling of cell lines with $^{35}SO_4$, the release of sulfated N-linked oligosaccharides by Peptide:N-Glycosidase F (PNGaseF), and the isolation of these molecules by gel filtration. Five mammalian cell lines (CHO-K1, CHO-761, BW-5147, CPAE and K-562) were metabolically labelled with $^{35}SO_4$ in a serum-free medium with altered sulfate, cysteine and methionine concentrations. In each case, the majority of N-linked oligosaccharides were specifically released by treatment with PNGaseF. The released sulfated oligosaccharides comprised 5 - 20% of the total $^{35}SO_4$-label incorporated into macromolecules in the cell lines. In a bovine pulmonary arterial endothelial cell line, more than 50% of the total released sulfated oligosaccharides were found in the matrix and the medium. The oligosaccharides were characterized by Sephacryl S200 gel filtration, QAE-Sephadex chromatography, various enzymatic and chemical treatments, and by serial lectin affinity chromatography. The oligosaccharides were also characterized in terms of distribution of charge among sialic acid, phosphate and sulfate groups. The results indicate the existence of two broad classes of sulfated N-linked oligosaccharides. Class I oligosaccharides are sialylated, complex-type chains bearing one to four negative charges. Class II oligosaccharides are more highly charged and unsialylated.

R 115 HIGHLY METASTATIC MOUSE LYMPHOMA CELLS SECRETE AN ENDOGLYCOSIDASE WHICH SPECIFICALLY DEGRADES MATRIX PROTEOHEPARAN SULFATE, Reinhard Schwartz[1], Ruprecht Keller[2], Volker Schirrmacher[1], [1]German Cancer Research Center, D-6900 Heidelberg FRG, [2]Aachen Technical University, D-5100 Aachen FRG.
Recently it has been shown that - similar to the B16 melanoma system - a highly metastatic variant (ESb) of a low metastatic T lymphoma (Eb) degrades heparan sulfate containing material of the extracellular matrix (ECM) of vascular endothelial cells more readily than the parental cell line Eb. These results support the concept that tumor cell associated enzymes are necessary for the penetration of blood vessel walls as an early step of the metastatic process. Here we studied the substrate specificity of the endoglycosidase described for the Eb/ESb tumor system (1) in an in vitro degradation assay using purified proteoglycans. Only the basement membrane proteoheparan sulfate from HR-9 cells (HR9-PHS) could be degraded by highly metastatic ESb cells, a proteochondroitin sulfate (PCS) and a proteoheparan sulfate (HSI) from bovine aortic endothelial cells (BAEC) remained uneffected. None of the proteoglycans used was degraded by the low metastatic cell line Eb and a low metastatic plastic adherent growing variant of ESb (ESb-MP). These results demonstrate that - within the system - the activity of an endoglycosidase which specifically recognized and degrades proteoheparan sulfate of the basement membrane is correlated with the metastatic capacity of tumor cells. Together with earlier observations our results suggest that the subendothelial ECM is the main target for tumor associated proteases and endoglycosidases.
(1) Vlodasky et al., Cancer Res. 43, 2704, 1983.

R 116 β-N-ACETYLGLUCOSAMINYLTRANSFERASE V (GnT V) ACTIVITY IN HAMSTER, Mohamed Shoreibah and Michael Pierce. Dept. of Anatomy and Cell Biology, Univ. of Miami Medical School, Miami, Fl. 33101

The glycosyltransferase GnT V has been implicated as a direct cause of the increase in the tri- and tetraantennary N-linked oligosaccharrides containing the sequence [GlcNAc-β(1,6)Man-α(1,6)Man] in baby hamster kidney (BHK) cells transformed by polyoma virus and rous virus. One approach to begin to analyze the mechanism(s) by which GnT V activity is specifically increased in these cells is to purify the enzyme and prepare antibodies to it. Therefore, we have screened several hamster tissues to locate a source of the enzyme with large amounts of activity. Assays were performed using solubilized membrane pellets prepared from whole organs that had been homogenized in ice-cold 0.1 M MES buffer, pH 7.5 using a Dounce homogenizer with Teflon-coated plunger. The homogenates, which contained protease inhibitors, were sonicated and spun at low speed (300G). The supernatants were then spun at 100,000G and the resulting pellet re-sonicated. These preparations were mixed with 1.0% Triton X-100, ADP (pyrophosphatase inhibitor), β-methylGlcNAc (β-hexosaminidase inhibitor), 6.0 X 10^6 cpm of UDP-[^{3}H]-GlcNAc (10,000cpm/nmol) and 40 nmol synthetic oligosaccharide acceptor (GlcNAc-β(1,2)Man-α(1,6)Man-β-O-$(CH_2)_8COOCH_3$) in a total volume of 0.020 ml. The assays typically contained about 0.25 mg of protein and were incubated for several hours at 37° C. The radioactive products were separated from used UDP-GlcNAc and its breakdown products by chromatography on a pellicular C-18 column. Specific activity of GnT V (pmol of GlcNAc transferred/mg-hr): kidney, 212; stomach, 188; small intestine, 90; ovary, 30; trachea, 26; brain, 22; heart, 8; liver, 0; lung, 0.

R 117 O-GLYCOSYLATION OF ASGP-1, THE CELL SURFACE SIALOMUCIN OF 13762 ASCITES TUMOR CELLS. J. Spielman, S.R. Hull and K.L. Carraway, Univ. of Miami School of Medicine, Miami, FL 33101.
ASGP-1, the major cell-surface sialomucin of the 13762 ascites tumor cells, contains 60-70% carbohydrate, all of which appears to be O-linked to the polypeptide via ser and thr. Glucosamine labeling followed by trypsinization shows that ASGP-1 reaches the cell surface within 5 min of terminal glycosylation. Amino sugar analysis reveals both GlcNAc and GalNAc present at this time, but GalNAc does not reach equilibrium with GlcNAc until 20 min after labeling, suggesting that at least some GalNAc is added earlier. This delay was not due to the time required for conversion of glucosamine to UDP-GalNAc, which reaches equilibrium in less than 5 min. To determine the earliest time at which GalNAc is added to the polypeptide, cells were labeled with thr and ASGP-1 was PNA (peanut lectin)-precipitated at intervals. Oligosaccharides were eliminated by mild alkaline borohydride treatment, which simultaneously converts thr to alpha-aminobutyric acid (abu). Thus the time at which α abu can first be detected corresponds to the time at which O-glycosylation is initiated. In the case of ASGP-1, this occurs within 5 min of translation. These results show that GalNAc addition (ie initiation of O-glycosylation) is occurring throughout biosynthesis of ASGP-1.

Developmental Expression; Cell Recognition and Adhesion

R 200 COMPARATIVE ANALYSIS OF MAMMALIAN SPLEEN LECTINS, Howard J. Allen, Mathew Cywinski, Jacob Lochnar, Rebecca Palmberg, and Richard DiCioccio, Roswell Park Memorial Institute, Buffalo, New York 14263.

A role for tissue lectins in cell adhesion, development, and metastasis has been suggested by several investigators. We have isolated galactoside-inhibitable lectins from rabbit, rat, mouse, pig, lamb, calf, and human spleens. Native molecular weight, subunit structure, pI, and hemagglutinating activity have been compared. The yields of lectin varied from 1.8 mg/kg for rabbit spleen to 79 mg/kg for lamb spleen. Pig, lamb, calf, and human lectins yielded single protein peaks when subjected to Superose 12 FPLC corresponding to native MMs of 33-34 kd. In contrast, rat and mouse lectin preparations were separated into three components ranging from 8.4 kd to 34 kd. FPLC analysis of rabbit spleen lectin revealed the presence of at least six components. Gradient slab gel SDS-PAGE showed the presence of single polypeptides for pig, calf, lamb, and human lectins of MM 14.0-14.5 kd. Multiple polypeptides were detected for the mouse, rat, and rabbit lectins. The MM of the major polypeptides were 15, 15, and 17 kd for rat, mouse, and rabbit, respectively. The presence of isolectins in all preparations was shown by isoelectric focusing. The major isolectins were acidc proteins with pI of 4.38-4.80. Hemagglutination and hemagglutination inhibition assays demonstrated similarities as well as differences among the lectin preparations. Hemagglutinating activity could not be demonstrated in rabbit spleen extracts nor for isolated putative lectin. Immunohistochemical data suggest that, in humans, the spleen lectin is present in the intercellular matrix of spleen and lymph nodes and is present at the periphery of REC's but is not present in normal or malignant lymphocytes of these organs.

R 201 MODULATION OF TUMOR CELL SURFACE GLYCOPROTEINS AS A POSSIBLE MECHANISM OF ACTION OF RETINOIDS, Brad Amos, Dafna Lotan and Reuben Lotan, Department of Tumor Biology, The University of Texas-M. D. Anderson Hospital and Tumor Institute at Houston, Texas 77030.

Retinoids, a group of vitamin A metabolites and synthetic analogs, can inhibit tumor cell growth and influence cell differentiation. It has been suggested that retinoids exert some of their effects by inducing changes in the glycosylation of membrane glycoconjugates. We have shown that retinoic acid (RA) increases the labeling of some membrane sialogalactoglycoproteins by methods that radiolabel specifically sialic acid or galactose/galactosamine residues that are exposed on the surface of viable carcinoma, embryonal carcinoma, melanoma, sarcoma, neuroblastoma and premonocytic leukemia cells. A further analysis of some of these cells revealed that RA increases the incorporation of monosaccharides including fucose, galactose and glucosamine into the same glycoproteins that are modified on the cell surface. These changes could be explained by our finding of increased activities of several glycosyltransferases in RA-treated cells. We propose that the initial effect of RA is the induction of de novo synthesis of glycosyltransferases which then increase the glycosylation of some endogenous acceptors in the Golgi apparatus. Different acceptors were found in the different cells that we have studied. RA treatment of melanoma cells that are sensitive to the growth inhibitory actions of RA enhanced the glycosylation of a M_r 160K cell surface glycoprotein whereas no such change could be detected in mutant clones selected for resistance to RA. Likewise, RA enhanced the glycosylation of two cell surface glycoproteins (280K and 400K) on sensitive human head and neck squamous cell carcinoma but not on insensitive cells. These results suggest that RA might modulate tumor cell growth by modifying surface glycoproteins.

R 202 PLASMA MEMBRANE GLYCOPROTEINS OF A HUMAN LEUKEMIA CELL LINE (K562) AND A CON A-RESISTANT VARIANT, Louise Bernier, Stephen MacDougall, and Arthur K. Sullivan, McGill Cancer Centre, McGill University, and Division of Hematology, Royal Victoria Hospital, Montreal Quebec, CANADA H3G 1Y6.

Natural killer cells (NK), a subpopulation of large granular lymphocytes, are able to recognize and kill certain other normal and tumour targets. Although the molecular components of the reaction are not characterized, it appears that oligosaccharides may be involved at one or more steps. In studies designed to determine how a tumour cell might escape NK, we have developed NK-resistant variants of the highly sensitive human leukemia line K562. One of these (Clone I), when made resistant to the toxic effects of Con A (ConA-R1 line), once again became sensitive to NK. When compared to Clone I, ConA-R1 showed a very similar pattern of membrane glycoproteins, as determined by blotting with lectins Con A, WGA, ricin, and soybean. However, studies with Ulex suggested a dramatic increase in fucosylation of a group of glycoproteins of very diffuse mobility ranging in apparent molecular weight from 30-200 kDa by SDS-PAGE. Also noted was a loss of staining with an antibody reactive with the lactoneofucopentaose III antigen (SSEA-like). These results support the possibility that cell surface oligosaccharides are relevant to early steps in NK reaction, and that tumour cells may modulate these structures to escape recognition.

R 203 MULTIVALENT BINDING OF COMPLEX TYPE OLIGOSACCHARIDES WITH D-GALACTOSE AND L-FUCOSE SPECIFIC LECTINS, Lokesh Bhattacharyya and C. Fred Brewer, Albert Einstein College of Medicine, Bronx, NY 10461

Tri- and tetraantennary complex type oligosaccharides containing terminal galactose residues precipitate the D-galactose specific lectins from E. indica, E. arborescens, and R. communis (Agglutinin I). Quantitative precipitin analyses indicate that the triantennary oligosaccharides are trivalent for protein binding, while the tetraantennary oligosaccharide is trivalent for binding the R. communis lectin, and tetravalent for the other two lectins. Thus, in most cases, the valencies of these oligosaccharides for the lectins is related to the number of terminal galactose residues in the molecule. Biantennary complex type oligosaccharides formed only soluble complexes with the lectins. However, biantennary complex type oligosaccharides with terminal fucose residues precipitate the L-fucose specific isolectin A from L. tetragonolobus. Quantitative precipitin analyses indicate that the oligosaccharides are bivalent for the lectin. These results, together with similar findings that the D-mannose/D-glucose specific lectin concanavalin A is also precipitated by certain asparagine-linked oligosaccharides, suggest that the multivalent binding properties of these carbohydrates and lectins are related to their biological activities.

R 204 GANGLIOSIDE-MEDIATED MODULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR Eric G. Bremer, Rush University, Chicago, IL 60612.

Gangliosides G_{M3} and G_{M1}, when added to cell culture medium, can inhibit the growth of A431 and KB cells. G_{M3} has the greater inhibitory effect on cell growth and also reduces EGF-stimulated phosphorylation of the EGF receptor (Bremer, et al., J. Biol. Chem. 261:2434, 1986). Further investigation of these phenomena indicated that gangliosides had differential effects on the EGF receptor. After Triton X-100 solublization, A431 membranes bound almost twice as much ^{125}I-EGF in the presence of G_{M3} as did control or G_{M1} additions; whereas, only small differences in binding were noted on intact membranes or whole cells. G_{M3} also reduced ^{32}P incorporation into the solublized EGF receptor. EGF binding sites were found in the Triton X-100 insoluble pellet, and the amount of ^{125}I-EGF binding to the pellet was reduced in the presence of G_{M1}. The binding of ^{125}I-EGF to detergent-insoluble receptor was repeated on A431 cells grown in the presence or absence of G_{M1} or G_{M3} prior to detergent extraction. In support of the previous experiment, those cells grown in the presence of G_{M1} showed a reduction in the number of detergent-insoluble EGF binding sites. In addition, G_{M1} reduced EGF-stimulated phosphorylation of detergent-insoluble material. These results imply that gangliosides may alter association of EGF receptors with cytoskeletal or matrix components. Association of EGF receptors with cytoskeletal elements which mediate aggregation and internalization has previously been suggested (Landreth, et al., J. Cell. Biol. 101:1341, 1985). The ability of G_{M1} or G_{M3} to alter this association may provide a clue to the mechanism of cell growth inhibition by these gangliosides.

R 205 BIVALENT BINDING OF ASPARAGINE-LINKED GLYCOPEPTIDES AND OLIGOSACCHARIDES WITH CONCANAVALIN A, C. Fred Brewer and Lokesh Bhattacharyya, Albert Einstein College of Medicine, Bronx, NY 10461.

We have previously reported that a high mannose type glycopeptide is capable of precipitating the jack bean lectin Concanavalin A (Con A) [L. Bhattacharyya and C.F. Brewer (1986) Biochem. Biophys. Res. Commun. 137, 670]. Using quantitative precipitin analyses and NMR techniques, we demonstrated that the glycopeptide possessed two Con A binding sites: one site on the $\alpha(1\text{-}6)$ arm and the other site on the $\alpha(1\text{-}3)$ arm of the core. Several other high mannose type glycopeptides have also been recently shown to be bivalent. In addition, certain bisected hybrid and bisected biantennary complex type glycopeptides and oligosaccharides possess two Con A binding sites and can precipitate the protein. However, the corresponding nonbisected complex analogs demonstrate only univalent interactions. In the presence of two different bivalent glycopeptides, Con A selectively precipitates each, indicating preferential crosslinking of the glycopeptides. These results have important implications for the binding interactions of the lectin with asparagine-linked carbohydrate receptors on the surface of cells.

R 206 CLUSTERING OF GANGLIOSIDES AT A MEMBRANE-MEMBRANE JUNCTION MEDIATES ADHESION AND A JUNCTIONAL INCREASE IN CONDUCTANCE, Gregory J. Brewer, Southern Il. Univ. Sch. of Med., Springfield, IL 62708.

Oncogenic changes in cell surface gangliosides suggest a possible role in modulating adhesion. A model membrane system was developed to study the function of isolated cell surface gangliosides. This novel system consists of two large spherical bilayer membranes attached to the tips of two syringes filled with physiological electrolyte. When membranes are made from 1% (w/v) egg phosphatidylcholine, 0.1% (w/v) beef brain gangliosides in n-decane, their apposition results in adhesion, and a three-fold increase in transjunctional electrical conductance (BBA 776:279). If gangliosides mediate the adhesion and conductance functions, they should be aggregated in the junctional region. The restricted motion expected from this aggregation was measured by fluorescence anisotropy. Membranes were made with 4 mole% gangliosides and 0.4 mole% of a synthesized fluorescent parinaryl derivative of GD1a. These membranes showed increased anisotropy in the junction and increased fluorescence in the junction. These results suggest considerable immobilization of gangliosides at an adhesive junction compared to relatively free mobility in non-adhering regions. Another consequence of aggregation would be increased surface charge density due to the negatively charged gangliosides. This was monitored with the K^+-ionophore nonactin which forms conducting carriers in proporation to the negative surface charge density. Measurements of junctional conductance were 70-80% higher than the conductance of non-junctional membranes made from 4 mole% gangliosides. These values suggest a three-fold higher concentration of gangliosides in the junctional region. (Supported by NIH Grant CA34145.)

R 207 LOCALIZATION OF GLYCOLIPIDS IN MDCK CELLS, Cecile M.B. Butor and Jean Davoust, EMBL, postfach 102209, 6900 Heidelberg, FRG.

The polarity of epithelial cells is associated with a difference in protein and lipid composition of the apical and basolateral domains of the cell plasma membrane. The two plasma membrane domains are separated by a junctional complex and constant sorting of membrane proteins occurs during exocytosis and transcytosis. We are interested in the distribution and the dynamics of glycolipids. Our model system is a polarized kidney cell line, MDCK. The glycolipid composition of two strains of this line is known (Hansson et al., 1986, EMBO J.,5 483.489). By immunolabelling of whole cells and of frozen sections we show a preferential apical localization for Forssman antigen, the galactocerebroside and the sulfatide in MDCK II. The glycolipids present on the apical domain can be patched by two layers of antibodies and are presumably mobile in the plane of the membrane. An internal glycolipid pool is also observed on semi-thin frozen section.

R 208 RETINOID TREATMENT IN VITRO REVERSIBLY MODULATES TUMOR CELL GLCOCONJUGATE EXPRESSION AND METASTATIC CAPACITY, John S. Coon, Marion J. Couch, Bendicht U. Pauli and Ronald S. Weinstein, Rush Medical College, Chicago, IL 60612.

KLN 205 murine squamous carcinoma cells were grown in medium supplemented with the retinoid 13-cis retinoic acid (RA) to study the relationship between RA-induced cell surface changes and alterations of the metastatic phenotype. Modulation of the cell surface glycoconjugate expression was measured by flow cytometric analysis of the RA-treated tumor cells stained with fluoresceinated lectins. RA treatment (5×10^{-6} M and 5×10^{-7} M) altered the glycoconjugate expression of KLN 205 cells in a selective, dose-dependent fashion. Tumor cells grown in RA-supplemented medium for more than 4 days demonstrated greatly increased binding of fluoresceinated Griffonia simplicifolia I lectin, peanut lectin, wheat germ lectin, Concanavalin A, and soybean lectin ($p < 0.001$), but the increased binding of Ulex europaeus lectin was of a much smaller magnitude ($p = 0.02$). After 15 days of growth in these non-cytotoxic or cytostatic concentrations of RA, malignant KLN 205 cells had a greatly decreased proclivity to metastasize as measured by the lung colony assay ($p = 0.0003$). Tumor cells grown in RA-supplemented medium (5×10^{-6} M, 15 days) and then transferred to control medium 3 days prior to injection into mice demonstrated much greater experimental metastatic behavior than RA-treated cells which were never transferred. Their ability to form lung colonies approached that of tumor cells that received no RA treatment. The recovery of metastatic behavior coincided in time with reversion of glycoconjugate expression in cells recovering from RA treatment, lending additional support for a mechanistic relationship between modulation of glycoconjugate expression and metastatic phenotype by RA. (Supported by NIH Grants CA 41040 and the Rayman Research Fund)

R 209 STUDIES ON SURFACE GLYCOPROTEINS OF SOME PROSTATE CANCER CELL LINES.

Björn Forsgren, Kerstin Andersson, Anita Billström, Elisabeth Kruse, Eva Sundberg and *Bo Ersson, AB LEO Res.Labs., Box 941, S-251 09 Helsingborg and *Biochemical Separation Center, BMC, Box 577, S-751 23 Uppsala, Sweden.

In our research on the prostate and prostate cancer we are studying the characteristics of some cell lines derived from primary and metastatic human and rat prostate tumors (1013L, DU 145, PC 3, LNCaP, Dunning G). As part of this work the binding by the cells of lectins with different carbohydrate specificity is examined. Two methods are used, one involving quantitative determination of ^{51}Cr-labeled cells attached to lectin-coated culture plates in absence or presence of various carbohydrates, the other quantitative determination by fluorometry of cell-bound fluorescein-labeled lectins after recovery by means of specific carbohydrates. Binding profiles of intact and neuraminidase-treated cells of different origin and with different growth properties will be presented.

R 210 EPIGENETIC CONTROL OF CARBOHYDRATE EXPRESSION BY EMBRYONAL CARCINOMA CELLS, Raymond J. Ivatt, Peter B. Harnett, and John W. Reeder, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Embryonal carcinoma and early embryonic cells express unusually large and complex carbohydrates. These glycans have been implicated in cellular adhesion and are lost in a programmed manner during differentiation. In addition to these characteristic glycans, embryonal carcinomas express a variety of glycans in common with teratocarcinoma-derived endodermal cells. We have found that these two classes of glycan are regulated independently. Developmentally regulated glycans are regulated by extrinsic signals; their expression is conserved during the cell cycle but modulated by cellular contacts. These glycans are localized to cell contacts and may be involved in intercellular functions. In contrast, the glycans common to both stem and endodermal cells are regulated by intrinsic signals. Their expression shows large, periodic changes during the cell cycle but is not altered by cell contacts. These glycans have a diffuse distribution across the cell surface and may be associated with household functions.

In addition to genetically programmed differences, tumor stem cells can be extremely heterogeneous in their carbohydrate expression and have rapidly changing phenotypes as a consequence of these epigenetic mechanisms.

This work was supported by grants CA-42650 from the National Cancer Institute and 1-972 from the March of Dimes Birth Defects Foundation.

R 211 SHEDDING OF EPITHELIAL CELL SURFACE PROTEOGLYCAN: A MECHANISM TO ALTER THE ASSOCIATION OF CELLS WITH THE MATRIX Markku Jalkanen*, Scott Saunders** and Merton Bernfield**, Turku University*, SF-20520 Turku, Finland and Stanford University**, Stanford, California 94305.

Mouse mammary epithelial (NMuMG) cells bear a cell surface proteoglycan (PG), that consists of a lipophilic membrane-associated domain and an ectodomain containing both heparan sulfate and chondroitin sulfate glycosaminoglycan chains. The ectodomain binds with high affinity to Types I, III and V fibrillar collagens and the C-terminal heparin-binding domain of fibronectin. Using the monoclonal antibody 281-2 directed against the PG core protein, confluent NMuMG monolayers show the cell surface PG solely on basolateral cell surfaces, co-localizing near the substratum with actin-rich filament bundles. At tissues, the 281-2 antibody localizes the PG to the basolateral surfaces of simple cuboidal and columnar epithelia cells and to the entire surfaces of stratified epithelia cells. Thus the cell surface PG may play an important role in the adhesion of epithelial cells to each others and to interstitial matrix. Expression of the PG may also be developmentally regulated because various terminally differentiated epithelial cells, e.g. apical cells of bladder, mucifed cells of vagina, acinar cells of several secretory organs, show no stain with 281-2. Culture medium conditioned by growing NMuMG cells contains a 281-2 reactive PG. This immunoreactive medium PG is indistinquishable from the intact ectodomain that is released from the cells by mild trypsin treatment: both contain heparan sulfate and chondroitin sulfate attached to a 53-kDa non-lipophilic core protein and a serum antibody directed against the medium PG recognizes the cell surface PG. The medium PG is derived from the cell surface PG by cleavage of its ectodomain from its lipophilic membrane-associated domain. This shedding of the ectodoamin is from the apical cell surface in subconfluent monolayers and is markedly enhanced by suspending the cells, which causes them to round up. Suspended cells lose their entire complement of ectodomain within 80 min and, although viable, fail to replace the PG. Because of the binding properties of the ectodomain, this shedding may provide a means by which epithelial cells loosen their association with the matrix and with other cells during normal epithelial development and the invasion of carcinomas.

R 212 BIOCHEMICAL PROPERTIES OF HUMAN LYMPHOCYTE HOMING RECEPTORS, Sirpa Jalkanen*, Markku Jalkanen*, Robert Bargatze** and Eugene Butcher**. Turku University*, SF-20520 Turku, Finland and Stanford University**, Stanford, CA 94305.
Lymphocyte-high endothelial venule (HEV) cell interaction that is mediated by specific receptors, is of critical importance during normal and malignant lymphocyte migration. Human lymphocyte homing receptors for peripheral lymph node HEV, mucosal HEV and (inflamed) synovial HEV consist of a family of functionally different but structurally closely related glycoproteins. This acidic (pI 4.2) 90 kD sulfated glycoprotein family is heavily modified postranslationally, containing both O-linked and (3-4) N-linked oligosaccharide side chains and possibly also ubiquitin side chain(s). During the processing, analyzed in methionine pulse-chase labeling, two major precursors of 64 and 76 kD molecular weights are seen. The 76 form is further processed to the mature 90 kD form and by adding chondroitin sulfate to 180-200 kD form. This higher molecular weight form incorporates iodine only to minor extent, and therefore, has not been detected in previous analyses performed with radioactive iodine. Chondroitin sulfate may be central in mediating cell-cell adhesion either at the endothelial cell level or later in time course, when lymphocytes enter the lymphatic tissue.

R 213 FURTHER STUDIES OF H-2 LINKED EFFECTS ON HEPATIC GANGLIOSIDE COMPOSITION, J.D. Kemp and T.A.W. Koerner, University of Iowa College of Medicine, Iowa City, IA 52242.
Altered glycosylation at the tumor cell surface may affect metastatic behavior and modulate the host anti-tumor immune response. In order to better understand the mechanisms for the expression of altered cell-surface carbohydrate we have tested the hypothesis that hepatic ganglioside distributions are affected by one or more genes linked to H-2, the major histocompatibility complex of the mouse. Quantitative analysis of the relative distribution patterns of GM3, GM2, GM1, GD3, GD1a, and GT1b (N-glycolated) gangliosides in the livers of congenic strains of mice have been obtained via high-performance thin layer chromatography (Schimadzu CS-930). The strains of mice tested were B10, B10.A, B10.M, B10.G, B10.A(1R), B10.A(2R), B10.A(3R), B10.A(4R), B10.A(5R), B10.A(18R), A.TFR 1, A.TFR 2, A.TFR 3, A.TFR 4, and A.TFR 5. Our studies have confirmed a prior observation about an H-2 linked effect on galactosyl transferase activity, but also suggest that the effect is more complicated than previously appreciated and that it may be modulated by background genes. Our studies also suggest that there may be additional H-2 linked effects on other glycosyl transferases and/or glycosidases. Very little is known about the regulation of cell-surface carbohydrate and we believe that an understanding of H-2 linked effect(s) on normal hepatic ganglioside expression will provide a valuable model system for the understanding of altered glycosylation in tumor cell membranes.

R 214 COMPLETE NUCLEOTIDE SEQUENCE AND BIOLOGICAL EXPRESSION OF HUMAN 4β-GALACTOSYLTRANSFERASE, V.J. Kidd, M.G. Humphreys-Beher, and B. Bunnell, University of Alabama-Birmingham, Birmingham, Alabama 35294. 4β-Galactosyltransferase is the most common of the galactosyltransferases in glycoprotein biosynthesis. This enzyme is involved in the addition of Galβ1→4 to GlcNac in glycolipids, proteoglycans and N-and O-linked oligosaccharides. A full-length human cDNA clone has been isolated from a human liver λgt-11 cDNA expression library. We have established the entire sequence of the mature unglycosylated protein, which in humans corresponds to 44,000 daltons. This clone contains a portion of the 5' untranslated region, a 3' untranslated region of approximately 180 bp and a normal polyadenylation signal. We have utilized this cDNA clone to express active recombinant human 4β-galactosyltransferase in COS M-6 cells. Using either the vector or vector plus 4β-galactosyltransferase in a negative transcriptional orientation, we see only background levels of 4β-galactosyltransferase activity due to endogenous cellular and serum protein. Conversely, when the vector contains the 4β-galactosyltransferase cDNA in a positive transcriptional orientation we observe three to four-fold increases in levels of the enzyme found in media and a two-fold increase in the enzyme found in cell lysates. Further experiments are now being done to demonstrate the possible role of 4β-galactosyltransferase in various cellular functions at the molecular level. These include possible involvement in cell growth and development as well as its function as a posttranslational-modification enzyme.

R 215 ENDOGENOUS LECTINS OF MURINE K-1735 MELANOMA CELLS: PURIFICATION AND PROPERTIES, Reuben Lotan, Leonid Meromsky, Tina Neira and Avraham Raz, Department of Tumor Biology, The University of Texas-M. D. Anderson Hospital and Tumor Institute at Houston, Texas 77030 [R.L., T.N.], and Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel [L.M., A.R.].
Extracts of various cultured tumor cells were found to contain galactose-inhibitable hemagglutinating activity. We have purified the endogenous lectins of a UV-induced murine melanoma K-1735P by affinity chromatography on immobilized asialofetuin using cell extracts prepared without detergents. All the hemagglutinating activity that was adsorbed on the affinity column could be eluted with lactose. The purified lectin represented 0.4% of the total protein and 42% of the hemagglutinating activity of the extractand it was purified about 100 fold. The isolated lectin was resolved into two protein components of apparent M_r of 14.5K and 34K, respectively, by polyacrylamide gel electrophoresis in the presence of mercaptoethanol and sodium dodecylsulfate. These two components were detected either by staining the gels for protein with Coomassie brilliant blue or by autoradiography of material isolated from [^{35}S]methionine-labeled cells. The conditioned medium of the labeled cells did not contain detectable lectin. Lectins with a similar electrophoretic migration were isolated also from murine B16 melanoma and UV-induced fibrosarcoma UV-2237 cells. Monoclonal anti-lectin antibodies prepared against the B16 melanoma lectins cross-reacted with lectins from the K-1735 cells as revealed by immunoblotting. These antibodies were used in indirect immunofluorescence staining and have demonstrated the presence of the lectins on the surface of the K-1735 cells as well as in the intracellular compartment.

R 216 CELL-SURFACE EXPRESSION OF 4βGALACTOSYLTRANSFERASE ACCOMPANIES PAROTID CELL TRANSITION TO GROWTH, Richard B. Marchase, Vincent J. Kidd, and Michael G. Humphreys-Beher, University of Alabama at Birmingham, Birmingham, AL 35294.
Rat parotid acinar cells stimulated to divide by isoproterenol demonstrate a dramatic increase in the synthesis of 4β-galactosyltransferase. Golgi-enriched fractions, however, show no increase in specific activity, but plasma membrane activity increases 40-fold. This selective increase at the cell surface was confirmed by immunofluorescence using a monospecific antibody prepared against the purified bovine milk galactosyltransferase. Following detergent-permeabilization staining of non-treated cells was seen only as groups of perinuclear vesicles, presumed to be Golgi apparatus. In isoproterenol treated and permeabilized cells both presumptive Golgi and cell surface staining was apparent. Enzyme assays performed on intact cells established that the enzyme's active site was oriented externally. The transferase could be detected as early as 3 hours after the primary challenge with isoproterenol and pretreatment with cyclohexamide prevented its appearance. The relocation to the cell surface was accompanied by an increase in a single 4β-galactosyltransferase mRNA species. The isoproterenol-induced hypertrophy of the parotid gland could be blocked *in vivo* by the 4β-galactosyltransferase modifier protein α-lactalbumin, by specific antiserum to bovine 4β-galactosyltransferase, and by UDP-galactose but no other nucleotides or nucleotide sugars. These agents also inhibited the incorporation of thymidine into isoproterenol-stimulated parotid cells in culture.

R 217 ALTERATION OF GLYCOLIPIDS IN *RAS* TRANSFECTED NIH3T3 CELLS, Gary R. Matyas, Membrane Biochem. Sect., Devel. & Metab. Neurol. Br., NINCDS, NIH, Bethesda, MD 20892.
Glycolipid alterations upon viral transformation are well documented. Transformation of 3T3 cells with murine sarcoma viruses resulted in marked reductions in the gangliosides GM1 and GD1a and an increase in GM3 and GM2 (Brady and Fishman, Biochim. Biophys. Acta (1974) 355, 121). Also, synthesis of $GgOse_3Cer$ was increased (Rosenfelder et al., Cancer Res. (1977) 37, 1333). The transforming oncogenes of the murine sarcoma viruses have been identified as members of the *ras* oncogene family. NIH3T3 cells transfected with the H-, K- and N-*ras* oncogenes were analized for their glycolipid composition and expression of cell surface gangliosides. Using conventional thin-layer chromatographic analysis, GM3 was elevated, GD1a was sightly reduced or unchanged and GM1 was present, but not in quantitatible levels. Cell surface levels of GM1 were determined by ^{125}I-cholera toxin binding to intact cells. GD1a also was determined by cholera toxin binding to cells treated with neuraminidase prior to toxin binding. All *ras* transfected cells had reduced levels of surface GM1 and GD1a as compared to logarithmically growing normal NIH3T3 cells. Levels of GM1 increased as the latter cells became confluent. Using a monoclonal antibody assay, $GgOse_3Cer$ was present in all *ras* transfected cells studied, but not in logarithmically growing untransfected cells. Interestingly, $GgOse_3Cer$ appeared when the latter cells became confluent. These results indicate that *ras* oncogenes are capable of inducing the alterations in glycolipid composition.

R 218 Changes in the glycosilation pattern of breast tissues as detected by α-tumor MAbs. S. Miotti, F. Leoni, M.G. Da Dalt, S. Canevari, M.I. Colnaghi. Experimental Oncology E, Istituto Nazionale Tumori, Milan, Italy.

Four MAbs (MOv2, MOv8, MOv15 and MBr1), raised against human ovary and breast carcinomas, were studied. All of them recognize distinct, non cross-reacting saccharide epitopes which on the respective immunizing tumors were found to be carried by glycolipids (MOv2, MOv15 and MBr1), glycoproteins (MOv2, MOv8 and MOv15) and mucins (MOv2). We analyzed, by SDS-PAGE and immunoblotting, the crude membrane preparations or soluble extracts from frozen sections of normal breast tissues (resting, pregnant and lactating breast), mammary adenomas, and mammary carcinomas. The distribution of high and intermediate M.W. glycoproteins, which reacted with the four monoclonal antibodies, was quite homogeneous in the different breast samples. On the contrary the expression of glycolipid structures, limited to certain hormone-stimulated or neoplastic conditions, suggested that, for the structures defined by our monoclonal antibodies, the kind of antigenic glycoconjugate more than the defined determinant could be specific for the differentiation phase and/or the transformation status of breast epithelial cells. In an attempt to understand the biological significance of differential expression on breast tissue, the possibile correlation of our markers with some parameters associated with breast carcinoma differentiation and proliferation are being investigated. Partially supported by grant no. 85.02103.44.

R 219 DECREASED C(5) EPIMERIZATION OF GLUCURONIC ACID IN THE GLYCOSAMINOGLYCANS OF THE STRONGLY METASTATIC RAT RHABDOMYOSARCOMA CELL LINES. E.Moczar, F.Redini and M.F. Poupon°. CNRS UA 1174, Faculté de Médecine, 94010 Créteil, ° IRSC du CNRS, BP 8, 94002 Villejuif.

Cell surface glycosaminoglycans (GAG-s) of strongly (RMS 0, RMS 6, RMS J_1) and weakly (RMS 8) metastatic rat rhabdomyosarcoma cell lines and of non tumorigenic rat myoblasts (L6) were studied. GAG-s were metabolically labeled in cell culture with (^{3}H)glucosamine and (^{35}S)sulfate. Pericellular proteoglycans and GAG-s were separated by trypsin treatment, purified and partially characterized. The incorporation of the labels in the strongly metastatic cells was by 70 to 80 % lower than the incorporation in the RMS 8 cells and by more than 90 % lower than the incorporation in the L6 myoblasts. The distribution pattern of the GAG-s was different in the highly metastatic and in the RMS 8 or in the L6 cells. The chondroitin sulfate (CS) to heparan sulfate (HS) ratio was about 2 folds higher and the dermatan sulfate to HS ratio 3-4 folds lower in the highly metastatic than in the RMS 8 cells or in the myoblasts. Degradation by heparitinase revealed that the HS of the weakly metastatic or of the non tumorigenic cells contained twice as much clusters of induronic acid-N-sulfated glucosamine disaccharides than the strongly metastatic cells. These findings were confirmed by the analysis of the nitrous acid degradation products of the HS. The results suggest, that the dermatan sulfate and/or the iduronic acid rich regions of the HS may be involved in the control of the expression of the metastatic phenotype of the rat rhabdomyosarcoma cells.

R 220 CELL-TO-CELL HETEROGENEITY IN THE EXPRESSION OF CARBOHYDRATE BASED ONCO-DIFFERENTIATION ANTIGENS WITHIN CLONAL POPULATIONS OF HUMAN MAMMARY CARCINOMA CELLS. Lenny Moss, Jack Bartley, Martha Stampfer, Gordon Parry, Lawrence Berkeley Laboratory, Berkeley, CA 94720. A panel of six monoclonal antibodies raised against purified human milk fat globule membrane binds to the high molecular weight mucin expressed on the surface of differentiated luminal mammary epithelial cells. The panel distinguishes at least four basic epitopes according to Western blot analysis, and these epitopes are at least largely defined by carbohydrates.

While all epitopes are expressed on a single species of a high molecular weight mucin of the lactating gland, epitope expression is heterogeneous in human mammary carcinoma 734B cells. When these cells are stained in culture, heterogeneity is seen with respect to the percentage of cells that stain for a particular epitope, and with respect to the intensity of staining. Neuraminidase treatment increases the percentage of cells showing some of the epitopes, only at the low level of staining. When 734B cells are subcloned, each population displays a heterogeneous and distinct profile of staining with the panel of antibodies. While each subclone population displays all of the epitopes, cell-to-cell heterogeneity within the population is striking, as individual cells display only one or two epitopes. Additionally, epitope expression appears to correlate with cellular morphology, suggesting that epigenetic influences upon a cell may directly influence the glycosylation pattern.

Glycolipids play a role in altered epitope expression. In normal human mammary cells from reduction mammoplasty, one of the epitopes is absent. When immortalized sublines are derived through chemical transformation, this epitope appears. Glycolipid analysis reveals the appearance of mucin epitopes on glycolipids derived from transformed cells.

R 221 UROMODULIN, A MODEL FOR DEFINING CARBOHYDRATE BINDING SPECIFICITIES OF RECOMBINANT CYTOKINES by A.V. Muchmore, J.M. Decker, and A. P. Sherblom NIH, Bethesda MD

Uromodulin is an immunosuppressive 85 kilodalton urinary glycoprotein which exhibits in vitro activity at 10^{-9} to 10^{-10}M. We have previously shown that uromodulin is a high affinity ligand for recombinant IL-1 and in a companion abstract we report that it also binds to recombinant tumor necrosis factor (TNF). Although uromodulin fails to bind to insulin, transferrin, albumin, and a number of other defined proteins, we now report that under appropriate conditions uromodulin also binds to rIL-2. Binding to these potent inflammatory/ immunostimulatory cytokines is dependent upon intact glycosylation of uromodulin. Furthermore, pronase resistant fragments of uromodulin or defined mono- and disaccharides compete with binding. Interestingly, IL-2 but not IL-1 or TNF binding is potently inhibited by several defined mannose derivatives. These observations lead us to propose that IL-2, TNF, and IL-1 may be examples of endogenous mammalian lectin like proteins. In vitro immunosuppression mediated by uromodulin is actually enhanced after either trypsin or pronase digestion of uromodulin and subsequent isolation of certain oligosaccharide fragments. This suggests that uromodulin may be a potent source of immunosuppressive oligosaccharides capable of disrupting normal cellular immunity which is dependent upon the activity and binding of naturally occuring endogenous lectins.

R 222 CHRONDROITIN-4-SULFATE DEPENDENT ADHESION OF A375 HUMAN MELANOMA TO SINUSOIDAL LIVER ENDOTHELIAL CELLS, M. Nakajima, P.N. Belloni, I.J. Fidler and G.L. Nicolson. The Departments of Tumor Biology and Cell Biology, The University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, 77030.

Cell-associated and released glycosaminoglycans (GAGs) of a human melanoma A375 parental line and sublines derived from metastases in lung, lymph node, and liver of athymic nude mice were examined. Cells were metabollically labeled with ^{35}S-sulfate and the proteoglycans expressed on the cell surface as well as those released in the media were analyzed for sulfated GAGs. The subline derived from liver metastasis (A375 Liver), expressed 4-8 times more cell-surface C4S than the other sublines. The A375 liver cells were highly adhesive to a monolayer of mouse hepatic sinusoidal endothelial cells (HSE), while they showed poor adhesion to mouse lung (LE-1) and bovine aortic (BAE) endothelial cells. The number of A375 Liver cells adherent to HSE monolayers was 2-3 fold greater than the number of adherent cells from other variant lines. Treatment of A375 Liver cells with chondroitinase ABC reduced their adhesiveness to HSE monolayers without significant loss of cell surface proteins. The A375 liver cell-associated C4S bound to HSE monolayers but not to BAE monolayers. HSE and LE-1 detergent cell extracts were fractionated by C4S-Sepharose affinity chromatography and the binding proteins were anayzed by SDS-PAGE. The HSE-derived high affinity fraction contained a single prominent band (M_r~43 Kd), while similar high affinty binding proteins were not detectable in the LE-1 cell extracts. These results suggest the possible involvement of C4S in A375 Liver/HSE cell adhesion. Supported by RO1-CA41524 to MN and RO1-CA42346 to GLN

R 223 IDENTIFICATION OF METASTATIC CELL-SURFACE DETERMINANTS BY GLYCOSYL-TRANSFERASES. *A. Passaniti and G.W. Hart* Johns Hopkins Sch. Med., Baltimore, MD 20205.

Highly-purified glycosyltransferases and glycosidases are being used to quantitatively probe surface saccharide topography of several lung-homing murine melanoma cells *(Fidler (1973), Nature* 242, *148).* Levels of cell-surface sialic acids are similar for three different lung-homing variants, B16-F1 (155 pmol/10^6 cells), B16-F10 (115 pmol/ 10^6 cells), B16-Flr (128 pmol/10^6 cells). No significant differences in total labeling or in specific glycoproteins labeled are found with three linkage specific sialyltransferases. However, B16-Flr (lymphocyte-resistant variant) label 2-fold higher than the other two variants with galactosyltransferase. Endogenous cell-surface galactosyltransferase(s) also exhibit a 2-fold elevation in this variant, predominantly labeling a glycoprotein of 90-95kDa.

In contrast, when similar experiments are performed on two subclones of subcutaneous metastatic variants *(Stackpole, et al. (1985), Inv. Met.* 5, *125),* substantial differences are found in specific sialic acid linkages. The non-metastatic variant, G3.26, incorporates 2.5-fold more Galß1-4GlcNAc linked sialic acid after neuraminidase treatment than the metastatic variant, G3.12. Likewise, the non-metastatic variant also incorporates 4-fold more Galß1-3GalNAc linked sialic acid than the metastatic variant. However, the total cell-surface levels of sialic acids are similar for each of these variants (G3.12, 147 pmol/10^6 cells; G3.26, 140 pmol/10^6 cells). Also, endogenous cell-surface galactosyltransferase(s) are 2-fold higher in the highly metastatic subclone. These two metastatic variants contain similar levels of iodinatable, periodate/borotritide, and galactose oxidase/borotritide accessible surface components. The data indicate that for these cells, the distribution of sialic acid among specific linkages may be a more important determinant of metastatic potential than total levels of cell-surface sialic acids. Supported by NIH CA 42486.

R 224 α-D-GALACTOSYLATION OF FUCOGLYCOCONJUGATE ON THE SURFACE OF MURINE MACROPHAGES DURING THEIR DIFFERENTIATION, Jerzy Petryniak, Thomas K. Huard* and Irwin J. Goldstein, Department of Biological Chemistry, *Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

Carbohydrates expressed on the macrophage cell surface may play a role in macrophage biological activities including macrophage-tumor cell interactions. Changes in the expression of carbohydrates on the surface of murine macrophages during their stimulation were monitored by binding of ^{125}I-labeled Evonymus europaea and Griffonia simplicifolia (GS) lectins to resident and thioglycollate-stimulated macrophages. The binding of GS I-B_4 isolectin provided a control for the removal of α-D-galactosyl residues. Digestion of stimulated macrophages with coffee bean α-galactosidase abolished completely the binding of GS I-B_4 isolectin, and changed the pattern of binding of Evonymus lectin. The affinity of Evonymus lectin for α-galactosidase-digested macrophages decreased 20-fold, from 1.6 to 32 μg/ml of lectin as measured by the lectin concentration required for binding at half saturation. Resident macrophages showed the same pattern of Evonymus lectin binding, with the same affinity, as α-galactosidase-digested, stimulated macrophages. From these results, as well as from the Evonymus lectin carbohydrate-binding specificity, the following conclusions can be drawn: a) α-D-galactosyl groups are part of the Evonymus lectin receptor on stimulated macrophages; b) the Evonymus receptor with a terminal nonreducing α-L-fucosyl group is present on resident macrophages; c) during macrophage stimulation α-D-galactosyl groups are added, presumably in an 1,3-linkage. The sequence of events may be represented as: LFucαDGal-R-resident macrophage→stimulation→DGalα1,3[LFucα]DGal-(R or R')-stimulated macrophage.

R 225 BINDING OF UROMODULIN TO TUMOR NECROSIS FACTOR: ROLE OF CARBOHYDRATE, A.P. Sherblom, J.M. Decker, and A.V. Muchmore, NCI, NIH, Bethesda MD

Uromodulin, an 85K immunosuppressive glycoprotein originally isolated from human pregnancy urine, has been shown to bind to interleukin 1 (IL-1). We now report that uromodulin also binds to tumor necrosis factor (TNF) or cachectin, a compound which shares many of the immunoregulatory properties of IL-1. This binding is monitored through an enzyme-linked immunosorbent assay (ELISA) for uromodulin adhering to plates coated with recombinant human TNF. The following observations suggest that carbohydrate present on uromodulin may be critical to the binding to TNF: 1) The binding is inhibited by 6-deoxyglucose (20 mM for 50% inhibition) or N,N'-diacetyl chitobiose (<1 mM), but not by a wide range of other sugars; 2) the binding is inhibited by wheat germ agglutinin (0.027 mg/ml), and the inhibition is reversed by addition of N-acetylglucosamine; 3) the binding is inhibited by fractions prepared by gel filtration following digestion of uromodulin with either N-glycancase or pronase, but not by control fractions prepared from digested albumin. The results suggest that TNF may demonstrate lectin-like activity in the binding to uromodulin. Both the regulation and activity of TNF may be dependent on its interaction with specific carbohydrate structures.

R 226 GANGLIOSIDES AS BIMODAL REGULATORS OF CELL GROWTH, Sarah Spiegel, Membrane Biochem. Sect., Devel. and Metab. Neurol. Branch, NINCDS, NIH, Bethesda, MD

The B subunit of cholera toxin, which binds specifically to several molecules of ganglioside GM1 on the cell surface, stimulated DNA synthesis and cell division in quiescent, non-transformed mouse 3T3 cells in a dose-dependent manner. In addition, the B subunit potentiated the response of the 3T3 cells to other mitogens such as epidermal growth factor, platelet derived growth factor and insulin. This synergistic effect suggests that the B subunit may not act identically to any of these growth factors but probably modulates a common effector system crucial for cell proliferation. In distinct contrast, the B subunit inhibited the growth of ras-transformed 3T3 cells (Ha-, Ki-, and N-ras) as well as rapidly dividing normal 3T3 cells. Thus, a bimodal response to the B subunit was demonstrated in the same cells just by varying their state of growth. In conclusion, endogenous gangliosides may be regulators of both positive and negative signals for cell growth. (Supported by a grant from FIDIA.)

R 227 GLYCOSYLATION DEFFECT IN A MURINE THY-1 NEGATIVE LYMPHOMA CELL LINE, Mei-hui Teng, Ross Basch and Joel N. Buxbaum, New York University and New York VA Medical Center, New York, NY 10010. Thy-1 is a major surface glycoprotein of T-lymphocytes and neurons. A Thy-1 negative variant (clone 4) was isolated from a Thy-1 expressing lymphoma cell line in our laboratory by immunoselection. Southern blot analysis for the Thy-1 gene showed no distinguishable difference between these two cell lines. The gene is transcribed into a 2kb message in the positive and negative clone. In the negative clone, the Thy-1 polypeptide could be labelled metabolically with radioactive amino acids but not with labeled sugars. No mature Thy-1 antigen was dectectable on the surface of clone 4 by immunofluorescence, iodination or galactose oxidase-sodium borohydride labelling. The results indicate that in clone 4 Thy-1 polypeptid is synthesized but not glycosylated or inserted into the membrane. Introduction of a cloned normal Thy-1 gene into this cell line by protoplast fusion failed to correct the defect, although murine myeloma cells transfected with the same construct expressed the protein on the cell surface. These data indicate that a gene(s) other than that coding for Thy-1 itself is (are) required for its expression and that these variants should be a useful tool for identifying the involved gene products.

R 228 SPECIFIC INHIBITION OF HUMAN NATURAL KILLER CELL-MEDIATED CYTOTOXICITY BY SIALIC ACID AND SIALO-OLIGOSACCHARIDES. Johanna Van Rinsum*, Lou A. Smets**, Henny Van Rooy** and Dirk H. Van Den Eijnden***. *Dept. of Bio-Organic Chemistry, University of Utrecht, **Div. of Cell Biology, The Netherlands Cancer Institute, Amsterdam, ***Dept. of Medical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands.
To identify carbohydrate structures involved in recognition and/or lysis of K562 target cells by human natural killer (NK) cells, inhibition studies were performed. When tested with monosaccharides, lysis of K562 cells was inhibited only by N-acetylneuraminic acid (NeuAc). Di- and trisaccharides and glycopeptides containing NeuAc or N-glycolylneuraminic acid all inhibited NK cell-mediated lysis. Among the non-sialylated carbohydrates tested, only Galβ(1-3)GalNAcol was effective. The inhibitory capacity appeared to be dependent both on the linkage type of the sialic acid residue and on adjacent sugar residues; carbohydrates containing α(2-6) linked sialic acid were more potent inhibitors than their α(2-3) isomers and NeuAcα(2-6)GalNAcol was more effective than NeuAcα(2-6)Galβ1-R (where R = glucose or oligosaccharide-peptide). The conjugation of target cells and NK effector cells was not inhibited by carbohydrates that effectively blocked the cytolytic responce. These results may indicate that cell-surface carbohydrates containing α(2-6) linked sialic acid are crucial structures in a post-binding event in NK cell-mediated lysis. Isolation and structural analysis of the target structure for human NK cells is in progress.
This work was supported by grants (NKI 84-16, UUKC 83-13) from the Netherlands Foundation for Cancer Research (KWF).

R 229 ACETYLATION AND DE-ACETYLATION OF SIALIC ACIDS IN NORMAL AND MALIGNANT CELLS Ajit Varki, Herman Higa, Sandra Diaz, Bruce Gill and Elaine Muchmore, University of California at San Diego, CA 92093.
Sialic acids can be O-acetylated at the 4,7,8, or 9 positions. We and others have shown that such substitutions are position- and tissue-specific, and are developmentally regulated in the neuroectoderm and the colon. Furthermore, the expression of O-acetylation can be aberrant in malignant cells (e.g. expression of 9-O-acetylated GD3 in malignant melanoma cells, and the loss of O-acetylation in colonic tumors).

We have studied various aspects of the biosynthesis of O-acetylated sialic acids in [3H]acetyl-Coenzyme A-labelled Golgi vesicles. Our evidence suggests that O-acetyl groups are transferred to the 7-position of sialic acid molecules, and subsequently migrate to the 9-position. We have also developed a highly sensitive, specific and facile assay for 9-O-acetyl-esterases. Using this assay, we have found evidence for the existence of several different sialic acid O-acetyl-esterases in mammalian cells and tissues. In the rat liver, studies of pH optima, latency to Triton X-100 and sensitivity to various inhibitors indicate the presence of at least three membrane-associated esterases and one cytosolic activity. All of the membrane-associated enzymes bind to Concanavalin A-sepharose, whereas the cytosolic esterase does not. All of the esterases are inactivated by di-isopropyl-fluorophosphate(DFP), showing that they are a family of serine-active-site enzymes. The existence of these multiple esterases implies that the regulation and turn-over of sialic-acid O-acetylation might be much more complex than previously imagined.

We have also developed methods for the selective inactivation of the esterase function of Influenza C virus. This allows the use of the virus as a specific probe for 9-O-acetylated sialic acid residues. Using this unique probe we have initiated studies of the topology, distribution, and developmental regulation of 9-O-acetylated sialic acid residues on the surface of normal and malignant cells.

R 230 DNA-MEDIATED TRANSFER OF LECTIN-RESISTANCE PHENOTYPE ACCOMPANIED BY ALTERED GLYCOSYLATION, Tien-wen Tao, Stanford University, Stanford, CA. 94305.

The wheat-germ-agglutinin-resistant (WGA-R) mutants selected from mouse melanoma cells express phenotypic alterations in glycosylation (reduced sialic acid residues in acidic complex N-glycosidic glycopeptides), cell adhesiveness (increased homotypic but decreased heterotypic adheisveness), and malignant potential (reduced tumorigenicity and metastatic potential). One major objective is to determine if these altered expressions share a common genetic basis and what the basis is. DNA-transfer experiments were carried out using the WGA-R phenotype as a selectable marker. High molecular weight cellular DNA isolated from the WGA-R mutant was co-transfected with a plamid containing a dihydrofolate reductase (DHFR) cDNA into DHFR-deficient Chinese hamster ovary cells (DG44). The controls included DG44 cells transfected with DG44 DNA and DHFR cDNA. Following selection and expansion in culture medium deficient in glycine, hypoxanthine, and thymidine to eliminate untransfected cells, the $DHFR^+$ transfectants were selected in WGA for the WGA-R population. WGA-R transfectants were obtained. Secondary transfections and selections were carried out with DNA isolated from the primary transfectants to obtain secondary WGA-R transfectants. The WGA-R transfectants which were tranfected with the WGA-R DNA showed not only greater resistance to WGA as compared to the controls but also a unique acidic glycoprotein complex of about 80kd. FACS analysis of these transfectants revealed a reduced cell surface binding to WGA characteristic of the DNA donor WGA-R cells. These results suggest strongly that a single gene (or a closely linked gene complex) is involved in conferring the WGA-R phenotype, thus the specific glycosylation alteration.

R 231 DECREASE IN POLYLACTOSAMINOGLYCANS DURING DIFFERENTIATION OF CaCo-2 CELLS, Adel Youakim and Annette Herscovics, McGill Cancer Centre, McGill University, Montreal, Quebec, Canada.

CaCo-2 human colonic adenocarcinoma cells undergo morphological and functional differentiation into polarized enterocytes containing active brush border enzymes, when maintained in culture for extended periods of time. We have investigated the changes in carbohydrate structure of glycoproteins during differentiation of these epithelial cells. The cells, at various stages of differentiation, were incubated for 24h with [^{3}H]fucose or [^{3}H]glucosamine, and then subjected to mild trypsin treatment to release cell surface components. The trypsinate and cell pellet residue were exhaustively digested with pronase and then fractionated on Bio-Gel P-6, followed by affinity chromatography on Datura Stramonium (DSA)-agarose, a lectin which binds polylactosaminoglycans. The proportion of labeled cell pellet and cell surface glycopeptides bound to DSA-agarose decreased 3 and 6 fold respectively, with differentiation of the cells. The DSA-bound glycopeptides were degraded by E.freundii endo-β-galactosidase, into a mixture of oligosaccharides, thus indicating that the polylactosaminoglycans were branched. These results show that differentiation of CaCo-2 cells is accompanied by a relative decrease in polylactosaminoglycans. (Supported by the Medical Research Council of Canada)

Diagnosis and Therapy

R 300 CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO PARAGLOBOSIDE (PG) AND SIALOSYL-PG (2,6-SPG), AND AN IMPROVED CHROMATOGRAM BINDING ASSAY FOR RAPIDLY IDENTIFYING ANTIBODIES TO TUMOR ANTIGENS, Byron Anderson, John Slota, Samar Kundu, Joseph Patrick, George Manderino, Harry Rittenhouse and Joseph Tomita. Northwestern University Medical School, Chicago, IL 60611 and Abbott Laboratories, North Chicago, IL 60064.

A mouse monoclonal antibody (designated 85/34) was generated in response to mucin glycoprotein immunogen isolated from the ascites of a cancer patient. The antibody was reactive with PG (Galβ1,4-GlcNAcβ1,3-Galβ1,4-Glcβ1, Ceramaide) and with mucinous glycoproteins prepared from ascitic fluids. Reaction with desialylated sialosylα(2,3) lacto-N-norhexaosylceramide and sialosylα(2,3) lacto-N-isooctaosylceramide suggested specificity for the Galβ1,4-GlcNAcβ1, R sequence similar to the I or i antigen determinants. An additional monoclonal antibody (S6P) was produced following immunization with monosialogangliosides purified from meconium and characterized as reactive with the NeuNAcα2, 6-Gal sequence of 2,6-SPG. The oncofetal nature of the antigen [Hakomori, et al., Biochem. Biophys. Res. Comm. 113, 791 (1983)] and elevated levels of 2,6-SPG in human liver and colon adenocarcinomas suggest further investigation of the clinical usefulness of the S6P antibody.

R 301 CHARACTERIZATION AND IMMUNOASSAY OF HUMAN TUMOR-ASSOCIATED GALACTOSYLTRANSFERASE ISOENZYME II, Alan E. Brandt, Richard C. Winant, Morito Uemura and Branimir I. Sikic, SRI International, Menlo Park, CA, 94025, Konishiroku Photo Ind. Co., Ltd., Tokyo, Japan, and Stanford University, Stanford, CA 94305.

Galactosyltransferase (GT) was purified 150,000-fold from human ovarian tumor effusion fluid or 350,000-fold from normal human serum by chromatography on α-lactalbumin and anti-human IgG affinity columns. Both preparations showed a single band of 48 kd on SDS-PAGE but nondenaturing PAGE of GT isolated from tumor effusion fluid revealed a series of oligomeric proteins possessing GT activity which were barely detectable in normal serum. Purified GT was used to immunize BALB/c mice for monoclonal antibody (MAb) preparation. Five MAb were isolated which reacted with GT. MAb 3872 (an IgG_1, pat. pend.) was determined to be specific for a cancer-associated GT isoenzyme (GT-II) by immunostaining of Western blots and nondenaturing PAGE of GT specifically eluted from a MAb 3872 affinity column. MAb 3872 was immobilized on 1,1'-carbonyldimidazole-activated trisacryl GF-2000 and used to assay normal control and cancer patient serum samples for GT-II. 29 Individual normal serum samples were assayed and found to contain 85.3 ± 30.9 mUnits of GT-II/ml serum (1 unit = 1 nmole galactose transferred/hr). Analysis of 77 serum samples from 38 ovarian tumor patients undergoing megestrol acetate therapy, identified 33/38 patients (87%) with GT-II in excess of 200 mUnits/ml with a range of 216 to 8,469 mUnits/ml. Serial samples obtained from the ovarian tumor patients suggested that serum GT-II levels reflected the tumor burden of the patient and offered a more accurate assessment of tumor burden than CA-125.

R 302 ALTERED O-GLYCOSYLATION OF PROTEINS IN THE NEOPLASTIC TROPHOBLAST, Laurence A. Cole, Yale University, New Haven CT 06510.

Human chorionic gonadotropin (hCG) is a glycoprotein composed of two dissimilar subunits, alpha and beta, and is produced by the normal (in pregnancy) and neoplastic trophoblast. hCG has 4 O-linked oligosaccharides, all on the beta subunit. Large free alpha (LFA) is an oversized (M_r = 24,000) hCG alpha subunit (M_r = 22,000) -like protein, also produced by the trophoblast. LFA can have a single O-linked sugar unit attached at Thr 39. O-linked sugar structures were compared on hCG and LFA from normal and neoplastic cells. Normal pregnancy trophoblast cells and JAr neoplastic trophoblast were cultured in fluids containing [3H-GlcN]. hCG and LFA in fluids were purified by immunoaffinity chromatography and O-linked sugar units released by beta-elimination. Radiolabeled oligosaccharides were separated by size on Bio-Gel P4 and elution volumes compared to those of standards of established structure. Similar peaks and distributions were observed in Bio-Gel P4 eluates from hCG and LFA released sugars, even though the former has 4 and the latter just 1 sugar unit. This suggested tissue- rather than peptide-specific glycosylation, and that the structures found may be representative of those on other trophoblast proteins. The larger proportion of released sugar units from neoplastic trophoblast molecules eluted in the volume of a hexasaccharide structure (51% of hCG and 55% of LFA oligosaccharides), NeuAc-Gal-GlcNAc(Neuac-Gal)-GalNAc-ol, the elution volume of only a small percentage (14% of hCG and 18% of LFA) of those from normal trophoblast molecules, which primarily emerged in the position of a tetrasaccharide structure NeuAc-Gal(NeuAc)GalNAc-ol. Similar differences in the occurrence of the hexasaccharide structure were observed in hCG preparations from urines of trophoblast malignancy (n=2) and pregnant (n=10) subjects.

R 303 BIOSYNTHESIS AND GLYCOSYLATION OF THE ANTIGEN RECOGNIZED BY MONOCLONAL ANTIBODY KS1/4, Philip D. Fernsten, Katherine W. Pekny, and Leslie E. Walker, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Monoclonal antibody KS1/4 has been shown to be an effective drug carrier and is currently being evaluated in phase I clinical trials. The antigen recognized by KS1/4 appears to be an excellent receptor for this type of therapy. KS1/4 recognizes an epitope expressed on the cell surface of human adenocarcinoma cells. From radiolabeled extracts of human lung tumor cells, KS1/4 precipitates a 40 kDa major glycoprotein component and a 42 kDa minor glycoprotein component. Both components are also detectable by Western blotting analysis of tumor cell extracts. When synthesis occurs in the presence of tunicamycin or when the immunoprecipitates are treated with peptide, N-glycosidase F, a single 37 kDa polypeptide component, is precipitated. Immediately post-translation, digestion of the 40 kDa and 42 kDa glycoproteins with endo-β-N-acetylglucosaminidase H (endo H) also yields a single 37 kDa polypeptide component. However, over a 3-hour period beginning at 10 minutes post-translation, a 39 kDa major component and a 41 kDa minor component gradually appear in the endo H digests as the 37 kDa component gradually disappears. This acquisition of partial endo H resistance by the 40 kDa and 42 kDa antigens indicates that at least one oligosaccharide on each component remains in the high mannose form. The post-translational processing of the other oligosaccharides is not inhibited in the presence of NH_4Cl or diethylcarbamazine. Non-equilibrium pH gradient electrophoresis (NEPHGE)/SDS-PAGE analysis and analysis of tryptic glycopeptides suggest that the 40 kDa and 42 Kda glycoprotein components differ only by the addition of one extra oligosaccharide to the 42 kDa component.

R 304 DEMONSTRATION OF ALTERED SIALIC ACID EXPRESSION IN HUMAN NEOPLASTIC COLON, Jeff T. Hutchins, J. Milburn Jessup and Christopher L. Reading, University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston TX 77030.
The purpose of this study was to compare the expression of O-acetylated sialic acids on normal colonic epithelial cells to that on primary and metastatic human adenocarcinoma of the colon and rectum. In 24 cases, the relative percentages of biosynthetically labeled non-, mono-, di-, and tri-O-acetylated sialic acids were measured after hydrolytic release, separation, and identification by paper chromatography. The chemical nature of these O-substitutions was confirmed to be acetyl groups by sodium periodate, mild alkali, and esterase studies coupled with determination of the ion molecular weight of these molecules by fast atom bombardment - mass spectrometry. Differences were observed in the expression of these sialic acids on normal colonic epithelium, "uninvolved" colon mucosa remote to a colonic adenocarcinoma, and colonic adenocarcinoma. The levels of mono- and tri-O-acetylated sialic acids accounted for the difference in the ratios of sialic acids expressed between normal and "uninvolved" colonic mucosa, while the total amount of O-acetylation was unchanged. However, no difference was observed in the relative amounts of non- and O-acetylated sialic acids between either fresh and tissue culture-established colon carcinomas, or fresh and tissue culture-established liver metastasis derived from carcinoma of the colon. The relative expression of these O-acetylated sialic acid molecules appears to vary according to tissue type. This study identifies a possible diagnostic tool for determining whether individuals genetically predisposed to develop adenocarcinoma of the colon express a field defect resulting in abnormal ratios of O-acetylated sialic acids.

R 305 ULEX EUROPUS AGGLUTININ (UEA) DETECTS CARCINOEMBRYONIC ANTIGEN (CEA) IN EXTRACTS OF HUMAN COLORECTAL CARCINOMA. J. Milburn Jessup, K. Kanellopoulos, K. Hickey, C. L. Reading, UT M. D. Anderson Hospital, Houston, TX 77030.
Recent interest has focused on fucosylated epitopes expressed on human neoplasms. The plant lectin UEA binds fucosylated oligosaccharides while UEA-reactive substances have a tissue distribution similar to CEA. We sought to determine if UEA reacted with CEA in 0.5M mannitol- 0.5% Triton X-100 extracts of fresh primary and metastatic colorectal carcinomas and extracts of paired normal colon or liver. Wester blots of the extracts were transferred to nitrocellulose membranes after SDS-PAGE in 10% polyacrylamide gels. The blots were then stained with peroxidase-conjugated UEA (UEA-P) or CEA (CEA-P). UEA-P reacted with a 170-190 kDa band in 10 of 15 primary tumors, all 8 metastases, and none of 13 normal colons and 6 normal livers. UEA-P also did not react with 3 villous adenomas, 1 breast carcinoma metastatic to bowel, or 1 focal nodular hyperplasia of liver. CEA-P displayed similar reactivity but detected CEA in 2 UEA negative tumor extracts. Fucose blocked finding of UEA-P to Western blots of tumor extracts. CEA-P reacted with a 170-190 kDa substance in tumor extracts eluted with fucose from a column of immobilized UEA. Thus, UEA reacts with oligosaccharides on most, but not all, species of CEA and is a useful adjunct to anti-CEA immunohistochemistry.

R 306 REDISTRIBUTION OF LECTIN BINDING SITES IN OVARIAN CARCINOMA
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Changes in surface carbohydrate presentation by malignant cells is one of the few surface properties which allows descrimination between benign reactive cells and malignant cells. I have investigated the lectin binding patterns on malignant ovarian and breast carcinoma cells in serous effusions. Cytocentrifuge preparations of malignant cell populations and normal mesothelial cells were probed for carbohydrate expression by staining with a panel of biotinylated lectins.The lectin binding directed at terminal carbohydrate residues was detected using the avidin peroxidase technique. The studies showed peripheral membrane binding of WGA, PNA and CON A with WGA and CON A also showing some cytoplasmic staining in selected cases. Inhibition of WGA binding showed both N-acetyl-glucosamine and ovomucoid but not neuraminidase could effectively inhibit binding.Binding patterns in non-inhibited cell populations showed heterogeneity of binding to groups and to cells within clusters. In cell clusters positive for WGA binding was around the periphery of the cluster and maintained a lumenal pattern of the cells. Within positive clusters 60 % of the cells were positive wheras greater than 80 % of the clusters were positive.The heterogeneity allows discrimination of tumor cell subpopulations based on surface carbohydrate expression.

R 307 PURIFICATION AND CHARACTERIZATION OF CA-549, A NEW CIRCULATING BREAST CANCER MARKER. Barry C. Kress, Laura A. Dollar, Joy E. Koda, and Pramod K. Gaur, Hybritech, Inc., San Diego, CA 92121

CA-549 is a circulating breast tumor-associated antigen that reacts with the monoclonal antibody BC4E549. Measurement of serum levels of CA-549 using a monoclonal antibody-based TAMDEM® immunoradiometric assay has been shown to be valuable in monitoring breast cancer.

CA-549 has been purified to near homogeneity from cytosol from T-417, a breast carcinoma cell line grown in nude mice, using a combination of Sephacryl S-400 gel permeation chromatography, immunoaffinity chromatography and wheat germ agglutinin-agarose lectin chromatography. Preliminary epitope analysis indicates that BC4E549 reacts with a periodate-sensitive, neuraminidase-insensitive site(s) on CA-549. Biochemical characterization of CA-549 revealed that it is a high MW (>900 kd) sialic acid-containing glycoprotein. On SDS-PAGE under reducing conditions the antigen exhibits two bands with apparent MW of approx. 500 and 400 kd. Similar MW species of CA-549 were observed in patients' sera after binding to monoclonal antibody-coated polystyrene beads and subsequent elution with SDS-dissociation buffer. Both bands stain poorly using either Coomassie blue or silver staining but can be detected with PAS. Neuraminidase treatment of the antigen decreases the migration of the subunit bands on SDS-PAGE without affecting reactivity with BC4E549. CA-549 interacts specifically with the lectins wheat germ agglutinin, Ricinus communis and peanut agglutinin but does not bind to concanavalin A.

R 308 GANGLIOSIDES SHED BY TUMOR CELLS ENHANCE TUMOR FORMATION IN VIVO, Stephan Ladisch, Shinichi Kitada, and Esther F. Hays, UCLA School of Medicine, Los Angeles CA 90024 Gangliosides are shed in significant quantities by some murine and human tumors. Furthermore, certain gangliosides shed by tumor cells in vivo have potent immunoregulatory activity (Cancer Research 43 3808, 1983). Thus, we hypothesized that the degree of ganglioside shedding may modulate the tumorigenicity of malignant cells, possibly by an immunologic mechanism. Therefore, we are studying the gangliosides and tumorigenicity of several related cloned cell lines, derived from spontaneous lymphoma 12 (SL12) of an AKR mouse. We found that the most highly tumorigenic clone, SL12.3, contained and shed substantial quantities of gangliosides (70 pmol/10^6 cells and 18 pmol/10^6 cells/24h, respectively). In contrast, a poorly tumorigenic clone, SL12.4, contained and shed markedly less gangliosides (1 pmol/10^6 cells and 0.2 pmol/10^6 cells/24h). The influence of shed gangliosides on tumorigenicity was next directly tested in vivo. If shed gangliosides "protect" tumor cells from host destruction, then gangliosides shed by the highly tumorigenic SL12.3 cells when co-injected with the poorly tumorigenic ganglioside-deficient SL12.4 cells might enhance tumor formation by the SL12.4 cells. In fact, 1-10 pmol purified SL12.3 cell gangliosides injected I.D. with $5x10^4$ SL12.4 cells into syngeneic normal mice increased the 2-week tumor incidence of these poorly tumorigenic cells from 8% to 83%. The findings suggest a direct relationship between ganglioside shedding and tumorigenicity, and identify tumor gangliosides as previously unrecognized potent enhancers of tumor formation in vivo.

R 309 PROPERTIES OF DU-PAN-2 EPITOPE. M. S. Lan, M. A. Hollingsworth, and R. S. Metzgar, Dept. Microbiology and Immunology, Duke Univ., Durham, N. C. 27710.
This work describes the molecular properties of a human pancreatic cancer-associated epitope defined by a murine monoclonal antibody (DU-PAN-2). Metabolically labeled DU-PAN-2 antigen was isolated from a pancreatic adenocarcinoma cell line (HPAF) by affinity chromatography and characterized to be a mucin type glycoprotein. Threonine, Ser, Pro, Gly, and Ala comprised more than 50% of the amino acid residues. The sugar moieties were O-glycosidically linked via GalNAc and contained Fuc, Gal, GlcNAc, GalNAc and NeuNAc. Tritiated glucosamine labeled DU-PAN-2 antigen was subjected to digestion with various proteases, neuraminidase, and alkaline reductive β-elimination. DU-PAN-2 antigen was susceptible to pepsin, pronase and papain digestion. Protease digestion fragmented the molecule and destroyed most of the antigenic activity. Neuraminidase treatment released N-acetylneuraminic acid from the DU-PAN-2 molecule, completely abolished the antigenic activity, and caused a dramatic conformational change from a filamentous to a globular structure as observed by electron microscopy. A lectin blocking assay indicated that WGA and Slug agglutinin partially inhibited antibody binding to the DU-PAN-2 antigen. Although the exact nature of the DU-PAN-2 epitope has not yet been defined, these results suggest that both protein component and sialic acid are essential for maintaining the structure of the epitope recognized by the DU-PAN-2 monoclonal antibody.

R 310 BIOSYNTHESIS OF THE CARCINOEMBRYONIC ANTIGEN (CEA): STUDIES ON THE GLYCOSYLATION PATHWAY, Patrizia Lorenzoni, Cristina Mottola and Costante Ceccarini, Sclavo Research Center, Siena, Italy.

Native CEA is a 180 kdalton membrane-associated glycoprotein widely used as a marker of gastrointestinal adenocarcinomas. This antigen's preponderant carbohydrate portion is believed to be responsible for its microheterogeneity. In order to gain some insight into this problem and to identify CEA precursors during its biosynthesis, we performed experiments with the glycosylation inhibitors tunicamycin and monensin on CEA producing cells. Human gastric adenocarcinoma MKN-45 cells were selected for this study after screening of several cell lines by immunofluorescence; a polyclonal antibody raised against purified CEA was employed. An aliquot of this antibody was also immobilized on sepharose and used for immunoadsorption of CEA at the end of the cell labelling procedure. The cells were incubated with 2 μg/ml tunicamycin or 1 μM monensin and then pulsed with ^{3}H - or ^{14} leucine and ^{3}H mannose; the solubilized material was immunoadsorbed on the resin and electrophoresed. The gel was in part sliced and counted, in part autoradiographed and the remainder used for immunoblotting. Tunicamycin greatly reduced the CEA peak while producing a 72 kdalton main band that may be considered the peptide portion of CEA. Monensin appeared to block the glycosylation process to a single 130 kdaltons molecular form. Experiments are underway to establish the nature of this precursor and the role of CEA sialylation in its membrane insertion.

R 311 SPECIFIC EXPRESSION OF GD3 IN CHILDHOOD T-CELL LYMPHOBLASTIC MALIGNANCIES, William D. Merritt and Gregory H. Reaman, The George Washington University Medical Center, Washington DC 20037 and Children's Hospital National Medical Center, Washington DC 20010.

The oncogenic transformation is consistently accompanied by alterations in glycosphingolipid biosynthesis. We have studied the particular alteration in gangliosides associated with T-cell childhood malignancy. Gangliosides were isolated from lymphoblasts from 7 children with T-cell lymphoblastic malignancies, 3 children with non-T,non-B acute lymphoblastic leukemia, and from non-malignant T-cells from human thymus. Thin layer chromatography of the isolated ganglioside fraction from T-cell lymphoblasts revealed only two major gangliosides. One ganglioside comigrated with GM_3, the major ganglioside of normal lymphoid tissue, and the other ganglioside comigrated with human brain GD_3, in three different solvent systems. Neuraminidase treatment of the latter ganglioside yielded GM_3 and lactosyl ceramide. Scanning densitometry revealed that whereas thymus cells contained 97% GM_3 and 3% GD_3, T-cell lymphoblasts contained from 22 to 86% GD_3 and a corresponding reduction in GM_3. The shift to increased GD_3 was observed in all 7 T-cell patients, but not in the non-T,non-B patients. Immunofluorescence microscopy with anti-GD_3 antibody (R24) showed that whereas lymphoblasts from 22 out of 27 patients with T-cell ALL were positive for GD_3, 0 out of 11 non-T,non-B patients were positive for this ganglioside. This difference in anti-GD_3 staining was confirmed by FACS analysis. The results demonstrate a consistent expression of GD_3 in T-cell ALL lymphoblasts when compared to non-T,non-B ALL and normal thymocytes, and suggests that GD_3 represents a tumor-associated antigen for the T-cell subclass of childhood lymphoblastic malignancy.

R 312 STRUCTURAL ANALYSIS OF SMALL CELL LUNG CARCINOMA GANGLIOSIDES, Lana Rittman-Grauer, Anne Dell[1], and Minoru Fukuda[2], Hybritech, Inc., San Diego, CA 92126, Imperial College of Science and Technology, London, Great Britain[1], and La Jolla Cancer Research Foundation, La Jolla, CA 92037[2].

Small cell lung carcinoma cell lines and gangliosides purified from small cell lung carcinoma (SCLC) tumors absorbed to acid-treated Sal. Minn. were immunized into Balb/c mice. Antibodies resulting from the fusion of the mouse splenocytes with the mouse myeloma line P3.653 were screened by ELISA on SCLC cell lines as well as gangliosides purified from SCLC tumors. Two antibodies, LS2D173 and LS2D220 reacted with 7/8 SCLC ganglioside preparations on immuno-TLC. The antibodies also reacted with a similar migrating ganglioside in melanoma, pancreatic and colon carcinoma gangliosides, but failed to react with gangliosides from lung adenocarcinomas, prostate and breast carcinoma, as well as, normal lung, kidney and colon. The monoclonal-reactive ganglioside was purified by HPLC and identified as GM2 by FABMS. A third antibody, LS2E090 reacted with 8/8 SCLC ganglioside preparations. By immuno-TLC analysis, two major bands of reactivity were seen. Gangliosides from a SCLC tumor were purified by HPLC, acetylated, and repurified by preparative HPTLC followed by HPLC. Structural analysis of the monoclonal reactive species by FABMS identified the slower migrating ganglioside as GD1a and the faster migrating ganglioside as GM1b. The levels of GD1a in small cell tumors appears to be 5-10 fold higher than that seen in gangliosides extracted from normal colon, lung, kidney, and liver. GM2, GD1a and GM1b all appear to be elevated in small cell lung carcinoma over levels in normal tissues.

R 313 ANTI-IDIOTYPE MONOCLONAL ANTIBODY DIRECTED TO ANTI-SIALYL Le^x-i, FH6,

Sadahito Shin, Tetsuya Tachikawa, Midori Abe, and Katsuyuki Nakajima, Otsuka Assay Laboratory, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan.

Monoclonal antibody directed to the specific cancer-associated carbohydrate antigen sialyl Le^x having a long type 2 chain with internal fucosyl residue (hereby called sialyl Le^x-i) was prepared previously and termed FH6 (Fukushi et al. (1984) J. Biol. Chem. 259, 10511-10517). We have now established the monoclonal anti-iodiotype antibody that inhibits FH6 binding to the antigen sialyl Le^x-i. Wistar rat was immunized with a purified FH6 antibody and complete Freund's adjuvant three times, and spleen cells were harvested from the immunized mouse myeloma cells P3U1. The hybridoma was screened by ELISA assay with FH6 and AH6 (anti-Le^y). A few clones which secrete antibody that specifically reacts with FH6 but does not react with other antibodies, including AH6, FH7 (anti-disialyl Le^a), and FH9 (anti-disialyl type 1 chain), were isolated. The antibody AId-6F (IgG2a) inhibits quantitatively the binding of FH6 antibody to sialyl Le^x-i antigen and is assumed to recognize the internal image of FH6 structure directed to sialyl Le^x-i epitope.