Glycosylation & Disease, 1994, **1**, 67–74

ABSTRACTS

Glycobiology: New Perspectives on Human Disease

Bethesda, MD, USA, 13–15 September 1993

The molecular biology of glycosyltransferases—exciting prospects for glycobiology

H. Schachter

Research Institute, Hospital for Sick Children, and Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

The functions of glycoconjugates have been called 'one of the last great frontiers of biochemistry' (G.W. Hart, *Curr Opin Cell Biol*, **4**, 1017, 1992). Those of us who have been in the game for 25 years remember the lonely sessions at general biochemistry meetings in those early days. The first revolution in our field began in the 1970s with the astonishing new techniques of fine structure analysis that revealed for the first time the tremendous diversity and complexity of protein and lipid-bound glycans. Structure led rapidly to complex enzymatic paths for the synthesis of *N*- and *O*-glycans and glycolipids. Although biosynthetic schemes were devised to explain oligosaccharide microheterogeneity (H. Schachter, *Biochem Cell Biol*, **64**, 163, 1986), we were left with a sense of total frustration as to the functional significance of all these many structures. A glimmer of hope was derived from the fact that structural variations were not totally random; there were recognizable patterns between species, interesting polymorphism's within species, and specific temporal and spatial changes within a single organism. The view emerged that glycoconjugates had many different roles and that 'all of the theories are correct' but that there are exceptions to all (A. Varki *Glycobiology*, **3**, 97, 1993). The diversity of structures seems to allow the organism to adapt with functional diversity, using a limited gene pool, to the stresses of evolution and a hostile world. But how can we distinguish crucial structures and functions from trivial ones and determine the precise functions of a particular structure? Analysis of mutants is a time-honored approach to this problem. Whereas perturbations of glycosylation appear to have a rather minimal effect on the ability of cells in culture to grow and survive, genetic defects in glycosylation are rare in the intact organism, suggesting that glycoconjugates play crucial roles in the latter. The most recent revolution in glycobiology, the cloning of glycosyltransferase genes (J.C. Paulson & K.J. Colley *J Biol Chem*, **264**, 17615, 1989; H. Schachter, *Curr Opin Struct Biol*, **1**, 755, 1991), may provide answers to these puzzles! To date, 21 genes have been cloned and insights into the control of tissue-specific/time-dependent glycosylation and Golgi targeting have been obtained. Several laboratories are attempting to create transgenic mice in which specific glycosyltransferase genes have been 'knocked out'. There are indeed exciting prospects ahead. (Support by MRC of Canada and the Protein Engineering Centre of Excellence).

Exploring the biological roles of oligosaccharides in development and differentiation: problems and prospects

A. Varki

Glycobiology Program, University of California, San Diego Cancer Center, University of California, San Diego, La Jolla, CA, USA

Many different theories have been made concerning the biological roles of the oligosaccharide units of glycoconjugates. The evidence indicates that while all of these theories are correct, exceptions to each can also be found. These biological roles span the spectrum from those that are trivial, to those that are crucial for the development, growth, function or survival of an organism. Elucidation of the more specific roles is difficult because most studies in glycobiology begin with the simple discovery of an interesting oligosaccharide structure. It is often difficult to predict the functions this oligosaccharide might be mediating, or its relative importance to the organism. Also, the same oligosaccharide sequence might mediate different functions at different locations within the organism, or at different times in its ontogeny or life-cycle. The more specific biological roles of oligosaccharides are often mediated by unusual sequences, unusual presentations of common sequences, or by further modifications of the monosaccharides. However, these are also more likely to be targets for recognition by pathogenic toxins and microorganisms, and hence more subject to intra- and interspecies variation, resulting from ongoing host–pathogen interactions. This talk will focus on four approaches to uncovering biological roles for oligosaccharides, with examples from our recent work:

1. *Studying natural mutants in glycosylation*. New data regarding a rare human genetic disease called Carbohydrate-Deficient Glycoprotein Syndrome (CDGS) will be presented. Taken together, our data indicate that, contrary to the prior hypotheses, CDGS does not exhibit a global change in the processing and elongation of *N*-linked oligosaccharides. It is more likely that the defect involves the earliest steps in the initial glycosylation of glycoproteins.

2. *Construction of glycosylation mutants in intact animals*. Interfering with the genetic and cellular machinery for glycosylation could result in an early lethal event that would abort the experiment. Alternatively, functional redundancy between transferases might result in lack of phenotype. As a first approach, we have chosen instead to eliminate certain oligosaccharides by expressing a specific degradative enzyme as a cell surface molecule. Specific promoters allow analysis of tissue-specific functions of oligosaccharides in later aspects of development. Some transgenic experiments currently under way (partly in collaboration with others) will be discussed.

3. *Finding specific oligosaccharide ligands for cell surface lectins*. CD22β is a glycoprotein on the surface of naive B cells believed to play a role in cell-cell interactions. We have found that CD22β recognizes multiple glycoproteins on surface target cells and that sialylation is essential for binding. *N*-linked oligosaccharides released by peptide N:glycosidase F interact with CD22β. Interaction requires α2,6-linked sialic acids, is affected by the number and location of these residues, does not require the tri-mannosyl core of *N*-linked oligosaccharides, and is abolished by truncation of sialic acid side chains or 9-*O*-acetylation of the α2,6-linked sialic acids.

4. *Tagging specific oligosaccharides to explore their biology*. We have synthesized a novel fluorescent reagent (BAP) that can tag oligosaccharides under non-degradative conditions with high efficiency. The adducts show excellent fractionation by reverse-phase HPLC with detection in the low picomole range. Stepwise sequencing of the sugar chains is possible. The interaction of the biotinyl group with multivalent avidin or streptavidin can be used to create the functional equivalent of neoglycoproteins carrying multiple copies of oligosaccharides of defined structure. These complexes allow production of IgG antibodies against the oligosaccharide chain. They can also harness the power of (strept)avidin–biotin technology for detection and isolation of oligosaccharide-specific receptors from native sources or recombinant libraries.

Synthesis and organization of hyaluronic acid in mammalian preovulatory follicles

A. Salustri, E. Tirone, G. Siracusa, A. Camaloni*, M. Yanagishita* and V. Hascall*

*Department of Public Health and Cell Biology, Second University of Rome, Rome, Italy and *Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA*

In the ovary of most mammals, human included, during the preovulatory period, granulosa cells surrounding the oocyte (cumulus cells) produce large amounts of hyaluronic acid (HA). This glycosaminoglycan is organized with glycoproteins in a mucilaginous matrix that causes a conspicuous volumetric expansion of the cumulus cell–oocyte complex (COC). The deposition of such matrix appears to be essential for normal ovulation, COC capture by oviductal fimbriae and preservation of oocyte fertilizability. By using mouse cumulus cell cultures and media conditioned by oocytes we were able to show that, in addition to gonadotropins, a soluble factor(s) produced by the oocyte is required to stimulate HA synthesis and consequent expansion of the cumulus. We also observed that cumulus cells cultured for a few hours before being stimulated lose the ability to synthesize HA, unless medium conditioned by oocytes is present during preculture. Thus, the oocyte also appears to have a role in preserving such metabolic activity of cumulus cells. The possibility should be considered that defects in the production of paracrine factor(s) by the oocyte might be involved in unexplained anovulatory cycles in mammals.

A serum glycoprotein, belonging to the inter-alpha-trypsin inhibitor (IaI) family, is required to retain HA in the extracellular matrix of *in vitro* stimulated COCs, and co-localizes with HA in the preovulatory follicle. We present evidence suggesting a structural role for such protein in organizing HA in the cumulus expansion process. The possibility that the proteinase inhibitory activity of the molecule might have a role in stabilizing COC extracellular matrix will be also discussed. IaI is present in normal human plasma and increases in pathological conditions such as cancer, rheumatoid arthritis, certain infections and inflammatory diseases, but its function in these processes is still unknown. The study of the COC expansion process might open new perspectives to understand the multiple roles of such a molecule.

Carbohydrate-mediated adhesion of gametes during mammalian fertilization

P.M. Wassarman

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ, USA

For both plants and animals, the ability of male and female gametes of the same species to recognize one another is a ubiquitous and essential feature of fertilization process. Among mammals, the unfertilized egg extracellular coat, or *zona pellucida* (ZP), is the site of 'sperm receptors' that, together with complementary 'egg-binding proteins' located on the sperm surface, support species-specific adhesion of gametes during the course of fertilization (Wassarman, 1987, 1990, 1993). The mouse sperm receptor, called mZP3, is a ZP glycoprotein (~83 kDa M_r) that consists of a ~44 kDa M_r polypeptide (402 amino acids; not including a 22 amino acid signal sequence), three or four complex-type *N*-linked oligosaccharides, and an undetermined number of *O*-linked oligosaccharides. Solely as a consequence of its oligosaccharides, mSP3 is quite acidic and exhibits considerable heterogeneity on SDS–PAGE. There are more than 10^9 copies of mZP3 in the ZP, located periodically along the filaments that make up the extracellular coat. Each sperm binds to as many as tens-of-thousands of copies of mZP3 at the ZP surface. Binding to mZP3 causes sperm to undergo a form of exocytosis, called the 'acrosome reaction', that enables bound sperm to penetrate through the ZP and to reach, and then fuse with, egg plasma membrane. mZP3 is inactivated as a sperm receptor shortly after gamete fusion. The sperm receptor function of mZP3 is attributed to certain of the glycoprotein's *O*-linked oligosaccharides. These oligosaccharides have an apparent M_r of ~3.9 kDa, have a galactose residue at their non-reducing terminus (alpha-linkage) that is essential for sperm receptor function *in vitro*, and at least certain of the biologically active oligosaccharides are located on the carboxy-terminal half of the mZP3 polypeptide. Embryonal carcinoma cells, stably transfected with the mXP3 gene, synthesize and secrete functional sperm receptors, suggesting that the glycoprotein is properly O-glycosylated in these cells. These cell lines, together with other experimental approaches, such as exon-swapping and site-directed mutagenesis, are being used in our laboratory currently to investigate *O*-linked glycosylation of mZP3 specifically and glycoproteins generally.

Wassarman, PM, *Science*, **235**, 553–560, 1987; *Development*, **108**, 1–17, 1990; *Seminars in Develop Biol*, **4**, 189–197, 1993.

In defense of the oral cavity: the role of salivary mucins

L.A. Tabak

Department of Dental Research and Biochemistry, University of Rochester, Rochester, NY, USA

Salivary mucins form a protective coating on the hard and soft tissues of the mouth which lubricate the oral cavity and form a barrier against exogeneous insult. In addition, mucins favor the attachment and subsequent proliferation of specific mircroorganisms while facilitating the clearance of others. Human saliva contains two structurally and functionally distinct mucins, MG1 (M_r >1000 kDa) and MG2 (M_r 120–150 kDa). MG1 is a heavily glycosylated (>75% carbohydrate by weight), oligometric macromolecule which by virtue of its unique rheological properties is largely responsible for tissue coating. MG2 consists of a single polypeptide backbone which is substituted with about 100 oligosaccharide side chains. Two glycoforms have been identified which differ in their level of fucose and sialic acid. The predominant acidic oligosaccharide of MG2, NeuAcα2,3Galβ1,3GalNAc, specifically interacts with microbial lectins on *Streptococcus gordonii* and *Streptococcus oralis*, whereas, the neutral oligosaccharide Galβ1,3GalNAc interacts with Streptococcus *mutans*. Since little MG2 appears to bind to the tissue coat *in vivo*, it is available to interact selectively with members of the oral flora, thereby masking their adhesions and effecting their clearance from the mouth. The elucidation of mucin structure/function relationships has provided a strong rationale for the development of future therapeutic modalities to treat oral disease. For example, over 3 million Americans suffer from some form of salivary hypofunction ('dry mouth'). Mucin-based artificial saliva has shown the greatest promise for treatment. However, given the structural complexity of mucins, it will be necessary to identify simple natural analogs of mucin, or produce peptide mimetics of mucin carbohydrate function. Oral infections such as dental caries and the periodontal diseases remain as the most prevalent human infections. Anti-adhesion strategies exploring adhesion-expression blockers or adhesion-binding blockers are approaches worthy of exploration.

GPI anchors of African trypanosomes: potential targets for anti-parasitic chemotherapy

T.L. Doering

Department of Molecular & Cellular Biology, University of California, Berkeley, CA, USA

Glycosyl phosphatidylinositol (GPI) structures anchor diverse eukaryotic proteins to the cell surfaces of organisms ranging from yeast to mammals. These anchors consist of a complex glycan core linking the protein C-terminus to phosphatidylinositol in the outer leaflet of the plasma membrane. The best-studied GPI-anchored protein is the variant surface glycoprotein (VSG) of *Trypanosoma brucei*, the protozoan parasite responsible for African sleeping sickness. The VSG anchor is unique in containing only myristic acid (a 14 carbon saturated fatty acid) in its lipid moiety, in contrast to the lipid mixtures present in most GPIs. This specificity is striking because myristate is relatively rare fatty acid, which is not synthesized *de novo* by the parasite.

We have been investigating various aspects of the biosynthesis of the trypanosome GPI anchor, most recently concentrating on the specific metabolism and incorporation of myristate. Myristate can be incorporated during initial anchor synthesis (Masterson *et al.*, *Cell*, **56**, 793, 1989), or via a newly discovered myristate exchange reaction occurring on VSG protein (L.U. Buxbaum and P.T. Englund, unpublished). We have used myristate analogs to study these processes. Several analogs containing oxygen heteroatoms in the alkyl chain are utilized for GPI synthesis both by intact trypanosomes and in a cell-free system. One of these is highly toxic to trypanosomes in culture, but it is not toxic to mammalian cells or to insect-form trypanosomes which bear non-myristoylated GPIs (Doering *et al.*, *Science* 1991: **252**; 1851). This selective toxicity may be related to the metabolism or function of the VSG GPI. We have now tested 267 myristate analogs which contain a range of substituent groups, desaturations, and heteroatoms; several of these are more potent than the oxygen-containing analogs previously studied. These compounds offer a potential approach to anti-trypanosomal chemotherapy.

The lipophosphoglycan of *Leishmania* parasites: a multifunctional virulence factor

S.J. Turco, A. Descoteaux, K. Ryan, L.A. Garraway and S.M. Beverley

Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY and Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Protozoan parasites of the genus *Leishmania* have the remarkable ability to avoid destruction in the hostile environments they encounter throughout their life cycle, especially in phagolysosomes of host macrophages. Lipophosphoglycan (LPG) is the major surface glycoconjugate of the promastigote form of these pathogens and is an essential virulence determinant. An intriguing function of this glycoconjugate is its inhibitory effect on protein kinase C-mediated activation of macrophages. Preincubation of macrophages with LPG was found to inhibit the production of oxidative burst, c-*fos* gene expression, chemotaxis, and IL-1 in response to activators of protein kinase C. The consequence of this inhibitory effect appears essential for successful intraphagolysosomal establishment by the parasite. Some of the functions that have been attributed to LPG were determined with the use of LPG-defective mutants of *Leishmania*. To explore the genetic aspects of LPG expression, and hence virulence, we have begun to undertake functional genetic complementation of these mutants defective in LPG biosynthesis.

Multiple attachments of Herpes viruses during cell entry

A.O. Fuller

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

Human herpes viruses cause widespread morbidity and mortality, particularly in immune incompetent individuals. Current therapies that affect genome replication

allow frequent occurrence of drug resistant mutants and establishment of virus latency. Elucidation of the molecular events in virus entry into cells focuses on one possible event that could be circumvented to prevent infection, or be exploited for design of effective virus vectors. We proposed and provided evidence for a model of Herpes simplex virus type 1 (HSV-1) entry through a cascade of events that allow direct fusion with the cell plasma membrane at neutral pH (*J Virol*, **66**, 5002, 1992). HSV-1 entry involves several types of attachment to ubiquitous heparan sulfate (HS) proteoglycans and to other non-HS cellular receptors (*Virology*, 1993, in press). Cells that are highly susceptible to HSV-1 have both HS and non-HS receptors (*J Virol*, **67**, 5088, 1993). We recently characterized a poorly susceptible cell line that is defective in HSV-1 entry, but otherwise is competent for virus replication. Biochemical analyses and the effect of sodium chlorate on virus infectivity show that the poorly susceptible cells contain functional HS, but lack functional non-HS stable attachment receptor(s) used by HSV-1 (Subramanian *et al.* 1993, Submitted). Highly susceptible and poorly susceptible cell lines have been used to demonstrate that the presence of HS increases the efficiency of HSV entry, but it does not appear to be sufficient, or required, for infection. Finally, susceptibility to HSV-1 infection can be transferred to poorly susceptible cells by transfection of human genomic DNA. This system provides a novel resource to identify and characterize the non-HS receptor on human cells that is required for efficient HSV-1 infection. Accumulating evidence shows that attachment to HS seems to be a common initial event in entry of HSV-1 and HSV-2, human cytomegalovirus, varicella zoster virus, pseudorabies virus and bovine herpes virus type 1 and type 4. This interaction may be part of a widespread mechanism of multiple attachments by which herpesviruses, and perhaps other viruses, mediate neutral pH entry and specific cell and tissue tropisms during lytic and latent infection.

Regulatory paradigm for new glycobiology therapies

K. Zoon

Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA

New biological products derived from biotechnology have been licensed for use as therapeutics and vaccines. These products are derived from diverse sources which include recombinant expression systems, hybridoma cell lines, and extracts from crude cell membranes. Licensed therapeutic products include glycoproteins derived from hybridoma cell culture. Effective polysaccharide vaccines are also derived from conjugation of purified polysaccharides with different carriers to provide effective immunity. The oligosaccharide structure of these adverse products can affect the physiochemical (solubility, stability, structure) and biological (pharmacokinetics, pharmacodynamics, antigenicity, specific activity, site of action) properties. Therefore, it is critical that biological products be produced consistently in order to ensure the safety, purity, and potency. It is difficult to unambiguously characterize the carbohydrate moieties of many biological products due to the microheterogeneity of oligosaccharide species and the current limitations in analytical technologies. However, the process for regulating the production of historical biological products as applied to products derived from new biotechnological processes has resulted in the licensure of safe and efficacious products. These issues will be discussed in the context of current and future biological products.

Human fucosyltransferase genes: structure, function and roles in selectin ligand synthesis

J.B. Lowe, D.J. Legault, S. Natsuka, Y. Natsuka, K.M. Gersten, P.L. Smith, R.J. Kelly, J.W. Homeister, P. Maly, A.D. Thall, B. Petryniak and N. Hiraiwa

The Howard Hughes Medical Institute and the Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

Fucosylated cell surface oligosaccharides are expressed with temporal and spatial precision during mammalian development. A subset of these molecules, represented by the sialyl Lewis x and sialyl Lewis a determinants, are known to function as counter-receptors for E- and P-selectin-mediated immune cell adhesion during inflammation. Related molecules have also been implicated in adhesive events during mammalian embryogenesis. We have begun to approach these issues by cloning and studying α(1,3)fucosyltransferase genes that determine the expression of such α(1,3)fucosylated molecules. We have used expression cloning and cross-hybridization approaches to isolate and characterize five such human genes. Comparison of their sequences indicates that their respective acceptor substrate specificities are determined in large part by peptide sequences within a 'variable' region located near their respective amino termini. This notion has been confirmed in experiments where we have constructed and tested chimeric recombinant α(1,3)fucosyltransferases, in which various subdomains taken from the variable regions have been exchanged among the different fucosyltransferases. Analysis of the catalytic activities of these enzymes expressed in transfected mammalian host cells indicates that discrete peptide segments within these variable domains play pivotal roles in substrate recognition. These cloned α(1,3)fucosyltransferase genes have also been used as tools to identify ligands for E-selectin, and to create sialyl Lewis x-containing oligosaccharides, that have in turn been used as anti-inflammatory compounds in animal models of selectin-dependent inflammation.

Alcohol-induced changes in glycobiology

W.E.M. Lands

Division of Basic Research, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD, USA

Alcohol-related research provides many opportunities for new research initiatives that apply methods in glycobiology to identify underlying mechanisms of alcohol induced tissue damage. Alcohol causes devastating effects in almost every organ system, producing liver disease, cardiovascular disorders, fetal abnormalities, and neuropathological illness. Alcohol claimed more than 108000 lives in 1989, and the years of potential life lost to alcohol-related events exceeded the years lost to

heart disease and approached the years lost to cancer. Chronic ingestion of alcohol impairs the integrity of glycoproteins and glycolipids in liver, brain, erythrocytes and intestine. For example, a carbohydrate-deficient transferrin (CDT) found in plasma is a reliable marker of alcohol abuse. Activities responsible for these alcohol-induced changes may include increased glycosidase and/or decreased glycosyltransferase. More research is needed to characterize the extent to which impaired immune functions in chronic alcoholics and alcohol-induced liver damage and birth defects may also be related to impaired integrity of the cellular glycoconjugates that regulate adhesion and intercellular signaling. This situation provides a clear invitation to glycobiologists to develop a better understanding of the scope and intensity of the molecular and cellular mechanisms whereby alcohol exerts its wide range of effects on tissue functions so that new interventions may be designed to ameliorate alcohol's impact.

Glycosylation and glycoprotein hormone function

J.U. Baenziger and S.M. Dharmesh

Department of Pathology, Washington University Medical School, St. Louis, MO, USA

Lutropin (LH) was the first glycoprotein demonstrated to bear Asn-linked oligosaccharides on its α and β subunits terminating with the sequence SO_4-4GalNAcβ1, 4GlcNAcβ1,4Mana. Subsequently other glycoproteins were described which bear these same structures including thyrotropin and pro-opiomelanocortin. A protein-specific GalNAc-transferase which recognizes the tripeptide motif ProXaaArg/Lys and a saccharide-specific sulfotransferase mediate the synthesis of these structures. In the absence of the GalNAc and sulfotransferase, galactose and sialic acid are added in place of GalNAc and sulfate. The presence or absence of sulfated oligosaccharides on LH dramatically affects its bioactivity by controlling hormone circulatory half life and activation of the hormone receptor. The effect on circulatory half life reflects clearance by a receptor in liver specific for the sulfated oligosaccharides on LH. LH synthesis increases dramatically at the time of the pre-ovulatory 'surge' and following ovariectomy. We have found that the levels of GalNAc- and sulfo-transferase in gonadotrophs change in response to estrogen levels in the same manner as LH synthesis. As a result the sulfated oligosaccharides are maintained on LH throughout the ovulatory cycle. As a result, the short circulatory half life of LH, which may be essential for its *in vivo* activity, is maintained throughout the ovulatory cycle.

Design of potential clinical glycoprotein analogs by recombinant DNA methods

I. Boime, F. Fares, M. Furuhashi, A.J.W. Hsueh*, P. LaPolt*, E. Perlas* and T. Shikone*

*Department of Pharmacology, Washington University Mededical School, St Louis, MO and *Stanford University, Stanford, CA, USA*

Human chorionic gonadotropin (hCG), luteinizing hormone, follicle-stimulating hormone (FSH) and thyrotropin are a family of glycoprotein hormones that share a common a subunit but differ in their hormone-specific β subunits. Gene transfer techniques together with site-directed mutagenesis have been valuable tools for elucidating functional determinants of the glycoprotein hormone family. Similar methodology provides a strong approach for designing therapeutic analogs of these hormones. FSH is used clinically to stimulate the ovarian follicles for *in vitro* fertilization and to initiate follicular maturation in anovulatory women with chronic anovulatory syndrome or luteal phase deficiency. hCG has been useful for the treatment of male hypogonadism. The CGβ subunit contains a hydrophilic carboxy-terminal extension with four serine *O*-linked oligosaccharides. We showed previously that this extension is important for maintaining the circulating half-life of hCG. One major issue regarding the clinical use of FSH is its relatively short half-life in circulation. To enhance the potency of FSH, the carboxy-terminal extension of CGβ was fused to the 3′ end of FSHβ coding sequence. This sequence did not affect assembly of the chimera with the a subunit or secretion of the dimer. The *in vivo* potency of FSH dimer containing a β-CTP chimera was increased over four-fold. In addition, although hCG did not induce ovulation in female rats primed with a single dose of wild-type FSH, hCG-induced ovulation was seen with animals primed with less than one-third of a single dose of the chimera. Since the a subunit is common to LH, FSH, hCG and TSH, the CTP was inserted in the subunit to increase the half-life of all four hormones (and derivatives thereof) with one change. When the CTP was fused to the carboxy terminus of the a subunit, receptor binding of the resulting dimers (FSH or hCG) was decreased; this is presumably due to the presence of determinants for receptor binding/signal transduction at the carboxy end of the α subunit. If inserted near the amino terminus of the α subunit, the CTP sequence of FSH or hCG did not effect receptor binding or steroidogenesis of FSH or hCG *in vitro*. The *in vivo* activity of these dimers are being tested. These data support the hypothesis that the *O*-linked region in the CGβ subunit is necessary for extracellular stability. The results show the potential of the FSH chimeras as therapeutic agents. In addition, the presence of the CTP sequence may represent a general method for enhancing the *in vivo* longevity of different proteins.

Targeted gene delivery and expression in hepatocytes

G.Y. Wu, J. Roy Chowdhury, N. Roy Chowdhury and C.H. Wu

Department of Medicine, Division of Gastroenterology–Hepatology, University of Connecticut School of Medicine, Farmington, CT and Albert Einstein College of Medicine, Bronx, NY, USA

We have developed a DNA carrier system by which genes can be targeted and expressed in mammalian liver *in vivo* based on the presence of asialoglycoprotein (AsG) receptors that are highly selective for these cells. An AsG was coupled to poly L-lysine (PL) to form a conjugate carrier capable of binding DNA in a non-covalent manner. The gene for human low density lipoprotein (LDL) receptor was complexed to an AsG-PL to form a soluble DNA complex. Injection i.v.

into Watanabe rabbits led to transient decreases in cholesterol and LDL. In Nagase analbuminemic rats, conjugates complexed to DNA containing the human albumin structural gene were injected i.v. into rats followed by 67% partial hepatectomy to stimulate hepatocyte replication. Plasmid DNA was found at a level of 1000 copies/cell 2 weeks after injection. Human albumin mRNA was also detected at this time. Circulating albumin levels were undetectable 24 hrs after injection of the DNA complex, but became easily measured at 5 μg/ml after 48 h, reached a maximum level of 34 μg/ml by 4 weeks post-injection and persisted for at least 11 weeks at this level. Persistence was determined to be temporally related to disruption of microtubules during regeneration following partial hepatectomy. Pre-treatment of rats with a microtubule disruption agent, colchicine, resulted in persistence of CAT gene expression for at least 4 weeks. Conclusions: A normal gene targeted by a soluble DNA carrier system and expressed in liver, can result in persistent secretion of a normal serum protein and partial correction of a genetic defect *in vivo*. Persistence of targeted gene expression can be achieved by pharmacological means. Supported in part by grants from NIH: DK42182 (GYW), March of Dimes 1-0786 (GYW); and TargeTech, Inc. (CHW).

Liver cell targeting through a route followed by sporozoites during malaria infection

C. Cerami, U. Frevert, B. Takacs* and V. Nussenzweig

*Michael Heidelberger Division of Immunology, Department of Pathology, New York University Medical Center, New York, NY, USA and *Pharmaceutical Research-New Technologies, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

During feeding by infected mosquitoes, malaria sporozoites are injected into the host's bloodstream and enter hepatocytes within minutes. The speed and the specificity of the invasion indicate that it is receptor-mediated. The plasma membrane of sporozoites is covered with the circumsporozoite protein (CS). We have shown that CS binds specifically *in vitro* to the regions of the plasma membrane of hepatocytes which are exposed to circulating blood in the Disse space (*Cell*, **70**, 1021, 1992). The ligand is the region II-plus of CS (EWxxCxVTCGx GxxRxK/R), an evolutionarily conserved region of the protein which has a striking homology to a cell-adhesion motif of thrombospondin. The hepatocyte receptor for region II-plus is a heparan sulfate proteoglycan tightly associated with the plasma membrane, which shares some properties with the syndecan family of proteoglycans (*J Exp Med*, **177**, 1287, 1993). We have now found that intravenously injected recombinant CS constructs bind rapidly and specifically to the basolateral surface of hepatocytes in the Disse space, provided that the recombinants contain region II-plus. These findings suggest that similar mechanisms are involved in the recognition of CS *in vitro* and *in vivo*, and provide a basis for development of inhibitors for possible use in the prevention of malaria infection. Outside the malaria field, the specificity of region II-plus for hepatocytes *in vivo* raises the intriguing possibility of utilizing this motif for targeting drugs or genetic material to those cells. It is conceivable that the incorporation of the region II-plus amino acid sequence in the envelope protein of a recombinant virus will enhance its capture by hepatocytes.

Diagnosis and characterization of lysosomal disorders resulting from defective glycoconjugate metabolism

D.W. Wenger

Departments of Medicine (Medical Genetics) and Biochemistry & Molecular Biology, Jefferson Medical College, Philadelphia, PA, USA

Lysosomal disorders figure prominently in the field of glycobiology for several reasons. All known lysosomal proteins are glycoproteins, many lysosomal enzymes are responsible for the catabolism of glycolipids, glycoproteins, oligosaccharides and glycosaminoglycans, and studies on patients with lysosomal disorders have led to major findings on the biosynthesis and processing of glycoproteins, on the range of glycoconjugates produced by humans and on the role of lysosomal membrane proteins in the export of sugars and other compounds from lysosomes. Although lysosomal glycosidases have strict specificity for the sugar moiety and its anomeric configuration, their activities can usually be easily measured using one of several synthetic substrates. These compounds make the diagnosis of lysosomal disorders relatively simple. However, these substrates are not successful in differentiating the clinical subtypes of a given disease. Essentially all of the genes coding for the lysosomal glycosidases have been cloned, and attempts at defining the regions critical for substrate specificity, function and stability are being made. The lysosomal disorders are also fortunate in having well characterized animal models available for comparison and for attempts at treatment by enzyme supplementation and gene therapy.

Glycobiology and glycopathology of Immunoglobulin G

A. Kobata, T. Endo*, N. Tsuchiya† and K. Furukawa*

*Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; *Institute of Medical Science and †Faculty of Medicine, University of Tokyo, Tokyo, Japan*

Immunoglobulin G (IgG) contains a biantennary sugar chain at Asn 297 of each heavy chain. Decrease in the galactose content of IgG was found in patients with rheumatoid arthritis (RA). Such galactose-deficient IgG showed lower affinity to C1q and human Fcg receptor type 1 than normal IgG, but showed the same affinity to polyclonal IgM-type rheumatoid factor and protein A as normal IgG. It was confirmed that the lower galactosylation in RA results from the reduced activity in B cells of a galactosyltransferase, which preferentially transfers galactose to asialo-agalacto IgG. Enzyme kinetic studies revealed that affinity of the galactosyltransferase in B cells from RA patients was lowered for UDP-Gal but not for asialo-agalacto-IgG. Although the phenomenon of galactose deficiency in the serum IgG is highly correlated with RA, more field work including family studies are necessary before applying this interesting phenomenon to the clinical field. Structural analysis of the sugar chains of purified IgG with use of hydrazinolysis affords the most accurate determination of the galactose content, but the method is not suitable for

clinical laboratories. We recently found that *Psathyrella velutina* lectin (PVL) reacts preferentially with the GlcNAcβ1,2Man group. An ELISA-based assay for the detection of agalactosylated IgG was developed using recombinant protein G and biotinylated PVL. Screening of patients revealed that binding of serum IgG to the lectin is highly correlated with the galactose deficiency determined by conventional structural analysis.

Role of heparan sulfates in angiogenesis

J. Folkman

Harvard Medical School, Boston, MA, USA

No abstract available.

Proteoglycans in vascular disease

T.N. Wight

Department of Pathology, University of Washington, Seattle, WA, USA

Proteoglycans (PGs) are enriched in the arterial intima and accumulate in vascular disease. The principle PGs present in arterial tissue and synthesized by vascular cells *in vitro* include large interstitial PGs containing chondroitin sulfate (versican) and heparan sulfate (perlecan) as well as small interstitial dermatan sulfate containing PGs (decorin and biglycan). The synthesis of these PGs are regulated in part by growth factors and cytokines. For example, both PDGF and TGF-β1 increase mRNA transcript levels for versican in cultured arterial smooth muscle cells while not affecting decorin mRNA. On the other hand, IL-1 decreases versican synthesis by these same cells while TGF-β1 but not PDGF, increases biglycan mRNA levels. In addition, these growth factors cause specific post-translational changes in the processing of the glycosaminoglycan (GAG) chains. PDGF and TGF-β1 cause elongation of the GAG chains attached to versican, decorin and biglycan, while PDGF and not TGF-β1, causes enrichment of the 6-sulfated disaccharide in the chondroitin sulfate chain of versican. These changes may influence the capacity of these PGs to interact with component molecules involved in vascular disease. For example, versican binds specifically to low density lipoproteins while perlecan binds with high affinity to amyloid. Decorin and perlecan interact with growth factors such as PDGF and bFGF. These interactions may regulate, in part, the accumulation and activity of these components in vascular disease.

Astenose—a drug for the prevention of restenosis

N.R. Ackerman

Glycomed, Incorporated, Alameda, CA, USA

The proliferation of smooth muscle cells is under the control of both growth stimulation and growth inhibitory factors. Growth promoters include platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and angiotensin II. The major endogenous growth inhibitory factor is heparan sulfate proteoglycan. This material is a part of the extracellular matrix of endothelial cells which are normally in contact with the quiescent (*i.e.* non-proliferating) smooth muscle cells. Addition of exogenous heparin to rapidly proliferating smooth muscle cells leads to a marked inhibition of their replication. Heparin is used clinically as an anticoagulant. This effect is manifested by a specific short sequence, consisting of five monosaccharide units, which specifically interact with endogenous anticoagulant antithrombin III (ATIII). This interaction of ATIII with heparin leads to its efficient binding to and subsequent inactivation of a number of serum proteases in the coagulation system. A different part of the heparin molecule is capable of blocking the proliferation of smooth muscle cells, hence one may generate a heparin molecule having minimal effects on the coagulation system yet be an effective anti-restenotic agent. Astenose has these properties. Astenose is produced by oxidizing heparin under mild reaction conditions. This resultant product retains most of the properties of heparin including the smooth muscle anti-proliferative actions without demonstrating significant anticoagulant activities. When it was evaluated in culture vs. proliferating rat and human smooth muscle cells, it blocked proliferation with essentially the same activity as did heparin. The anticoagulant (APTT) activity, however, was reduced by >90%. Astenose was also evaluated in rat and rabbit models of restenosis. In both species, Astenose significantly protected against the smooth muscle response to balloon angioplasty. These results demonstrate that Astenose has a high level of activity in models of smooth muscle cell proliferation and restenosis and is virtually devoid of anticoagulant activity. The effects noted in the angioplasty-induced restenosis models may in part be due to the anti-proliferative action of the material. In addition, heparin and Astenose have an anti-thrombotic effect as well as an ability to complex a variety of growth factors such as PDGF and bFGF. The high level of efficacy observed with Astenose may be due to multiple activities of a heparin based therapy. An IND is scheduled for early 1994 for a restenosis indication.

Selectins and leukocyte adhesion in human disease

J.C. Paulson

Cytel Corporation and The Scripps Research Institute, San Diego, CA, USA

The selectins represent a family of cell adhesion proteins whose function in leukocyte trafficking is well understood and represents a paradigm for the biological role of a mammalian carbohydrate binding protein. The selectin family consists of three known members which are involved in the recruitment of neutrophils and other leukocytes to sites of inflammation. Two of these, E-Selectin and P-Selectin, are expressed on vascular endothelium of inflammatory sites initiating the tethering of the cells to the blood vessel wall, allowing them to literally roll under the shear force of the blood flow. Both of these selectins recognize the carbohydrate, Sialyl Lewis X (SLe^x), present on glycoproteins of the neutrophil. Direct evidence for the importance of the selectin-SLe^x interaction in recruitment of leukocyte neutrophils to sites of inflammation has come from a genetic deficiency in the carbohydrate ligand known as leukocyte adhesion deficiency type II (LAD II; Etzioni *et al. New*

Engl J Med, **327**, 1789, 1992). Analysis of these patients suggests that the selectin mediated rolling of neutrophils increases efficiency of recruitment to inflammatory sites by 10- to 20-fold. For these reasons, the endothelial cell selectins may be suitable targets for development of anti-inflammatory agents that prevent neutrophil mediated damage by blocking their exit from the blood into tissues. Indeed, both anti-selectin antibodies and small molecule carbohydrate analogs of SLe^x have been found to reduce injury in multiple animal models of disease. The elucidation of the biological role of the selectins and resultant progress in development of carbohydrate-based therapeutics stands as an example for the future applications of glycobiology to problems in human disease.

Approaches to the identification, synthesis and modeling of natural ligands for ELAM-1

R.B. Parekh, T.P. Patel, C.C. Campion, M.R. Wormald* and C.J. Edge*

*Oxford GlycoSystems, Abingdon and *Oxford Glycobiology Institute, Oxford, UK*

An early event in the recruitment of leukocytes to sites of inflammation is the adhesion of carbohydrate(s) on circulating leukocytes to the lectin ELAM-1, whose expression is induced on endothelial cells by inflammatory cytokines. The importance of the ELAM-1 dependent adhesion pathway has now been shown *in vivo*, and much interest centers around the identification of the ELAM-1 carbohydrate ligand. Many of the approaches taken towards identifying this carbohydrate have indicated that the blood group determinant sialyl Lewis X (NeuNAcα2,3Galβ1,4[Fucα1,3]GlcNAc) can bind to ELAM-1. While the sialyl Lewis X (and sialyl Lewis a) structures may prove useful as pharmacophores for the development of more potent antagonists, relatively few attempts have been made to characterize the 'natural' set of ELAM-1 carbohydrate ligands. Towards this end, we have performed a systematic study of the *N*- and *O*-glycans associated with the total cell-surface glycoproteins of various cell lines, including human PMNs, and correlated the expression of individual *N*- and *O*-glycan structures to the ability to bind cells expressing ELAM-1. The results indicate that the 'natural' set of glycoprotein-associated glycan ligands for ELAM-1 consist of multi-antennary (tri- and tetra-) complex type *N*-glycans, one or more of whose outer-arms terminate in 3-sialyl poly Lewis X. Subsequent *de novo* chemical synthesis of 3-sialyl Lewis X, 3-sialyl Lewis a and related carbohydrates as well as a variety of myristic acid conjugates has been performed. This is allowing some of the important details of ELAM-1-carbohydrate binding to be addressed, including the relative potency of individual carbohydrates as competitive inhibitors of ELAM-1 dependent adhesion, and the effects of multivalent presentation of carbohydrate ligand on the potency of synthetic inhibitors. In addition, analysis of solution conformations (using 600 MHz one- and two-dimensional ^{1}H-NMR), and modeling studies on 3-sialyl Lewis X, 3-sialyl Lewis a and related structures has allowed the identification of structural epitopes that appear to be conserved in carbohydrates that are able to bind ELAM-1. These epitopes are proving useful in the design of non-carbohydrate, synthetic antagonists of ELAM-1 binding.

Glycosylation of membrane proteins and cell adhesion

K. Olden

National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

No abstract available.

Glycosylation and the metastatic phenotype

J.W. Dennis, B. Korczak and A. Datti

Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada

Neoplastic transformation in both murine and human cells is commonly accompanied by structural alterations in *N*-linked oligosaccharides, in particular, the expression of highly branched complex-type oligosaccharides, polylactosamine and Lewis antigens. Studies on tumor cell glycosylation mutants and drugs which inhibit oligosaccharide processing suggest that expression of branched complex-type *N*-linked oligosaccharides are required for efficient tumor cell metastasis. Lactosamine-containing oligosaccharides on the tumor cells surface function as ligands for mammalian lectins and can enhance the retention of blood-born tumor cell in the microvasculature of host tissues. Expression of embryonic carbohydrate sequences (*i.e.* polylactosamine, extended-chain Lewis X and Y) in tumor cells which may play a role in organ retention is dependent upon regulated expression of specific glycosyltransferases. These include the β1-6GlcNAc-branching of *N*- and *O*-linked core oligosaccharides; *N*-acetylglucosaminetransferase V (GlcNAc-TV), and core 2 GlcNAc-T, respectively. In several cell culture models of transformation and differentiation, the activity of GlcNAc-TV and core 2 GlcNAc-T are regulated and constitute an important rate limiting step in the biosynthetic pathway of polylactosamine and associated Lewis antigens. Studies with somatic glycosylation mutants and with the processing inhibitor swainsonine suggest that multiple cellular phenotypes are affected, including tumor cell invasion and proliferation. These observations prompted our recent investigation to determine whether the loss of sialylated complex-type *N*-linked oligosaccharide in tumor cells affects the expression of genes which, in turn, could influence the malignant phenotypes. In both human and rodent tumor cells, we observed a selective increase in c-*jun* and tissue inhibitor of metalloproteases (TIMP) mRNA levels under conditions where *N*-linked processing was inhibited either by the alkaloid swainsonine or by stable glycosylation mutations. This suggests that *N*-linked oligosaccharide processing may be an integral element of the cellular phenotype controlling expression of select genes. Altered glycosylation of growth factor, lymphokines and/or their receptors on the cell surface are possible mediators of phenotype charge; an avenue which is currently being investigated.