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International Glycoconjugate Organization Award 2003

Pamela Stanley, Ph. D.

Dr. Pamela Mary Stanley (nee Fetherstonhaugh) was born in Melbourne, Australia. She graduated from the University of Melbourne and obtained her Ph.D. degree from this University in 1972 with a thesis on the Influenza Virus Proteins. In 1972 she took the position of postdoctoral fellow of the Medical Research Council in the Department of Medical Genetics, University of Toronto, Canada and from 1976 she was research associate. With Dr. Siminovitch she started in Toronto her groundbreaking work on the isolation and characterization of 'Lectin Resistant Hamster Ovary Cells'. In 1977 she moved to the Department of Cell Biology at the Albert Einstein College of Medicine, Bronx, New York, U.S.A. as Assistant Professor. Since 1986 she holds the position of Professor at that Institute.

In the framework of the research of Dr. Siminovitch to expose mammalian cell lines, like CHO cells, to various toxic agents in order to select mutant cell lines that were resistant to these toxins, Dr. Pamela Stanley studied the effects of a variety of toxic lectins. This work led to the isolation of a number of lectin-resistant CHO lines. It was concluded that these cell lines must have different glycosylation defects. Subsequently, it was found that one of these cell lines was defective in *N*-acetylglucosaminyltransferase I. To determine the molecular consequences for the glycoprotein structures, Dr. Pamela Stanley collaborated with Dr. D. Summers and set up the VSV G glycoprotein system for the analysis of N-glycan structures.

After moving to the Albert Einstein College, she focused her research on determining the biochemical basis of several mutants and on designing selections to get new CHO glycosylation mutants. Ultimately, she identified many of the genetic glycosylation defects of these cells. In addition, she observed the occurrence of 'gain-of-function' mutants that arose from the *de novo* expression of developmentally regulated glycosyltransferases.

Dr. Pamela Stanley contributed to the elucidation of aspects of glycosylation pathways and to the cloning of key glycosyltransferase genes. Impressive were her achievements in the creation of knockout mice with deficiencies in glycosyltranferases. She found that complex or hybrid N-glycans are required for development beyond mid-gestation. Chimeric mice pointed to the need for complex or hybrid glycans in the formation of the organized layer of bronchial epithelium.

In recent years, Dr. Pamela Stanley has shown in collaboration with other groups that Fringe is a β1,3-*N*-acetylglucosaminyltransferase involved in both Fringe and Notch signaling during development. Over the years Dr. Pamela Stanley has been most generously sending her cell lines to a large number of researchers. Many of these cell lines have played a vital role in the research of numerous glycobiologists. Dr. Pamela Stanley received several awards, has published about 100 papers in leading journals and has authored 16 review papers. Furthermore, she has mentored a large number of young glycobiologists.

At present, Dr. Pamela Stanley is one of the leading glycobiologists. Clearly, she is in the middle of a most productive scientific career. Therefore, the members of the selection committee all agree that Dr. Pamela Stanley should be selected as the recipient of the International Glycoconjugate Award 2003.

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Abstracts

Oral presentations

Keynote Address

Targeting glycosylation as a therapeutic approach

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Increased understanding of the role of protein – and lipid-linked carbohydrates in a wide range of biological processes has led to interest in drugs that target the enzymes involved in glycosylation. But given the importance of carbohydrates in fundamental cellular processes such as protein folding, therapeutic strategies that modulate, rather than ablate, the activity of enzymes involved in glycosylation are likely to be a necessity. Two such approaches that use imino sugars to affect glycosylation enzymes now show considerable promise in the treatment of viral infections (such as hepatitis B, hepatitis C, Dengue and Japanese encephalitus virus) and in glucosphinglolipid storage disorders (such as Gaucher disease).

The data from 36 month clinical trials in Type I Gaucher disease will be presented, as will studies in mouse models, with CNS storage. The use of imino sugars as anti-virals in appropriate animal models will be described for hepatitis B, Dengue and Japanese encephalitis virus. Additionally, a new class of imino sugars which exert their anti-viral effects by a different mechanism will be described and initial data for their evaluation in Hep C patients as an anti-viral will also be presented.

International Glycoconjugates Organization Award

From Lec 1 to Notch signaling – a glyco-evolutionary tale

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Mice that lack *N*-acetylglucosaminyltransferase 1 (GlcNAc-TI) encoded by the *Mgat1* gene die at mid-gestation. However, the null mutation is not expressed in Mgat1$^{-/-}$ blastocysts due to the presence in all blastocysts of maternal Mgat1 mRNA that allows production of GlcNAc-TI and the synthesis of complex *N*-glycans. Therefore, to determine whether complex *N*-glycans are required for pre-implantation development, oocytes that lack GlcNAc-TI are necessary. To obtain chimeric females with oocytes derived from *MgatI*$^{-/-}$ ES cells, 3.5 dpc blastocysts have been injected with MgatI$^{-/-}$ ES cells. *In situ* hybridization will determine whether ES cells lacking GlcNAc-TI can develop into oocytes. In another approach, female mice with a "floxed" *Mgat1* gene have been crossed with mice carrying a Cre transgene under the control of the Zp3 promoter in order to obtain oocyte specific-elimination of the *Mgat1* gene. Female mice with oocytes surrounded by a zona pellucida (ZP) that does not bind the lectin L-PHA, and thus lacks complex *N*-glycans have been obtained. Histochemical analyses and Con A staining indicate that these oocytes have a thin zona pellucida (ZP). Antibodies against mouse proteins Zp1, Zp2 and Zp3 are being tested for binding to the oocytes. In addition, females with oocytes lacking GlcNAc-TI are being mated to *Mgat1*$^{+/+}$ males. These experiments will define the earliest stage of development at which complex *N*-glycans are required.

Glyco XVII Lecture

Humanization of glycoprotein production in insect cells

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Production of glycoproteins in insect cells has some advantages over that in animal cells, especially with respect to cost and safety. However, glycoproteins produced in insect cells also have shortcomings with respect to the nature of *N*-glycan structures. Most of the established insect cell lines typically produce high-mannose type N-glycans rather than complex-type or paucimannosidic type, and sialic acids are extremely rare. Therefore, production of glycoproteins containing *N*-glycans similar to those found in humans requires substantial modification of biosynthetic schemes. This modification involves the introduction of glycosyl transferases required to fabricate complex-type *N*-glycans (e.g., β-galactosyl transdferase, sialyl transferase). Some sugar nucleotides lacking in insect cells must be supplemented either by genetic engineering and/or substrate manipulation. The presence of Fucα(1-3) linked to GlcNAc adjacent to Asn is potentially allergenic, and may be problematic. Moreover, a specific β-*N*-acetylglucosaminidase, which prevents proper extension of the glycan chains must be considered as well.

1.Tomiya, N, Howe, D, Aumiller, JJ, Pathat, M, Park, J, Palter, K Jarvis, DL, Betenbaugh, MJ, and Lee, YC, *Glycobiology*, in press

Plenary Lectures

PL#1

Evolutionary explorations of sialic acid biology

A Varki
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The sialic acids are a family of nine-carbon acidic sugars displayed at the outer ends of sugar chains on cells and soluble proteins of vertebrates and certain higher invertebrates (1). In our group, we study the recognition of sialic acids by lectins that are endogenous or exogenous to the organism synthesizing them (1-3), the role of sialic acids in tumor spread (2), and differences in sialic acid biology between humans and great apes (4,5). This lecture will provide an overview of some of these efforts, with an emphasis on evolutionary considerations (1,3,4,5). Topics discussed will include analysis of new genomic data to understand the origins of sialic acids (1); the apparent involvement of sialic acids in an evolutionary arms race between vertebrates and their pathogens (1,3,4,5); and potential implications of the human-ape differences for microbial disease susceptibility, the innate immune response, diseases associated with meat consumption, and for the evolution of the human brain.

1. Angata T, and Varki A. *Chemical Reviews* **102**, 439-469 (2002)
2. Varki N and Varki A. *Seminars in Thrombosis and Hemostasis* **28**, 53-66 (2002).
3. Angata T, Kerr SC, Greaves DR, Varki NM, Crocker PR, and Varki A, *J.Biol.Chem.* (in press).
4. Angata T, Varki NM and Varki A, *J.Biol.Chem.* **276**, 40282-40287 (2001).
5. Varki A, *Yearbook of Physical Anthropology* **44**, 54-69 (2002).

PL#2

The role of glycosylated phosphatidylinositols in microbial pathogens

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Surface glycoconjugates are important virulence determinants in many pathogenic microorganisms. In the medically important parasitic protozoa, the major surface glycoconjugates are characteristically glycosyl-phosphatidylinositol (GPI) anchored glycoproteins and proteoglycans and/or free GPI glycolipids. Recent studies in our laboratory have focussed on understanding how the complex surface coats of *Leishmania* parasites are assembled and the precise role of individual coat components. We have found that stage-specific changes in the levels of expression of GPI enzymes, which determine the composition of the surface coat, are regulated at the level of protein trafficking and turnover in a novel lysosomal compartment. Information of the role of different classes of GPI-anchored macromolecules and free GPIs in *Leishmania* surface coat has been obtained by the targeted deletion of genes encoding specific glycosyltransferases, sugar transporters, or enzymes involved in sugar nucleotide synthesis. Analysis of these mutants has confirmed that these molecules are important virulence factors, but has also revealed a high degree of functional redundancy and raised the prospect that intracellular polysaccharides may be equally important virulence factors. Finally, GPI-related glycolipids are also synthesized by some prokaryotic pathogens. We have investigated the role of the mycobacteria GPIs (the PIMs and LAM) that are major components of both the plasma membrane and unusual cell wall of these bacteria. Analysis of mycobacterial mannose or inositol auxotrophs suggest that one or more of these glycolipids may play a key role in mycobacterial cell wall biogenesis and cell division.

PL#3

Plant lectins: Folds, quaternary association and carbohydrate specificity

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The structures of different forms and complexes of peanut lectin, winged bean lectins (acidic and basic), the two lectins from jackfruit seeds (jacalin and artocarpin), garlic lectin and snake gourd lectin, analysed in this laboratory, exemplify the folds, modes of quaternary association and strategies for generating carbohydrate specificity adopted by plant lectins. The minimal structural requirements of the legume lectin fold and the variability in the quaternary association of legume lectins, have been elucidated. The β-prism I fold has been established as a structural motif characteristic of *Moraceae* lectins. Bulb lectins could be dimeric or tetrameric. Lectins from the *Cucurbitaceae* family have a fold similar to that found in plant toxins such as ricin and gelonin, but without their toxicity. The study also revealed several strategies for generating carbohydrate specificity. They involve water-bridges, post-translational proteolysis, interactions involving aromatic residues and oligomerisation.

PL#4

Cell adhesion and signaling controlled by membrane glycosylation domain (glycosynapse)

S Hakomori
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Glycosphingolipids (GSLs) are clustered and noncovalently associated with signal transducer molecules (TDs) and hydrophobic proteins (proteolipids) to form membrane domains involved in GSL-dependent cell adhesion coupled with signal transduction through activation of TDs such as cSrc, Src family kinases, small G-proteins, and growth factor receptors [1]. Such structural membrane units are termed "glycosynapses" [2]. Examples will be described for cell adhesion mediated by GSLs through their carbohydrate-to-carbohydrate interaction, or stimulation of GSL by its antibody, followed by remarkable phenotypic changes, *e.g.*, differentiation and cell motility, through initial activation of cSrc or FAK. Recent studies of globo-series GSLs strengthened this concept, and showed that diversity of signaling pathways depends on type of cells and consequent linkage of signaling molecules with or without involvement of proteolipids. This concept, *i.e.*, noncovalent organization of adhesion molecules with TDs, has been applied to various glycosyl epitopes carried by O-linked glycoproteins associated with Src family kinases in cholesterol-rich membrane domain (type 2 glycosynapse). Since the majority of selectin epitopes are present in O-linked form in such microdomains, the possibility is opened for cell adhesion coupled with activation of Src family kinases leading to phenotypic changes, particularly in defining tumor cell malignancy.

1. Hakomori S, Handa K (2000), in: Ernst B, Hart G, Sinay P, eds. Oligosaccharides in chemistry and biology. Wiley-VCH, Weinheim, pp. 771-781.
2. Hakomori S (2002) PNAS 99(1): 225-232.

PL#5

**Glycosphingolipids:
Cell biology, functions and pathobiochemistry**

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Sphingolipids are building blocks of the plasma membranes of eukaryotic cells. Knockout mice embryos deficient in the biosynthesis of most glycosphingolipids (GSL), die at day E 6.5. Mice deficient in the biosynthesis of most of the neuronal gangliosides (missing GM2/GD2-Synthase or GD3-Synthase) show only a mild phenotype. However, mice which express only ganglioside GM3 are severely sick and can die from audiogenic seizures, whereas mice deficient in ganglioside GM3 biosynthesis do not form any of the neuronal gangliosides but instead produce gangliosides of the 0-series normally not occuring in mice and human brain. GSL of the plasma membrane reach the lysosomal compartment for degradation on intralysosomal vesicle and membrane structures. The analysis of the molecular pathogenesis of neurodegenerative diseases demonstrates that catabolism of membrane-bound sphingolipids - as catalyzed by water-soluble lysosomal exohydrolases - is synergystically stimulated by two additional components, lysosomal activator proteins and BMP [bis(mono-acylglycero)phosphate].The discovery of normal components in the skin, ω-hydroxylated glucosylceramides esterified with linoleic acid and covalently protein-bound ω-hydroxylated glucosylceramides as precursors of protein-bound ω-hydroxylated long chain ceramides, leads to a new concept of the topology and the biochemical pathway for the generation of the extracellular water-permeability barrier in the stratum corneum of land dwelling animals.

PL#6

Hyaluronan-SHAP covalent complex is an active form of hyaluron

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Hyaluronan (HA) is a widely distributed glycosaminoglycan with the simple structure of repeating disaccharide units. However, HA associates with various proteins and proteoglycans to form a so-called "HA-rich matrix" with a variety of functions, so that it affects cell behaviors not only in embryogenesis but also in some pathological events such as inflammation and cancer cell metastasis. Therefore, it is important to learn what molecules participate in the formation of HA-rich matrix and how the assembly is regulated for each event. SHAPs (Serum-derived Hyaluronan-Associated Proteins), the heavy chains of plasma inter-alpha -trypsin inhibitor (ITI) family, are so far only the proteins covalently bound to HA. The physiological significance has not been studied, but is of great interest because ITI is abundant in plasma, and the SHAP-HA complex is formed wherever HA encounters plasma. We abolished the formation of the SHAP-HA complex in mice by targeting the gene of bikunin, the light chain of the ITI family, which is essential for their biosyntheses and, consequently, for the SHAP-HA complex formation. Mice with the null mutation showed a severe female infertility. Histological and biochemical studies revealed the defect in the cumulus masses due to the impaired HA-rich matrix formation. Another analysis has led to the finding that the mice are significantly resistant to induced inflammations. Thus, the SHAP-HA complex plays a critical role in some functions of the HA-rich matrix.

PL#7

Fucosyltransferase 9 (FUT9) as a Lewis X synthase

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The α1,3-fucosyltranferase family comprises six human enzymes. They are named FUT3, 4, 5, 6, 7 and 9. All members catalyze an α1-3 linkage by transferring a fucose toward the GlcNAc residue of type 2 chain. A phylogenetic tree of the six α1,3-FUTs showed that there are four clusters in the vertebrate *FUT* gene family, the *FUT9* gene subfamily being the first to diverge from the ancestral gene.

We found that FUT9 preferentially fucosylated the distal GlcNAc residue of the polylactosamine chain resulting in the synthesis of Lewis x (Le^x) epitope, while the other four α1,3-FUTs, FUT3, 4, 5 and 6 preferentially fucosylated the inner GlcNAc residue. FUT9 exhibited very strong activity for Le^x synthesis, i.e. the 15-20 fold stronger activity than FUT4. FUT4 is ubiquitously expressed in all tissues in a body, and has been believed to be an essential enzyme for Le^x expression in the tissues. However, Le^x is detected in limited tissues in mouse, such as early embryos, brain, stomach, kidney and leukocytes. The expression profile of Le^x is almost perfectly co-localized with the FUT9 expression.

Mouse has three functional *α1,3Fut* genes. Fut9-deficient knockout mice have been established and analyzed for the Le^x expression. Wild mice expressed abundant Le^x epitopes on proteins in stomach, kidney and leukocytes. These Le^x epitopes on proteins in the three tissues almost disappeared in the KO mice. Brain tissue in wild mice expressed abundant Le^x-carrying glycolipids. KO mice lack Le^x-positive glycolipids in brain. Thus, FUT9 can synthesize the Le^x-epitope both on proteins and glycolipids. FUT9 functions a major role for the Le^x expression *in vivo*, while FUT4 may participate in the Le^x expression when it is enormously expressed in tissue and in the cultured cells.

KO mouse lacking Fut9 showed the following phenotypes. 1) The SSEA-1 antigen disappeared in embryo. However, there was no effect on embryogenesis. 2) Glandular stomach of Fut9-KO mouse showed mucous cell hyperplasia. 3) Disappearance of Le^x in brain of KO mouse resulted in emotional disorder of mice. 4) Leukocytes of KO mouse showed aberrant rolling phenomenon on endothelial cells. Thus, KO-mice looks healthy, however, they showed some interesting phenotypes. The important roles of Le^x will be discussed.

PL#8
Sialidases: from viruses to humans

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Sialidases are expressed in viruses, bacteria, protozoa and vertebrates. Viral and bacterial sialidases display about 35% homology in their primary structure, and share some specific motifs like the F(Y)RIP motif, located toward the aminoterminal region, and the repetitive motif SXDXGXXT/W in the active site region. In mammalian (including human) organs and tissues sialidases are present with different subcellular location (the lysosomes, plasma membrane and cytosol) and different substrate specificities with particular reference to sialoglycolipids (gangliosides) and sialoglycoproteins.

The lysosomal, cytosolic and plasma membrane sialidases, also from the same animal, have different protein structure and different cDNAs. These share common motifs that are the same ones as those occurring in viral and bacterial sialidases. Presently, the following mammalian sialidases have been cloned: the cytosoluble sialidases, including that of human origin, the lysosomal sialidase, including the human one, the plasma membrane bound sialidase from bovine and human origin. Recombinant sialidases, as well as properly “engineered” enzymes, constitute powerful tools for studying the structure-function relationships of mammalian, and human, sialidases.

An important feature exhibited by some plasma membrane bound sialidases is the attachment to the membrane via a glycan-phosphoinositide (GPI) anchor, possibly indicating their location in particular plasma membrane "domains" (caveolae, lipid rafts, GEMS), that could be also enriched in sialoglycoconjugates.

Sialidases were shown to be directly involved in the differentiation of human and murine neuroblastoma cells, and murine myoblasts. In the latter case the block of sialidase expression led to inhibition of the cell differentiation towards myocytes. All this strongly suggests a role of these enzymes in myogenesis. The implication of sialidases in human erythrocytes ageing under physiological and pathological conditions was also recently suggested.

Concurrent symposia

1.Structural basis of the carbohydrate specificities of lectins and glycosyltransferases

I#1

Structural basis of carbohydrate recognition by legume lectins

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Protein-carbohydrate interactions are the language of choice for inter-cellular communication. The specific recognition of an oligosaccharide by a protein poses some specific problems. On sugars, only a limited number of functional groups are available to guide recognition. There is an enormous abundance of OH groups and small alifatic patches. Except these, there is only the occasional N-acetyl group and rarely a carbohydrate. In addition, the glycosidic linkage is flexible in solution, setting limits on the affinity that can be obtained because of large unfavourable entropic term. The legume lectins form a large family of homologous proteins that exhibit a wide variety of carbohydrate specificities. The legume lectin family is therefore highly suitable as a model system to study the structural principles of protein-carbohydrate recognition.

The crystal structures of a vast number of legume lectins with different carbohydrate specificities are available. These allow us to rationalize the structural basis of carbohydrate specificity within this protein family. Recent structures of lectins from Ulex, Maackia, Griffonia, Dolichos and Pterocarpus species have not only confirmed known principles, but also illustrated new and unexpected binding modes. In combination, the available structural knowledge should allow us to rationally design lectins with altered specificity.

I#2

Carbohydrate mimicry: Structural basis and immunological implications

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The immunological implications and the structural basis of carbohydrate mimicry were addressed using concanavalin A, the mannose-specific multivalent carbohydrate binding protein. Structures of concanavalin A complexed with several independent carbohydrate-mimicking peptides [Jain et al. (2000) *J Biol Chem* 275:16098; Jain et al. (2001) *Biochemistry* 40:12059; Jain et al. (2001) *Biophys J* 80:2912], revealed plasticity in specific interaction of the peptides with concanavalin A at the carbohydrate binding site. The aromatic ring structures in the amino acid side chains were critical for peptide-carbohydrate mimicry. An added dimension to molecular mimicry of carbohydrate moieties was deciphered from the comparison of the crystal structure of meso-tetrasulphonatophenylporphyrin complexed with concanavalin A [Goel et al. (2001) *J Biol Chem* 276: 39277]. The interactions of the porphyrin moiety exhibited functional equality with those of α-D-methyl mannopyranoside although these two ligands exhibit no obvious structural or chemical similarity. The immunogenicity and antibody cross-reactivity of the carbohydrate and peptide ligands, the two chemically different but structurally equivalent molecular mimics, were analyzed by presenting them to the immune system in different modes [Kaur et al. (1997) *J Biol Chem* 272:5539; Kaur et al. (2001) *Vaccine* 19:3124]. The carbohydrate-peptide mimicry appears to exhibit topological quasi-equivalence reflected differently in terms of antibody response during maturation.

I#3

Structural mechanism of a retaining glycosyltransferase

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UDP-galactose: β-galactosyl α-1,3 galactosyltransferase (α3GT) catalyzes the transfer of galactose from UDP-α-D-galactose into an α-1,3 linkage with β-galactosyl groups in glycoconjugates. The enzyme is expressed in many mammalian species, but is absent from humans, apes and old world monkeys as a result of the mutational inactivation of the gene; in humans, a large fraction of natural antibodies are directed against its product, the α-Gal epitope. It represents a family of enzymes, including the histo blood group A and B transferases, that catalyze retaining glycosyltransfer reactions of unknown mechanism. We have determined highly ordered structures for α3GT in the presence of UDP (1, 2), with donor substrate, UDP-galactose, UDP-glucose and two acceptor substrates, lactose and *N*-acetyllactosamine, at resolutions up to 1.46Å (3). These structures provide a clear depiction of the catalytic site of the enzyme. In addition, structural and calorimetric binding studies suggest an obligatory ordered binding of donor and acceptor substrates, linked to a donor-substrate-induced conformational change, and the direct participation of UDP in acceptor binding. The monosaccharide-UDP bond is cleaved in the structures containing UDP-galactose and UDP-glucose, producing noncovalent complexes containing buried β-galactose and α-glucose. The location of these monosaccharides and molecular modeling suggest that binding of a distorted conformation of UDP-galactose may be important in the catalytic mechanism of α3GT.

1. Zhang, Y., Wang, P.G., and Brew, K. (2001) *J. Biol. Chem.* **276**, 11567-11574.
2. Boix, E., Swaminathan, G.J., Zhang, Y., Natesh, R., Brew, K., and Acharya, K.R. (2001). *J. Biol. Chem.* **276**, 48608-48614.

3. Boix, E., Zhang, Y., Swaminathan, G.J., Brew, K., and Acharya, K.R. (2002) *J. Biol. Chem. in press.*
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I#4
Glycosyltransferase structure and mechanism

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The x-ray crystal structures of a number of glycosyltransferases have begun to shed light on fundamental structure-function relationships such as the molecular basis for substrate binding specificity. In addition, these structures have also provided a framework for furthering our understanding of the mechanistic basis for catalysis. N-Acetylglucosaminlytransferase I (GnT I) is a key control point in the biosynthesis of N-linked oligosaccharides. It is a manganese dependent enzyme found to possess an ordered sequential Bi-Bi kinetic mechanism. A combination of structural studies, enzyme kinetics experiments and surface plasmon resonance-based binding studies, has led to a model for catalysis in which protein conformational changes are important for both catalysis and the ordered binding and release of substrates and products. Comparison with other glycosyltransferases suggests that similar mechanisms may be a common feature of these nucleotide sugar-dependent enzymes.

2. Organic and Enzymatic Synthesis of Complex Glycans

I#5
Telling glycosyl donors where to go

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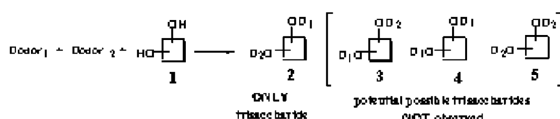
The importance of surface carbohydrates in molecular recognition in biological systems (see for example; Sharon and Lis Scientific American, January 1993, 82-89) provides the impetus for developing approaches for chemical synthesis of the bio-regulators. Unfortunately the idiosyncracies encountered in these tasks can frustrate the assembly of even small oligosaccharides, much less the complex structures found on cell surfaces. Current attempts at overcoming these challenges focus heavily on a growing array of glycosyl donors. Yet Paulsen's 20-year old warning of the need to "match" each donor with each acceptor remains valid.

Recent studies in our laboratory show that glycosyl donors can be "told where to go". For example, when equimolar amounts of acceptor diol, symbolised as **1**, is simultaneously presented to TWO donors, only a SINGLE trisaccharide, e.g **2,** is produced, with no evidence of the other possible trisaccharides, **3**, **4** or **5**, that could also have been formed.



I#6
Towards the total synthesis of *Leishmania* lipophosphoglycan

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Leishmania parasites have remarkable ability to survive and proliferate in hostile microbicidal environments during its digenetic lifecycle. For this the parasite has evolved a family of specialized glycoconjugates termed phosphoglycans [1] including the most abundant GPI-anchored Lipophosphoglycan (LPG), and secreted proteophosphoglycans (PPG). LPG/PPG are antigenic multifunctional virulence-factor essential for parasite infectivity and survival and inhibit PKC dependent signaling and gene-expression of the host macrophages. Intriguing structure of LPG consists of four distinct domains: alkyl-*lyso*-GPI-anchor, conserved glycan-core with internal Gal_f residue, variable phospho glycan (PG), and neutral oligomannose cap. Unique feature of LPG is the variable PG domain made of repeating phosphodisaccharides [6Galp-β1,4-Manp-α1-phosphate]$_n$ linked to each other by chemically labile anomeric phosphodiester groups. In our ongoing efforts [2-7] on organic-synthesis and biosynthesis of *Leishmania* GPI glycoconjugates, we have designed and synthesized all four LPG structural-domain (GPI anchor, glycan-core, repeating phosphoglycans, and neutral oligomannose cap). We have designed and executed efficient iterative synthesis of *Leishmania* phosphoglycans of desired length, by solution, solid-phase and block-polymerization approaches; either of which does not involve any glycosidation step. This approach has laid foundation for the total synthesis of LPG and its structural and functional mimics.

1. Turco SJ, Spath GF, Beverley SM (2001) *Trends Parasitol* **17**: 223-226
2. Sahai P, Vishwakarma RA (1997) *J Chem Soc Perkin Trans I*: 1845-1849
3. Tyagi AK, Vishwakarma RA (1998) *Tetrahedron Lett* **39**: 6069-6072

4. Upreti M, Vishwakarma RA (1999) *Tetrahedron Lett* **40**: 2619-2622
5. Upreti M, Ruhela D, Vishwakarma RA (2000) *Tetrahedron* **56**: 6577-6584
6. Sahai P, Chawla M, Vishwakarma RA (2000) *J Chem Soc Perkin 1*: 1283-1290
7. Ruhela D, Vishwakarma RA (2001) *Chem Commun*: 2024-2025

I#7

Artificial glycoconjugates with enhanced lectin cross-linking abilities

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Multivalent carbohydrate-containing clusters with varied numbers of ligands and shapes were synthesized using a combination of transition metal-catalyzed reactions. Thus, olefin self- and cross-metathesis with ruthenium carbenoid catalyst [$Cl_2Ru(PCy_3)_2$=CHPh], were used successfully from either O- and C-alkenyl glycosides to generate a wide range of clusters and their precursors. Oxidative dimerization of terminal alkynes such as 2-propynyl glycopyranosides was accomplished with palladium and copper-catalyzed homo- and cross-coupling reactions. Furthermore, Sonogashira-type cross coupling between 2-propynyl and 4-iodophenyl glycosides afforded novel "sugar-rods" useful in studying multivalent carbohydrate-protein interactions.
With the above goals in mind, together with deciphering factors involved in the increased binding properties of these glycodendrimers, we have established that polymannosides were potent ligands for plant lectins such as Concanavalin A and *Dioclea grandiflora*, as well as with fimbriated *E. coli*. Moreover, we showed that clustered lactosides can trigger Galectin-3 cross-linking. A comparative study between tetravalent plant lectins and divalent IgG antibodies using both T-antigen conjugates and Galili antigen was undertaken. The cross-linking behavior of small carbohydrate clusters is heavily dependent on the system involved and on their topography.

I#8

Synthesis and proinflammatory effects of peptidoglycan-derived neoglycopeptide polymers

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Based on recent estimates, microbial sepsis is associated with 100,000 human deaths annually in the United States. In recent years, the incidence of sepsis due to gram-positive bacteria has increased dramatically. Peptidoglycan (PGN), a major structural component of the cell wall of gram-positive bacteria, initiates the production of multiple host-derived inflammatory mediators, including cytokines (e.g., tumor necrosis factor; TNFα), which in turn cause the deleterious effects of gram-positive sepsis. The first step in the production of these mediators entails binding of peptidoglycan to cluster differentiation antigen CD14 on mononuclear phagocytes. Cell-signaling also involves interaction with Toll-like receptor 2, a transmembrane receptor protein that transmits the peptidoglycan signal to the interior of the cell.

We have unraveled the molecular mechanisms controlling the interactions between PGN and CD14 and Toll-like 2 receptors by the biological evaluation of a series of synthetic mono- and multivalent PGN-derived ligands.[1] Structure/function studies with human monocytic cells revealed the structural features of PGN for binding to CD14 and Toll-like receptor 2, activation of NF-κB, and stimulation of TNFα production. The specificity of the receptors responsible for cellular activation was studied by using human cells transfected with CD14 and/or Toll-like receptor 2. These studies have identified ligands that antagonize the proinflammatory effects of peptidoglycan.

1 Siriwardena, S., Jørgensen, M., R., Wolfert, M. A., Vandenplas, M. L., Moore, J. H., and Boons, G.-J. *J. Am. Chem. Soc.*, **123**, 8145-8146, (2001).

3. Glycosaminoglycans, integrins and extracellular matrix

I#9

Glycosaminoglycans and diabetic nephropathy

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The onset of diabetic nephropathy in streptozotocin treated rats involves a transient mesangeal cell proliferation (1,2), an upregulation of TGF-beta and prostaglandins (3-8), and an influx of inflammatory cells into the glomeruli (1). Further, confluent, but not pre-confluent mesangeal cells in culture produce heparan sulfate oligosaccharides that are anti-mitogenic to the pre-confluent cultures (9), and heparin treatment has been shown to ameliorate the time course of diabetic nephropathy (10,11). We observed that mesangeal cell cultures exposed to high glucose concentrations, typical of diabetes, synthesize and retain in the cell layer significantly higher levels of hyaluronan than cultures exposed to normal levels of glucose. Further, this additional hyaluronan was organized into cable-like structures that specifically bind mononuclear leukocytes by a mechanism similar or identical to that previously observed when colon

smooth muscle cells are exposed to a virus or a viral mimetic, poly I:C, in medium with normal glucose levels (12). We then observed that hyaluronan in glomeruli increases 3-4 fold during the first week after treating rats with streptozotocin to induce a diabetic response, a time frame coincident with the influx and activation of inflammatory cells, and that the heparan sulfate fine structure is significantly different at later times. Further, passage of mesangeal cells five times in medium with normal glucose supplemented with heparin yielded cells that no longer synthesized the adhesive hyaluronan structures when exposed to high glucose, but still did so when exposed to normal glucose in the presence of poly I:C. In contrast, mesangeal cells passaged in medium with normal glucose without heparin produced the adhesive hyaluronan structures in response to both treatments. These results indicate that two distinct intracellular signaling pathways are responsible for inducing the adhesive hyaluronan structures in response to high glucose versus a viral stimulus. They also suggest that the early inflammatory response in diabetic nephropathy involves the formation of an aberrant hyaluronan-based matrix that engages and activates surveillance inflammatory cells, and that therefore the organization of the extracellular matrix in the diabetic response is instructive in this pathological process.

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1. Young BA et al. *Kidney Int., 47*, 935-944 (1995).
2. Mahadevan P et al. *Diabetes, 45*, 44-50 (1996).
3. Sharma K and Ziyadeh FN. *Diabetes, 44*, 1139-1146 (1995).
4. Nakamura T et al. *Diabetes, 42*, 450-456 (1993).
5. Shankland SJ et al. *Kidney Int., 46*, 430-442 (1994).
6. Schambelan M et al. *J. Clin. Invest., 75*, 404-412 (1985).
7. Craven PA et al. *Metabolism, 36*, 95-103 (1987).
8. Derubertis FR and Craven PA. *Diabetes, 43*, 1-8 (1994).
9. Wang A et al. *J Cell Physiol, 159*, 295-310 (1994).
10. Gambaro G et al. *Kidney Int., 42*, 285-291 (1992).
11. Gambaro G et al. *Kidney Int., 46*, 797-806 (1994).
12. de la Motte CA et al. *J. Biol. Chem., 274*, 30747-30755 (1999).

I#10

Regulation of matrix metalloproteinases by proteoglycans

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Matrix metalloproteinases play a crucial role in remodeling of extra cellular matrix (ECM). By regulating the activity of the MMPs, the composition and nature of the micro environment of the cell is modified. Data on changes in the level of glycosaminoglycans and MMPs during mammary gland ontogeny, inhibition of MMPs by GAG, and upregulation of PG in lactating mammary gland where the MMP activity is low, suggested the occurrence of a novel regulatory mechanism in the control of MMPs in mammary gland. A CS/HS PG has been isolated from lactating mammary gland that inhibited MMP2 in zymographic assay. Higher levels of the PG in lactating stage where the secretory glandular structure is maintained and immunocytochemical localization on the cell periphery and ECM, suggest that this PG may contribute to maintaining proper cell matrix ratio during lactation. It suggests that the PG, a major component of ECM itself can influence the composition and structure of ECM by regulating the activity of MMPs that cause degradation of matrix in extracellular surface.

I#11

Assigning biological function to hyaluronan binding protein 1 (HABP1), a multiligand protein located on human chromosome 17

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Hyaluronan binding protein was purified initially from rat tissue using hyaluronan as matrix and characterized as a cell surface glycoprotein. This protein was reported to be involved in cellular transformation and signalling. As it was immunologically crossreactive with human tissue, the cDNA encoding hyaluronan binding protein was fished out by immunoscreening human fibroblast expression library, cloned and overexpressed in different systems. Sequence analysis of this protein confirms its multifunctional nature, as it is identical with p32, the protein copurified with splicing factor and the receptor for globular head of C1q; the complement protein. In addition to the localization of the functional gene for HABP1 on human chromosome 17, pseudogenes for HABP1 have been reported on human chromosome 4,11,15 and 21. Recently, HABP1 has been shown to be a bonafide MAP Kinase substrate, which translocates to the nucleus upon mitogenic stimulation. The promoter analysis of HABP1 ! sequence suggests the presence of regulation by Sp1 site and cAMP. In order to assign the biological function of HABP1, we reported the oligomeric transition of the native and its variants with functional implication and differential cellular localization. As a part of this programme, we have also examined the phenotypic changes by altering the constitutive level of expression of HABP1 in simple eukaryotes like *S. po*mbe and mammalian cells like fibroblast. We have found that overexpression of HABP1 in S. pombe leads to multinucleation, abnormal cell septum formation and growth inhibition, whereas the overexpression of HABP1 in fibroblast results in growth inhibition and vacuole formation. Using the *S. pombe* transformants and the stable transfectants of fibroblast cell lines overexpressing HABP1 as a model system, the regulatory role of HABP1 in cell cycle is our current interest, which will be discussed in detail.

I#12

Demonstration of novel biological functions for chondroitin sulfate/dermatan sulfate and cloning of glycosyltransferases essential in the biosynthetic pathway of the sugar backbone

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Previously, we found dendrite and axon outgrowth promoting activities for shark cartilage oversulfated chondroitin sulfate (CS-D) and squid cartilage oversulfated CS-E, respectively. Recently, we discovered neurite outgrowth promoting activities for

oversulfated dermatan sulfate (DS), an IdoUA-containing variant of CS. The DS preparations used included CS-H (or DS-E) from hagfish notochord, sea urchin eggs and ascidians. On the other hand, CS-E was demonstrated to interact specifically with various heparin-binding growth factors including midkine, pleiotrophin and some FGF family members with high affinities, which may be involved in the growth factor signaling and the neurite outfrowth by oversulfated CS and DS. To investigate the regulatory mechanism of these functions of CS a nd DS, it was essential to clone the responsible genes for the biosynthesis of CS and DS. We succeeded in cloning and identifying the cDNAs of human glycosyltransferases essential in the biosynthetic pathway of the chondroitin backbone, the precursor of the biologically active CS/DS. They are chondroitin GalNAc transferase and chondroitin synthase involved in the chain initiation and elongation, respectively. Homologues were also identified in *D. melanogaster* and *C. elegans*. Evidence for a novel biological function of chondroitin synthase was also obtained by disruption of the gene expression *in vivo.*

4. Gangliosides in neuronal functions and cellular signaling

I#13

Gangliosides, ligands for myelin-associated glycoprotein (MAG), are vital for myelin-axon stability and regulate nerve regeneration

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Gangliosides, prominent determinants on nerve cells, and are ligands for the myelin-axon recognition protein myelin-associated glycoprotein (MAG), a member (Siglec-4) of the sialic-acid dependent immunoglobulin-like lectin family. MAG binds to gangliosides having the "NeuAc α3 Gal β3 GalNAc β4" terminus, such as GD1a and GT1b. Mice lacking the ganglioside-specific GalNAc transferase responsible for initiating that terminus lack all complex gangliosides (e.g. GD1a, GT1b) and instead express truncated gangliosides (GM3, GD3). These mice display axon degeneration and myelination defects similar to those found in MAG knockout mice, supporting the conclusion that gangliosides, as MAG ligands, function as myelin-axon recognition molecules essential for long-term nerve stability. MAG is also an inhibitor of nerve regeneration, limiting recovery after central nervous system (e.g. spinal cord) injury. This occurs via MAG binding to nerve cell surface gangliosides, in that MAG-mediated inhibition of nerve regeneration is reversed *in vitro* by neuraminidase, by inhibitors of glycosphingolipid biosynthesis, or by specific anti-ganglioside antibodies. Clustering of nerve cell surface gangliosides mimics MAG-mediated inhibition, providing a mechanism by which the control of nerve regeneration is initiated.

Supported by the NIH and the National Multiple Sclerosis Society.

I#14

Regulation of myelinogenesis and myelin function by galactosphingolipids

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Oligodendrocytes and myelin are enriched in galactocerebroside (GalC) and sulfatide. We have hypothesized that they play important roles in the regulation of oligodendrocyte and myelin development and function by acting as sensors and transmitters of environmental information and/or organizers of membrane proteins. Consistent with this hypothesis, we have shown that perturbing GalC and sulfatide with antibodies, or in mouse models with genetic deletion of the genes coding for their synthesis, oligodendrocyte terminal differentiation is blocked or stimulated, respectively. This suggests that these glycosphingolipids function as negative regulators of differentiation. In addition, in these GalC and/or sulfatide deficient mice, there is a loss of normal localization of paranodal and juxtaparanodal proteins of the axon, altered gene expression of specific myelin proteins, and altered partitioning of select myelin proteins into glycosphingolipid-cholesterol microdomains. These data suggest that these glycosphingolipids also function in the organization of the myelin membrane and as a result, that of the adhering axolemma. Finally, antibody cross-linking of the myelin-specific myelin protein MOG results in its repartitioning and is accompanied by alterations in the phosphorylation state of specific proteins. This suggests that these glycosphingolipids are important in the regulation of signal transduction. Together these data speak in favor of an extensive role for this family of lipids in the regulation of this important aspect of central nervous system development and function.

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I#15

Neuronal dysfunction in glycosphingolipid storage diseases

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Despite years of study of the genetic and clinical basis of lysosomal glycosphingolipid storage diseases, no satisfactory molecular explanations exists to explain the pathophysiological mechanisms that cause the neurological symptoms and the early demise of patients suffering from some types of these diseases. Over the past 2-3 years, work from our laboratory has begun to provide clues that may provide molecular descriptions for the neuropathophysiology in two of these diseases, namely neuronopathic forms of Gaucher disease and Sandhoff disease. These studies are being performed using cultured neurons and brain tissue obtained from genetic (mouse) models of the diseases. Briefly, we have detected changes in calcium homeostasis, and changes in glycerolipid metabolism that may be of relevance for understanding neuronal dysfunction and death.

In a mouse model of Gaucher disease (the Gba mouse), in which the simplest glycosphingolipid, glucosylceramide,

accumulates, there is a significant increase in the rate of calcium release from the endoplasmic reticulum (ER), via the ryanodine receptor, resulting in elevated cytosolic calcium levels, which leads to enhanced sensitivity to agents that induce apoptotic cell death via the release of calcium from intracellular stores. The rate of glycerolipid, particularly phosphatidylcholine (PC) synthesis, is also elevated in neurons cultured from Gba-/- mice, due to direct activation of the rate limiting enzyme in PC synthesis, cytidylyltransferase (CT) by glucosylceramide. In addition, rates of axonal growth are faster in Gba neurons. In contrast, in a mouse model of Sandhoff disease, the Hexb mouse, rates of axonal growth are slower, and rates of PC synthesis are also reduced. Cytosolic calcium levels are also elevated in Hexb neurons, but in contrast to Gba neurons, this is caused by changes in the rate of uptake of calcium into the ER rather than by changes in the rate of calcium release. As a consequence, Hexb neurons are more sensitive to thapsigargin-induced cell death, suggesting that elevated cytosolic calcium may contribute towards apoptotic cell death in Hexb neurons. Based on these data and studies underway using DNA microarrays, we will present an integrated model that may begin to explain the molecular basis of the neurological symptoms observed in glycosphingolipid storage diseases.

1. Korkotian, E., Schwarz, A., Pelled, D., Schwarzmann, G., Segal, M. and Futerman, A.H. (1999) *J. Biol. Chem.*, **274**, 21673-21678.
2. Bodennec, J., Pelled, D., Riebeling, C., Trajkovic, S. and Futerman, A.H. (2002) *FASEB J.* in press
3. Pelled, D., Riebeling, C., van Echten-Deckert, G., Sandhoff, K., and Futerman, A.H. Submitted for publication.

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I#16

Tumor gangliosides, cell signaling and tumor development

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Modulation of host cell function by gangliosides, which are shed by tumor cells into their microenvironment, has been well defined with respect to the immune system. Inhibition of tumor specific immune responses *in vivo* and *in vitro,* by tumor gangliosides, has been confirmed. Most recent evidence demonstrates that exogenous gangliosides also affect normal human fibroblasts and human vascular endothelial cells, enhancing their biological responses to growth factors. Recent insight into the mechanisms by which growth factor mediated signaling pathways are affected by ganglioside enrichment of the cell membrane will be presented. The consequences of these ganglioside effects in the tumor microenvironment on tumor formation, elucidated in part by the strategy of specific depletion of cellular gangliosides, will be described. The findings illuminate the concept that shedding of tumor gangliosides, and the association of these shed molecules with normal cells in the tumor microenvironment, markedly influence tumor formation and progression. Conversely, downregulation of ganglioside shedding may abrogate these tumor-enhancing effects of tumor gangliosides.

5. Bangalore structural glycobiology highlights

O1.

Signature of quaternary structure in the sequences of legume lectins

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Legume lectins exhibit a wide variety of oligomerization and sugar specificity while retaining the characteristic jelly-roll tertiary fold. An attempt has been made to understand and identify relationships, if any, within various classes of carbohydrate specificities and modes of oligomerisation and to find whether this diversity is reflected in their primary structures by constructing phylogenetic trees. Dendrograms based on sequence alignment showed clustering related to the oligomeric nature of legume lectins. Though the clustering primarily follows the oligomeric states, it also appears to correlate with different sugar specificities indicating an interdependence of these two properties. Analysis of the structure based alignment and the alignment of the sequences of the carbohydrate binding loops alone also revealed the same features. Legume lectins are either dimers of various types or tetramers that can be considered as dimers of dimers. Each tetramer has three types of interfaces. All these interfaces have varying degrees of similarity, ranging from very close to broad, with those found in the dimeric proteins. All the interfaces involve the six-stranded back β-sheet of the monomer and it is possible to describe each of them in terms of the mutual disposition of the β-sheets in the two participating subunits. By a close examination of the interfaces of the various oligomers it was possible, in some cases, to pinpoint a few key residues responsible for the stabilization of the interfaces.

O2.

Development of an integrated lectin knowledge-base

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Lectins are now widely recognized to play a range of crucial roles in many cell-cell recognition events triggering several important cellular processes. Lectins, a class of carbohydrate binding proteins encompasses different members that are diverse in their sequences, structures, binding site architectures, quaternary structures, carbohydrate affinities and specificities as well as their larger biological roles and potential applications. It

is not surprising therefore that the vast amount of experimental data on lectins available in the literature is so diverse, that it becomes difficult and time-consuming to comprehend the advances in various areas. In order to achieve an effective use of all the data towards understanding the function and their possible applications, an organization of these seemingly independent data into a common framework is essential. An integrated knowledge base together with appropriate analytical tools has therefore been developed initially for plant lectins by collating and integrating diverse experimental data. The database has been implemented using MySQL server on Linux platform as back-end and has been web-enabled using Perl-CGI as front-end. Data for each lectin pertains to its taxonomic, botanical, biochemical, molecular and structural details as well as carbohydrate and hence blood group specificities and any known applications including those in biotechnology, immunology and clinical practice. Extensive links have also been provided for relevant bioinformatics resources and analytical tools.

O3.

Thermodynamic interactions of calreticulin with its substrate

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Calreticulin is a molecular chaperone found in the endoplasmic reticulum in eukaryotes, whose interaction with N-glycosylated polypeptides is mediated by the glycan, $Glc_1Man_{7-9}GlcNAc_2$, present on the target glycoproteins. We have studied this interaction by isothermal titration calorimetry to determine the thermodynamic parameters of its interaction with mono-, di-, tri- and tetrasaccharide, which are truncated versions of the glucosylated arm of $Glc_1Man_{7-9}GlcNAc_2$. The studies demonstrate that calreticulin has only one site per molecule for binding its complementary glucosylated ligands. While the binding of glucose by itself is barely detectable, it increases strikingly when glucose occurs in α-1,3 linkage to ManαMe as in Glcα1-3ManαMe. The binding constant increases by 25 fold in going from di- to trisaccharide and doubles in going from tri- to tetrasaccharide, demonstrating that the entire Glcα1-3Manα1-2Manα1-2ManαMe structure of the oligosaccharide is recognized by calreticulin. Thermodynamic parameters thus obtained are supported by modeling studies, which show that increased number of hydrogen bonds and van der Waals interactions occur as the size of the oligosaccharide is increased.

O4.

Role of N-linked glycans in unfolding of *Erythrina corallodendron*

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In this study we examine the role of glycosylation in a dimeric protein (ECorL) from *Erythrina corallodendron*, a legume lectin, composed of 239 amino acids and two carbohydrate groups per monomer. The glycosylated form shows a two-state unfolding profile. Its Tg as obtained from isothermal melts is 74ºC at pH 7.4. On the other hand the non-glycosylated protein (rECorL) shows a non two-state unfolding profile. Both gel filtration and DSC studies show the presence of at least one intermediate. It also shows reduced stability compared to ECorL. rECorl shows similar secondary and tertiary structures as ascertained by circular dichroism and fluorescence as well as sugar binding activity as the native protein thus suggesting that the final monomeric structure of the protein does not change upon deglycosylation. The drastic difference in the folding behavior can be partly explained by the fact that at least one of the two carbohydrate groups is unusually structured and forms many hydrogen bonds with the protein unlike sugar groups in most other glycosylated proteins. Minimization studies on ECorL, with the sugars removed show that there is little change in secondary structure and although the Cα traces do not overlap exactly, the overall structure shows insignificant alteration. It therefore appears that while the covalently linked sugar does not contribute appreciably to the final folded structure of ECorL, it does alter its folding process in a significant manner.

O5.

Structural features of *Moraceae* lectins

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Lectins are carbohydrate-binding proteins, which mediate various biological processes. The β-prism fold was first discovered in the galactose specific lectin jacalin from jackfruit seeds. Structural studies on the lectin, carried out in this laboratory, also established post translational modification as a strategy for generating carbohydrate specificity. The homologous artocarpin, a second lectin from jackfruit seeds, also assumes a β-prism fold. The absence of post translational modification and the replacement of aromatic residues in the binding site, make it mannose specific. Similar structures determined in other laboratories include *Maclura pomifera* agglutinin (MPA), *Helianthus tuberosus* lectin (heltuba), domain II of δ-endotoxin and the vitelline membrane outer layer protein. These structures among them, define the structural features of the β-prism fold as indeed those of *Moraceae* lectins. Among the proteins of this structural family, the structures belonging to the lectin family (jacalin, MPA and heltuba) have much more similarity amongst themselves than those belonging to other types of proteins. Also, among the lectins, the similarity is higher within the *Moraceae* family (with jacalin and MPA), even when their carbohydrate specificities are different.

6. Glycomics

I#17

Screening of oligosaccharide mixtures using frontal affinity chromatography coupled to mass spectrometry (FAC/MS)

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Many methods exist for evaluating the binding of oligosaccharides to proteins. Almost all of these methods involve the measurement of the interaction of a single oligosaccharide species (at a known concentration) with the protein of interest. The research presented here will demonstrate the use of FAC/MS for characterizing the interactions of mixtures of oligosaccharides competing for the same protein. FAC/MS (Angew. Chemie **34**, 3383, 1998), Anal Biochem **299**, 173, 2001) can provide estimates of the dissociation constants for individual compounds present in a mixture while simultaneously confirming the molecular weights of each of the active species. We routinely screen mixtures of 10-30 oligosaccharides against an immobilized protein. The experiment requires at most 0.5 micrograms of each sugar. An extraordinary power of FAC/MS is that the concentration of individual compounds present in a mixture does not affect their order of elution from the column of immobilized protein. This means that even if we have no idea of the relative concentrations of the individual compounds present in a mixture, we can still establish the tightest binding compound. Examples will be given on the use of FAC/MS for screening the binding of mixtures to lectins and glycosyltransferases.

I#18

An approach to deciphering the information in the glycome

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Knowledge that the human genome encodes less than 50 thousand proteins has served to emphasize the importance of oligosaccharide chains as diversifiers and modulators of the functions of proteins in health and disease, through recognition processes mediated by carbohydrate-recognizing receptors. The great challenge is to determine roles of the oligosaccharides as ligands. The neoglycolipid (NGL) technology [1] for generating lipid-linked oligosaccharide probes has many features that render it adaptable for this challenge. We have explored the potential of this technology as the basis of a carbohydrate microarray system that is applicable both to structurally defined oligosaccharides and to those derived from biological sources, glycoproteins, proteoglycans, cells and even a whole organ (submitted for publication). We observe that such repertoires of NGLs are robust probes when presented on nitrocellulose membranes, thus permiting sensitive and potentially high throughput detection of ligands for carbohydrate-binding proteins. We show that carbohydrate-recognizing proteins single-out their ligands, not only in the arrays of homogenous oligosaccharides, but also in arrays of heterogeneous oligosaccharides. The unique feature is that deconvolution strategies are included with TLC and mass spectrometry for sequencing the ligand-positive components. In conjunction with advanced protein expression systems, mass spectrometry and bioinformatics, the principle of constructing oligosaccharide arrays from desired sources could form the foundation for surveys to identify oligosaccharide-recognizing proteins in the proteome, and to map the repertoire of complementary recognition structures in the glycome.

1 Feizi,T., Stoll,M.S.,Yuen,C.-T., Chai,W. & Lawson, A.M. *Methods Enzymol.* **230**, 484-519 (1994).

I#19

Carbohydrate and protein-based microarrays as a new generation of glyco- and immunological tools

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Here we present a biochip platform that is suitable for the large-scale production of carbohydrate-based as well as protein-based microarrays. We demonstrate that polysaccharides, glycolipids, glycoproteins, glycosaminoglycans, semi-synthetic glycoconjugates and a broad range of protein molecules of distinct structural characteristics can be stably immobilized on a surface-modified glass slide without chemical conjugation. With this procedure, a large repertoire of proteins, carbohydrate containing macromolecules, lectins, antibodies, and other carbohydrate binding molecules can be patterned on a single micro-glass slide, providing a powerful tool to investigate the structural basis of carbohydrate-mediated molecular recognition and the anti-carbohydrate immune response. We summarize here our recent progress in applying this high throughput technology to explore the molecular heterogeneity and antigenic diversity of carbohydrate-containing macromolecules as well as in developing diagnostic biochips for the simultaneous detection of a wide range of microbial infections. Theoretical and technical considerations of the similarities and differences between this carbohydrate and protein microarray platform and other established biochip technologies will be summarized.

I#20

Lectin-based glycomics

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Glycomics is an emerging field of glycobiology, because protein glycosylation is a critical issue of post-genome science [1]. Though most of secreted and membrane-anchored proteins are predicted to be glycosylated, it is totally unknown about “whether they are actually glycosylated or not”, “what types of glycans they have”, and “what their biological functions are”. In this context, we recently developed a novel affinity technique named a glyco-catch method, which enables us to identify systematically genes that encode glycoproteins based on lectin affinity chromatography and *in silico* database search [2]. Moreover, the method also clarifies glycosylation sites and structural types of glycans, i.e., high mannose type and complex type by specific use of lectins. Recent application of the method to both simple *Caenorhabditis elegans* [3] and more complex *Mus musuclus* (submitted), proved its practical validity, though the method still needs further improvement in particular in throughput and sensitivity. As regards characterization of glycan structures, effective use of

various lectins is also promising because lectins can discriminate subtle differences in glycan structures, e.g., terminal modification, linkage, unit numbers, etc. For this purpose, we recently reinforced the system for frontal affinity chromatography, by which interaction with a number of pyridylaminated oligosaccharides can be rapidly and sensitively analyzed. Recent application of this quantitative affinity chromatography to 13 galectins derived from diverse origins, such as mammals, bird, nematode, sponge and mushroom, revealed significant difference in fine specificity of these galectins [3]. In its high potential, the reinforced system should be able to determine 10,000 interactions in terms of K_d's for 100 lectins and 100 glycans in the near future (defined as "hect-by-hect" project). In this context, a versatile innovating method to specify glycan structures should hopefully be developed.

1. Hirabayashi, J and Kasai, K (2000) *Trends Glycosci. Glycotechnol,*. **12**:1-5
2. Hirabayashi, J and Kasai, K (2002) *J. Chromatogr. B* **771**:67-87
3. Hirabayashi J et al (2002*) J. Biochem. (Tokyo)* in press

I#21

Differential expression of *O*-acetylated sialoglycoconjugates for monitoring childhood acute lymphoblastic leukemia (ALL)

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Exploiting the selective binding affinity of Achatinin-H whose lectinogenic epitope has been defined as 9-*O*AcSA $\alpha2\rightarrow6$ GalNAc, the presence of 9-*O*-acetylated sialoglycoconjugates (9-*O*AcSG's) was demonstrated on lymphoblasts of ALL patients of both T and B lineage, minimally present in normal individuals (1-4). They were affinity purified and characterized by SDS-PAGE analysis, Western blotting, isoelectric focusing and flow cytometric studies. These glycotopes induced high antibody titres (5) and caused enhanced alternate complement mediated lysis. Among the several synthetic analogues of sialic acid, 9-*O*-AcSA showed the highest inhibition with the affinity-purified antibody. Differential expression of these antigens (120kDa and 90kDa) and variation in their receptor density in different phases of treatment was utilized for prediction of relapse. Similarly, anti *O*-AcSGs have a prognostic relevance as high levels were found at presentation that decreased with remission induction and importantly reappeared with clinical relapse. We therefore propose that expression of these disease specific glycotopes may serve as an economical yet effective index for long-term monitoring of ALL.

1.Mandal C, *et al., Br J Haemato* **110**, 801-812 (2000)
2.Sinha, D. e*t.al,. Leukemia* **13**, 119-125(1999a)
3.Sinha, D. *et.al.*, *Leukemia* **13,** 309-312 (1999b)
4.Sinha, D. *et.al.*. *Leukemia Res*. **23**, 433-439 (1999c)
5.Pal S. *et.al*., *Glycobiology* **10**, 539-549 (2000)

7. Glycochemistry

I#22

From an anti-tuberculosis drug ethambutol to genes encoding arabinosyltransferases in *Mycobacteria*

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Despite the widespread use of ethambutol, as a frontline anti-tuberculosis drug, neither the mechanism of its action nor the genetic basis for resistance is fully understood. Three contiguous genes embCAB, encoding the cellular target(s) of ethambutol have been recently identified [1-4]. These genes are likely to be organized in an operon in the order of embC, embA and embB. The EmbCAB proteins are predicted to be typical membrane proteins with 12-14 transmembrane domains and a C-terminal globular region of approximately 400 amino acids of predicted periplasmic location.

We have been able to show that these homologous genes are distinct in their functions, such that embC is responsible for the synthesis of arabinan of lipoarabinomannan (LAM)-a key molecule, widely implicated in the immunopathogenesis of tuberculosis and leprosy, and embA and embB are responsible for the arabinan of cell wall arabinogalactan (AG).

Construction of a series of fusion proteins [hybrids containing the N-terminal of EmbC and the C-terminal of EmbB] and complementation in the embC mutant, followed by detailed biochemical analyses of the products formed is now showing the N-terminal region of the hybrid polypeptide (from EmbC) is responsible for the recognition of, and binding to lipomannan rather than the galactan. We hope these studies will lead to better insights about the pathway of the arabinan formation in mycobacteria.

1. A. E. Belanger, G. S.Besra, M. Ford, K. E. Mikusova, J. T.Belisle, P. J Brennan, J. M. Inamine. *Proc. Natl. Acad. Sci. U S A,* **93** (1996) 11919-24.
2. F. Alcaide, G. E. Pfyffer, A, Telenti. *Antimicrob. Agents Chemother*. **41** (1997) 2270-2273.
3. K.-H. Khoo, J.-B. Tang, and D. Chatterjee, *J. Biol. Chem*., **276** (2001) 3863-3871.
4. V. E., Escuyer, M. A., Lety, J. B. Torrelles, K. H. Khoo, J. B.Tang, C. D. Rithner, C. Frehel, M. R., McNeil, P. J., Brennan, D. Chatterjee, *J. Biol. Chem*., **276**, 48854-62.

I#23

Arabinogalactin segment of *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a dreadful disease and is caused by a unique *Mycobacterium (M.) tuberculosis.* It is estimated that every year 8 million people are contracted with and 3 million die of TB infection worldwide. India has the largest TB infected population and with AIDS becoming epidemic, the situation is alarming.

Mycolic arabino-galactin (AG) complex present on the cell wall surface of *M. tuberculosis* has unique structural features particularly the furanoside rings which are more conformationally mobile and largely linked through primary hydroxyl groups. These characteristics enable the crowded AG complex to adopt a structure in which mycolic acids are closely arranged in parallel arrays. The AG complex is critical for the survival of *M. tuberculosis*. The hydrophobic AG complex acts as a barrier for the passage of antibiotics into cell and therefore plays an important role in developing resistance of mycobacteria to many antibiotics.

The drug choice for TB namely ethambutol blocks the biosynthetic pathway of arabinose. This inhibition of biosynthetic pathway is considered as an attractive strategy for drug development against *M. tuberculosis*.

The motifs of AG complex have been isolated and structurally elucidated. The arabinose residues exist in furanose forms and their syntheses are challenging endeavors.

I#24

Studies on the structure of glycoproteins

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The determination of the structure of glycoproteins requires the unraveling of the primary structures of the protein and glycan moieties, including the glycan heterogeneity at each glycosylation site. The availability of limited amounts of material demands for a miniaturization of the analytical methods. For the analysis of the epitope diversity of the N-glycans from Bovine Peripheral Myelin Glycoprotein P0, the combination of mass spectrometry and nano probe magic angle spinning ^{1}H NMR spectroscopy proved to be a powerful approach. Although only a single N-glycosylation site is present in P0, a huge glycan heterogeneity is present. Surprisingly, the natural killer cell epitope HNK-1 is prominently occurring, raising interesting questions as to the functional importance of this epitope. It leads to the suggestion that the protein to which the glycan is attached is essential for exerting its function.

Another striking example of glycan heterogeneity is found in Tamm-Horsfall glycoprotein and in its homologue in pregnant women, uromodulin. It could be shown that the glycosylation pattern of this glycoprotein is genetically determined, but between individuals there is a considerable variability possible. Obviously, these findings complicate studies focused on the biological role of individual glycans.

The next step in the structure analysis is the determination of the three-dimensional structure of the integral glycoprotein in solution and finally the structure in the interaction with complementary proteins.

I#25

Sugar amino acids in designing new molecules

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Sugar amino acids represent an important class of conformationally constrained templates that have been used extensively in recent years in many glycomimetic and peptidomimetic studies and have emerged as versatile synthetic monomers leading to many *de novo* oligomeric libraries [1-4]. The rigid furan and pyran rings of these molecules make them ideal candidates as non-peptide scaffolds in peptidomimetics where they can be easily incorporated by using their carboxyl and amino termini utilizing well-developed solid-phase or solution-phase peptide synthesis methods. At the same time, they allow efficient exploitation of the structural diversities of carbohydrate molecules to create combinatorial library of sugar amino acid based molecular frameworks predisposed to fold into architecturally beautiful ordered structures with valuable properties. The protected/unprotected hydroxyl groups of sugar rings provide additional leverage to manipulate the hydrophobic/hydrophilic properties of such designer molecular assemblies. Detailed work from our laboratory will be presented.

1. Chakraborty TK, Ghosh S, Jayaprakash S, *Curr. Med. Chem.* **9**, 421-35 (2002).
2. Gruner SAW, Locardi E, Lohof E, Kessler H, *Chem. Rev.* **102**, 491-514 (2002).
3. Schweizer F, *Angew. Chem. Int. Ed.* **41**, 230-53 (2002).
4. Peri F, Cipolla L, Forni E, La Feria B, Nicotra F, *Chemtracts–Organic Chemistry* 481-99 (2001).

I#26

Synthesis of high mannose, LacNAc and Lewis b oligosaccharide structures and dendrimer and glycoconjugates thereof to study interactions with bacterial, human and plant lectins

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To study carbohydrate-protein interactions the access to well-defined synthetic oligosaccharide structures presented as monomers or in a multivalent fashion as dendrimers or glycoconjugates are often a necessity and almost always an advantage. The synthesis of three variant oligosaccharide structure types and results from their interaction studies with lectins will be presented, namely:

1) Synthesis of high mannose structures [1] and their interaction with various plant lectins and type 1 fimbriae adhesins from *E.coli* strains.
2) Synthesis of LacNAc oligomers [2] and dendrimers to study their interactions with Galectins.
3) Synthesis of Lewis b structures [3] and glycoconjugates thereof and the interaction with *Helicobacter pylori* bacterial adhesins.

1. S. Oscarson and U. Tedebark, *Carbohydr. Res.*, **278** (1995) 271-287.
2. T. Buskas, P. Konradsson and S. Oscarson, *J. Carbohydr. Chem.,* **20** (2001) 569-583.
3. S. Oscarson, A. Tchernyak, and D. Turek, *Carbohydr. Res.*, **329** (2000) 309-316.

8. Glycosyl transferases and glycosidases

I#27

Structural basis of catalysis and specificity in α-1,3 galactosyltransferase

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α-1,3 Galactosyltransferase (α3GT), which catalyzes the synthesis of the α-Gal epitope, is a model for a family of homologous retaining glycosyltransferases with varying substrate specificity. To develop an understanding of the structural basis of catalytic activity, we have utilized mutagenesis together with ITC, kinetic characterization and structural analysis of interactions of α3GT with substrates and inhibitors (1-3). α3GT utilizes an ordered sequential mechanism in which UDP-galactose is bound prior to acceptor substrate, reflecting a conformational change required for acceptor substrate binding. UMP binds to α3GT with an affinity similar to UDP, but the ΔH of binding is much lower, apparently reflecting a distinct conformational state reflected in an inability to bind acceptors. In the UDP-galactose/α3GT complex, the galactose - UDP bond is cleaved forming β-galactose that is buried by the conformational change. Modeling studies indicate that the enzyme can only accommodate a distorted conformation of UDP-galactose in its active site that forms similar galactose-enzyme contacts to those made by the cleaved β-galactose (3). Consistent with the model, mutagenesis of His[280] which forms contacts with the galactose, perturbs UDP-galactose binding and catalytic efficiency. Glu[317] is essential for catalytic activity and is located in a position to stabilize a positive charge on C1 of galactose. The model helps to explain how differences in specificity for UDP-sugars between members of the α3GT family are regulated at the level of catalytic efficiency.

1. Zhang, Y., Wang, P.G., and Brew, K. (2001) *J. Biol. Chem.* **276**, 11567-11574.
2. Boix, E., Swaminathan, G.J., Zhang, Y., Natesh, R., Brew, K., and Acharya, K.R. (2001). *J. Biol. Chem.* **276**, 48608-48614.
3. Boix, E., Zhang, Y., Swaminathan, G.J., Brew, K., and Acharya, K.R. (2002) *J. Biol. Chem. in press.*
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I#28

Brain-type sugar chains and β1-4galactosyltransferase

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We previously detected in mouse neural tissue two brain-type N-linked sugar chains with bisecting GlcNAc and Fucα1-6 residues, BA-1 (n=0) and BA-2(n=1): $(GlcNAc\beta1\text{-}2)_n$ Manα1-3 (GlcNAcβ1-2 Manα1-6) (GlcNAcβ1-4)Manβ1-4 GlcNAcβ1-4 (Fucα1-6) GlcNAc [S. Shimizu et al. 1993]. When sugar chains in the brain were analyzed, a biantennary sugar chain and a biantennary sugar chain with a Fucα1-6 residue were detected; however, neither Sia-Gal nor Gal was found on the GlcNAc residues of BA-2. These findings led us to analyze the substrate specificity of mouse brain β1 4galactosyltransferase(s) (GalT) by using four pyridylamino (PA) derivatives of an agalactobiantennary sugar chain with structural variations in the bisecting GlcNAc and Fucα1-6 residues as acceptor substrates [S. Nakakita et al. 1999]. While the GalT in mouse liver and kidney could utilize all four oligosaccharides as substrates, the brain GalT(s) was not able to utilize the agalactobiantennary sugar chain having both bisecting GlcNAc and Fucα1-6 residues, but could utilize the other three acceptors. The β1-4galactosyltransferase activity of adult mouse brain thus appears to be responsible for producing the brain- type sugar chains and to differ from that of the GalT in liver and kidney (β1-4 galactosyltransferase 1)

I#29

Biosynthesis and functions of selectin ligands

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The leukocyte ligand for P- and L-selectin is PSGL-1, which is a membrane-bound dimeric mucin containing multiple O-glycans and a very small number of *N*-glycans. The important determinants for selectin recognition of PSGL-1 resides at the extreme N-terminus and consists of a specific tripartite arrangement of *O*-glycan, peptide, and tyrosine sulfate determinants that dictate the high affinity interactions. To define the molecular determinants required, we have used recombinant glycosyltransferases and synthetic glycopeptides to generate a large number of derivatives. The results demonstrate the unique specificity of P- and L-selectin for a sialylated and fucosylated core-2 based *O*-glycan and underscore the importance of peptide and carbohydrate determinants for high affinity binding. Most importantly, in the course of studying the enzymes required for PSGL-1 biosynthesis, we defined a key regulatory enzyme for O-glycan biosynthesis, the core 1 β3 galactosyltransferase. This unique enzyme activity is encoded by a single gene and genetic changes resulting in altered expression of the core 1 β3 galactosyltransferase activity dramatically alter cellular glycosylation with unexpected consequences. In addition, the expression of the core 1 β3 galactosyltransferase is highly dependent on a unique molecular chaperone specific for this enzyme. This talk will highlight the unique molecular controls of selectin ligand biosynthesis and especially the regulatory factors for *O*-glycan biosynthesis.

I#30

Identification of the gene encoding endo-β-*N*-acetyl-glucosaminidase, an enzyme involved in processing of free oligosaccharides in the cytosol

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Formation of oligosaccharides (OSs) occurs both in the cytosol and in the ER. Lumenal OSs have to be transported into the cytosol so that they do not interfere with glycan-dependent quality control machinery for glycoproteins in the ER. An endo-β-*N*-acetylglucosaminidase (ENGase) is a key enzyme involved in the processing of free OSs in the cytosol. Here we report the identification of the gene encoding a cytosolic ENGase in human. The enzyme was first purified to homogeneity from hen oviduct, and several internal amino acid sequences were analysed. Based on the internal sequence, we identified the human orthologue of the purified protein. By expressing the cDNA of the putative human ENGase in COS-7 cells, the enzyme activity of the protein was confirmed. Gene database surveys revealed the occurrence of ENGase homologues in wide variety of eukaryotes. Moreover, this gene was widely expressed in human tissues, suggesting that the enzyme is involved in basic biological processes in multicellular organisms. Supported by Mizutani Foundation for Glycosciences.

I#31

Control of free oligosaccharides in yeast and mammalian cells

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In mammalian cells, free oligosaccharides (fOS) are generated in both the endoplasmic reticulum (ER) and cytosol during glycoprotein biosynthesis. ER lumenal free OS are exported into the cytosol where they, as well as their cytosolically generated counterparts, are trimmed in order to be imported into lysosomes for final degradation. The physiological relevance of this pathway is highlighted by results which demonstrate that blocking fOS export from the ER into the cytosol causes Golgi-dependent secretion of sialic acid containing fOS into the extracellular space. Blocking lysosomal import of fOS provokes a substantial accumulation of small fOS in the cytosol. Radio-iodinatable, photoaffinity chito-oligosaccharide probes are being used in order to identify the proteins (transporters and lectins) responsible for this novel subcellular trafficking pathway.

We have initiated studies on fOS regulation in the yeast *Saccharomyces cerevisiae*, in which many of the early events of glycoprotein biosynthesis are similar to those of mammalian cells. We show that the bulk of yeast fOS is generated by peptide:*N*-glycanase (PNGase: *PNG1* gene product) during exponential growth when glycoprotein biosynthesis is maximal. By contrast, PNGase-generated fOS are stable in exponentially growing yeast, and are only degraded by the vacuolar mannosidase (*AMS1* gene product) when cells enter the stationary phase. The yeast vacuolar mannosidase has many similarities to the mammalian cytosolic mannosidase and the role of these enzymes in controlling fOS metabolism will be discussed.

9. GPI anchors, mucins and heparan biology

I#32

Subunit assembly and subcellular targeting of GPI biosynthetic enzymes

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Glycosylphosphatidylinositol (GPI)-anchored proteins are synthesized in the endoplasmic reticulum (ER) for eventual display at the cell surface. Both the GPI moiety and the pro-protein to be GPI-anchored are assembled in the ER membrane. The GPI structure is the product of a topologically complex series of reactions in which phosphatidylinositol (PI) is glycosylated (with *N*-acetylglucosamine and mannose), acylated and modified by phosphoethanolamine residues to yield a mature GPI structure. The reaction sequence is initiated in the cytoplasmic leaflet of the ER through the action of a multi-subunit *N*-acetylglucosaminyltransferase (GPI-GnT; composed of at least five membrane-bound subunits of which one, PIG-A/Gpi3p, resembles a retaining glycosyltransferase) that generates *N*-acetylglucosaminyl-PI (GlcNAc-PI). GlcNAc-PI is de-*N*-acetylated by PIG-L/Gpi12p to yield GlcN-PI; this reaction occurs primarily in an ER domain that appears to be closely apposed to mitochondria. Later reactions in the pathway occur on the lumenal face of the ER after a GPI biosynthetic intermediate, possibly GlcN-PI or its inositol-acylated derivative, is flipped across the ER membrane. Mature GPIs are attached to ER-translocated pro-proteins bearing a carboxy-terminal, GPI-directing signal sequence. Attachment is mediated by a multi-subunit transamidase (GPIT; composed minimally of 5 membrane-bound subunits of which one, Gpi8p, is the likely catalytic subunit).

Data will be presented concerning the physical organization of the subunits of GPI-GnT and GPIT, and the identification of sorting signals within GPI-GnT and GPIT subunits, as well as PIG-L/Gpi12p, that confine these proteins and protein complexes to the ER and ER domains.

1. McConville, M.J., and Menon, A.K. (2000) *Molec. Membr. Biol.*, **17**, 1-16.
2. Vidugiriene, J., Sharma, D.K., Smith, T.K., Baumann, N.A., and Menon, A.K. (1999) *J. Biol. Chem.* **274**, 15203-15212.
3. Kostova, Z., Rancour, D.M., Menon, A.K., and Orlean, P. (2000) *Biochem. J.*, **350**, 815-822.
4. Vidugiriene, J., Vainauskas, S., Johnson, A.E., and Menon, A.K. (2001) *Eur. J. Biochem.,* **268**, 2290-2300.
5. Vainauskas, S., Maeda, Y., Kurniawan, H., Kinoshita, T., and Menon, A.K. (2002) *J. Biol. Chem.,* **277**, 30535-30542.

I#33

Structure-activity relationship of heparan and heparan sulfate glycosaminoglycans

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Heparin and heparan sulfate glycosaminoglycans [HSGAGs] are complex acidic polysaccharides that are characterized by a disaccharide repeat unit of α-D-glucosamine [1→4] linked to uronic [α-L-iduronic /β-D-glucuronic] acid. These complex

sugars coat the surface of every eukaryotic cell. Recent advances in developmental biology, cancer biology and other fields have resulted in a dramatic increase in the number of important roles for extracellular HSGAGs in critical biological processes including development, angiogenesis, tumor metastasis, viral invasion and anticoagulation [1]. An emerging paradigm in this field is that unique HSGAG sequences specifically bind to a wide range of proteins including morphogens, growth factors and enzymes and influence the physiological state of the cells and tissues. This presentation will provide an overview on the structure-activity relationships of HSGAG and provide a few examples to illustrate the complexity and challenges of this emerging field.

1. Sasisekharan, R., Shriver, Z., & Narayanasami U., (2002) *Nat. Rev. Cancer.; 8*2, 521-8.

I#34

Altered expression of mucin genes (MUC1, MUC5 B and MUC8) in endometerial carcinomas and studies of alternate splicing of MUC genes in female reproductive tissues and carcinomas

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Mucin glycoproteins are an important class of tumor-associated antigens and are known to be differentially expressed in certain carcinomas. Recently, our laboratory has identified a novel human airway mucin gene, MUC8 (Am.J. Respir. Cell Mol. Biol. 16, 232-241 (1997). The objectives of this work was (1) to quantitate the expression of MUC8 and four other MUC genes, MUC1, MUC2, MUC5AC and MUC5B in reproductive tissues and carcinomas and (2) to determine whether MUC1 and MUC8 genes undergo alternate splicing in the reproductive tissues and carcinomas. The first aim was investigated using Northern and slot-blot analysis of the RNA isolated from the control and carcinomas tissues. The second aim was investigated by RT-PCR using primers specific to certain regions of MUC1 and MUC8. Five MUC genes were expressed in the reproductive tissues studied. Levels of expression of MUC1, MUC5B and MUC8 were considerably higher in endometrial adenocarcinomas as compared to that in control tissue. Of these three MUC genes, MUC1 level was the highest in the reproductive tumor tissues. The RT-PCR data showed that all regions of MUC1 studied and certain regions of MUC8 showed no tissue-specific or tumor-specific processing of the respective genes. In contrast, RT-PCR experiments showed that amplification of the repeat regions of MUC8 gene gave only 8 or 9 repeats compared to 22 repeats reported earlier by us in human tracheal tissue. These data suggest tissue-specific processing of MUC8 transcript in reproductive epithelia. Supported in part by NIH grant HL34012.

I#35

Glycosaminoglycan specificity in the binding and regulation of growth factors

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A large number of growth factors bind to glycosaminoglycans (GAGs), in particular to heparan sulphate / heparin, and a significant number require GAG as a co-receptor. Elucidating the details of such molecular recognition, i.e. which GAGs are biologically relevant, what are the important and necessary structural features, and how large an oligosaccharide is required for binding, as well as for activation, are important questions. The answers will not only help in understanding the physiological role of these GAGs, but also could provide important information for the design of therapeutic agents with potential for modulating growth factor activities.
We have been particularly interested in hepatocyte growth factor / scatter factor (HGF/SF) which, unlike fibroblast growth factor (FGF-2), binds with high affinity to dermatan sulphate as well as to heparan sulphate / heparin. Both growth factors require GAG as a co-receptor for activation of their respective tyrosine kinase receptors, with evidence for the formation of ternary signaling complexes. Also, in both cases, the minimal length of heparin oligosaccharide that will activate is a tetrasaccharide. However, these two proteins clearly recognise different structural features in GAGs, even though the basic mechanism of action of GAGs may be similar for both.

I#36

NDSTs in heparan sulfate biosynthesis

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During biosynthesis of heparan sulfate in the Golgi compartment, the first modification enzyme starts to work on the growing heparan sulfate (HS) polysaccharide chain. The enzyme, glucosaminyl N-deacetylase/N-sulfotransferase (NDST) makes the overall design of the sulfation pattern which will determine the ability of the HS chain to interact with target molecules. NDST removes acetyl groups from glucosamine residues and replaces them with sulfate groups. These N-sulfate groups are essential for further modification during biosynthesis; without N-sulfation no O-sulfation or conversion of glucuronic acid into iduronic acid will occur. Four NDST isoforms, transcribed from four genes, have been identified. Much of our work is concentrated on how the enzymes are organized within the Golgi compartment and identification of interacting partners. In addition, in collaboration with Erik Forsberg we have generated mice where the genes encoding NDST-1 and NDST-2, respectively, have been knocked out. The NDST-1 knockout mice with altered HS structure die at birth due to lung failure. Lack of NDST-2 instead results in abnormal mast cells. Since NDSTs have a key role in HS design (see above), these mice can be used to study HS function. Areas of interest are cell differentiation, growth, inflammation, cancer, lipid metabolism and microbial infection.

10. Glycoconjugates in development and immune responses

I#37

Genetic approaches to the roles of glycoconjugates in development and disease

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The combined use of genetics, biochemistry and cell and molecular biology has recently shown that posttranslational modifications such as glycosylation and sulfation of secreted and membrane proteins play pivotal roles in normal development of multicellular eukaryotes such as *C. elegans, Drosophila* and humans. This lecture will focus on recent results on the roles of Golgi nucleotide transporters on carbohydrates and sulfate moieties of glycoproteins and proteoglycans during development of the above organisms. The consequences of genetic defects in these functions including molecular causes of novel diseases such as Leukocyte Adhesion Deficiency Syndrome II will be discussed.

Supported by NIG grants GM 30365 and 34396.

I#38

Lectin-mediated innate immunity

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Mannan-binding protein (MBP) also called mannose-binding protein (MBP) or mannan-binding lectin (MBL), is a C-type lectin, which was first isolated from rabbit liver and then from rabbit serum, and designated as liver-type MBP (L-MBP) and serum-type MBP (S-MBP), or MBP-C and MBP-A, respectively. S-MBP was shown to trigger carbohydrate-mediated complement activation Lectin pathway . This pathway leads to the deposition of C3b and opsonization as well as the formation of a membrane attack complex. S-MBP was also shown to serve as a direct opsonin, and to mediate the binding and phagocytosis of bacteria by mono- cytes and neutrophils. In addition, MBP functions as a beta-inhibitor of the influenza virus. A marked correlation was found between low serum levels of MBP and immune opsonic deficiency. Such patients display susceptibility to recurrent bacterial and fungal infections. More recently MBP was shown to have an anti-tumor activity *in vivo*, which we proposed to term MBP-dependent cell-mediated cytotoxicity (MDCC) (1). In this presentation, recent studies on the structure and functions of MBP will be summarized focusing on its roles in host defense through triggering of carbohydrate-specific innate immune responses.

1. Y. Ma et al., *Proc. Natl. Acad. Sci. USA,* 96 (2), 371-375 (1999)

I#39

Molecular diversity of the macrophage galactose-type C-type lectin: a dendritic cell/macrophage receptor for glycoconjugate uptake and cellular trafficking

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The most diversified family of carbohydrate recognition molecules, lectins, is Ca^{2+}-dependent and termed C-type. MØs and related cells such as dendritic cells are known to express several subfamilies of C-type lectins: type 1 multilectin such as MØ mannose receptor and lectins having type II transmembrane configurations such as MØ galactose-type C-type lectin (MGL). We have previously made contributions by demonstrating the role of MGL+ cells in the pathogenesis of contact hypersensitivity, tumor metastasis, and other disease processes. MGLs seem to function as regulators of cellular trafficking and uptake of glycoconjugates. In this presentation, the significance of diversity of MGLs will be discussed. We recently discovered that mice have two highly homologous MGL genes, MGL1 and MGL2, and the products have distinct carbohydrate specificity. Human MGLs seemed to contain a variety of spliced products from a single gene. A single nucleotide polymorphism influencing the amino acid residue was also identified. MGL were shown to serve as a marker for the immature dendritic cells differentiating from monocytes (humans) and bone marrow cells (mice), and intermediate stage of macrophage differentiation (humans).

1. Higashi et al, *J Biol Chem*, 277:20686 (2002)
2. Higashi et al, *Int Immunol*,14:545 (2002)
3. Tsuiji et al, *J Biol Chem*, published online (2002)
4. Denda-Nagai et al, *Glycobiology*, 12:443 (2002).

11. Glycosphingolipids and intracellular trafficking

I#40

Trafficking and sorting of (glyco)sphingolipids and proteins in polarized liver cells

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Hepatocytes display membrane polarity, the sinusoidal membrane representing the basolateral surface, while the bile canalicular (BC) membrane typifies the apical domain. SAC, an endosomal organelle, fulfills a prominent role in BC biogenesis.

Here, sorting and redistribution of (glyco-)sphingolipids occurs, regulated by protein kinase A (PKA), and involving membrane transport, in which rab-11 appears to play a role. A clue for a molecular mechanism that couples the biogenesis of a plasma membrane domain to the regulation of intracellular transport in response to an extracellular stimulus, was inferred from data showing involvement of a cytokine-induced signal transduction pathway, which promotes BC biogenesis. Specifically, oncostatin M promotes polarity development via the gp130 subunit receptor, which is recruited into detergent-resistant membrane microdomains, thereby activating a sphingomyelin-marked pathway to the apical membrane. Distinct detergent-resistant rafts are ubiquitously present in hepatic cells, and operate in the direct and transcytotic pathway, both pathways being exploited for delivery of apical proteins. Cholesterol and raft-cargo, likely distinguishing single (e.g.GPI-linked) versus multispanning membrane anchors, rather than rafts *per se* (co-)determine the sorting pathway of apical proteins in liver cells.

I#41

Caveolae as portals of microbial entry

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Many pathogenic microbes persist in the host by entering host cells with limited loss of microbial viability. A portal of entry shared by some bacteria, viruses and parasites are caveolae (lipid rafts), which are involved in the import and intracellular translocation of macromolecules in host cells. A requirement for caveolae-mediated phagocytosis of microbes appears to be that the respective receptor is a constituent of caveolae or must move to caveolae following ligation.

I#42

Functional organization of GPI-anchored proteins in membranes of living cells

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Glycosyl-phosphatidylinositol-anchored proteins (GPI-APs) are a class of cell surface proteins, which are anchored in membranes via a complex glycolipid. The GPI-anchor appears to direct the sorting of GPI-APs to the apical surface of polarized epithelia and into distinct endocytic pathways, result in their retention in endocytic pathways, and facilitate transbilayer signaling. Many of these properties are dependent on the level of cholesterol and sphingolipid levels in cell membranes. The observation of phase segregated regions enriched in cholesterol and sphingolipids in artificial membranes, has lead to the proposal that the mechanism of sorting or signaling via GPI-APs involves their association with specialised domains or 'rafts' in membranes, regulated by specific lipid-lipid or lipid-protein interactions. We have utlized a novel energy transfer (FRET)-based microscopy which relies on the dependence of fluorescence polarization on the extent of energy transfer to observe nanometer scale heterogenieties in fluorescently-tagged GPI-AP distribution in living cell membranes. These experiments have revealed that multiple fluorescently-tagged GPI-APs are organised in cholesterol-dependent sub-micron sized domains. In an attempt to understand the structure of functional rafts containing GPI-anchored proteins in living cells we have extended the same FRET-methodology to further probe the functional organization of GPI-APs in living cell membranes.

12. Sialic acids

I#43

Challenges of the research on the enzymatic sialic acid *O*-acetylation

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Sialic acids (Sia) in glycoproteins and gangliosides are frequently *O*-acetylated at C-4 or the glycerol side chain, from the echinoderms to man and in some microorganisms. These modifications much contribute to the chemical diversity and to the manifold functions of Sia. These include, influencing Sia-recognising-enzyme activity and the modulation of Sia-mediated receptor interactions. Also in tumour biology, Sia-*O*-acetylation seems to play a role, since its expression was found to be upregulated in various neuroectodermal tumours and impaired in colon cancer.

Although the activities of acetyl-coenzyme A:sialate 4-*O*- and 7(9)-*O*-acetyltransferases have been detected in various tissues and bacteria, none of the enzymes involved could be purified or cloned so far. We report on the localization in the Golgi compartment, the solubilization, the enrichment and the characterization of *O*-acetyltransferases from guinea-pig liver, bovine submandibular gland, and human colon mucosa. Remarkably, the enzyme from guinea-pig requires a low molecular mass activator. With regard to substrate specificity, all these *O*-acetyltransferases modify both free and bound Sia at various degrees. The colon enzyme prefers CMP-Neu5Ac and that from guinea-pig liver gangliosides. In colon, including cancer, the level of Sia *O*-acetylation was found to be dependent on the activities of both the *O*-acetyltransferase and the Sia esterase.

I#44

KDN studies: current status and future directions

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During the last more than two decades of research on sialic acids in glycoconjugates, the important achievements were (a) the discovery of about 30 modified sialic acids; (b) the elucidation of their structures and positions in specific glycoconjugates; and (c) the clarification of their biosynthetic pathways. That modified sialic acids play an important role in cellular processes and cellular recognition is indicated in many publications. In 1986 we found the natural occurrence of a new sialic acid, 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) in fish eggs, Among the modified sialic acids the structure of KDN is unique because the *N*-acyl group in Neu5Acyl is substituted by a hydroxyl group. Our previous studies using chemical and biochemical methods showed the occurrence of KDN, mostly in its free form, if not all, in cells from human origin and elevated expression in cancer cells. These findings of the elevated expression of KDN in ovarian cancer and in ascites cells prompted us to investigate the occurrence of bound form of KDN in human ovarian teratocarcinoma cells. In this symposium we discuss the significance of the discovery of unprecedented sialic acid and current status and future directions of KDN glycoscience.

I#45

Analysis of polysialylation in cultured cell lines during growth and differentiation, and in embryonic and adult chicken brains by a newly developed highly sensitive chemical method

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$\alpha 2 \rightarrow 8$-linked PSA (polysialic acid) is a characteristic feature of embryonic NCAM (neural cell adhesion molecules) and functionally important in neurogenesis. Studies using anti-PSA antibodies have shown that PSA expression on NCAM in animal brain is progressively attenuated from a week onwards after birth, and almost vanished in the adult brain except for the regions where persistent remodeling of the tissue is taking place. PSA is an oncodevelopmental antigen. Neuroblastomas and cell lines derived from them express PSA-NCAM and used as *in vitro* models of studies on biosynthesis and function of PSA. We noted that in such studies, information on DP (degree of polymerization) has been ignored or incorrectly evaluated, despite such information is important in understanding both mechanism and biological significance of its regulated expression. Recently we have established a highly sensitive fluorescence-assisted HPLC method for DP analysis of PSA. The sensitivity of the method is almost equivalent to immunochemical methods. We used the new method for analyzing growth-stage dependent and retinoic acid-induced differentiation-dependent changes of PSA expression in human neuroblastoma (IMR-32) and rat pheochromomytoma (PC-12) cells. We also analyzed DP changes of NCAM-PSA in chicken brain during late embryonic stages, postnatal periods and in adult.

13. Lectins and ER quality control

I#46

BIP regulates UDP-Glc:glycoprotein glucosyltransferase activity by hiding hydrophobic amino acid residues in acceptor glycoproteins

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The UDP-Glc glycoprotein glucosyltransferase (GT) is a key element of the quality control of glycoprotein folding in the ER mediated by the interaction of monoglucosylated glycans and ER-resident lectins (calnexin and calreticulin) as GT only glucosylates high mannose-type oligosaccharides linked to incompletely folded protein moieties. To characterize structures recognized by GT in misfolded conformers we synthesized neoglycoproteins by chemical coupling $Man_9GlcNAc_2$-Asn to fragments of a modified (L8C) chymotrypsin inhibitor II (CI-2) having 25, 40, 54 or 64 (full length) amino acid residues. We found that the 1-54 fragment had the highest glucose acceptor capacity (Km= 60 micromolar), followed by the 1-40 fragment (Km= 250 micromolar). The 1-25 and full-length (1-64) fragments only had residual acceptor capacity. Structural analysis showed that the 1-54 neoglycoprotein had a molten globule conformation, whereas the 1-40 and 1-25 fragments lacked structure. The full length CI-2 had a properly-folded glycoprotein conformation. The main difference between fragments 1-25 and 1-40 resides in a stretch of hydrophobic amino acids occurring between amino acids 27 and 40 that also display a putative BiP binding capacity. Both the 1-40 and 1-54 fragments expose those hydrophobic amino acids, as revealed by the lack of tertiary structure of the former and the capacity of ANS (8-anilinonaphtalene-1-sulphonate) binding of the latter. It was concluded that GT recognizes hydrophobic amino acids but preferentially those in patches exposed in molten globule conformers. Moreover, BiP binds preferentially to the 1-40 fragment (Km of fragment= 70 micromolar) than to the molten globule structure (Km higher than 500 micromolar) as revealed by enhancement of ATPase activity and, simultaneously, preferentially inhibits GT when 1-40 fragment is used as acceptor substrate. It was concluded that BiP regulates UDP-Glc:glycoprotein glucosyltransferase activity by hiding hydrophobic amino acid residues exposed in acceptor glycoproteins.

I#47

Quality control of protein folding – biology and pathology

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The early secretory pathway provides a folding environment for newly synthesized proteins with UDP-Glc: glycoprotein glucosyltransferase (GT), glucosidase II (Gls II), calnexin, calreticulin and various chaperones playing important roles in this quality control [1]. Failure of rescue of misfolded proteins occurs in a spectrum of congenital human diseases, the protein folding/retention diseases. Although it is assumed that the ER is the site of this fundamental cellular process, the subcellular distribution of GT and Gls II suggests that the pre-Golgi intermediates are prominently involved in this process [2, 3]. Our studies on renal diabetes insipidus caused by aquaporin 2 mutants revealed remarkable differences in the degradation kinetics for the glycosylated and non-glycosylated T126M AQP2 ($t_{1/2}$ = 2.0 hrs versus 0.9 hrs) and demonstrated that both were efficiently degraded by the proteasome. In electron microscopic studies of a congenital diabetes mellitus in mice, the misfolded proinsulin was found to principally accumulate in 5.2-fold enlarged pre-Golgi intermediates and to a lesser extent in dilated ER subdomains. This demonstrates the involvement of pre-Golgi intermediates in a protein folding disease.

1. Roth J. (2002) *Chem Rev* **102**:285-303.
2. Zuber C, Spiro MJ, Guhl B, Spiro RG, Roth J (2000) *Mol Biol Cell* **11**:4227-4240.
3. Zuber C, Fan JY, Guhl B, Parodi A, Fessler JH, Parker C, Roth J (2001) *Proc Natl Acad Sci USA* **98**:10710-10715.

I#48

Cytoplasmic peptide: *N*-glycanase (Pngase) in eukaryotic cells: its structure and potential functions

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A cytoplasmic peptide: *N*-glycanase has been implicated in the proteasomal degradation of newly synthesized misfolded glycoproteins exported from the endoplasmic reticulum. The gene encoding this enzyme (Png1p) has been identified in yeast. Based on sequence analysis, Png1p was classified as a member of the 'transglutaminase-like superfamily' that contains a putative catalytic triad of amino acids (cysteine, histidine, and aspartic acid). More recent studies in yeast indicate that Png1p can bind to the 26S proteasome through its interaction with the DNA repair protein Rad23p. A mouse homologue of yeast Png1p (mPng1p) bound not only to the mRad23 protein, but also to various mouse proteins related to ubiquitin and/or the proteasome through an extended amino-terminal domain. This amino terminal extension of mPng1p, which is not found in yeast, contains a PUB domain predicted to be involved in the ubiquitin-related pathway. We are testing the hypothesis that association with the proteasome may be a feature in common with various enzymes and that this serves as a mechanism for regulating their activity.

14. Glycan synthesis

I#49

Large-scale production of carbohydrate-based nutraceuticals and pharmaceuticals (zstatflu®, linear b-vi tri, tetra and pentasaccharides and other oligosaccharides)

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The need for pre-clinical and clinical quantities of oligosaccharides necessitated the development of synthetic processes suitable for large-scale production. The complete chemical synthesis of oligosaccharide glycosides is a difficult task involving protection and deprotection strategy of hydroxyl groups leading to multi-step chemical synthetic procedure. Generation of crystalline intermediates, cost effective synthetic processes, high yield reactions, elimination of chromatography, minimization of manipulation of intermediates to reduce the number of steps and high purity are desirable elements for the production of these carbohydrates in multi-kilogram quantities.

For example, we developed a novel process (Srivastava et al., US Patent 6,303,764,B1) for the synthesis of a chromogenic and N-fluorogenic 4, 7-disubstituted N-acetylneuraminic acid substrate and a chromogenic derivative of 4-O-methyl Neu5Ac, and produced it in multi-kilogram quantities. These molecules were discovered at ZymeTx, Inc. and used as substrates in their ZstatFlu® diagnostic kit, which distinguishes between influenza Type A, and B viruses and neuraminidases from other viral and bacterial pathogens. The new process we developed is more cost effective, has higher yield reactions, fewer synthetic steps, reduces chromatography, uses more crystalline intermediates, and maintains high purity.

A rational approach for the production of ZstatFlu®, linear B-VI tri, tetra and pentasaccharide and other oligosaccharides will be discussed in detail.

I#50

Photoactive multivalent sugar clusters and their lectin binding properties

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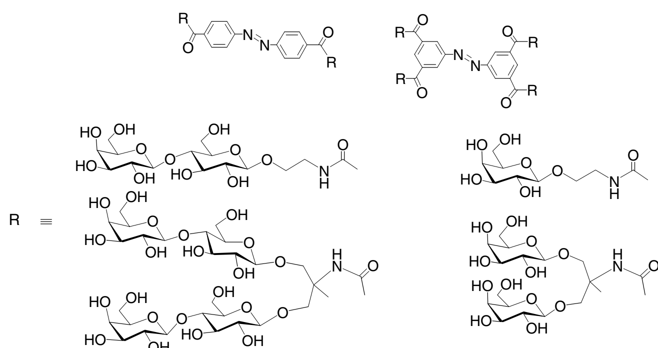
Clustered patches of carbohydrate ligands are known to be a requirement in order to exhibit high binding affinities with lectins [1]. While the critical increase in the density of sugar units allows to observe the so-called multivalent 'glycoside cluster effect', it is also known that the factors such as orientation of the saccharides and secondary interactions at high surface densities are important in endowing specificity in carbohydrate – protein interactions [1, 2]. We have undertaken an effort to understand how changes in the orientation of the sugar clusters, as modulated by a covalently linked non-sugar unit, will affect its binding affinity to a lectin. We have synthesized a number of β-galactosyl- and β-lactosyl sugar clusters (Figure), which are appended to a photoisomerizable azobenzene unit and elucidated their photoisomerization behavior in aqueous solutions. Preliminary lectin binding studies with lectin pea nut agglutinin (PNA) reveals the presence of not only the anticipated enhancements in the binding affinities, but also an anomalous 'cooperative effect' in the case of the binding of a β-lactosyl sugar derivative to the lectin PNA [3]. Our efforts to rationalize the 'cooperative effect', which is hitherto unknown in synthetic carbohydrate ligand – protein interactions, will be discussed in the Lecture.



1. (a) Lee, YC, *Carbohydr. Res.* **67**, 509 (1978). (b) Lee YC, Lee RT, *Acc. Chem. Res.* **28**, 321 (1995).
2. (a) Strömberg N, Nyholm P-G, Pascher I, Normark S, *Proc. Natl. Acad. Sci.* **88**, 9340 (1991). (b) Horan N, Yan L, Isobe H, Whitesides GM, Kahne D, *Proc. Natl. Acad. Sci.* **96**, 11782 (1999).
3. Srinivas O, Mitra N, Surolia A, Jayaraman N, *J. Am. Chem. Soc.* **124**, 2124-2125 (2002).

I#51

On the choice of GlcNAc and Asn as the linkage region constituents in n-glycoproteins : a crystallographic investigation using models and analogs

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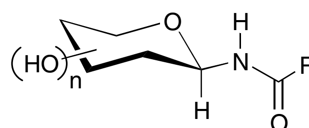
The oligosaccharide components of glycoproteins function as recognition determinants as well as modulators of physiological attributes including folding of nascent polypeptides. Understanding the molecular basis of these vital roles is a challenging problem in glycobiology. All eukaryotic N-glycoproteins have a conserved linkage region – GlcNAcAsn. While a few exceptions (Glc, GalNAc and Rha) to GlcNAc are found in prokaryotes, none whatsoever for Asn from any sources.



1: **Glc**: R = Me, **2**: **Glc**: R = Pr, **3**: **GlcNAc**: R = Me, **4**: **GlcNAc**: R = Pr, **5**: **GlcNAc**: R = Bz, **6**: **Gal**: R = Me, **7**: **Man**: R = Me, **8: Xyl**: R = Me, **9**: **Galβ(1-4)Glc**: R = Me, **10**: **L-Rha**: R=Me

The present X-ray crystallographic study explores, for the first time, the effect of systematic changes in the attached sugar and aglycon structure on the linkage region glycosidic torsion using models of the conserved and variant linkages and their analogs. Detailed analysis has revealed significant variations in glycosidic torsion, as much as 30^0, among these compounds compared to a value of -161^0 reported for GlcNAcAsn. Interestingly, the analogs derived from Man and Xyl which represent the attached sugars in O-glycoproteins show maximum deviation. The conformational preferences and molecular packing observed for these compounds in the solid state will be elaborated.

15. Lectin-carbohydrate recognition

I#52

***Artocarpus hirsuta* lectin: an unusual galactose specific lectin of the jacalin family**

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The α-galactoside binding *Artocarous hirsuta* lectin has two subunits, α and α' having identical N- terminal sequence for the first 10 residues. The binding of sugar to the lectin is enthalpically driven. The lectin shows insectisidal activity against the larvae of red flour beetle. The unfolding and inactivation of the lectin by Gdn HCl was partially reversible but thermal denaturation was irreversible. Exposure of hydrophobic patches, distorted secondary structure and formation of insoluble aggregates of the thermally denatured lectin probably leads to the irreversible inactivation. The *A.hirsuta* lectin complexed with methyl-α-galactose crystallized in two orthorhombic and two hexagonal forms. The crystals were found to have unusually high solvent content. The carbohydrate specificity of *A.hirsuta* lectin is due to the creation of free N-terminus by post-translational modification. *A.hirsuta* lectin has a hydrophobic pocket at the

face opposite to the side where the sugar-binding site is located. The sequence and the structures of *A.hirsuta* lectin on comparison with other jacalin related lectin structures reveal differences responsible for differing sugar specificity.

1. Gurjar M.M., Khan M.I., and Gaikwad S.M. *Biochim. Biophys. Acta.* **1381**, 256-264. (1998)
2. Gaikwad. S.M., Gurjar M.M., and Khan M.I. *Biochem. Mol Biol. Int,* **46**, 1-9 (1998)
3. Rao. K.N., Gurjar. M.M., Gaikwad S.M., Khan. M. I., and Suresh C.G. *Acta Cryst.* **D55,** 1204-1205 (1999)
4. Gurjar. M.M., Gaikwad S.M., Salokhe, S.G., Mukherjee.S., and Khan, M.I. *Invert. Reprod. Dev.* **38,** 95-98 (2000)
5. Gaikwad. S.M., Gurjar M.M., and Khan M.I (2002). *Eur. J. Biochem.* **269,** 1413-1417.

I#53
From *Pseudomonas aeruginosa* galactose and fucose-specific lectins (PA-IL and PA-IIL) to *Ralstonia solanacearum* fucose-binding lectin RSL

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Pseudomonas aeruginosa, the human pathogen, produces two potent lectins, PA-IL and PA-IIL (12.76 x 4 and 11.73 x 4 kDa), that are associated with its virulence. PA-IL binds D-galactose while PA-IIL exhibits high fucose affinity and binds D-arabinose and D-mannose. Based on terminal amino acid sequence, PA-IL gene *lec-A* was isolated and sequenced enabling inference of the lectin 121 amino acid sequence. PA-IIL gene *lec-B* and its 114 amino acid sequence were derived based on its N-terminus and MS analyses. Alignment searches revealed high homology between PA-IIL and a putative protein (113 amino acids) in the genomic data of *Ralstonia solanacearum.* No PA-IL homologue was found in the R. *solanacearum* genomic repertoire. Examination of the cell extracts of that bacterium, which causes severe wilt in many plant crops resulting in heavy agricultural losses, revealed a lectin RSL resembling PA-IIL only in its sugar specificity: L-fucose> D-arabinose>D-mannose, but diplaying lower affinities. The lectin (close to 10 kDa) sequence differs from that of PA-IIL, but shows surprising similarity to *Aleuria aurantia* fucose-binding lectin. The herein reported lectin activities in the above phylogenetically related bacteria might be associated with their different host ranges.

I#54
Specificity and kinetics of oligosaccharide recognition by RSL, a fucose-binding lectin from the plant pathogen *Ralstonia solanacearum*

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Ralstonia solanacearum is a worldwide distributed plant aggressive pathogen which causes lethal wilt in many crops. Its extracts contain a fucose-binding lectin that has been recently purified and characterized [1]. Its 90 amino acid sequence contains two repeating domains, with strong similarity to the fucose-binding lectin of the mushroom *Aleuria aurantia* (AAL), which is also a soil inhabitant. Surface plasmon resonance experiments demonstrate that the lectin binds strongly to fragments of fucose-containing xyloglucan polysaccharide purified from plant cell walls. This binding can diversely be inhibited by fucose and fucose-containing oligosaccharides. Best inhibition was obtained with oligosaccharides containing an αFuc(1-2)Gal terminal disaccharide, particularly XG9 (Glc_4 Xyl_3 Gal Fuc) which is a structurally well determined plant oligosaccharide that has previously been demonstrated to have biological activity in plants [2]. Our results are in agreement with the assumption that RSL plays a role in binding of the bacterium to specific oligosaccharides that are present in the primary cell wall of the root hairs of host plant.

1. Sudakevitz D, Imberty A and Gilboa-Garber N *J Biochem*, in press
2. Darvill A *et al* (1992) *Glycobiology* **2**:181-198

16. Neoglycoconjugates and gene targeting

I#55
Neoglycoconjugates as tools and beyond

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The presentation deals with the tendencies in design of multivalent neoglycoconjugates for glycobiology research and HTP profiling technologies including cellular phenotyping. Soluble polyacrylamide (PAA) conjugates are remarkable due to a variety of possibilities for synthesis and application. PAA is soluble and stable. The molecule is flexible - PAA-tethered ligands are capable of adjusting to the receptor during multiple-point interactions, PAA does not bind to the cell surface. Synthesis provides unlimited diversity of the probe types (*biot, flu, allyl, dig,* ^{3}H, *I**), glyco-particles, glyco-surfaces, multiarrays, immunogens etc.

Dynamic systems. The selectin ligands immobilized on the surface as Sug-PAA made possible to study the kinetics of rolling in the model system. Carbohydrate ligands covalently attached to the chip as Sug-PAA- are of use with the SPR method.

Pseudoglycoprotein. Some questions arise regarding the biologically active GPs - is a carbohydrate or peptide fragment responsible for the activity? We have proposed the approach that promotes to answer this and other questions. The pool of oligosaccharides spitted off GP is attached to PAA resulting in a pseudoGP.

Virtual (dynamic) glycotope. Receptor-ligand recognition such as P-selectin with PSGL-1 frequently involves molecular interactions at two distinct sites. Using P-selectin as a model, we developed an approach to discover novel ligands. PAA was synthesized with multiple ligands (L^1 = SiaLe^x, L^2 = tyrosine sulfate). A marked synergistic inhibitory effect was observed.
New generation. New type of neoglycoconjugates is presented, where units are self-assembled.

I#56

Nuclear import of glycoconjugates upon microinjection into the cytosol of living cells

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The nuclear import of proteins larger than M_r 40,000 depends either on the presence of a short peptide sequence called nuclear localization signal (NLS) or on defined sugars. The glycodependent nuclear import was previously evidenced by using glycosylated proteins, introduced into the cytosol of cells either by electroporation or by digitonin-permeabilization.

In this work, we studied by confocal microscopy the intracellular localization of fluorescent glycoconjugates delivered into the cytosol by microinjection. We confirmed that large proteins (such as BSA or streptavidin) substituted with caryophilic sugars (α-**D**-glucopyranosides, β-di-*N*-acetylchitobiosides or α-**D**-mannopyranosides) are transported into the nucleus while the sugar-free or lactose-bearing proteins are maintained in the cytosol. The sugar-dependent nuclear uptake is inhibited by wheat germ agglutinin (WGA), a lectin known to block the nuclear pore by binding *O*-glycosylated glycoproteins.

The microinjection procedure was applied to various cell types, including quiescent cells (mouse macrophages, rat hepatocytes), unsynchronized and synchronized dividing cells (HeLa cells). Quiescent cells actively import BSA substituted with caryophilic sugars, showing that the sugar-dependent import does not require a mitotic activity. Furthermore, the nuclear import activity, assessed with HeLa cells synchronized upon a double thymidine pulse, was found to be cell cycle-dependent. In addition, we show that neoglycoproteins bearing an import signal are selectively retained within nucleus upon cell division.

This work was supported by grants from Vaincre la mucoviscidose, Association de Recherche sur le Cancer, Ligue Nationale du Cancer and Agence Nationale de la Recherche sur le SIDA.

I#57

Glycofection: gene transfer with the help of by cationic glycopolymers

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Glycosylated cationic polymers, called glycofectins™, such as glycosylated polylysines (Mr ~25 000) interact with plasmid to give a glycoplex™, a compacted form of a polymer/DNA complex. We recently developed a simple method to convert reducing saccharides (mono-, di- or oligosaccharides) into glycosynthons (glycoamino-acids or glycopeptides) in a very high yield within a day. Such glycosynthons are easily either converted into clusters or used directly to synthesize glycoconjugates including neoglycoproteins and cationic glycopolymers. A glycoplex, prepared with a given glycofectin, is preferentially taken up by the cells which express, at their surface, a lectin which recognizes the carbohydrate moieties born by that glycofectin and usually leads to an enhanced gene expression, up to thousands times that obtained with glycoplexes prepared with glycofectins bearing irrelevant carbohydrate moieties or with polyplexes prepared with the sugar-free polymer. However, in some cases, there is an inverted relationship between the uptake extent of the glycoplex and the gene expression level. It is, for instance, what happens with cystic fibrosis airway epithelial cells. These cells actively take up neoglycoproteins as well as glycoplexes containing α-mannopyranosyl residues but they very poorly take up glycoplexes containing lactosyl (Galβ-4Glcβ-) moieties; while lactosylated glycoplexes lead to a quite high gene expression, mannosylated ones lead to an extremely poor gene expression. These paradoxical results will be discussed on the basis of a differential intracellular trafficking of glycoplexes.
Supported by *Vaincre la Mucoviscidose* grants.

17. Plant glycoconjugates

I#58

Study of role of amylose-molecule in stability of rice starch granule

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The role of amylose-molecule in stability of rice starch granule is obscure. In this study amylose contents in more than 30 kinds of waxy, non-waxy, and low amylose containing rice starches were measured, and those rice starch samples were also subjected to staining with 1.5M KI and 0.4M I2 solution. It was known that staining of waxy starch granules with a solution of 1.5M KI and 0.4M I2 showed that the granules of all kinds of cereal starches changed to a ghost-like appearance (1). Waxy and low amylose containing rice starch granules changed to ghost-like appearance, however, the rate of change to the ghost-like appearance were various, and then which was measured from photographs of KI/I2 stained and non-stained rice starch granules. From data of amylose contents of rice starch samples and their rates to the ghost-like appearance, it was known that the ghost-like appearance of rice starch granules was observed when amylose contents decreased to around 10%, which suggests that the presence of more than 10% amylose-molecule in rice starch granule is necessary to maintain the stability of rice starch granule. Same results of the role of amylose-molecule in stability of starch granules were shown in wheat and barley starch.

1. M. Seguchi et al: *Starch/Starke* **53** (2001), 140-146.

I#59

Chemical glycosylation of genistein

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Flavonoids and other polyphenols of plant origin usually occur in nature as glycosides, which are not always stable enough to withstand standard isolation procedures, hence observed differences in availability, in favour of aglycones . In case of Genistein, which became an important molecular probe, because of its pleiotropic biological activity, several natural glycosides are known, but scarcely available as a research material. Since there are some lasting controversies concerning pharmacokinetics and pharmacodynamics of free Genistein versus its glycosides and their esters, it is important to gain an access to some structural variety of these isoflavones. Particularly, synthetic glycosides can be of great help in verification of basic concepts related to the way in which Genistein and other flavonoids should be administered for achieving a chemopreventive or therapeutic effect.

In order to achieve efficient chemical derivatization of polyphenolic substrates we have introduced a novel concept of stoichiometric tetraalkylammonium phenolate salts, which are amenable to a variety of selective synthetic transformations.

We have tested numerous procedures for chemical glycosylation of Genistein, its salts an selectively protected derivatives, using the following types of glycosyl donors: acylated halogenoses, protected 1-OH monosaccharides and their 1-O-silylated analogs, anomeric esters, thioglycosides, carbamates as well as glycals and carbonates of unsaturated pyranoses. In most cases, difficulties in achieving efficient nucleophilic substitution at the anomeric center under basic conditions were experienced. In contrast, anomeric exchange and glycal addition reactions based on acid type activation, afforded expected glycosyl derivatives in reasonable yields.

Interestingly, majority of newly obtained Genistein derivatives exhibit pronounced cytostatic and cytotoxic activity against model tumor cell lines, considerably higher than that observed for parent isoflavonoid aglycone.

I#60

Biosynthesis of glycoprotein *N*-glycans in plants

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In eukaryotic cells. a complex biosynthetic pathway accounts for the posttranslational modification of newly synthesised proteins with *N*-glycosidically bound oligosaccharides. While the initial steps during protein *N*-glycosylation are highly conserved between animal and plant cells, significant differences exist in the late processing events that lead to the formation of complex-type *N*-linked glycans. In particular, complex plant N-glycans are frequently modified with β1,2-xylose and α1,3-fucose residues that are immunogenic and allergenic for humans. So far, four key glycosyltransferases involved in the biosynthesis of complex plant *N*-glycans have been cloned in my department: N-acetylglucosaminyltransferase I, *N*-acetylglucosaminyltransferase II, β1,2-xylosyltransferase and core α1,3-fucosyltransferase. Recombinant forms of the enzymes were produced to define the substrate specificities of these glycosyltranferases *in vitro*. GFP fusion proteins were used to determine the subcellular localisation of N-acetylglucosaminyltransferase I and β1,2-xylosyltransferase in plant cells. Furthermore, the domains responsible for proper intracellular targeting of these two enzymes were identified. Our results show that N-glycan processing in plants is controlled by the enzymatic properties as well as the subcellular compartmentation of the glycosyltransferases involved therein. These studies also provide clues to customise plant protein *N*-glycosylation for the production of recombinant glycoproteins suitable for therapeutic applications.

18. Glycan synthesis and biosynthesis

I#61

Initiation of polysialic acid biosynthesis in *Escherichia coli* K92

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We are using *Escherichia coli* K92 which produces the alternating structure α(2-8)NeuNAc α(2-9)NeuNAc as a model for studying the biosynthesis of bacterial capsular polysaccharides. We recently demonstrated that polysialyltransferase encoded by the neuS gene can elongate existing polysialic acids and synthesize both α(2-8)NeuNAc and α(2-9)NeuNAc linkages. The enzyme cannot initiate chain synthesis without the exogenous addition of polysialic acid (1). To determine which regions of the K92 gene cluster are essential for chain initiation, we have constructed strains that could potentially complement the neuS gene and assayed membranes prepared from these strains for their ability to initiate polysialic acid synthesis. We have also investigated the nature of the acceptor for initial transfer of sialic acid residues. We demonstrated that the K92 polysialyltransferase transfers sialic acid to a membrane associated acceptor. This acceptor has been labeled with a 9-modified ^{14}C-sialic acid analogue and appears to be hydrophobic. We have extracted a fraction that supports initiation of chain synthesis from the membranes of a sialic acid negative *E. coli* K1 mutant. Our results suggest that K92 polysialyltransferase probably synthesizes polysialic acid attached to a membrane associated glycolipid like acceptor.

1. McGowen, *et. al.* (2001) *Glycobiology* **11**: 613-620.

I#62

New insights into lignin biosynthesis

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We have detected and identified three glucosides in the xylem of transgenic poplars, two of them do not occur in the wild type plant. These sugar derivatives allow to obtain new insights in the biosynthetic proces of the lignin monomers. Knowledge of the biosynthetic pathway will allow engineering lignin, so that its extractability from pulp becomes easier.

Angiosperm lignin is predominantly built of two cinnamyl alcohol units, namely conyferyl alcohol and sinapyl alcohol. Several possible biosynthetic pathways for these lignin monomers are proposed in the literature. In order to find out the correct flux through the pathway we considered following points: 1) We studied *in vivo* processes, 2) Some compounds synthesized further on the pathway act as inhibitors, so that certain reactions do not contribute to the synthesis of the lignin monomers and 3) we have isolated for the first time three glucosides from the xylem of transgenic plants (for down-regulation of caffeoyl-CoA-*O*-methyltransferase). We show how consideration of these glucosides allow to exclude certain proposed pathways. Our conclusions are confirmed by feeding experiments.

I#63

Design of new glycosyltransferase inhibitors

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Oligosaccharides play an important role in various cellular recognition and signal transduction processes. Therefore, control of the biosynthesis of the structurally diverse oligosaccharides is of great interest for biological studies. An important control mechanism is specific inhibition of the various glycosyltransferases.

In glycosyltransferase catalyzed oligosaccharide synthesis, a glycosyl donor (commonly a nucleoside mono- or diphosphate) and an acceptor are coupled, under loss of a nucleoside mono- or diphosphate residue. Substrate analogues of the donor and of the acceptor have been investigated as inhibitors, but with limited success. Transition state analogues of the donor have also been constructed. They are often derived from the corresponding glycals, because it is assumed that an oxocarbenium ion is structurally closely related to the geometry of the transition state. Disubstrate analogues that contain the glycosyl donor and acceptor in a steric arrangement that simulates the transition state may be of particular interest. Such compounds are expected to have a high affinity for the active site, thus leading to a higher specificity.

Our recent results, particularly on sialyltransferase and α-(1-3)-galactosyltransferase inhibition[1,2] will be discussed.

1. Schwörer, R. and Schmidt, R. R. (2002) *J. Am. Chem. Soc.* 124: 1632-1637
2. Waldscheck, B., Streiff, M., Notz, W., Kinzy, W., and Schmidt, R. R. (2001) *Angew. Chem. Int. Ed.* 40: 4007-4011.

19. Ligand recognition by C-type lectins and siglecs

I#64

Novel patterns of sugar recognition by C-type animal lectins

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Many C-type animal lectins recognise endogenous mammalian glycoprotein ligands and mediate cell adhesion and signalling events or clearance of glycoproteins from serum. Individual carbohydrate-recognition domains (CRDs) in these receptors bind with low affinity to specific sets of monosaccharides, while intact lectins usually bind with high affinity and specificity to larger oligosaccharides. This selectivity can be achieved by a combination of extended binding sites and multivalent interactions with clusters of CRDs in oligomeric lectins. Our goal is to examine the specificity of these interactions in as much structural detail as possible. Recent advances in our understanding of binding specificity are illustrated by the SIGN cell adhesion receptors that are found on dendritic cells and other cell types. The CRDs in these receptors facilitate human immunodeficiency virus infection of T cells by interacting with branched high mannose oligosaccharides through primary and secondary binding sites. This mechanism contrasts with the binding of similar ligands to the several CRDs in the single mannose receptor polypeptide. Endocytosis of glycoproteins bearing terminal galactose residues involves a different mechanism, in which multiple CRDs are brought together in receptor oligomers. For example, multiple CRDs in the hepatic and macrophage asialoglycoprotein receptors and the Kupffer cell receptor bind to multi-antennary complex glycans. These studies help to define the natural ligands for these receptors and hence their biological functions.

1. Taylor ME, Drickamer, K Introduction to Glycobiology (Oxford University Press, Oxford, 2002)

I#65

Sialoside modulation of siglec function

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The Siglec family of sialoside binding proteins comprise 11 human and 6 murine genes. Most Siglecs contain ITIM (and ITAM) motifs in their cytoplasmic domains implicating them as regulatory molecules in cell signaling. Family members exhibit considerable differences in affinity towards sialoside sequences that terminate the carbohydrate chains of glycoproteins and glycolipids. Unlike some mammalian carbohydrate binding proteins, Siglecs are documented to recognize and bind sialoside sequences expressed either on the same cell (*cis* interactions) or on opposing cells (*trans* interactions). Our goal is to understand the role of ligand binding (*cis* and *trans*) inmodulating Siglec function. We have conducted a detailed specificity analysis of human Siglecs and have begun to develop novel multivalent sialoside probes specific for each of them. Focusing on lymphocyte Siglecs, including CD22 (Siglec 2), we have begun to analyze the relative contributions of *cis* and *trans* ligand interactions on their functions. Results suggest that binding of multivalent probes to cell surface Siglecs before and after sialidase treatment can measure the degree of *cis* ligand binding ('masked'), but may not predict the ability of Siglecs to participate in *trans* interactions.

I#66

Sialic acid-dependent interactions mediated by siglecs

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The Siglecs are a family of more than 10 immunoglobulin-like proteins which mediate cellular interactions by binding to sialylated glycoconjugates. Several functions have been proposed for Siglecs[1]. These include the regulation of the immune system and the maintenance of myelinated axons. However, the contribution of the sialic acid-recognition to such biological functions are difficult to demonstrate, especially if a cell expresses more than one Siglec.

All Siglecs described share characteristic features in their N-terminal sialic acid binding domain, such as a conserved arginine residue and hydrophobic amino acid side chains at critical positions. Their contribution to sialic acid recognition have been demonstrated by X-ray crystallography of Siglec-1 complexed with 3´-sialyllactose[2].

Based on this crystal structure and previous inhibition data[3] we have developed effective and specific competitive inhibitors containing hydrophobic substituents at position 9 of 9-amino-9-deoxy-5-N-acetyl-neuraminic acid. These bind several hundredfold better to Siglecs than Neu5Ac and are suitable tools for investigations on the role of Siglecs as recently demonstrated for CD22[4].

1. Crocker,P.R. & Varki,A. (2001) *Immunology*, **103,** 137-145.
2. May,A.P., Robinson,R.C., Vinson,M., Crocker,P.R., & Jones,E.Y. (1998) *Mol. Cell*, **1,** 719-728.
3. Kelm,S., Brossmer,R., Gross,H.J., Strenge,K., & Schauer,R. (1998) *Eur. J. Biochem.*, **255,** 663-672.
4. Kelm,S., Gerlach,J., Brossmer,R., Danzer,C.P., & Nitschke,L. (2002) *J. Exp. Med.*, **195,** 1207-1213.

20. Carbohydrates and malaria pathogenesis

I#67

Automated solid phase synthesis of glycoconjugates to address biomedical problems

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The importance of cell surface oligosaccharides and glycosaminoglycans in signal transduction processes of biomedical significance is now well established. A major impediment to the rapidly growing filed of molecular glycobiology is the lack of pure, structurally defined carbohydrates and glycoconjugates. Detailed biophysical and biochemical studies of complex carbohydrates require sufficient quantities of defined oligosaccharides.

Described is the application of an automated solid-phase oligosaccharide synthesizer we developed recently to the preparation of complex *N*- and *O*- linked oligosaccharides from monosaccharide building blocks. Applications of this new synthetic strategy, that should be attractive to the non-specialist, to the assembly of a variety of oligosaccharides involved in cancer, infectious diseases, tropical diseases and HIV will be discussed.

Particular emphasis will be placed on the development of an anti-toxin malaria vaccine using the automated synthesizer to prepare complex oligosaccharides.

I#68

Adherence of *Plasmodium falciparum* infected erythrocytes in human placenta

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Adhesion of *Plasmodium falciparum*-infected red blood cells (IRBCs) in the vascular capillaries of various organs is central to malaria pathogenesis. In pregnant women, IRBCs

opportunistically accumulate in high density in the intervillous spaces of the placenta, causing placental malaria. Chondroitin 4-sulfate has been shown to mediate the placental IRBC adhesion. Our study demonstrates that an unusually low sulfated chondroitin sulfate proteoglycan (CSPG) in the intervillous spaces of the placenta is the receptor for IRBC adhesion. The CS chains of the placental CSPG contains on an average ~8% sulfate groups. Detailed studies revealed that the placental CSPG is a mixture of two differentially sulfated species with 2-4% and 12-18% sulfate groups; the CS chains of both CSPGs are exclusively 4-sulfated. Despite their low sulfate contents, the CSPGs can efficiently bind IRBCs. However, optimal binding requires two 4-sulfated and four non-sulfated disaccharide repeats within a dodecasaccharide minimum structural motif. Enzymatic degradation and IRBC-adhesion inhibition studies showed that the sulfate groups are clustered in certain regions of the CS chains of the placental CSPGs to provide the necessary structural elements for IRBC adhesion. CSPG-IRBC adhesion inhibition studies using sera from people in malaria endemic areas showed that women develop the IRBC-adhesion inhibitory antibodies during pregnancy in a gravidity-dependent manner.

I#69

Glycobiology of the malaria parasite

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Malaria is caused by infection with a protozoan parasite of the *Plasmodium* genus. Four species affect man, with *P. falciparum* responsible for most infections and almost all mortality. Characteristics of the human infection include highly specific recognition of hepatocytes (by sporozoites) and erythrocytes (by merozoites) each of which involves carbohydrates on the surface of the human cells. The erythrocytic stage of an infection (merozoites) has little or no oxidative metabolism and relies almost exclusively on glucose utilization for energy production. Surprisingly, the surface proteins of the parasite are neither N-, nor O-glycosylated although most are stabilized in the parasite outer membrane through glycosylphosphatidylinositol anchors. The failure to glycosylate parasite proteins is not attributable to the amino acid sequence since parasite proteins expressed in mammalian cells utilize available consensus N-glycosylation sites as expected.

The glycolipid anchors have a characteristic structure with an additional mannose residue, are essential for parasite function and represent an attractive target for drug development. The parasite biosynthesizes UDP-GlcNAc and GDP-mannose but lacks some component of the pathway that completes the conversion of these precursors to saccharides covalently attached to protein. An additional unusual feature is the lack of galactose or fucose, sugars normally present in eukaroytes. Associated with disease pathology is the adherence of infected erythrocytes to either capillary endothelial cells, (again mediated by carbohydrate recognition) or, in pregnant women, to low-sulfated chondroitin sulfate chains present in placental proteoglycans. Thus, key steps in the parasite life cycle are carbohydrate-dependent but the parasite itself functions with truly minimal saccharide components.

21. Bacterial Cell Surface and Vaccines

I#70

Glycosylation of bacterial surface layer proteins

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Surface layers (S-layers) constitute the outermost cell boundary in many bacteria. Chemical analyses of S-layer proteins have shown that some of them are glycosylated. Within the domain Bacteria, S-layer glycoproteins have been exclusively observed on members of the Bacillaceae. Carbohydrate analyses by degradation experiments, NMR spectroscopy and MALDI-TOF MS have demonstrated that the glycans usually consist of identical repeats. One of the most frequent sugars in S-layer glycans is L-rhamnose; in addition, sugars such as D-glycero-D-manno-heptose, D-rhamnose, 3-N-acetyl-D-fucosamine and quinovosamine have been observed. The latter sugars are well-known constituents of lipopolysaccharide (LPS) O-antigens of Gram-negative bacteria.

Recent molecular analyses of S-layer glycan biosynthesis clusters from different strains allowed the characterization of genes involved in the biosynthesis of the nucleotide activated forms of L-rhamnose (dTDP-b-L-rhamnopyranose), D-rhamnose (GDP-b-D-rhamnopyranose) and D-glycero-D-manno-heptose (GDP-D-glycero-a-D-manno-heptopyranose). Based on these findings, the pathway of heptose formation LPS of *E.coli* could be identified. Generally, NDP-heptose is encoded by four genes and includes a kinase/ phosphatase step instead of the previously proposed mutase reaction. Currently we are investigating the remaining genes of the S-layer glycan biosynthesis clusters of *Geobacillus stearothermophilus* and *Aneurinibacillus thermoaerophilus*, including glycosyltransferases, polymerase, ligase, etc., to understand the formation of glycan chains from S-layer glycoproteins of Gram-positive bacteria at the molecular level. These investigations are complementary to chemical analyses of export and incorporation of S-layer glycans into S-layer polypeptides. The long-term perspective of our research is the functionalization of bacterial cell surfaces for relevant

applications of prokaryotic glycoproteins in biotechnology, medicine, and nanotechnology.

I#71

Selection by phage display of llama conventional VH fragments specific for anti-carbohydrate antibodies and their NMR studies

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Heavy chain antibodies from camelid species lacking light chains have antigen combining sites that are formed only by the three CDRs of the heavy chain variable domain (1) .These heavy –chains antibodies are missing the entire CH1 domain. In addition, the variable domains contain consistently have remarkable substitution (Gly44Glu, Leu45Arg or Cys, and Trp47Gly of amino acids located at the surface of the variable domain (VHs) , where they contact normally CH1 and the VL (2). These marker amino acid substitution reshape the surface considerably and render the isolated domain more soluble.

We have generated a phage display library of VH fragments (3) from nonimmunized llama and have isolated anti-idiotypic VHs to Yst9.1 a monoclonal antibody that recognize the *brucella O*-polysaccharide, and 1B1, a monoclonal antibody that is specific for the group B *Streptococus* capsular polysaccharide. Surprisingly, all of these dAbs are conventional antibody VH sequences i.e. they do not have the key "camelid" residues such as Glu, Arg orCys and Gly at positions 44.45 and 47 respectively. These are produced in high yield in *Escherichia coli,* are highly soluble have excellent thermal stability profiles and do not display any aggregation tendencies (4). The three- dimensional structure of BrucD4.4 has been established by use of solution NMR. In constrast to the murine and human VHs, BrucD4.4 has sufficient solubility, monomeric in solution and display high-quality NMR spectra characteristic of well-structured proteins (5). Overall, the surface characteristic of BrucD4.4 can be situated in between the human VH domain from Fv Pot and the llama VHH fragment HC-V with respect to hydrophobicity, which may explain its enhanced solubility allowing NMR structural analysis.

1. Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N., and Hamers, R. *Nature*, **363**, 446-448 (1993).
2. Desmyter, A., Transue, T.R., Ghahroudi, A., Thi, M., D., Poortmans, F., Hamers, R., Muyldermans, S., and Wyns, L. *Nature Structural Biology*, **3**, 803-811 (1996).
3. Tanha, J., Xu, P., Chen, Z., Ni, F., Kaplan, H., Narang, S.A., and MacKenzie C.R. *J. Biol. Chem.*, **276**, 24774-24780 (2001).
4. Tanha, J., Dubuc, G., Hirama, T., Narang, S.A., MacKenzie, C.R. *J. Immunol. Meth.* (2002) in press.

I#72

Utilization of polysialic acid epitopes in vaccines against meningitis and cancer

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Group B meningococci (GBM) and *E. coli* K1 share a common α(2-8) polysialic acid capsular antigen, which because of its similarity with human tissue antigens, is poorly immunogenic. However the N-propionylated form of PSA (NPrPSA) is immunogenic and is a potential vaccine candidate against meningitis caused by above pathogens. NPrPSA-specific antibodies are cytotoxic for GBM by virtue of their ability to bind to unique cell-associated PSA structure on the surface of GBM, and it has been demonstrated these protective antibodies are only induced by extended helical segment of NPrPSA.

Some human tumors also express PSA, while extended NPrPSA antibodies also bind to leukemic cells *in vitro*, they are not cytotoxic. Therefore to better target cells, they are biochemically engineered to express NPrPSA, by incubating them with NPr mannosamine. This strategy induced antibody-dependent cytotoxicity of the cells both *in vitro* and *in vivo*.

22. Glycosylation and glycoproteins

I#73

New forms of congenital disorders of N-glycosylation

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Congenital disorders of glycosylation (CDG) represent a rapidly expanding group of inherited disorders affecting N- or O-glycosylation. Presently 12 congenital defects of N-glycosylation are known impairing the synthesis of nucleotide-linked monosaccharides, the assembly of dolichol linked mono- or oligosaccharides (CDG type I), the trimming or elongation of asparagine linked oligosaccharides or the import of nucleotide linked monosaccharides into the Golgi (CDG type II). The latest additions to the group of CDG-type I defects are the deficiencies of the mannosyltransferases elongating either dolichol linked $GlcNAc_2$-Man_7 (CDG-Ig, 1-3) or dolichol $GlcNAc_2Man_1$ (CDG-Ih, 4). The former is utilizing Dol-P-Man as donor at the lumenal face, the latter GDP-mannose at the cytoplasmic face of the ER-membrane. The CDG type II forms have recently been extended to the deficiency of β-1,4 galactosyltransferase (CDG-IId, 5) involved in the elongation of N-linked oligosaccharides.

1. Chantret, I. et al. (2002) *J. Biol. Chem.* 277, 25815-25822
2. Thiel, C. et al. (2002) *Biochem. J.* 367, 195-201
3. Hennet, T. et al. (2002) *Mol. Hum. Genet.* 11, 2331-2339
4. Hanßke, B. et al. (2002) *J. Clin. Invest.* 109, 725-733
5. Thiel, C. et al., manuscript in preparation

I#74

Synthetic studies towards a glycoconjugate vaccine against *Shigella sonnei*

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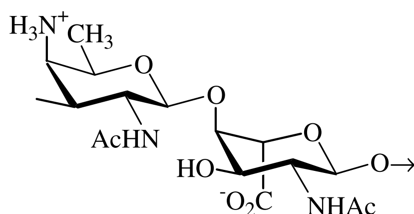
Shigellosis is a severe disease that afflicts 200 million people in many parts of the world. Symptoms of this disease may include dysentery, bacteremia, hemolytic uremic syndrome and post-

infectious arthritis. The Gram-negative bacterium *Shigella sonnei* is a major contributing organism.

An important cell surface component of this bacterium is its zwitterionic O-specific polysaccharide (O-SP, **1**) that is the outermost, serodeterminant domain of the highly complex cell-surface lipopolysaccharide. The O-SP is an essential virulence factor of all Shigellae: only strains that have their O-SP fully expressed are virulent. Additionally, the O-SP is related to host immunity in that protection against infection is a correlate of the IgG antibody level against the O-SP. Structurally, the O-SP consists of alternating, α-linked 2-acetamido-2-deoxy-L-altruronic acid and 2,4,6-trideoxy-2-acetamido-2-amino-D-galactose residues. Clinical trials have confirmed that protein conjugates of the O-SP elicited IgG levels in adult volunteers that were similar to those in patients convalescent from shigellosis.(1)



1

We surmised that an improved vaccine might be synthesized from chemically defined, extended fragments of the O-SP, provided that the geometry of the synthetic fragments allows the expression of its main conformational features. In order to test this hypothesis we have initiated a synthetic project to prepare a series of mono- and oligosaccharides containing the altruronic acid and the trideoxy-diamino-galactose moieties. The lecture will report our synthetic approaches as well as conjugation experiments.

1. Ashkenazi, S.; Passwell, J. H.; Harlev, E.; Miron, D.; Dagan, R.; Farzan, N.; Ramon, R.; Majadly, F.; Bryla, D. A.; Karpas, A. B.; Robbins, J. B.; Schneerson, R. *J. Infect. Dis.*, **1999**, *179*, 1565.

I#75

Inhibition of $Glc_3Man_9GlcNAC_2$-PP-Dolichol biosynthesis is anti-angiogenic and induces apoptosis in capillary endothelial cells through *unfolded protein response*

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The biological spectrum of oligosaccharides in (N-linked) glycoproteins span from development, growth, function to survival of an organism. Availability of $Glc_3Man_9GlcNAc_2$-PP-Dol (OSL) at the ER, together with the primary, secondary, and tertiary structure of the protein, controls the kinetics of the catalytic transfer of OSL to the asparagine nitrogen. We have shown earlier that increased OSL biosynthesis upon β-adrenergic stimulation was due to a phosphorylation activation of DPMS (1). The objective of the present study is to delineate the role of OSL in angiogenesis, i.e., the proliferation and differentiation of endothelial cells into blood capillaries. We have observed that OSL biosynthetic inhibitor tunicamycin interfered with ER function and developed ER stress and *unfolded protein response (UPR)* in an immortal capillary endothelial cell line. As a result, cells were arrested in G1 phase and induced apoptosis as judged by flow cytometry, DNA laddering, cellular morphology (2,3), Annexin-V binding, and caspase-3 and -9 activity. Reduced *Bcl*-2 expression, and enhanced grp-78/Bip (an ER chaperon) and caspase-12 expression supported UPR. It is therefore concluded that maintaining ER function is essential for the angiogenic process, and its down-regulation leads to apoptosis.

1. Banerjee, D.K., DaSilva, J.J. and Bigio, B. (1999) Biosci. Reports, **19**, 169-177.
2. Martínez, J.A., Torres-Negrón, I., Amigó, L.A. and Banerjee, D.K. (1999). Cellular Mol. Biol. **45**, 137-152.
3. Martínez, J.A., Torres-Negrón, I., Amigó, L.A., Roldán, R.A., Mendéz, A., Banerjee, D.K. (2000). In: Angiogenesis: From the Molecular to Integrative Pharmacology (Ed., M. Maragoudakis), pp. 197-208, Kluwer Academic/Plenum Publishers, New York.

23. Galectins and C-type lectins

I#76

Galectin affinities, inhibitors, and cellular activities.

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Galectins, a protein family with 14 reported mammalian members, are defined by a characteristic carbohydrate recognition domain (CRD) with affinity for β-galactosides (about 130 amino acids). Galectins cause a variety of cellular responses, and have been implicated in inflammation and cancer.

We are comparing the properties of different types of galectins: galectin-1 – a non-covalent dimer of one CRD, galectins- 4 and –8 – monomers with two CRDs in one peptide chain, and galectin-3 – a chimera of one CRD and another domain. First, we have analyzed the binding to small saccharides (by fluorescence polarization) and cells (by flow cytometry) of individual CRDs and intact galectins. For example, the two CRDs of galectin-8 have remarkably different affinities for small saccharides but show a strong synergism for binding to cell surfaces. Synthetic galectin inhibitors are being designed resulting so far in monovalent inhibitors with submicromolar K_d for galectin-3, some of which bind galectin-1 also whereas others are selective for galectin-3. Finally, we have studied fast cellular responses to the CRDs and galectins, such as agglutination, endocytosis, phosphatidyl serine exposure, and neutrophil oxidative burst where both similarities and marked differences between the galectins are found.

I#77

Multiple functions of the macrophage mannose receptor

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The macrophage mannose receptor recognizes carbohydrate structures found on pathogenic microorganisms and harmful soluble glycoproteins. The extracellular region of the mannose receptor consists of 8 C-type carbohydrate recognition domains (CRDs), a fibronectin type II repeat and an N-terminal R-type CRD. Functions in the innate immune response and glycoprotein homeostasis have been proposed for the receptor. Experiments to evaluate these roles and additional functions have been undertaken. Evidence for a new role for the receptor in mediating collagen clearance or cell-matrix adhesion has been obtained. Cell adhesion assays and collagen-binding experiments show that the receptor binds collagen through the fibronectin type II domain. A model system has been developed to provide direct evidence for a proposed role of the receptor in enhancing processing and presentation of glycosylated antigens. The results suggest that the mannose receptor does not enhance presentation of glycoprotein antigens indicating no general role for the receptor in the adaptive immune response. Genetic studies show that a major susceptibility locus for leprosy maps to the mannose receptor gene on human chromosome 10 (1). The effects of polymorphic amino acid changes on mannose receptor structure and function are being studied in an attempt to explain the role of the mannose receptor in increased susceptibility to leprosy.

1. Siddiqui MR et al. A major susceptibility locus for leprosy in India maps to chromosome 10p13. *Nat. Genet.* **27**, 439-441 (2001).

I#78

Galectin-8 functions as a matricellular modulator of cell adhesion

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Objective Galectin-8 is a family member of animal lectins which selectively binds beta-galactoside residues (1-3). In the present study we undertook to characterize the signaling cascades which are activated upon ligation of integrins by galectin-8 and confer upon cells adherent to this lectin a unique cytoskeletal organization.

Results Cell adhesion to galectin-8 triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK, p130Cas, MAP kinase, protein kinase B (Akt) and p70S6 kinase. Activation of signaling pathways downstream of phosphatidyl-inositol 3-phosphate (PI3K) is required to further propagate the adhesive process induced by galectin–8, because wortmannin, a selective inhibitor of PI3K, partially blocks galectin-8-mediated adhesion. Truncation of the C-terminal half of galectin-8, including one of its two CRDs, largely abolishes its ability to modulate cell adhesion, indicating that both CRDs are required to maintain a functional form of galectin-8.

Conclusions The above results and additional findings implicate galectin-8 as an ECM protein that triggers a unique repertoire of integrin-mediated signals, leading to a distinctive cell adhesion, spreading and cytoskeletal organization.

1. Hadari, Y. R., Paz, K., Dekel, R., Mestrovic, T., Accili, D., and Zick, Y. *J. Biol. Chem.* **270**, 3447-3453 (1995)
2. Hadari, Y. R., Goren, R., Levy, Y., Amsterdam, A., Alon, R., Zakut, R., and Zick, Y., *J. Cell Sci.* **113**, 2385-2397, (2000).
3. Levy, Y., Arbel-Goren, R., Hadari, Y. R., Eshhar, S., Ronen, D., Elhanany, E., Geiger, B., and Zick, *J. Biol. Chem.* **276**, 31285-31295, (2001)

24. Novel modes of carbohydrate recognition

I#79

F-lectins: a family of fucose-binding animal lectins with a characteristic sequence motif and structural fold

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Fucose-binding lectins are present in tissues and fluids from invertebrate and vertebrate species. Well-characterized examples, such as the lectin CPL-III from the tunicate *Clavelina picta* and the fucose-binding mammalian collectins, clearly belong to the C-lectin type. Others, such as FBP32 from the striped bass *Morone saxatilis*, the agglutinin from *Anguilla anguilla* (AAA), and serum "fucolectins" from *A. japonica*, lack a typical sequence motif present in any of the lectin families described so far. Furthermore, because of their specificity for carbohydrate moieties present on potential microbial pathogens, and their inducibility upon infectious or inflammatory challenge, these lectins are considered as recognition factors in innate immunity. We have characterized the biochemical properties and primary structure of the carbohydrate recognition domain (CRD) of FBP32 and by comparison with other related lectins, identified the sequence motif that defines a lectin family (F-lectins) which includes members present in organisms ranging from insects (*Drosophila melanogaster*) to ectothermic vertebrates (*Xenopus laevis*). F-lectins exhibit considerable diversity in organization, with CRDs present as single units, such as in AAA, organized in multiple homologous tandem repeats, or associated to other recognition domains. The crystal structure of the AAA enabled the characterization of a structural fold (F-lectin fold), which is shared not only with other lectins from this family, but also with a glycosidase, a glycooxidase, and a human clotting factor.
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I#80

Oligochitin elicitor –binding protein, a putative receptor, from plasma membrane of rice cells

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N-Acetylchitooligosaccharides (DP 6-8, $GlcNAc_{6-8}$) is a potent elicitor for suspension-cultured rice cells, inducing a set of defense-related responses such as membrane depolarization, ion fluxes, production of reactive oxygen species, expression of defense genes and phytoalexin synthesis (1,2). Putative receptor for this elicitor, high affinity binding site/protein was identified in the plasma membrane (PM) of rice cells by binding studies as well as affinity labeling (3).

The oligochitin elicitor binding protein (EBP), a 75 kDa plasma membrane protein, was effectively solubilized with Triton X-100 while maintaining the ability to bind elicitor active sugars specifically. The EBP was purified by affinity chromatography with newly designed affinity matrix. The purified EBP showed specific binding activity to ^{125}I-labeled $GlcNAc_8$ –APEA derivative as proved by the affinity crosslinking with glutaraldehyde. SDS-PAGE followed by silver-staining as well as affinity labeling showed the presence of two protein bands, corresponding to 75 and 55 kDa. Both bands retained the elicitor binding activity, suggesting that EBP was partially cleaved with endogenous protease during purification. The survey of the EBP gene based on the partial amino acid sequence of EBP is in progress.

Using mutant rice cells lacking functional G-protein α-subunit, oligochitin elicitor was shown not to couple to heterotrimeric G-protein, one of the most important signal transducer from the cell surface to down-stream in various cellular responses. Furthermore, affinity labeling experiments indicated the occurrence of elicitor-induced dimerization of EBP, which is typical for various receptor protein kinases, on the plasma membrane. These results may suggest that the oligochitin receptor may belong to a family of receptor protein kinases.

Plasma membrane EBP was not only found in suspension-cultured rice cells but also found in rice leaves and roots, suspension-cultured cells of barley, carrot, soybean and wheat. These results indicate that the defense system based on oligochitin recognition distributes widely among plant as a kind of “innate immune system in plant”.

1. N. Shibuya, E.Minami, *Physiol. Mol. Plant Pathol*., in press
2. T. Yamaguchi, Y. Ito, N. Shibuya, *Trends Glycosci. Glycotechnol.,* 64(2000), 113-120
3. Y. Ito, H. Kaku, N. Shibuya*, Plant J.,* 12(1997) 347-356.

I#81

Yet another way to bind mannose: the bacterial adhesin FimH

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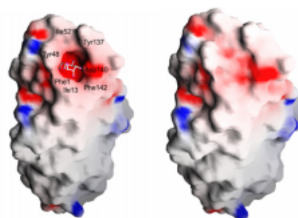
The first step in the colonization of the human urinary tract by pathogenic *Esherichia coli* is the mannose-sensitive binding of FimH, the adhesin of type 1 pili, to the bladder epithelium. We elucidated crystallographically the interactions of FimH with D-mannose. The mannose is buried in a unique site at the tip of the receptor-binding domain, an elongated eleven-stranded β-barrel, in a deep and negatively charged pocket. The solvent accessible surface buried (368 Å^2) and the number of hydrogen bond interactions are unusually large for binding of the single mannose. The binding pocket was probed using site-directed mutagenesis. All mutants but one examined had greatly diminished mannose binding activity and had also lost the ability to bind human bladder cells. Our structure/function analysis demonstrated that α-D-mannose is the primary bladder cell receptor for uropathogenic *E. coli* and that this event requires a highly conserved FimH binding pocket. Several new FimH crystal structures are currently under investigation to gain a better insight into the mechanisms of function of FimH – α-D-mannose interactions in the urinary tract.



Electrostatic potential surface of the FimH receptor- binding domain without (left) and including (right) the bound α-D-mannose (red is neg., blue is pos., white is neutral). See version on journal's webpage for color.

25. Glycoconjugates in development and malignancy

I#82

Molecular mechanism for glycoconjugate modulation in malignant cells

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Cell adhesion mediated by selectins and their ligands, sialyl Lewis X and sialyl Lewis A, plays an important role in hematogenous metastasis of cancer. Expression of these carbohydrate ligands is markedly enhanced in solid tumors, but the molecular mechanism that leads to cancer-associated expression of sialyl Lewis X/A is not well understood. Two principal mechanisms are involved in the accelerated expression of sialyl Lewis X/A in cancer; one is "incomplete synthesis" of

normal carbohydrate determinants, and the other is "*de novo* synthesis." As to incomplete synthesis, we have recently found further modified forms of sialyl Lewis X and sialyl Lewis A in non-malignant colonic epithelium, which carry additional 6-sulfo or 6-sialo residues. The impairment of GlcNAc 6-sulfation and 6-sialylation upon malignant transformation leads to accumulation of sialyl Lewis X/A in colon cancer cells. As to the mechanism called *de novo* synthesis, cancer-associated induction of some glycosyltransferases has been assumed to affect expression of the determinants. Recent studies, however, have indicated that cancer-associated alterations in the sugar transportation and intermediate carbohydrate metabolism also play important roles. Cancer cells are known to exhibit a metabolic shift from oxidative to elevated anaerobic glycolysis (Warburg effect), which is correlated with the increased gene expression of sugar transporters and glycolytic enzymes induced by common cancer-specific genetic alterations. The increased sialyl Lewis X/A expression in cancer is a link in the chains of these events, because our recent results indicated that these events accompany transcriptional induction of a set of genes closely related to sialyl Lewis X/A expression.

I#83

Targeted cytosolic delivery of hepatitis x gene into mouse hepatocytes *in vivo* through sendai viral envelopes : modulation of mitogenic signaling cascades

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Transcriptional activation of diverse cellular genes by the X protein (HBx) of hepatitis B virus (HBV) has been suggested as one of the mechanisms for HBV-associated hepatocellular carcinoma (HCC). However, such functions of HBx have been studied using transformed cells in culture and have not been examined in the normal adult hepatocytes, a natural host of HBV. Using an efficient hepatocyte-specific viral-based gene delivery system developed in our laboratory earlier, we studied the HBx action in vivo. We demonstrate that following virosome-mediated delivery of HBx DNA, a large population (>50 %) of hepatocytes express HBx protein in a dose-dependent manner which induces a significant increase in the activity of extracellular signal-regulated kinases (ERKs) in the liver of HBx-transfected mice.Inhibition of HBx-induced ERK activation following intravenous administration of PD98059, a MEK inhibitor, confirmed the requirement of MEK in the activation of ERKs by HBx. HBx induction of ERK activity was sustained up to 30 days. Interestingly, sustained activation of c-Jun N-terminal kinases (JNKs) up to 30 days was also noted. Such constitutive ERK and JNK activation as a consequence of continued HBx expression, also led to sustained stimulation of further downstream events such as increased levels of c-Jun and c-Fos proteins along with the persistent induction of AP-1 binding activity. Our data suggest a critical role of these molecules in HBx-mediated cell transformation. Further, we identified the regions of HBx that are crucial for activating such signaling cascades in vivo. A truncated mutant incorporating regions C to E (aa 58 to 140) was as effective as the full length HBx in activating MAPKs and enhancing AP-1 binding activity. While deletion of region C (aa 58 to 84) or D (aa 85 to119) led to a drastic loss of function, region E (aa 120 to 140) was dispensable for the activation of signaling cascades. These findings provide the first evidence for the requirement of domain 58 to 119 of HBx in transmitting mitogenic signals to the nucleus *in vivo*.

1. Nijhara et. al., *J. Virol*. (2001) vol.**75**, pp. 10348-10358.
2. Nijhara et. al., *FEBS Letts*. (2001) vol.**504**, pp.59-64.

I#84

Detecting of le^{y} antigen expression of individual blastocyst and exploring its functional significance

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Le^{Y} antigen is specifically expressed during 8 cells till blastocyst stages in the mouse embryo. Earlier we found that Le^{Y} not only plays a vital role as a mediator between the uterus-embryo recognition and adhesion in implantation of the mouse [1,2], but also may function as a signal molecule involved in regulation net work affecting on other implantation related factors, such as LIF, EGF, MMPs and ECM expression [3,4]. To investigate Le^{Y} expression in individual embryo and its significance for embryo development, highly sensitive and reliable methods were established to detect the Le^{Y} secretion (by Dot-blot analyzed with AKP labeled immunoblotting or ECL-HRP labeled immunoblotting) and gene expression (with LightCycler RT-PCR analysis) of single embryo scale. Using these new methods embryos at day4 of p.c. were detected and found that the secretion of Le^{Y} was different for individuals although they were in the same developing stage and had the same morphology under the microscopy. According to their Le^{Y} secretion level, embryos were divided into two groups and the results showed correspondence in FUT1 gene expression with Le^{Y} secretion by real-time PCR analysis. The developing situation of the two groups was observed *in vitro*, the primary results showed that the developing rate of the group with high level of Le^{Y} was faster than that of with low Le^{Y} level. In summary, the results indicated that Le^{Y} secretion of individual embryo was regulated by its gene transcription, and it might be a functional marker of embryo development. The detection of Le^{Y} secretion might be helpful for improving the implantation rate of IVF and transgenic animals.

The work is supported by NSFC(30170226) and preliminary research grant of national important basic research program.

1. Zhu ZM et al.(1995) *Biol. Reprod*. **52**:903-912
2. Zhu and Wang XQ (1998) *Mol.Hum.Reprod*.**4**:735-738
3. Ge CH et al. (2002) *Acta Biochim. Biophy.Sinica* **34**:45-49
4. Kong Y et al. (2002) *Prog. Biochem. Biophys*. **29**:278-282

26. Carbohydrate-carbohydrate and lectin-sugar recognition

I#85

Nuclear/cytoplasmic chito-oligosaccharide binding lectin from tobacco leaves: a novel class of regulatory proteins?

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In contrast to animal lectins, no evidence has been presented for the occurrence of plant lectins, which recognize and bind ‘endogenous’ receptors and accordingly are involved in recognition mechanisms within the organism itself. Recently we have shown that the plant hormone jasmonic acid methyl ester induces in leaves of *Nicotiana tabacum* the expression of a lectin that is absent from untreated plants (Chen et al., 2002). The jasmonate-induced *Nicotiana tabacum* agglutinin (called Nictaba) is a dimer consisting of two identical subunits of approximately 19 kDa. Hapten inhibition assays revealed that Nictaba exhibits an exclusive specificity towards oligomers of N-acetylglucosamine, the inhibitory potency of which increases with chain length up to 4 residues. Immunocytochemical localization studies have shown that the lectin is exclusively located in the cytoplasm and the nucleus. A search in the databases revealed that Nictaba shares the highest sequence similarity with putative phloem specific lectins from *Arabidopsis thaliana* and with some previously cloned Cucurbitaceae phloem lectins.

The identification of a major jasmonate-induced protein as a lectin with an exclusive specificity towards oligomers of N-acetylglucosamine is important because it demonstrates that tobacco cells express a potential receptor for chito-oligosaccharides as a response to treatment with jasmonate. Both the subcellular location and specificity indicate that the *Nicotiana tabacum* agglutinin may be involved in the regulation of gene expression in stressed plants through specific protein-carbohydrate-interactions with regulatory cytoplasmic/nuclear glycoproteins.

1. Chen Y, Peumans WJ, Hause B, Bras J, Kumar M, Proost P, Barre A, Rougé P, Van Damme EJM (2002) *FASEB J* (April 23, 2002) 10.1096/fj.01-0598fje

I#86

Macromolecular structures and interactions of cell wall polysaccharides

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Plant cells are distinguished from animal cells by the presence, around the plasmalemma, of a wall within which complex physicochemical and enzymatic phenomena progress. During cell growth the dimensions of the wall vary according to the type of macromolecule of which it is composed. Young plant cell walls represent a structure that is simultaneously rigid and dynamic. Rigidity is required to counterbalance the effect of turgor pressure on the plasmalemma. To allow cell extension to occur the cell wall structure must be deformable. This dual functionality of cell walls is achieved through the mixture of polysaccharides and proteins.

The presentation will focus on the description of the three-dimensional and architectural features of these cell-wall polysaccharide components. A description of their molecular structures and interactions is now available, at different levels of structural organisation, from the constituting monomers to the complex assemblies of macromolecular chains and their interactions. This has been achieved, throughout the development of novel molecular modelling procedures, in conjunction with the accumulation of experimental results derived from high-resolution NMR spectroscopy and diffraction studies. Emphasis will be given to the family of pectic components, with the very recent finding about the three-dimensional structure of one of the most complex polysaccharide in the plant kingdom: rhamnogalacturonan II.

I#87

Pseudomonas aeruginosa lectin PA-IIL displays a novel carbohydrate recognition mode: structural analysis of complexes with fucose and oligosaccharides

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Pseudomonas aeruginosa galactose (PA-IL) and fucose-binding (PA-IIL) lectins contribute to the virulence of this pathogenic bacterium. Determination of the crystal structure of PA-IIL complexed with fucose demonstrates a tetrameric structure. Each monomer displays a nine-stranded antiparallel β-sandwich arrangement and contains two calcium cations in one binding site. In each monomer, the calcium binding pocket is formed by two β-strand-connecting loops together with the C-terminal extremity of the adjacent monomer. The fucose-lectin interaction is mediated by the two calcium ions. Such a binding mode is unique in carbohydrate-protein recognition. Three of the fucose hydroxyl groups participate in the coordination spheres of the two calcium ions. Experimental binding studies together with theoretical docking of fucose-containing oligosaccharides are consistent with the assumption that antigens of the Lewis A series might be the preferred ligands of this lectin. Precise knowledge of the lectin binding site, should allow for a better design of new antiadhesive glycoderived or glycomimetic drugs.

I#88

Structure-function relationship of the salivary glycoproteins: agglutinin and mucins

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The mucous layer covering an organism's sensitive epithelia provides a protective barrier against potential noxious environments. Human saliva contains three types of glycoproteins that are crucial for keeping the microflora under control.

MUC5B, an oligomeric mucin, MUC7, a monomeric mucin, and salivary agglutinin are all three glycoproteins, of which 50-80% of their total mass consists of carbohydrate structures. The difference in genetic organization, protein structure as well as post-translational modifications may be suggestive for how these molecules achieve their protective role on a molecular level. The high-M_r mucins possess a tremendous heterogeneity in oligosaccharide side chains, including blood group active antigens, which may function as a selective colonization barrier for micro-organisms. On the other hand, the low-M_r mucins act as soluble receptor analogs for bacterial adhesins to promote oral clearance of micro-organisms. Salivary agglutinin is identical to gp-340, a member of the Scavenger Receptor Cysteine-Rich (SRCR) superfamily and is known for its bacteria agglutinating properties. The specific structures involved in bacteria binding will be illustrated on the basis of the interaction of the glycoproteins to cariogenic *Streptococcus mutans*, the periodontitis-associated *Actinobacillus actinomycetemcomitans*, and the gastric pathogen *Helicobacter pylori* as examples.

27. Glycosphingolipid receptors

I#89

_N_-glycans of sphingosine 1-phosphate receptor, Edg-1, regulate ligand induced receptor internalization

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Endothelial differentiation gene-1 product (Edg-1) is a G-protein coupled receptor (GPCR) for the platelet derived bioactive lipid mediator sphingosine 1-phosphate (Sph-1-P). Recent studies have shown that, in response to Sph-1-P, Edg-1 mediates various signaling pathways through downstream signaling molecules, such as MAP kinase and calcium, via trimeric G proteins.

In this study, we found, for the first time, that ddsdg-1 is glycosylated in its N-terminal extracellular portion, and further identified the specific glycosylation site as asparagine 30 by creating a non-glycosylated mutant of Edg-1 (N30D-Edg-1) and transfecting it into cell lines. The non-glycosylated mutant receptors, resembling their wild type controls, were predominantly expressed in the plasma membrane. Although there was no difference in ligand-binding ability and ligand-induced MAP kinase activation in the wild type and mutant receptors, non-glycosylated Edg-1 was much less responsive for ligand-induced internalization. Furthermore unlike the wild type receptor, which was associated with the caveolae, non-glycosylated N30D-Edg-1 was dispersed broadly in the membrane fractions separated by sucrose density gradient centrifugation, suggesting that internalization and microdomain localization of N-glycosylated Edg-1 are closely related. Although the precise molecular mechanism of the internalization of the N-glycosylated Edg-1 localized in the microdomain remains to be examined, the present studystrongly suggested that the presence of N-linked glycan in the receptor may play a regulatory role in the receptor dynamics in the ligand-stimulated mammalian cells.

I#90

Aglycone modulation of globotriosyl ceramide(gb_3)-verotoxin receptor function

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The Gb_3 fatty acid chain length/saturation significantly affects recognition by the *E. coli* verotoxin family. This may explain the differential cytotoxicity of VT1 and VT2 *in vitro* and *in vivo*. We now report that fatty acid hydroxylation can augment Gb_3 receptor function, particularly, for VT2. Differential cytotoxicity in the mouse correlates with binding in the lung which acts as a selective sink for VT1. Mouse renal Gb_3 is highly hydroxylated which may explain the VT2 preference for kidney binding, and lower LD50. Mouse renal and lung Gb_3 are different. These effects make the generation of soluble inhibitory Gb_3 analogues difficult. We have made a soluble Gb_3 mimic by exchanging the fatty acid for an adamantan frame. The behaviour of adamantylGb_3 in discontinuous sucrose gradients suggests that adamantylGb_3 in some way, mimics Gb_3 within lipid microdomains or rafts.

We have modeled a Gb_3 binding site within the VTB pentamer at the monomer interface-site 1. Subsequent co-crystalography using the lipid-free oligosaccharide identified three Gb_3 binding, sites, of which, site 2 was preferred. To further address which binding site accommodates Gb_3 glycolipid, we synthesized adamantylGb_3 containing amino substitutions at hydroxyl groups important in VT1 recognition. Neither the co-crystal site 2 or the modeled site 1 complex could fully explain the results obtained. The data however, were consistent with both complexes, particularily when solvation effects were considered. The binding results were, however, inconsistent with the co-crystal site 3.

I#91

Carbohydrate structural units in glycosphingolipids as receptors for Gal and GalNAc reactive lectins

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Glycosphingolipids (GSLs) contain many carbohydrate epitopes or crypto-glycotopes for Gal and GalNAc reactive lectins. Many of them are in the nervous system, and function as important receptors in various life processes. During the past two decades, eleven mammalian structural units have been used to express the binding domain of applied lectins. They are:

F, GalNAcα1→3GalNAc; **A**, GalNAcα1→3Gal; **T**, Galβ1→3GalNAc; **I**, Galβ1→3GlcNAc; **II**, Galβ1→4GlcNAc; **B**, Galα1→3Gal; **E**, Galα1→4Gal; **L**, Galβ1→4Glc; **P**, GalNAcβ1→3Gal; **S**, GalNAcβ1→4Gal and **Tn**,

GalNAcα1→Ser(Thr). Although ten of them occur in GSLs, only three (**L**$_\beta$, **S**$_\beta$, and **T**$_\beta$) are found in human brain, and two (**L**$_\beta$ and **II**$_\beta$) are present in the inner structures of human blood group active GSLs. In the families of gangliosides, **L**$_\beta$ and **II**$_\beta$ represent 55% of the total structural units, while the other three units (**T**$_\beta$, **P**$_\alpha$, and **S**$_\beta$) constitute the rest. To facilitate the selection of lectins that could serve as structural probes, the carbohydrate binding specificities of Gal/GalNAc reactive lectins have been classified according to their highest affinity for the structural units and their binding properties expressed by decreasing order of reactivity. Hence, the binding relation between GSLs and Gal/GalNAc specific lectins can be established.

1. Wu AM, Song SC, Tsai MS, Chen YY, Nechama GG (2000) *J. Biol. Chem.* **275**:14017-14024.
2. Wu AM (2001) *Adv. Exp. Med Biol.* **491**:55-64.
3. Wu AM, Song SC, Tsai MS, Herp A (2001) *Adv. Exp. Med Biol.* **491**:551-586.

I#92

Lactosylceramide: Its role in cell proliferation, cell adhesion in atherosclerosis

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Although several established such factors such as oxidized low density lipoproteins, platelet derived growth factor, nicotine and shear areas and a proinflammatory cytokine such as tumor necrosis factor-α are known to contribute to atherosclerosis the role/involvement of glycosyltransferases and glycosphosphingolipids in this disease is not known.

We have found that in human aortic smooth muscle cells oxidized LDL, PDGF and nicotine activate LacCer synthase to produce LacCer. In turn, LacCer activated NAD(P) H oxidase to produce superoxide ion O_2 - that transduced an "oxygen-sensitive signal transduction cascade involving the phosphorylation of mitogen-activated protein kinase, and a protooncogene c-fos expression ultimately leading to cell proliferation. Nicotine stimulated the expression of an immediate early gene; monocyte chemoattractant protein (MCP-1). Both cell proliferation and the expression of MCP-1 was inhibited by preincubation of cells with D-PDMP, an inhibitor of LacCer synthase.

In human umbilical vein endothelial cells (HUVEC's) TNF-α and shear stress also stimulated the activity of LacCer synthase and the oxidant-sensitive signal transduction cascade involving Nf-KB expression and intercellular cell adhesion molecule (I-CAM-1) expression to stimulate the adhesion of monocytes.

In human monocytes and neutrophils LacCer activated phospholipase A_2 to produce arachidonic acid that in turn stimulated the expression of platelet endothelial cell adhesion molecule (PECAM-1) and CD11b/CD18 (Mac-1), respectively to stimulate cell adhesion and cell migration.

In sum, by converging upon LacCer synthase and producing LacCer, various risk factors activate a molecular/ signal transduction mechanism that contribute to critical phenotypic changes in human vascular cell such as adhesion, migration and cell proliferation that collectively accelerate atherosclerosis.

28. Glycolipids in cell death and activation

I#93

Glycolipids of parasitic metazoa: structures and biological relevance

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Glycolipids play important roles in metazoan host/parasite interactions. Using parasitic and free-living nematodes (e.g., *Ascaris suum*, *Caenorhabditis elegans*) as well as two trematode representatives (*Schistosoma mansoni*, *Fasciola hepatica*) as model organisms, we have structurally characterized their glycolipids. The results revealed that Nematoda express *arthro-series* glycolipids with phosphorylcholine substituents, thus representing highly conserved glycoconjugate markers. As a characteristic feature, these molecules induced human peripheral blood mononuclear cells to release a distinct set (TNF-α, IL-1, IL-6) of pro-inflammatory cytokines demonstrating the immunomodulatory potential of this type of glycoconjugate [1]. In contrast, analysis of glycolipids from *S. mansoni* and *F. hepatica* revealed the expression of host-specific carbohydrate epitopes, Lewis X (CD15) and globotriaosylceramide (CD 77), respectively [2,3], in agreement with the concept of molecular mimicry. Hence, the glycolipids of parasitic metazoans may fulfill multiple tasks in their different survival strategies.

1. Lochnit G *et al* (2000) *Biol Chem* **381**:839-847
2. Wuhrer M *et al* (2000) *Glycobiology* **10**:89-101
3. Wuhrer M *et al* (2001) *Biol Chem* **382**:195-207

I#94

GM3 as a component of a multimolecular signaling complex involved in T cell activation

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Gangliosides, sialic acid containing glycosphingolipids, are ubiquitous constituents of cell membranes and have been implicated in a variety of biological events occurring at the cell surface. In human peripheral blood lymphocytes GM3 is the main ganglioside constituent of cell plasma membrane, where it is localized in glycosphingolipid (GM3)-enriched microdomains (GEM). The presence of tyrosine kinase receptors, mono- (Ras, Rap) and heterotrimeric G proteins, Src-like tyrosine kinases (lck, lyn, fyn), PKC isozymes and GPI anchored proteins allows to consider these portions of the plasma membrane as "glycosignaling domains" In this concern, we demonstrated that GM3 is the main ganglioside constituent of microdomains in

human lymphocytes. Immunoelectron and scanning confocal microscopy revealed a clustered GM3 distribution over the cell surface and an intracellular localization, resembling specific cytoplasmic compartment(s). However, in lymphocytes, not only GM3 and cholesterol, but also CD4 and $p56^{lck}$ are selectively recovered in GEM. T cell activation does not promote a redistribution of GEM, but induces Zap-70 translocation to the selective membrane domains in which the phosphorylation protein Zap-70 may interact with GM3. Coimmunoprecipitation experiments revealed that only after costimulation through CD3 and CD28, GM3 was immunoprecipitated by anti-Zap-70. Therefore, GM3 is a component of a multimolecular signaling complex involved in the regulation of T cell activation in which several proteins, including Srk kinases, receptors and cytoskeleton proteins cooperate in triggering the signal transduction pathway.

I#95

Elevated expression of monosialoganglioside GM3 modulates availability of viral receptors: studies with mouse melanoma skin fibroblast B16 cells expressing HIV-1 receptors CD4, CXCR4 and/or CCR5

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Target membrane glycosphingolipids (GSLs) are essential for HIV-1 infection provided that CD4 and chemokine receptors are expressed at physiological levels. To evaluate molecular interactions between GSLs and the HIV-1 receptors, we examined fusion activity of B16 cells, which express glucosyl ceramide plus ***exceptionally high levels*** of GM3.

We report here that B16 cells bearing CD4, CXCR4 and/or CCR5 ***do not*** support fusion with CD4-*dependent* HIV-1 Envelope glycoprotein (Env)-expressing cells. In contrast, B16 cells bearing CD4 and cognate coreceptors readily fused with (a) CD4-*dependent* $HIV\text{-}2_{SBL6669}$ and SIV_{mac} Envs; and (b) CD4-*independent* HIV-1mutant Envs, 8X and 8X-V3BaL. Therefore, inability of B16 cells to promote CD4-mediated HIV-1 fusion was not due to altered expression of CD4 and coreceptors.

GM3 has been implicated in binding and down-modulation of cellular CD4. To disrupt potential lateral CD4-GM3 association, we pre-incubated B16 cells with (a) influenza virus which binds to GM3; (b) PPMP to deplete cellular GSLs; and (c) unsaturated fatty acids to disrupt plasma membrane microdomains. Our first studies show that influenza-treated B16 cells became highly susceptible to subsequent fusion with $HIV\text{-}1_{Env}$-expressing cells without affecting surface expression of receptors.

We propose that ***elevated levels*** of GSLs in the plasma membrane can engage viral receptors and provide a novel mechanism by which host cells can escape viral infections.

I#96

Galβ1-3GalNAcβ as a trigger of anti-tumor macrophage activity

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The macrophages (Mϕs) is considered the first line of defense in immune response to tumor growth. Mϕs employ a number of mechanisms to inhibit tumor growth, destroy and engulf tumor cells. Recently we have shown that Galβ1-3GalNAcβ ($T_{\beta\beta}$-disaccharide) is crucial for phagocytosis of apoptotic bodies by monocyte originated THP-1 cells. Taking into consideration these data we studied endogeneous lectin involvement in the recognition of tumor apoptotic bodies by infiltrating Mϕs in leukosis model. The leukosis was generated from spontaneous tumor in BYRB mouse. Using a panel of fluorescein-labeled glycoconjugates (Sug-PAA-flu), the expression of siglecs and galectins on the peritoneal Mϕs in tumor-bearing and intact mice BYRB was studied. Our results showed increase of galectin and siglec ligands binding to i.p. tumor infiltrating Mϕs comparing with intact cells. Infiltrated Mϕs displayed strong binding to $T_{\beta\beta}$, asialoGM1. Furthermore, $T_{\beta\beta}$ as synthetic glycolipid was obtained and incorporated into apoptotic bodies generated from tumor-bearing BYRB mice. Loaded apoptotic bodies were injected i.p. in BYRB mice bearing the same tumor in advanced form. We observed survival improvement tendency of treated animals as compared with control tumor-bearing mice (treated with intact apoptotic bodies).

29. Sialic acids in health and disease

I#97

Cleavage of polysialic acid by endosialidase: dissociation of the substrate binding and catalytic activities and design of a substitute antibody

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Polysialic acid, a cell surface polysaccharide shared by bacteria and eukaryotic cells, is cleaved by bacteriophage-derived endosialidase. By first selecting bacterial mutants resistant to such phages, and then phages infecting the mutant bacteria, mutant phages were obtained. These lacked endosialidase activity but still bound to polysialic acid.

The mutant phages in combination with anti-phage antibodies and secondary antibodies proved to be useful reagents in the detection of polysialic acid of eukaryotic cells in binding

assays, dot and Western bolts, as well as light and fluorescence microscopy.

In order to determine the basis for the lost catalytic activity, the genes coding for the endosialidases of the PK1A and PK1E phages were cloned and sequenced. The mutations were revealed as changes at the single amino acid level. The mutant endosialidase was engineered to a fusion protein, which was found to be a superior single-step reagent for the detection of polysialic acid.

Thus, the substrate-recognition specificity of an enzyme was converted to the detection specificity of a molecular beacon. More generally, this approach could be applied in the design of enzyme-derived substitute antibodies and reagents in biological research.

I#98

Sialyl linkages and molecular species of sialic acid as the determinant of host range of influenza viruses

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Receptor specificity of influenza virus hemagglutinin is linked to the host species from which the virus is isolated. We found that the sialyllacto/sialyl*neo*lacto-series sugar chains, SA(sialic acid)α2-3(6)Galβ1-3(4)GlcNAcβ1-, in glycoproteins and glycolipids are the common and functional receptor sugar chains for influenza A and B viruses from human and animals. The change of the receptor binding specificity (Neu5Ac2-6Gal → Neu5Ac2-3Gal) appears as the substitution of the amino acid (Leu226 → Gln) located in the receptor binding pocket of the viral hemagglutinin (HA). A set of 2 amino acid substitutions, Leu226 →Gln; Ser228 → Gly in human influenza virus HA molecules are responsible for the change of the receptor binding specificity from the virus directed to human (Neu5Ac2-6Gal) to duck and horse (Neu5Gc2-3Gal). These results provide the possible mechanism for the transmission of the influenza viruses between humans and animals in nature, and also the first evidences of biological effect of different sialyl linkages and different sialic acid species in different animals. We found the evidence that the receptor binding specificity of H3 human influenza A viruses has changed since the time of its introduction from birds into the human population. We also found that all duck isolates tested universally retain sialidase activities under low pH conditions independent of their neuraminidase (NA) subtypes. In contrast, the sialidase activities of most isolates from humans and pigs practically disappear below pH 4.5, with the exception of four human pandemic viruses isolated in 1957 and 1968. This finding suggests that the low pH stability of duck influenza A viruses NA may be a critical factor for the replication in the intestinal tract through the digestive tract of ducks, and that the properties of NAs are important for understanding the epidemiology and the host range of influenza viruses.

I#99

Plasma membrane-associated sialidase is up-regulated in human prostate cancer

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To elucidate the molecular mechanisms and significance of aberrant sialylation in cancer, our studies have been focused on sialidase. We previously demonstrated increased expression of plasma membrane-associated ganglioside sialidase (Neu3) in human colon cancer. Here we report that Neu3 is also up-regulated in human prostate cancer. A marked increase (3- to 45-fold) in Neu3 mRNA level was detected in the most cases for cancer as compared with non-cancerous tissues. The expression level was significantly correlated to the corresponding PSA level ($p < 0.0001$) in the cancer tissues, and inversely to differentiation grade of the cancer. A prostate cancer cell line, androgen-insensitive and apoptosis-resistant PC-3 cells showed higher expression of the sialidase than androgen-sensitive LNCap cells, in the activity as well as in the mRNA levels. The sialidase expression of LNCap cells was down-regulated by induction of differentiation with sodium butyrate treatment. PC-3 cells increased Bcl-2 protein level by Neu3 overexpression. These results suggest that up-regulation of Neu3 sialidase may be related to malignant properties in prostate cancer and that the selective inhibitors of Neu3 may be useful for the treatment of prostate cancer.

1. Kakugawa Y. et al (2002) *Proc Natl Acad Sci USA* **99**: 10718-10723

I#100

Unusual glycosphingolipids in polyagglutinable NOR erythrocytes

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NOR erythrocytes contain two major unique neutral glycolipids (designated NOR1 and NOR2) stained on TLC plates with *G. simplicifolia* IB4 lectin and human anti-NOR antibodies. The structure of NOR1 has been previously established as globoside with additional Gal residue α1,4-linked to GalNAc [1]. To determine the structure of NOR2, this glycolipid was partially purified by silica gel column and preparative TLC chromatography. The ion trap mass spectrometry studies showed that NOR2 is NOR1 elongated by Hex1-4HexNAc1-3 unit. Total NOR neutral glycolipids fractionated by TLC showed the presence of a band migrating between NOR1 and NOR2 and strongly stained with soybean lectin. This band, absent from control erythrocytes, was likely to be a glycolipid intermediate between NOR1 and NOR2 (Nor_{int}). NOR2 was totally digested with α-glactosidase to give NOR_{int} which, in turn, was partially transformed into NOR1 by β-hexosaminidase treatment. These data allowed to conlude that NOR erythrocytes contain the following glycolipids:

Galα1-4GalNAcβ1-3Galα1-4Lac-Cer (NOR1)
GalNAcβ1-3Galα1-4GalNAcβ1-3Galα1-4Lac-Cer (NOR_{int})
Galα1-4GalNAcβ1-3Galα1-4GalNAcβ1-3Galα1-4Lac-Cer (NOR2)

Our results suggest that NOR phenotype is connected with expression of unusual Gal-T able to form the Galα1-4GalNAc linkage.

1. Duk M. *et al.,* (2001) *J. Biol. Chem.* **276**: 40574-82.

30. Glycome and glycotechnology

I#101

Ultra-high sensitivity mass spectrometry in glycomics and glyco-proteomics

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Ultra-high sensitivity mass spectrometric strategies for defining the primary structures of highly complex mixtures of glycopolymers are revolutionizing structural glycobiology in the post-genomic era (1). MS strategies incorporating FAB-MS, MALDI-MS and ES - MS/MS are applicable to diverse glycopolymers including natural and recombinant glycoproteins, glycosaminoglycans, glycolipids and polysaccharides, and mapping strategies lie at the heart of many of our protocols (2). They enable very complex mixtures from biological extracts or glycopolymer digests to be screened thereby revealing the types of glycans present and, importantly, providing clues to putative novel structures. Data will be presented from our knockout mouse and *C. elegans* glycomics research programmes (3,4) where we have identified a number of novel glycosylation pathways. We will also report on some of our proteomics and glyco-proteomics research including studies of glycosyltransferases in *Dictyostelium*, tyvelose-containing glycoproteins in *Trichinella*, novel glycoproteins in *Campylobacter*, and T-cell derived glycoproteins.

1. A.,Dell, H.R.Morris, *Science,* 291 (2001) 2351-2356.
2. Sutton-Smith, H.R. Morris, A. Dell, *Tetrahedron Assymetry* 11 (2000) 363-369.
3. M. Sutton-Smith, H.R. Morris, J.E. Hewitt, P.K. Grewal, R.E. Bittner, R.Schiffmann, A.Dell, *Annual Symp. Biochem. So*c., in press.
4. S.M. Haslam, D. Gems, H.R. Morris, A. Dell, *Annual Symp. Biochem. So*c., in press.

I#102

Oligosaccharides and oligolysine-based clusters: selective recognition and endocytosis by membrane lectins of mammalian cells.

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Dendritic cells (DC) are the most potent antigen-presenting cells; they express several membrane lectins including ManR, DEC205 and DC-SIGN. On these bases and in search of highly specific ligands, oligosaccharide were converted into glycosynthons (Os) and Os were used to prepare oligolysine-based saccharides, Os-$[Lys(Os)]_n$ -Ala-Cys-NH_2 with $n = 1$ to 5 , were synthesized (optimal activity for $n = 4$, Frison *et al*., submitted). The thiol group of the appended cysteine residue allows a fluorescent tagging or the linkage to an antigen.

Surface plasmon resonance was used to determine the affinity of the different glycosylated compounds to ManR and DC-SIGN. The uptake of fluoresceinyl-labeled compounds was studied by flow cytometry and confocal microscopy analysis.

Dimannosides clusters were efficiently taken up by human DC (Bédouet *et al*., 2002, Bioscience Reports) and fibroblasts expressing the ManR. The affinity of dimannoside clusters was higher for the ManR than for DC-SIGN and depended on the structure of the dimannosides, while clusters bearing fucosyl residues were better recognized by DC-SIGN. As controls, lactosyl clusters were efficiently taken up by HepG2 which express a galactose-specific lectin, while they were neither recognized by the ManR and DC-SIGN nor taken up by DC cells . These results demonstrate that such disaccharide clusters are highly specific. ManR, ManR expressing cells and DC-SIGN were respectively from M. Taylor and K. Drickamer, Oxford UK. Plasmid encoding DC-SIGN was from E. Soilleux, Cambridge, UK.

I#103

Studying carbohydrate-protein and carbohydrate-carbohydrate interactions

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The study of biomolecular interactions is of general importance for understanding the processes of molecular recognition and biological functioning. Traditionally, techniques such as immunoassays, equilibrium dialysis, affinity chromatography, and spectroscopic methods have been used for measuring interactions between biomolecules. Recent developments in biosensor technology based on surface plasmon resonance (SPR) have greatly simplified the measurement of binding characteristics of biomolecules. Although the applications of SPR in studying protein-protein interactions are multifold, the usefulness of this technology in analyzing carbohydrate-protein and carbohydrate-carbohydrate interactions is still under-exposed. Besides SPR, also transmission electron microscopy (TEM), making use of carbohydrate-conjugated gold nanoparticles, receives more and more attention in studying carbohydrate-ligand interactions.

Here, typical SPR and/or TEM examples of lectin-carbohydrate (different plant lectins), antibody-carbohydrate (antibodies recognizing synthetic fragments of capsular polysaccharides of pathogenic bacteria), and carbohydrate-carbohydrate (synthetic fragments of the self-aggregation factor of the marine sponge *Microciona prolifera*) interactions will be presented. Additionally, a method will be introduced based on SPR combined with HPLC profiling for the screening of the binding of oligosaccharides, present in complex mixtures, to complementary molecules.

I#104

Biases and complex patterns in the residues flanking protein *N*-glycosylation sites

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Protein glycosylation is the most common and most complex post-translational protein modification reaction. N-Glycosylation occurs at the Asn residue of the sequon NXT/S, only about two thirds of which is glycosylated. Little is known about the influence of residues at the X position, or of those flanking the

sequon, on the efficiency of N-glycosylation. We used bioinformatics to analyze the relation between N-glycosylation and X, as well as the flanking residues M_2M_1 and P_1P_2 in the extended seven-mer sequon, M_2M_1NXS/TP_1P_2. Biases at single positions, and at multiple ones, were studied. The dataset was derived from SwissProt, and consisted of 602 well-characterized, non-redundant N-glycoproteins, which contain 1186 glycosylated and 717 non-glycosylated sequons.

The single site analysis revealed that glycosylated sequons had an under-representation of Pro and over-representation of Gly in the X position as suggested earlier, and an over-representation of Leu and under-representation of Pro in the P_1 position. For non-glycosylated sequons, over-representation of Ser was found in M_2, Asp in M_1, Lys and Pro in X, Tyr in P_1 and Gly in P_2; under-representation of Leu was observed in position P_1.

The multiple site analysis showed the presence of two patterns. When two or three residues at positions M_{1-2}, X, and P_{1-2} matched the extended sequon D/ESNGTLT, glycosylation invariably occurred. In contrast, when two or three residues at the same positions matched the sequence SDNKS/TYG, no glycosylation was found.

31. Glycosyltransferases and glycosidases trafficking and function

I#105

A functional glycomic approach of *N*-acetylglucosaminyltransferases III and V, and alpha1-6 fucosyltransferase: Identification and characterization of glycoprotein functions

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The glycosylation represents a critically important post-translational modification reaction and is a target for future proteomic research. However, the actual *in vivo* target proteins of each glycosyltransferase are not known with certainty. Even if the glycosyltransferase gene(s) could be knocked out and phenotypic changes observed in the gene targeted mice, the real target proteins *in vivo* would still remain unidentifiable, because those changes might be due to a secondary effect, derived from the glycosylation of some proteins. Therefore, a functional glycomic approach involving the identification of likely target proteins and the characterization of their functional changes due to glycosylation is essential for understanding the role of sugar chains on these molecules. We have transfected alpha1-6 fucosyltransferase or N-acetylglucosaminyltransferases (GnT) III or V into various cell lines, in an attempt to identify the target proteins in vitro and in vivo. Especially it is a relatively simple task to identify two enzymatic products, alpha 1-6 fucosylated proteins and bisected GlcNAc added glycoproteins using lectin binding analyses because these sugar chains are almost a " stop codon" and are no longer modified by other glycosyltransferases. In the GnT-III transfectants various glycoproteins such as E-cadherin underwent glycosylation and functional changes were observed. We also show some data for the case of alpha 1-6 fucosyltransferase gene transfectants and knock out mice in terms of functional glycomics.

I#106

Trafficking and localisation of resident golgi glycosyltransferases: evidence for the segregation of glycosyltransferases into distinct membrane subdomains

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The mechanisms that determine the steady-state distribution of resident Golgi glycosylation enzymes may include aggregation, lipid mediated sorting and/or retrograde transport pathways [1]. To investigate if the *medial*-Golgi enzyme, *N*-acetylglucosaminyltransferase I (GlcNAc-TI) is transported to the late Golgi, a modified GlcNAc-TI bearing an *N*-glycan site on the C-terminus was constructed. The modified GlcNAc-TI was demonstrated to be functionally active *in vivo*, and was localised to the Golgi stack of transfected cells. In stable CHO cell clones, the *N*-glycosylated GlcNAc-TI carried sialylated complex *N*-glycan chains. Pulse-chase studies showed that the majority of GlcNAc-TI was sialylalated within 60 min of synthesis [2]. Treatment of transfected CHO cells with brefeldin A resulted in the glycosylated GlcNAc-TI bearing Endo-H resistant chains, however, the sialylation of glycosylated GlcNAc-TI was dramatically reduced. These data imply that, in CHO cells, newly synthesized GlcNAc-TI is transported rapidly through the Golgi stack to the *trans*-Golgi network, suggesting that GlcNAc-TI continuously recycles from the late Golgi. Furthermore, by perturbation of distinct regions of the TGN, we have demonstrated that the recycling GlcNAc-TI and the TGN-resident sialyltransferase are segregated from the TGN membrane protein TGN38. These data strongly suggest that glycosyltransferases are partitioned into distinct membrane domains of the TGN.

1. A.S. Opat, F. Houghton and Paul A. Gleeson, *J. Biol. Chem.*, 275 (2000) 11836-11845.
2. A.S. Opat, F. Houghton and P.A. Gleeson, *Biochem. J.*, 358 (2001) 33-40.

I#107

Six hyaluronidase-like sequences in human and mouse genomes

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The human genome contains six hyaluronidase-like genes, two clusters of three each at 3p21.3 and 7q31.3. The genes code for Hyal-1, -2, and –3 on chromosome 3, and Hyal-4, a pseudo gene, and PH-20 (the sperm-specific enzyme) on chromosome 7. Extensive homology between all these genes suggests two ancient gene duplication events, followed by *en masse* block duplication, all occurring before the emergence of modern mammals. A model is proposed suggesting that Hyal-1 and –2 are the major somatic hyaluronidases acting in concert to degrade high molecular mass hyaluronan. Hyal-2 occurs as a GPI-linked enzyme on cell surfaces that degrades hyaluronan to intermediate sized fragments. Hyal-1, a lysosomal enzyme, then degrades these fragments to tetrasaccharides. Hyaluronan has different important biological functions at each stage of its degradation. The mouse genome contains similar clusters of these six sequences in chromosome regions syntenic with the human genome. Preliminary evidence suggests Hyal-4 is a chondroitinase, the first to be described in vertebrate tissues. *C. elegans* contains only one hyaluronidase-like sequence. However, the nematode has no hyaluronan, but does contain chondroitin. A chondroitinase may be the original proto-enzyme, which upon duplication, was modified to degrade hyaluronan also. This may explain why Hyal-4 degrades only chondroitin (and chondroitin sulfate), while hyaluronidases degrade both hyaluronan and chondroitin sulfates, albeit at slower rates.

I#108

A proposal of a common eukaryotic origin for cytidylyltransferases

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Cytidylyltransferases (CT) catalyze the activation of a single monomer, commonly a glycid, into the nucleotide-monophosphoryl-glycid form. This is not an ordinary reaction, since the most common product is the diphosphoryl-glycid form. Moreover, the substrate is not a glycid-1-phosphate, but a non-phosphorylated form.

We present here an analysis of the phylogeny of the KDO-CT and of the Neu-CT. These enzymes respectively activate KDO and NeuAc prior to its incorporation into the corresponding polymer. The CT family comprise three trees, one of them including all Neu-CT from both bacteria and animals, and another covering all the KDO-CT from bacteria and plants. A third cluster of heterogenous prokaryotic ORFs corresponding to COG#1861 also belongs to this family.

It is proposed an ancestral eukaryotic origin for an original CT gene, which gave rise to Neu-CT in animals and KDO-CT in plants. Later, two horizontal gene transfer event would have occurrd. On of them towards an ancestor of *Chlamydiae*, and subsequently expanded as a KDO-CT in gram-negative bacteria. The second one, towards some microorganisms, either animal parasites or animal symbionts, which would have accepted the Neu-CT gene as a part of their strategy to evade the host immune response.

32. Glycans in cell adhesion and disease

I#109

Increased expression of *N*-linked β(1,6) glycans affects cell adhesion and migration by multiple mechanisms

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Transcriptional upregulation of GnT-V and N-linked β(1,6) glycans during oncogenesis causes reduced cell-matrix and cell-cell adhesion, leading to increased invasiveness and migratory activity. Alpha5beta1 and alpha4beta1 integrin-mediated cell adhesion to fibronectin are both reduced by increased β(1,6) branching, and this effect appears to be due to inhibition of integrin clustering induced by contact with fibronectin. Changes in N-linked β(1,6) branching appears to be limited to the beta1 integrin subunit, although many N-linked glycans are found on the alpha subunits. Second, adhesion of cells that over-express GnT-V to the domain of fibronectin that binds specifically to heparan sulfate is also markedly reduced. Experiments to test the mechanism by which this inhibition in cells with increased β(1,6) branching occurs have implicated ~ 80% reduction of syndecan-4 transcription. In HT1080 cells, syndecan-4 expresses the majority of heparan sulfate found in these cells. Pre-treatment of GnT-V over-expressing cells with swainsonine, which eliminates N-linked β(1,6) branching by effects on upstream alpha-mannosidase II processing, returns syndecan-4 levels to normal levels. There appears to be a mechanism, therefore, by which up-regulation of β(1,6) branching causes reduction of syndecan-4 and heparan sulfate-mediated adhesion to fibronectin. Third, some human tumor cells, including breast carcinoma, express the homotypic adhesion molecule, N-cadherin, which is responsible for calcium-dependent cell-cell adhesion. Increased β(1,6) branching inhibits by 50% the rate of calcium-dependent HT1080 cell aggregation, and N-cadherin shows concomitant increases in β(1,6) branching. No changes in levels of cell-surface N-cadherin are observed after GnT-V over-expression. These and other results suggest that the reduction of cell-cell calcium-dependent adhesion after increased N-linked β(1,6) branching involves changes in N-cadherin-mediated adhesion. Experiments are in progress to determine the intracellular signaling pathways through which each of these changes affects cell migration activity.

I#110

Mammalian *O*-mannosyl glycan and relationship to muscular dystrophy and neuronal migration disorder

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We found the *O*-mannosyl glycan in α–dystroglycan, a cytoskeleton-linked extracellular matrix receptor. *O*-Mannosyl glycosylation is known as a yeast-type modification. However, a key difference between mammalian and yeast-type *O*-mannosyl glycans is that those in mammals have the GlcNAcβ1-2Man linkages. Analysis of the biosynthetic pathway of the *O*-mannosyl glycans in mammalian cells is important for elucidating not only the regulation of expression but also the biological functions of these glycans. We isolated a human cDNA for protein *O*-mannose β-1,2-*N*-acetylglucosaminyltransferase (POMGnT1) that participates in the synthesis of *O*-mannosyl glycan. Muscle-eye-brain disease (MEB) is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities, and lissencephaly. We identified six independent mutations of the *POMGnT1* gene in six patients with MEB. Expression of most frequent mutation revealed a great loss of the enzymatic activity. We also found a selective deficiency of α-dystroglycan in MEB patients. These findings suggest that α–dystroglycan is a potential target of POMGnT1 and that interference in *O*-mannosyl glycosylation of α-dystroglycan is a new pathomechanism for muscular dystrophy as well as neuronal migration disorder.

I#111

Development of *biogeneric* human glycoprotein therapeutics from mammalian cell lines

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Recombinant human glycoprotein therapeutics from mammalian cells have found their way into routine clinical treatment over the past decade. Nonetheless, there is no universal host cell line available which guarantees the optimal glycosylation or more general the optimal posttranslational modification repertoire for any therapeutic under development.

In the area of biotechnologically prepared *generic* products which are expected to be made available in the next few years (around 2006-2010 : e.g. *IFN-ß, EPO, antibody-cytokine-fusion proteins*). Apart from the unresolved regulatory issues associated with these biogeneric therapeutic glycoproteins a major difficulty in the development of *biogeneric products* lies the fact, that for most researcher at biotech companies it seems almost impossible to reproduce the exact posttranslational modification of an already marketed product and thus to identically reproduce its in-vivo poperties.

Data will be presented describing the identification/selection of new host cell lines using e.g. adenoviral vectors, the genetic engineering of cell lines and analytical and screening procedures for a rational development of recombinant glycoprotein products at the level of both upstream and downstream processing that should lead to *tailored modifications* of novel therapeutics as well as biogeneric products.

I#112

Molecular dance of β-1, 4-galactosyltransferase and lactose synthase

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Structural investigations on the β-1,4-galactosyltransferase-1 (Gal-T1) and lactose synthase (LS) have revealed that they are akin to an exquisite mechanical device with two well-coordinated flexible loops that are contained within the Gal-T1 catalytic domain. The smaller one has a Trp residue (Trp314) flanked by glycine residues. The larger one comprises amino acid residues 345 to 365. During the catalytic cycle, upon substrate binding, the Trp314 side chain moves to lock the sugar nucleotide in the binding site, while the large loop undergoes a conformational change, masking the sugar nucleotide binding site, and creates: 1) a metal ion binding site by repositioning the residue His347; 2) the oligosaccharide binding cavity; and 3) a protein-protein interacting site for the LS partner, α-lactalbumin (LA). Only in conformation II do Gal-T1 and LA form the LS complex, where LA sits at the extended sugar binding-site of Gal-T1 and holds Glc in its acceptor binding-site. Gal-T1 then maximizes the interactions with Glc, thereby making it a preferred acceptor in the LS reaction. Among the residues, Asp254, Met344 and His347 of Gal-T1, which coordinate metal ion, only Asp254 and His347 are strong metal ligands. Mutation of Trp314 to Ala slows the change to conformation II and abolishes the binding to UDP-Gal or GlcNAc. In the sugar nucleotide binding-pocket of Gal-T1, side chain hydroxyl group of Tyr289 acts as a molecular brake for the transfer of the sugars from the less preferred sugar nucleotides. LA stabilizes the sugar-nucleotide-Gal-T1 (conformation II) complex, thus kinetically enhancing the sugar transfer, even from the less preferred sugar nucleotides. The conformational change that masks the sugar nucleotide binding-site, can also be induced by the sugar acceptor alone, thus making it possible for each Gal-T family-member to act as a specific lectin.

33. Modulation of cellular functions by glycosylation

I#113

Apoptosis of human colon and breast carcinoma cells by inhibitors of glycolipid biosynthesis

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Apoptosis, or programmed cell death, plays an important role in many physiological and diseased conditions. Detection of apoptotic cancer cells, monitoring the cell's progression to apoptosis, and searching mechanism of the apoptotic pathway in the human carcinoma Colo-205 and breast cancer MD7 cells are the goals for our present investigation. In our present studies, we have employed several drugs: *cis*-platin (which inhibits DNA

polymerase-α), L-PPMP and L-PDMP (which inhibit GlcT-glycosyltransferase), Tamoxifen (cancer drug) and Melphalan to initiate apoptosis in Colo-205 cells in culture in the presence of ^{3}H-TdR (for DNA biosynthesis), and ^{3}H-L-Serine/or ^{14}C-L-Serine (for Ceramide and Glycolipid, GSL biosynthesis). Our analyses showed that the above mentioned drugs initiate apoptosis in a dose dependent manner and showed a pleiotropic effect and phenotypic changes in those cells. Incorporation of ^{3}H-L-Serine in Ceramide increased at low concentrations (2-4 μM) of L-PPMP and D-PDMP but decreased in ^{3}H-Ceramide. However, no such changes were observed with even higher concentrations of *cis*-platin (25-200 μM). It appears that the apoptosis of these tumor cells occurs by two separate pathways initiated by *cis*-platin (an inhibitor of DNA biosynthesis) and L-PPMP and D-PDMP (inhibitors of initiation of glycosylation of ceramide). Previously, we have established that *cis*-platin binds to the Zn-binding domain of the DNA Polymerase (Bose, R., Li, D., Yang, W-W, and Basu, S. (1999) J. Biomol. Struct. Dynamics,16, 1075-1084). The probable relationship of the inhibition of DNA and glycolipid biosyntheses to the apoptotic process in these tumor cells is being investigated.

I#114

Cell surface expression as well as the function of the epidermal growth factor receptor is impaired by mutation of specific glycosylation sites

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The present study was initiated to investigate the role of specific N-linked glycosylation sites in the functioning of the epidermal growth factor receptor (EGFR), a transmembrane protein with intrinsic tyrosine kinase activity. EGFR contains 12 N-linked glycosylation sites in its extracellular region subdivided in four domains. Core glycosylation is needed to induce a conformation critical for EGF binding. Ligand binding activates the kinase by converting the monomeric receptor to its dimeric state. However, we have demonstrated recently that a naturally occurring truncated EGFR expressed in glioblastomas is constitutively active due to its ligand-independent dimerization. This results in deregulation of signaling leading to oncogenesis. Interestingly, such receptor-receptor self-association, like EGF-induced dimerization, is also highly dependent on glycosylation. This led us to identify the glycosylation sites that are important in inducing a receptor-active confirmation. By *in vitro* mutagenesis, we have generated Asn→X mutants. When all four acceptor sites in a particular domain were mutated, the resulting tetra-substituted mutant lacks EGF binding activity; in addition, the receptor is trapped in the endoplasmic reticulum. However, neither maturation nor EGF binding is affected by single Asn→X substitution. The significance of these and related findings in the context of receptor-mediated signal transduction and structure-function relationship of the EGFR will be discussed.

I#115

The role of glycosphingolipids in HIV-1 entry

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HIV-1 infection is triggered by interactions of the HIV-1 envelope glycoprotein (Env) with CD4 and co-receptors such as CXCR4 and CCR5 on the host cell surface. Treatment of primary lymphocytes with inhibitors of GSL biosynthesis prevents HIV-1 infection of these cells. Similar results were obtained by treatment of lymphocytes with cholesterol–sequestering reagents. We hypothesize that GSL play a role by recruiting HIV-1Env-CD4-coreceptor complexes into GSL-enriched membrane domains (GEMs/rafts). Cells over-expressing co-receptors become independent of cholesterol and GSL levels in their susceptibility to HIV-1 entry, indicating that GSL and cholesterol do not play an intrinsic role in the CD4-dependent fusion process. However, CD4-independent entry pathways in non-lymphoid cells (e.g. kidney and brain) may require specific GSL (e.g. Gb3). These molecules may be involved in signaling events leading to the cytoskeletal changes and formation of the co-receptor clusters that are required to trigger the HIV-1 fusion process.

I#116

Glycolipids of apicomplexan parasites

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Apicomplexan parasites such as *Plasmodium falciparum* (*P. falciparum*) and *Toxoplasma gondii* (*T. gondii*) synthesise a variety of glycolipids. A predominant class are glycosylphosphatidyl-inositols (GPIs) which serve to anchor membranes to the surface of the parasites and have been identified to play an important role as pathogenicity factors. In addition, we could demonstrate that both, *P. falciparum* and *T. gondii* synthesise (glyco)sphingolipids. Recently, the formation of galactolipids of apicoplast origin has been shown for both parasites. This group of lipids has formerly been extensively described in plants. Key enzymes of apicomplexan GPI biosynthesis have been cloned and characterised. Complentation in yeast was used for functional analysis. Synthesis of dolichol-phosphate mannose (Dol-P-Man) is a prerequisite for GPI biosynthesis and some steps in N-glycosylation. DPM-1 of *P. falciparum* has been cloned and functionally characterised. Presence or absence of N-glycosylation in *P. falciparum* is still controversial.

Poster presentation

1. Glycoproteins, glycosidases and glycosyl transferases

P#1

Alkaline treatment has contrasting effects on structure of deglycosylated and glycosylated forms of glucose oxidase: *compaction of native conformation and partial unfolding*

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X-ray crystallographic studies on Glucose oxidase showed strong interaction between carbohydrate and protein moiety of the glycoprotein. However, experimental studies under physiological conditions reported no influence of carbohydrate moiety on the structural and functional properties of glucose oxidase. We have carried out a detailed comparative study on the pH induced structural changes in the native and deglycosylated forms of Glucose oxidase. Our studies demonstrate that at physiological pH both the forms of enzyme have very similar structural and stability properties. Acid denaturation also showed similar structural changes in both the forms of enzyme. However, on alkaline treatment contrasting effects on the structure and stability of the two forms of enzyme were observed. The glycosylated enzyme undergoes partial unfolding with decreased stability at alkaline pH whereas, a compaction of native conformation and enhanced stability of enzyme was observed for the deglycosylated enzyme under similar conditions. This is the first experimental demonstration for influence of carbohydrate moiety on structure and stability of Glucose oxidase. The studies also indicate the importance of pH studies in evaluation effect of carbohydrate moiety on structural and stability properties of glycoprotein.

P#2

The N-terminal stem regions (SR) of bovine and human β1,4-Galactosyltransferase I (β4Gal-T1) increases the *in vitro* folding efficiency of their catalytic domain by 3- to 4-fold.

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β1,4-Galactosyltransferase (β4Gal-T1) is a type II Golgi-resident enzyme that has a short cytoplasmic tail, a transmembrane domain, followed by a stem region, and a globular catalytic domain (CD) that faces the Golgi lumen. The CD domain transfers galactose from UDP-Galactose (Gal) to *N*-acetlylglucosamine (GlcNAc) residues of glycoproteins and glycolipids, generating a β1–4-linkage between Gal and GlcNAc. The cDNA clones encoding the CD domain of bovine β4Gal-T1 were expressed as inclusion bodies in *E. coli* and folded *in vitro* in the presence of oxido-shuffling reagents to generate the native and enzymatically active protein. In this study, human and bovine proteins, with and without their corresponding stem regions, were generated and also expressed as inclusion bodies in *E. coli*, purified and folded *in vitro*. The stem region fused to the catalytic domain increases the folding efficiencies, from the inclusion bodies, by 3- to 5-fold, compared to the proteins having the catalytic domain alone. Furthermore, the folding of the CD domain protein alone or fused with the stem, is enhanced another 3- to 4-fold by adding PEG 4000 (0.055%) and L-Arg (0.55M) in the folding buffer, resulting in 90% folding efficiency of the native protein. The results show the positive effect of the stem domain, acting as a chaperone, during the *in vitro* folding of the catalytic domain of both human and bovine β4Gal-T1.

P#3

Modulation of CD22 function by sialoside ligands

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Siglec-2, or CD22, is a negative regulator of B lymphocyte function. Mice deficient for this protein exhibit a hyperimmune phenotype as indicated by increased calcium flux and antibody production, including anti-DNA antibodies[1,2]. CD22 recognizes the sialoside sequence Siaα2,6Gal, which is synthesized *in vivo* by the enzyme ST6Gal I. Mice deficient in ST6Gal I, and therefore unable to make the CD22 ligand, exhibit a significantly different phenotype than the CD22 null mice. These mice are immunosuppressed, as indicated by lower calcium flux and little antibody production following innoculation with T dependent or independent antigens[3]. To investigate this difference in phenotypes we have begun a multifaceted approach. Included in this is the generation of the novel strain of mice deficient in both CD22 and the enzyme ST6Gal I. Initial observations have indicated that these mice are viable, breed normally, and that no major differences in B and T cell populations exist. Studies are underway to determine if these mice exhibit the immune phenotype of the CD22 null mice or that of the ST6Gal I null mice. These studies will be important in evaluating the role of the CD22 -sialoside interaction in modulating its role in immune cell function.

(1) Nitschke *et al* (1997) Current Biology, **7**, 133
(2) O'Keefe *et al* (1999) J Exp Med, **189**, 1307
(3) Hennet *et al* (1998) Proc Natl Acad Sci USA, **95**, 4504

P#4

Synthesis of glycopeptides containing tumor-associated carbohydrate antigens

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Mucin represents a family of cell-surface glycoproteins often associated, in aberrant glycoforms, with tumors of epithelial tissues. In epithelial cancers, such as those affecting the breast, ovary, lung and colon, low expression of β1,6-GlcNAc transferases together with increased levels of sialyltransferases

result in the formation simpler O-linked carbohydrates such as Tn, T, STn, and ST. Over-expression of any of these structures has been correlated to the aggressiveness of cancer. Syntheses of glycopeptides carrying these carbohydrate antigens are of importance for developing synthetic vaccines. In order to achieve this objective we have synthesized glycopeptides (i, ii and iii) derived from the tandem repeat domain of the mucin MUC1 (peptide 1) and neoglycosylated derivatives of a T-cell stimulating viral peptide (peptide 2) using a chemoenzymatic approach. Various strategies and synthetic protocols used will be discussed.

1. Kuduk, S. D.; Schwarz, J. B.; Chen, X.-T.; Glunz, P. W.; Sames, D.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1998**, *120*, 12474-12485
2. George, S. K.; Schwientek, T.; Holm, B.; Reis, C. A.; Clausen, H.; Kihlberg, J. *J. Am. Chem. Soc*. **2001**, *123*, 11117-11125
3. Coltart, D. M.; Royyuru, A. K.; Williams, L. J.; Glunz, P. W.; Sames, D.; Kuduk, S. D.; Schwarz, J. B.; Chen, X. T.; Danishefsky, S. J.; Live, D. H. *J. Am. Chem. Soc*. Web Release Date: 27-Jul-2002; (Article) DOI: 10.1021/ja020208f

P#5

Analysis of mechanisms underlying stabilization of matriptase with oligosaccharide modification

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Oligosaccharide moieties of glycoproteins are structurally altered during development, carcinogenesis, and malignant transformations. It is well known that beta1-6 GlcNAc branching, a product of UDP-GlcNAc alpha-mannoside beta1-6-N-acetylglucosaminyltransferase (GnT-V), is associated with malignant transformation as the results of such alterations. However, the mechanism by which beta1-6 GlcNAc branching is linked to metastasis remains unclear, because the identification of specific glycoprotein(s) that are glycosylated by GnT-V and its biological function have not been examined. We herein report that matriptase, which activates both urokinase-type plasminogen activator and hepatocyte growth factor, is a target protein for GnT-V. The overexpression of GnT-V in gastric cancer cells leads to severe peritoneal dissemination in athymic mice, which can be attributed to the increased expression of matriptase. This increase was due to the acquired resistance of matriptase to degradation. Purified matriptase from GnT-V-overexpressed cells had a beta 1-6 GlcNAc branched N-glycan. These results suggested that beta 1-6 GlcNAc branched oligosaccharides prevent degradation of matriptase, which could promote cancer metastasis with oligosaccharide modification.

P#6

Interaction of mouse PNGase with two other cytoplasmic mouse proteins MS4 and MHR23b

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Endoplasmic Reticulum Associated Degradation (ERAD) is utilized to degrade misfolded proteins/glyproteins by transferring them from the ER to the cytosol followed by degradation by the proteasome. A failure in ERAD mechanism could lead to the accumulation of misfolded proteins/glycoproteins in the ER and finally in the generation of a variety of disorders. One key factor involved in the degradation of glycoprotein is an enzyme peptid*e:N*-glycanase (PNGase) which cleaves the N-linked glycan before it is degraded by proteasome. The gene PNG1 encoding for the deglycosylating enzyme has been identified and found to be well conserved throughout eukaryotes. Recently, yeast and mouse PNGase (mPNGase) have been studied with respect to their structural features and interaction with other proteins (1-3). mPNGase has been shown to interact, using two hybrid library screening, with mHR23B (Rad 23 homolog in yeast), mS4 (19S proteasome subunit), mY33K and mAMFR proteins (2). In the present work, *in vitro* binding of mPNGase with mS4 and mHR23B is being studied. A detail analysis using mutagenesis will be made on the mechanism of interaction of mPNGase with mS4 and mHR23B. Finally, the focus will be on the ATP dependent binding of mS4 with mPNGase.

1 . Katiyar S, Suzuki T, Balgobin BJ, Lennarz WJ. *J Biol Chem*. 2002, **277**, 12953-12959.
2. Park H, Suzuki T, Lennarz WJ. *Proc Natl Acad Sci* U S A. 2001, **98**, 11163-11168.
3. Suzuki T, Park H, Kwofie MA, Lennarz WJ. *J Biol Chem*. 2001, **276**, 1601-1607.

P#7

Role of carbohydrate in formaecin I, a glycopeptide antibiotic, can be compensated by appropriate minor substitutions in its amino acid sequence

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Formaecin I, a 16-residue glycosylated antibacterial peptide, carries a monosaccharide moiety, N-acetyl galactosamine, attached to a threonine. It is an insect defense molecule, induced rapidly in response to bacterial challenge as part of the innate immune response. The non-glycosylated version of formacin I shows significantly lower activity compared to the native glycosylated peptide. The comparative antibacterial activity studies of formaecin I and its nonglycosylated form against various strains of gram negative bacteria showed a differential pattern of activity for different strains. However, the extent of contribution of the sugar was comparable in each case. The conformational studies by circular dichroism of formaecin I and its nonglycosylated analog revealed no evidence of regular structure for both the peptides in aqueous solution. We have synthesized formacin I and several designed nonglycosylated analogs and subjected them to structure-activity analysis to delineate the residues that may be responsible for the antibacterial activity. On the basis of this analysis, a nonglycosylated analog was identified which exhibited formaecin I - like activity. Thus, it is evident that the pharmacophore characteristics incorporating glycosylation could be mimicked by making a couple of amino acid substitutions in the native peptide without sugar.

P#8

Chemical modifications of α-6-fucosyltransferase define amino acid residues of catalytic importance

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Genomic and domain structure of core α-6-fucosyltransferase (FucT-VIII) as well as its amino acid sequence have been established (1). Less information is available on structure/function relationship of the enzyme. Using a site-directed mutagenesis

approach arginine 365 was identified as absolutely essential for enzyme activity while arginine 366 was implicated in GDP-Fuc- and acceptor substrate-binding (2). Both arginine residues are located in a highly conserved 361-370 stretch of FucT VIII. In the present study we subjected FucT VIII from human platelets (3) to the action of phenylglyoxal (PLG), pyridoxal-5'-phosphate/NaBH4 (PLP), and diethyl pyrocarbonate (DEPC) the reagents that selectively modify the structure of amino acids arginine, lysine and histidine, respectively, as well as to N-ethylmaleimide (NEM), mersalyl and p-chloromercuribenzoate (pCMB) that react with sulfhydryl group of cysteine. In addition, we treated the enzyme with β-mercaptoethanol, a reagent that disrupts disulfide bonds. All reagents except NEM significantly inactivated FucT VIII at 10 mM concentration. Protection against the action of PLG, PLP and organomercurials was offered by GDP-Fuc, GDP, and the acceptor substrate, a transferrin-derived biantennary glycopeptide with terminal GlcNAc residues. Neither donor nor acceptor substrate offered any protection against inactivation by DEPC or β-mercaptoethanol. We conclude that arginine, cysteine and lysine residues are present in, or closely by, the donor and acceptor substrate binding domains of the enzyme, whereas histidine may be a part of its catalytic domain. However, the primary structure of FucT VIII does not show cysteine residues in proximity to the postulated GDP-Fuc- and acceptor substrate-binding site of the enzyme. To rationalize our results we postulate that platelet FucT VIII is folded through disulfide bonds that bring together the donor/acceptor binding site and cysteine containing region of the molecule.

1.Miyoshi E, Noda K, Yamaguchi Y, Inoue S, Ikeda Y, Wang W, Ko JH, Uozumi N, Li W, Taniguchi N (1999) *Biochim Biophys Acta* **1473**: 9-20.
2. Takahashi T, Ikeda Y, Tateishi A, Yamaguchi Y, Ishikawa M, Taniguchi N (2000) *Glycobiology* **10**: 503-510
3. Kamińska J, Glick MC, Kościelak J (1998) *Glycoconjugate J* **15**: 783-788.

P#9

The human cytosolic sialidase Neu2 recognizes different packed and mono-molecular dispersed ganglioside

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Here we report the biochemical characterization of a novel human cytosolic sialidase encoded by the *NEU2* gene. The gene was isolated from a human genomic library using a sequence homology-based approach. *NEU2* encodes a protein of 380 amino acids, with a calculated molecular weight and isoelectric point corresponding to 42.23 kDa and 6.82, respectively. Recombinant Neu2 was produced by *E. coli* cells using the GST gene fusion system and purified to homogeneity by two FPLC steps. The enzyme protein was found to be stable and to cleave sialyllactose, sialo-glycoproteins and gangliosides, with a pH optimum of 5.6, besides MU-NeuAc. The highest enzymatic activity was found on α2-3 sialosyl linkage, followed by the α2-8 linkage; in contrast α2-6 linkage was cleaved only when present in sialo-glycoproteins, which appeared to be anyway, the poorest substrates. The enzyme was activated by detergents only in the presence of ganglioside substrates. Moreover, Neu2 was able to hydrolyse sialic acid from a monomeric dispersion of GM1 ganglioside with typical Michaelis-Menten kinetic. To the best of our knowledge, these results are the first evidence of a mammalian sialidase activity on GM1 ganglioside in its monomeric form.

P#10

Sp1 gene family and transcriptional suppression of Core 2 GlcNAc-transferase-1 in pre-B cell differentiation

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Sialyl-Le^X (sLe^X) antigen is highly expressed in human pre-B leukemia cells, and the down-regulated expression is observed and controlled by core 2 GlcNAc-transferase-1 (C2GnT-1) during differentiation. In the present study, transcriptional regulation mechanism of C2GnT-1 has been investigated using B cell lines. We analyzed transient transcriptional activity of the promoter region (nucleotides –1742 to +57) using luciferase reporter constructs, and found that a fragment –304 to +57 had 42-fold activity in sLe^X-positive KM3 compared with sLe^X-negative Raji cells and only 1/10-fold activity in TPA-treated KM3 compared with non-treated cells. The fragment carries a TATA box-like sequence and an Sp1 binding consensus. Destruction of Sp1 site resulted in significant reduction (40%) of transcriptional activity before differentiation and in marked increase (13-fold) after TPA treatment. RNAblot, immunoblot, and EMSA revealed characteristic expression and DNA-binding capability of Sp1 family genes and proteins, and it was strongly suggested that C2GnT-1 transcription at least in the early stage of human pre-B cell differentiation is controlled by Sp1 family transcription factors.

P#11

Structure-function relationships in blood group synthesizing glycosyltransferases

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Human blood group *N*-acetylgalactosaminyltransferase (GTA, E.C. 2.4.1. 40) catalyzes the synthesis of the blood group A determinant, GalNAcα(1-3)[Fucα(1-2)]Galβ-OR, by transferring GalNAc from an UDP-GalNAc donor to precursor Fucα(1-2)Galβ -OR substrates. The blood group B synthesizing galactosyltransferase (GTB, E.C. 2.4.1.37) is highly homologous differing from GTA at only four amino acids of 354. These changes alter the donor specificity from UDP-GalNAc to UDP-Gal. GTA and GTB are therefore ideal glycosyltransferases to investigate structure-function relationships. High-resolution crystal structures of GTA and GTB, native and complexed with substrates are available and reveal a structural basis for donor specificity. A series of mutant enzymes with interchanges of the four critical amino acids have been prepared to further probe substrate binding. These include *cis*-AB enzymes capable of utilizing both donors at comparable rates. Mutagenesis of potential active site residues has also been carried out to identify enzyme groups involved in catalysis.

P#12

Regulation of Le^x, Le^y and related fucosyltransferases gene expression by estrogenic hormones in endometrium of mouse and human

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Lewis oligosaccharides (Le^X, Le^Y) play important roles in the implantation of blastocyst onto the receptive endometrium which is controlled by estrogenic hormones. To study the effects of estrogen (E_2) and progesterone (P_4) on Le^X and Le^Y expressions, as well as the genes of synthetic enzymes (FUT ̄ and FUTIV for LeY, FUTIX for LeX) in mouse and human, immunochemistry, Western blot and RT-PCR were used. The endometrial samples from mouse were of E(100ngE_2), P (500µgP_4) and E+P (500µgP_4+10ngE_2) treatment group after ovariectomy. The human endometrium of proliferative phase (Pro) or with Mifepristone administration (Pro+M), and secretory phase (Sec) or with Mifepristone administration (Sec+M) were collected. The results showed that the expressions of Le^X and Le^Y increased after E treatment and decreased after P treatment in immunostaining. By Western blot analysis, we got Le^Y (+) bands of 60-90kD, and single Le^X(+) band at 70kD which changed up and down along with E and P treatment, respectively. It indicated the antagonist effect between E and P. Le^X and Le^Y were distributed in glandular epithelium and lumenal epithelium of the human endometrium, and showed more intense staining in endometrium of Sec than that of Pro by immunostaining. Oral administration of Mifepristone caused significant inhibition on oligosaccharide expression, especially on the endometrium of Sec+M. RT-PCR analysis of FUT I, FUTIV and FUTIX showed similar alterations with relatively higher transcription level at Sec compared with Pro, suggesting oligosaccharide synthetase genes were up-regulated by P and down-regulated by E in human endometrium. The study indicates that estrogenic hormones control Lewis oligosaccharide expression. Although the existence of species difference, the regulation is mainly by influencing the transcription level of the related fucosyltransferases.
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P#13

Cloning of an α2,3-sialyltransferase cDNA that directs synthesis of stage-specific embryonic antigen-4 (SSEA-4)

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Monosialosyl globopentaosylceramide (MSGb5), originally described as stage-specific embryonic antigen-4 (SSEA-4), is expressed in testicular germ-cell tumors and in aggressive cases of human renal cell carcinoma (RCC). Clarification of the molecular mechanisms regulating synthesis of MSGb5 is very important to understand testicular carcinogenesis and the malignant progression of human RCC. For this purpose, we investigated α2,3-sialyltransferase involved in the synthesis of MSGb5. We used the method of expression cloning combined with polymerase chain reaction targeted to sialylmotif to isolate a cDNA clone from RCC cell line ACHN library, and obtained Gb5ST cDNA. A soluble recombinant form of the protein in COS-1 cells showed an enzyme activity of α2,3-sialyltransferase toward globopentaosylceramide (Gb5). Transient transfection of COS-1 cells with this cDNA induced a slight increase of MSGb5, while stable transfection of antisense Gb5ST cDNA inhihited expression of MSGb5 in ACHN cells. However, level of Gb5ST mRNA was not necessarily consistent with that of MSGb5 in various RCC cell lines. This study indicates that Gb5ST is a MSGb5 (SSEA-4) synthase, but level of MSGb5 may also be regulated by the other glycosyltransferases or sialidase.

P#14

Characterization and functional purification of a β-xylosidase from potato

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Xylose, a common component of many plant and some gastropod Nglycans, has been shown to play an important role in forming allergenic epitopes. A specific xylosidase is a necessary requisite for the analysis of these structures and for the modification of potential allergens.

Here we present the functional purification and detailed characterisation of a β–xylosidase from potato. The enzyme was purified by ammonium sulphate precipitation, hydrophobic interaction chromatography, affinity gel blue, ion exchange and size exclusion chromatography yielding a protein with a molecular weight of 39-40 kDa . The N-terminus of the electrophoretically homogenous product was sequenced and compared with the data from other enzymes.

The β-xylosidase was characterised by its substrate specificity and its biochemical parameters. Detailed studies on its stability and its optimal incubation conditions were carried out in order to optimise its use for analytical purposes. A new inhibitor specific for xylosidases was synthesized and tested with the enzyme. Examples are given for the use of the β-xylosidase in structural analysis of gastropod glycans.

P#15

Novel sialidase inhibitors

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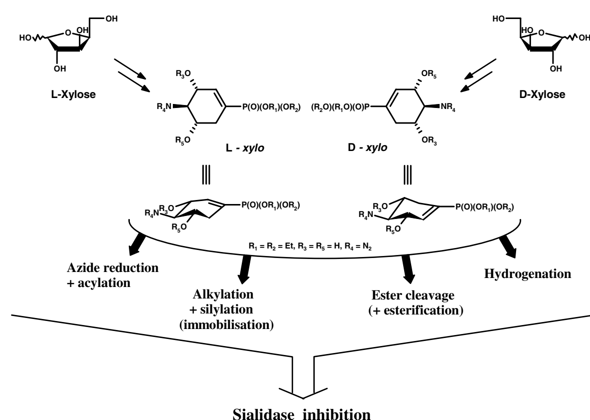
Sialic acids, derivatives of neuraminic acid, are the most commonly occuring mono-saccharides at the non-reducing end of oligosaccharides of the complex type where they take part in a wide variety of physiological processes.[1] These range from recognition phenomena to protective group functions and consequently, sialic acid binding and metabolizing proteins such as sialidases or lectins have been identified as interesting targets for drug design.[2] In contrast to the enzyme from influenza virus[3], effective inhibition of sialidases from bacteria or protozoa has proved to be somewhat more difficult to achieve. Our synthetic approach, starting from the enantiomeric xylofuranoses, provides access to isomeric cyclohexene-phosphonates, the synthesis and binding of which to selected bacterial sialidases we recently demonstrated.[4,5]

Selective modifications of the carbocycles to yield anologues both of the substrate and the transition state of the

sialidase reaction and further data on their inhibitory activity towards sialic acid recognizing proteins will be presented.



1. Traving C, Schauer R, *Cell. Mol. Life Sci.* **54**, 1330-1349 (1998).
2. Roberts NA, *Prog. Drug Res.* **56**, 198-237 (2001).
3. Dyason J., von Itzstein M, *Austr. J. Chem.* **54**, 663-670 (2001).
4. Streicher H, Meisch M, Bohner C, *Tetrahedron* **57**, 8851-8859 (2001).
5. Streicher H, Bohner C, *Tetrahedron*, submitted.

P#16

Role of different domains of classical swine fever virus glycoproteins in the formation of functional complexes

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Classical swine fever virus (CSFV) is a pestivirus which can be used as a surrogate model to elucidate the role of envelope glycoproteins of closely related hepatitis C virus (HCV). The necessity to use the surrogate models for HCV is due to the fact that this virus cannot be grown in *in vitro* cultures. CSFV genome codes for two major antigenic glycoproteins which are located in the same cluster of genes; they are designated as E2 and E0 (E^{rns}). Both glycoproteins form heterodimeric and homodimeric complexes on the external part of viral particles. It is generally accepted that envelope glycoproteins play a major role in the initial stages of viral infection both for CSFV and HCV. Formation of complexes is needed to effectively infect host cells. We have investigated the formation of E2 and E0 homodimers by immunoperoxidase monolayer assay and by immunoblotting (Western blotting). Immunoblotting is a very useful technique in these studies because the complexes are formed via cysteine-cysteine disulphide bonds and they are retained during SDS-PAGE under non-reducing conditions. By modifying the glycoprotein genes and by arresting N-glycosylation of E2 and E0 we have investigated what are the crucial regions which influence the formation of homodimers.

P#17

A lectin domain of UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase 1 (GalNAc-T1) is involved in *O*-glycosylation of a polypeptide with multiple acceptor sites

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Mucin type *O*-glycosylation is initiated by the transfer of GalNAc to serine and threonine residues on acceptor proteins, a reaction catalyzed by a family of UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases (GalNAc-transferases). The GalNAc-transferases are characterized by a C-terminal $(QXW)_3$ repeat domain consisting of three tandem repeats, α, β, and γ. These repeat sequences are believed to have lectin-like activity based on their predicted conformational similarity to the ricin B-chain. However, it has been reported that the $(QXW)_3$ domain of GalNAc-T1, has no measurable function. In this report, we have reevaluated the role of the GalNAc-T1 $(QXW)_3$ domain. The importance of the domain was demonstrated by a complete loss of activity upon deletion from the enzyme molecule. We also found that GalNAc-T1 has two enzymatic activities that can be distinguished by inhibition with free GalNAc: one activity is sensitive to GalNAc inhibition, the other is resistant. Site-directed mutagenesis of the $(QXW)_3$ domain selectively reduced the GalNAc sensitive activity, and identified Asp^{444}, in the α repeat, as the most important site for GalNAc recognition. Further reduction of the GalNAc sensitive activity was observed in a triple mutant, D444A/D484A/D525A, in which Asp^{444}, as well as the corresponding aspartate residues in the β and the γ repeats, have been mutated to alanines. This indicates a cooperative involvement of each repeat unit in the $(QXW)_3$ domain in the glycosylation of the polypeptides with multiple acceptor sites.

P#18

Cloning and characterization of an *O*-glycan core 3 synthase (β3Gn-T6)

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The core 3 structure of the *O*-glycan, GlcNAcβ1-3GalNAcα1-peptide, an important precursor in the biosynthesis of mucin-type *O*-glycans, is synthesized by UDP-*N*-acetylglucosamine: GalNAc-peptide β1,3-*N* acetylglucosaminyltransferase (β3Gn-T; core 3 synthase). The core 3 structure is restricted in its occurrence to mucins from specific tissues such as digestive organs. We cloned a new β3Gn-T member which having the β1,3-glycosyltransferase motifs and named β3Gn-T6. The soluble form of β3Gn-T6 expressed in insect cells showed β3Gn-T activity to GalNAcα-pNp and GalNAcα1-serine/threonine, with β1,3-linkage. β3Gn-T6 effectively transferred a GlcNAc to the GalNAc residue on MUC1 mucin, resulting in the synthesis of a core 3 structure. The tissue distribution of β3Gn-T6 transcript was restricted mainly to the digestive organs. We concluded that β3Gn-T6 is the most logical candidate for the *O*-glycan core 3 synthase.

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P#19

Biochemical and molecular characterization of GPI anchors in *Entamoeba histolytica*

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Proteophosphoglycans (PPG) are the most abundant glycoconjugates on the cell surface of *Entamoeba histolytica* . They are composed of an acidic polypeptide which is GPI-anchored and extensively glycosylated at serine residues through a phosphodiester bond. PPG is absent in other species of *Entamoeba*, making it a likely candidate for a role in pathogenesis.

So far there is no information about the GPI biosynthetic pathway in *E. histolytica*. We have started to unravel GPI biosynthetic pathway in *E. histolytica* by functionally identifying the different steps in the pathway starting with the first two intermediates, namely the formation of GlcNAc-PI and GlcN-PI . Our results suggest that both the enzyme activities are present in *E. histolytica*.

In an *in vitro* reaction, a membrane preparation of *E. histolytica* was incubated with UDP-GlcNAc [C^{14}] and the products were analyzed by HPTLC in chloroform:methanol:ammonium hydroxide solvent system. Two bands with Rf values close to GlcNAc-PI and GlcN-PI isolated in other systems were observed.

The nature of these two intermediates was validated by chemical analysis like deamination by HNO_2, acetylation with acetic anhydride, enzymatic digestion by PI-PLC, hydrophobic chromatography and detergent phase separation in order to confirm their structures. We could also demonstrate flipping of these intermediates from the cytoplasmic to the lumenal side of *Entamoeba* vesicles.

Two putative *E. histolytica* homologues of PIG-A, the catalytic component of GPI-Gnt complex and PIG-L (GPI-N-Acetylglucosamine deacetylase) were identified using bioinformatics tools. Functional characterization of these genes is being carried out by complementation analysis in yeast mutants deficient in these enzymes and by antisense mediated gene silencing in *E. histolytica* trophozoites.

P#20

***N*-acetyl-ß-D-glucosaminidase isoenzymes in membrane and cytosol of human erythrocytes**

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This report provides evidence of the occurrence in human erythrocyte plasma membrane and cytosol of two N-acetyl-ß-D-glucosaminidase (GlcNAc-ase), characterized by different acidic optimum pHs. The more acidic form, "acidic GlcNAc-ase", is prevalent, either in the cytosol or in the membrane, where is oriented forward the extracellular environment. The less acidic form, "neutral GlcNAc-ase", little expressed on the membrane (about 5-10 %), is more abundant in the cytosol, where represents about 30-40% of the total cytosolic activity. Due to its relatively low levels, it was not possible to ascertain its orientation in the membrane. The isoenzyme patterns presented by membrane and cytosolic enzymes are similar and superimposable to that presented by plasmatic form, including the I_2-P isoenzyme, till now considered typical of the plasma. Only the membrane isoenzymes increase during pregnancy, evidencing a close correlation between plasmatic forms. Among the cytosolic isoenzymes, of particular interest is the isoform C, that, for some properties and characteristics (optimum pH, high thermal lability and insensitivity to GalNAc inhibition) has been identified with the O-GlcNase, active on the particular glycoproteins, in which a single residue of GlcNAc is attached in O-linkage, recently discovered also in the erythrocyte cytosol and involved in an important regulatory system.

P#21

Molecular cloning and characterization of a novel member of the human UDP-GalNAc: polypeptide N-Acetylgalactosaminyltransferase family, pp-GalNAc-T12

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The repertoire of pp-GalNAc-Ts expressed in a cell regulates the initial step of *O*-glycosylation, thus determined the number and sites of *O*-glycan attachments. Here we report the cloning and expression of a novel member of human pp-GalNAc-T family, designated pp-GalNAc-T12. The deduced amino acid sequence of pp-GalNAc-T12 is predicted to comprise a typical type membrane protein, and contains the GT1, Gal/GalNAc transferase, and lectin-like motifs that are conserved in pp-GalNAc-Ts. The pp-GalNAc-T12 transcripts were expressed mainly in digestive organs such as stomach, small intestine and colon. The expression level in cancerous tissue in colon was down-regulated compared to the noncancerous tissue. The soluble form of pp-GalNAc-T12 transferred GalNAc to tandem repeats of mucins, which were mainly produced in the digestive organs. The catalytic specificities of pp-GalNAc-T12 showed preference for peptides derived from Muc1a, Muc5AC, EA2, and the GalNAc-Muc5AC glycopeptides. We estimated that pp-GalNAc-T12 plays an important role in the initial step of mucin-type oligosaccharides biosynthesis.

This work was performed as a part of the R&D Project of Industrial Science and Technology Frontier Program (R&D for Establishment and Utilization of a Technical Infrastructure for Japanese Industry) supported by New Energy and Industrial Technology Development Organization (NEDO).

P#22

T cell activation in sialidase (Neu3) transgenic mice

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Neu3 is a unique sialidase localized in plasma membrane and hydrolyzing specifically gangliosides. This sialidase has been implicated in modulation of signal transduction as well as ganglioside catabolism because of its unique properties. We previously found that mouse homologue was up-regulated during neuronal differentiation (1) and T cell activation. Neu 3 was also found to be within caveolae microdomain with close association with caveolin-1, indicating Neu3 as a signal molecule (2). To explore the function, we analyzed the role of Neu3 during T cell activation in a transgenic (Tg) mouse overexpressing Neu3. The mouse was engineered to express human Neu 3 cDNA under control of human β-actin promoter and, as a result, showed marked increase in ganglioside sialidase activity in all of the tissues including spleen. Weights of spleens of Tg mice were

slightly heavier than those of control mice. In response to anti-CD3 antibody or Con A, Tg splenic T cells showed enhanced growth compared with control T cells. On the other hands, stimulation by ionomycin plus PMA resulted in only slight difference for growth promotion between Tg and control mice. These results suggest that in splenic T cells, Neu3 enhances growth possibly through distinct signal transduction pathways. Molecular mechanism of the response is now under investigation.

1. Hasegawa T *et al* (2000) *J. Biol. Chem.*, **275**, 8007-8015

2. Wang Y *et al* (2002) *J. Biol. Chem.*, **277**, 26252-26259

P#23

Specific activity for the synthesis of Tn epitope of human UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase, pp-GalNAc-T10

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UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase (pp-GalNAc-T) forms a super family and transfers GalNAc to serine and threonine to initiate *O*-glycan biosynthesis. To date, nine distinct members have been identified in human. Each isoforms exhibits different tissue distribution and substrate specificity. We cloned pp-GalNAc-T10 and –T1 from human. The *pp-GalNAc-T10* gene contains 10 exons and a remarkable homology, 84.3%, to pp-GalNAc-T1 at the deduced amino acid level. The recombinant forms of pp-GalNAc-T10 and -T1 were expressed in an Insect-Baculo system. They exhibited *in vitro* activity toward unmodified Muc5Ac and MUC7 peptides and etc. In particular, pp-GalNac-T10 exhibited much stronger activity than did pp-GalNAc-T1. pp-GalNAc-T10 was able to form a Tn-epitope, three consecutive GalNAc-Ser/Thr structures, on peptides encoded in syndecan-3, while pp-GalNAc-T1 did not. pp-GalNAc-T10 appears to be a major enzyme responsible for the synthesis of *O*-glycan and essentially for the Tn-epitope synthesis.

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P#24

Folding and assembly of retinol binding protein in the endoplasmic reticulum

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Retinol binding protein (RBP) is a carrier of retinol from the liver to the target organs in the human body. It is a small protein of molecular weight 21 kDa (183 amino acids). The mature protein has three intra-molecular disulfide bonds. It is known that the binding of ligand, retinol, is a pre-requisite for effective secretion of RBP from the liver. In the absence of retinol, apo-RBP is retained within the Endoplasmic Reticulum (ER) of liver cells.

Our lab is interested in studying the mechanisms of ligand-dependent secretion of RBP and the selective retention of apo-RBP in the ER. Towards this goal, we have initiated efforts to dissect the oxidative folding, maturation and association of RBP with the chaperone proteins in the ER. Previously it was reported that human RBP, despite the lack of N-glycans, associates with calnexin during its maturation in the ER. Also, a Carp homolog of human RBP has been shown to possess two N-linked glycosylation sites and is known to be glycosylated. To address the potential involvement of calnexin binding and the role of N- linked glycosylation in the folding and maturation of RBP, we have generated two mutants of human RBP containing N-linked glycosylation sites. Our results with the *in vitro* translation, translocation, oxidative folding and maturation of the wild type as well as of the glycosylation mutants of RBP will be described.

P#25

Glycoprotein hormones: structure-function

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The Glycoprotein hormones, LH, FSH, TSH and hCG are interesting molecules, not only because of their roles in reproduction and overall physiology, but also because of their unique structural features. These hormones are heterodimeric molecules with an identical α subunit associated non-covalently with the hormone specific β subunit. The principal objective of the laboratory is to investigate structure-function relationship of these hormones. Towards this goal, different experimental strategies such as use of characterized antibodies, recombinant expression of the hormones and site-directed mutagenesis have been employed. Using the *Pichia pastoris* expression system, hyperexpression and purification of all human glycoprotein hormones have been achieved. Using the same expression system, single chain derivatives of hCG were expressed in which the C terminus of either the wild type or mutated α subunit was translationally fused to the N terminus of the β subunit. The wild type hCGαβ was folded properly as judged by immunological criteria, could bind to the receptor and also elicit biological response. The mutations introduced in the α subunit of this single chain molecule resulted in production of molecules that were either superagonist or partial agonist. The fusion of two β subunits resulted in a molecule (hCGββ) that had structure similar to the heterodimeric hormone as judged by immunological criteria, could bind to the receptor, but did not elicit any response. However, it could block response to hCG suggesting that hCGββ was an antagonist of the hormone. Thus, the strategy of fusing two subunits has led to production of hormone molecules with different biological properties. (Supported by grants from Department of Biotechnology, Government of India and Indian Council of Medical Research, India).

2. Lectins

P#26

Binding of phosphorylcholine and L(-) fucose by the major protein from bovine seminal plasma, PDC-109

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PDC-109 – the major protein of bovine seminal plasma – binds specifically to choline phospholipids on the sperm plasma membrane at ejaculation and modulates sperm capacitation. Binding of PDC-109 to spermatozoa leads to cholesterol and choline phospholipid efflux, an important step in the capacitation process. In addition, PDC-109 also binds to L(-) fucose, a component of the Le^a trisaccharide present on the oviductal epithelium. Previous ESR studies from our laboratory have shown that PDC-109 exhibits a high selectivity for choline phospholipids and that upon binding, penetrates into the hydrophobic interior of the membrane up to the 14th C atom [1]. In this study we have investigated the binding of phosphorylcholine and L(-) fucose to PDC-109. Difference absorption spectroscopic studies gave a K_a value of 81 M^{-1} for the binding of phoshorylcholine to PDC-109 at 20 °C. Fluorescence spectral studies on the binding of L(-) fucose yielded the association constant as 2.9×10^2 M^{-1} at 25 °C. The thermodynamic parameters for the binding of these two ligands were obtained from the temperature dependence of the K_a values.

1. Ramakrishnan M, Anbazhagan V, Pratap TV, Marsh D, Swamy MJ *Biophys. J.* 81, 2215-25 (2001).

P#27

Structural basis of the multifunctional nature of human Hyaluronan Binding Protein, HABP1/p32/gc1qR

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Hyaluronan binding protein HABP1/p32/gc1qR, a multi-ligand binding multifunctional protein (pI 4.10) with asymmetric charge distribution along its faces has been reported at different sub-cellular locations like its ligand HA. HABP1/p32/gc1qR exists in different oligomeric forms, namely, trimer and disulfide linked dimer of trimers in solution [**Eur. J. Biochem., 2002, 269, 298-306**]. Under *in vitro* conditions, the protein exhibits structural plasticity, regulated by the ionic environment around the molecule near physiological pH. HABP1/p32/gc1qR exists in a highly expanded conformation, similar to a molten globule like structure, at low ionic strength around neutral pH. However, presence of salt stabilizes the oligomeric structure making it more compact. There is a gradual increase in the thermodynamic stability of the molecule with increasing salt concentration. It is apparent that the presence of threshold amount of salt is essential for any relevant HA-HABP1 interaction; but salt requirement does not seem to be essential for its interaction with other reported ligands. It is likely that the combination of high net charge due to the low isoelectric point and the relatively low intrinsic hydrophobicity of HABP1/p32/gc1qR results in a more open structure, as reflected in its marginal stability at neutral pH. Thus, the presence of counter ions in the molecular environment of HABP1/p32/gc1qR results in minimizing intra-molecular electrostatic repulsion leading to stable conformations regulating its multi-ligand affinity and accounts for its multifunctional nature.

P#28

Binding of carbohydrate ligands to sialoadhesin and MAG as studied by NMR experiments and computer modeling

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The siglec family, sialic acid-binding Ig like lectins (1) provides a group of cellular recognition molecules that are characterized by sequence homology with members of the IgSF family. Sialoadhesin and Myelin-associated glycoprotein (MAG) are members of this family, and are responsible for inflammation (sialoadhesin) (2) and neurite outgrowth inhibition (MAG) (3). This leads to the hope that inhibitors of MAG could be beneficial for the treatment of injuries in the central nervous system. Here, we describe NMR experiments that allows us to define the binding epitope of the oligosaccharide ligands (STD NMR (4)), and the conformation of the ligands when bound to the protein (trNOESY (5)). These experimental results are then compared with computational docking models, using the X-ray crystal structure for sialoadhesin (1QFO) (6) and a homology model for MAG. Combining the computational docking data with the NMR-results leads to an improved understanding of the requirements for binding of oligosaccharide ligands to sialoadhesin and MAG.

1. Kelm et al., *Curr. Biol.* (1994), **4**, 965-72
2. Crocker et al., *Glyconj. J.* (1997), **14**, 601-9
3. Mckerracher et al., *Neuron*, **13**, 805-811
4. Mayer and Meyer, *Angew. Chem. Int. Ed.* (1999), **38**, 1784-87
5. Maaheimo et al., *Biochemistry* (2000), **39**, 12778-88
6. May et al., *Mol. Cell*, **1**, 719-728

P#29

Role of plant root lectin in recognition of host specific *Rhizobium*

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Recognition of cell surface carbohydrates by lectins has wide implications in various biological processes including plant-*Rhizobium* symbiosis. Lipopolysaccharides (LPS) present on the surface of these microbes (rhizobia) are found to bind specifically with the host lectins. Our earlier studies with the peanut plant indicate that root lectin PRAII binds to peanut specific *Bradyrhizobium* through polysaccharide portion of LPS present on the cell surface of peanut specific bradyrhizobia. To understand the molecular mechanisms of this interaction LPS mutants were

developed by transposon Tn5 mutagenesis of peanut specific *Bradyrhizobium* strain GN17. Two mutants identified out of 700 transconjugants were found to have altered cell surface carbohydrates as determined by high performance anion exchange chromatography. Mutants showed higher affinity towards PRAII as analyzed by sugar inhibition, Enzyme-linked lectin binding assay and FACS analysis.

1. Kalsi G, Babu CR, Das RH, *Glyconjugate J* 12, 45-50 (1995)
2. Jayaraman V, Das HR, *Biochim Biophys Acta* 1381, 7-11 (1998).
3. Das H, Jayaraman V, Bhattacharya I, *Biosci Rep* 19, 219-25 (1999).

P#30

Interactions of the gastrotropic bacterium *Helicobacter pylori* with the selectins – novel clues to the persistence of gastritis

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Helicobacter pylori, a gram negative bacterium, infects the gastric tissue of about 25% and 90% of humans in developed and developing countries, respectively, and can persist for decades. The deleterious effects are largely the result of a vigorous chronic inflammatory response, and include chronic gastritis, ulceration and gastric cancer. The lipopolysaccharides (LPSs) at the surface of most *H. pylori* strains contain carbohydrate sequences related to the Lewis (Le^x or Le^a) antigens, which encompass ligands for the leukocyte-endothelium adhesion molecules, the E-, L, and P-selectins. We have considered the possibility that carbohydrate-mediated interactions between the selectins and the LPSs shed by *H. pylori* may contribute to the non-remitting inflammation. Here, we have examined forty *H. pylori* isolates from patients with chronic gastritis, duodenal ulcer and gastric cancer, for their binding to the E-, L- and P-selectins. Our results show unequivocal evidence of binding by isolates from each of the diagnostic groups to the E-, and L-selectins. The selectin binding is distinct from the previously reported carbohydrate-mediated interactions of *H. pylori,* and is inhibitable by known carbohydrate ligands for the selectins**.** We propose that LPSs shed by *H. pylori* may actively contribute to persistent inflammation by mediating a leukocyte recruitment drive through interactions with the selectins, and suggest that inhibition of these interactions, intragastrically, could form the basis of new approaches to therapy of *H. pylori* infection.

P#31

A macrophage-surface lectin-like protein involved in the recognition of oxidized and apoptotic cells

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We have previously isolated a 50 kDa lectin-like protein from the membranes of THP-1 cells differentiated into macrophages (THP-1 macrophages) as a candidate for the macrophage receptor involved in the carbohydrate-mediated recognition of oxidized cells [Eda, S. *et al*. Arch. Biochem. Biophys., **385**, 186-193(2001)]. Now, we found that the N-terminal amino acid sequence of the 50 kDa protein is highly homologous to a 110 kDa nuclear protein. Antibody against this sequence bound to 50, 60, 70, 90 and 110 kDa proteins of THP-1 macrophages on immunoblots, suggesting that the originally isolated 50 kDa protein was a fragment of the 110 kDa protein. The antibody also bound to the cell surface of THP-1 macrophages, as detected by immunofluorescence microscopy, suggesting that the nuclear protein or its fragments were expressed on the cell surface. Recognition of oxidized and apoptotic Jurkat T cells by THP-1 macrophages was inhibited by the antibody. In addition, a soluble recombinant protein corresponding to 50 kDa truncated form of the 110 kDa protein blocked the binding of apoptotic Jurkat cells to THP-1 macrophages. These results suggest that the 110 kDa protein or its fragments play a role in the carbohydrate-mediated recognition of oxidized and apoptotic cells on macrophage surface.

P#32

Structural studies on jacalin - carbohydrate complexes

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Jacalin is a 66kDa tetrameric lectin from the seeds of jackfruit (*Artocarpus integrifolia*). Each subunit consists of two chains, a long α chain and a short β chain, produced by post-translational proteolysis. The structure of the lectin-bound to Me-α-galactose was earlier solved in this laboratory. The structure revealed a novel lectin fold and the use of post-translational modification as a strategy for generating specificity for galactose at the primary binding site. At the disaccharide level it is specific to tumour-related Galβ1-3GalNAc, known as T-antigen. We have now prepared and solved the structures of the complexes of the lectin with Gal, Me-α-GalNAc, T-antigen, Me-α-T-antigen and GalNAcβ1-3Gal-O-Me. In the complexes with T-antigen and Me-α-T-antigen, the primary site is occupied by the GalNAc residue. The interactions of the Gal residue with the protein are confined to water bridges. The methyl group in Me-α-T-antigen has favourable interaction with an aromatic residue, accounting for its increased affinity to the lectin. In the complex with GalNAcβ1-3Gal-O-Me, however, the primary site is occupied by the Gal residue presumably on account of the favourable interaction of the methyl group with the residue. The structures of the complexes provide a reasonable explanation for the available thermodynamic data of jacalin-carbohydrate interactions.

P#33

Oligosaccharide ligands on human colon cancer cells associated with the anti-tumor activity of a serum lectin, MBP

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Mannan-binding protein (MBP), a Ca^{2+}-dependent mammalian lectin specific for mannose, N-acetylglucosamine and fucose, is an important serum component associated with innate immunity. Recently, we found that MBP has a growth-inhibitory activity

against human colorectal carcinoma cells *in vivo* through the mechanism which is called MBP-dependent cell-mediated cytotoxicity (MDCC). We analyzed the oligosaccharide ligands on SW 1116 cells that bind to MBP. The MBP-binding oligosaccharides fraction consisted mainly of galactose, N-acetylglucosamine and fucose on N-glycans. The endo β–galactosidase digestion, or the selective removal of fucose residues of the ligands resulted in a great reduction of the binding activity to the MBP column together with the decrease of their molecular sizes. FITC-MBP binding to SW1116 cells was inhibited by fucose-specific plant lectins such as AAL but not by mannose-specific lectins such as Con A. FITC-MBP binding to the cells was inhibited by mAbs against Lewis A and B blood group antigens but not by those recognizing Lewis X and Y antigens. These results indicated that MBP ligands had high molecular size poly N-acetyl- lactosamine structures with high fucose content.

P#34

Chickpea (*Cicer arietinum L.)* lectin: a lectin with complex sugar specificity

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The chickpea (*Cicer arietinum L.*) seed lectin was purified to homogeneity by ammonium sulfate precipitation and ion exchange chromatography on DEAE -Cellulose and SP-Sephadex chromatography. The molecular weight of the lectin as determined by gel filtration was 37,000 Da and it consisted of two identical subunits of 20,000 Da.The N-terminal amino acid sequence of the lectin is Gly-Lys-Ser-X-Tyr. It is a basic glycoprotein with a pI of 9.0 and 4.5% content of neutral sugar.

Cicer lectin agglutinates pronase treated rabbit and human erythrocytes, but fails to agglutinate untreated erythrocytes. The purified lectin showed a specific activity of $5x10^4$ U/mg. CD analysis revealed the presence of 34% alpha, 28%beta, and 38% random coils as the secondary structure elements in the lectin. The lectin is stable upto 50°C for 10 min. The maximum pH stability shown by lectin was in the pH range of 5-8. The lectin is not inhibited by mono and di-saccharides. It shows affinity for complex oligosaccharides and glycoproteins containing these oligosaccharides.

P#35

Structural basis for N-Acetylgalactosamine specificity of basic winged bean lectin

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Winged beans (*Psophocarpus tetragonolobus)* contain a basic lectin (WBAI) (pI>9.5) and an acidic lectin (WBAII) (pI~5.5). These two lectins show high affinity for methyl-α-D-galactose at the monosaccharide level but have entirely different affinities for oligosaccharides. WBAI is an N-glycosylated homodimeric protein with MW 58,000 Da having 241 amino acid residues. The crystal structure of WBAI complexed with methyl-α-D-galactose (ComplexI) was solved earlier in this laboratory. Similar to the other Gal/GalNAc specific lectins, WBAI also exhibits higher affinity to GalNAc, compared to Gal. In order to understand the recognition of GalNAc by WBAI at the atomic level, WBAI has been crstallized in the presence of methyl-α-D-GalNAc (ComplexII). Intensity data to a resolution of 2.75 Å have been collected and the structure has been refined. The sugar molecule is clearly visible in the electron density maps. No conformational change occurs in the protein compared to ComplexI. All the interactions observed between the sugar and the protein in ComplexI are conserved in ComplexII as well. The higher affinity of WBAI for GalNAc can be attributed to the following interactions between the acetamido group of the sugar and the lectin: (i) formation of a new hydrogen bond between the carbonyl oxygen and the nitrogen atom of Gly105, (ii) a water bridge between the nitrogen atom and ND2 of Asn128 and (iii) stabilization of the methyl group in a hydrophobic pocket.

P#36

Sialoadhesin as a specific ligand for macrophage galactose-type C-Type lectin involved in the sensitization phase of contact hypersensitivity

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In the sensitization phase of contact hypersensitivity, dermal macrophages, which express galactose-type C-type lectin (MGL), migrate from the dermis and localize to the subcapsular sinus, interfollicular regions, and the areas surrounding high endothelial venules in the regional lymph nodes (LNs). Because the areas histologically stained with recombinant MGL (rMGL) are also restricted to those areas in LNs, the ligands for MGL were suggested to regulate the trafficking of MGL^+ cells in the sensitization process. However, little is known about the molecular natures of these ligands. In this report, a ligand for MGL in LNs was identified as sialoadhesin (Sn). Immobilized rMGL was prepared and bound fractions from lysates of murine LNs were recovered. Among several components eluted with EDTA, the 200 kDa protein was most prominent. This component was digested with Lys-C and the fragments were analyzed by MALDI-TOF MS. The mass numbers of two fragments were identified to be delivered from Sn. This component was further confirmed to be Sn by the Western blotting using anti-Sn mAb. MGL bound Sn *in vitro* by ELISA depending on the N-glycan of Sn. MGL also bound cells expressing Sn by flowcytometry. These results and the distribution of Sn within LNs strongly suggest that Sn is a functional ligand for MGL in murine LNs. The interaction should regulate the trafficking of MGL^+ cells, that lead to DTH response.

P#37

***Parkia biglandulosa* seed mannose/glucose-specific lectin : biochemical and physiological perspectives**

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Most studies on legume lectins involved in members of Fabaceae family, while investigations on other two families i.e. Caesalpiniaceae and Mimosaceae, are scarce. Amongst Mimosaceae members *Parkia* is the only genus from which four lectins have been characterized from seeds of four different

species. We have isolated a mannose/glucose-specific lectin by affinity chromatography on Sephadex G100 from the seeds of *Parkia biglandulosa* Wight & Arn., locally known as Ball-badminton tree. The purified lectin showed a single band on SDS-PAGE in presence and absence of β-mercaptoethanol, and the apparent molecular weight is 48 kDa. Glycoprotein nature of this lectin was confirmed in SDS-PAGE gel by periodic acid – Schiff's reagent stain and the carbohydrate content 1.6%. The lectin agglutinated rat erythrocytes but not those of humans and the agglutination inhibited by D-glucose, D-mannose, D-fucose, maltose and *N*-acetyl-D-glucosamine. The haemagglutinating activity and quantity of the lectin found to increased with a concomitant decrease of galactomannans in germinating seeds. The biochemical and physiological aspects of the seed lectin will be discussed.

P#38

Structural studies on crystals of peanut lectin grown in the presence of oligopeptides

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The structure of the tetrameric galactose specific peanut lectin was first solved as a complex with lactose using orthorhombic crystals grown at neutral pH. Its unusual quaternary structure was retained even at pH 4.6. In the low pH monoclinic and triclinic crystal forms, the carbohydrate binding site in some subunits is occupied by a loop from a neighboring molecule, indicating the possibility of molecular mimicry. To explore this possibility, crystallization experiments were carried out at low pH in the presence of two nonapeptidies and two decapeptides with sequences related to that in the loop. One set of experiments were carried out using protein solutions containing lactose and another set using solutions without it. All the experiments except one yielded crystals, although their diffraction quality varied widely. Normally peanut lectin crystals grow only in the presence of sugar. This is the first instance when oligopeptides have been seen to induce crystallization of the lectin. While crystals grown in the presence of lactose and one or the other of the nonapeptides, were orthorhombic as those grown at neutral pH, the remaining ones were monoclinic. The binding sites were occupied by lactose molcules when the sugar was present in the crystallizing medium. However, intriguingly, at this stage of analysis, the oligopeptides do not appear to bind to the lectin. Further work on the structures is in progress.

P#39

Calreticulin-glycoprotein interaction: chaperone with moderate affinity for the substrate

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Calreticulin is a lectin-like molecular chaperone of the endoplasmic reticulum in eukaryotes. Its interaction with N-glycosylated polypeptides is mediated by the glycan, $Glc_1Man_9GlcNAc_2$, present on the target glycoproteins. In this work, binding of monoglucosyl IgG (chicken) substrate to calreticulin has been studied using real time association kinetics of the interaction with the biosensor based on surface plasmon resonance (SPR). By SPR, accurate association and dissociation rate constants were determined, and these yielded a micromolar association constant. The nature of reaction was unaffected by immobilization of either of the reactants. The Scatchard analysis values for K_a agreed well with the one obtained by the ratio k_1/k_{-1}.The interaction was completely inhibited by free oligosaccharide, $Glc_1Man_9GlcNAc_2$, whereas $Man_9GlcNAc_2$ did not bind to the calreticulin-substrate complex, attesting to the exquisite specificity of this interaction. The binding of calreticulin to IgG was used for the development of immunoassay and the relative affinity of the lectin-substrate association was indirectly measured. The values are in agreement with those obtained with SPR. In conclusion, these studies clearly show that calreticulin binds to its substrate through its lectin function only and that protein-protein interactions do not stabilize these reactions. Since protein-protein interaction between the chaperone and the glycoprotein substrate do not seem to contribute to the binding reaction, calreticulin, if at all, has a very shallow and highly solvent exposed region for binding to the protein part of the substrate. Consequently, calreticulin acts as a chaperone by sequestering and sterically excluding interactions between the unfolded polypeptide chains of the nascent glycoproteins.

P#40

Computational analysis of multivalency in lectins: structures of garlic lectin-oligosachharide complexes and their aggregates

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The computational analysis of garlic lectin addresses multivalency as well as the structural basis for marked differences in affinities and specificities for various oligosachharides. Oligomannosides ranging from two to nine units, were systematically docked into each of the six crystallographically identified mannose binding sites of garlic lectin dimer. Their binding affinities vary from a 8-fold increase over monomeric mannose for a di-mannoside to ~14000-fold increase for a nona-mannoside. Computation of their interaction indices suggested that differences in affinities for various oligosachharides by the protein can be explained if these sugars were to interact with multiple binding sites on the protein, but from multiple protein molecules simultaneously. A systematic generation of such arrangements, indicate that trimannosides and larger sugars are capable of cross-linking multiple protein molecules resulting in the formation of protein lattices held together through oligosachharide interactions. Hepta-mannosides and larger sugars are capable of inducing double cross linking. The study suggests that this may be a strategy adopted by lectins in general for achieving high affinity for oligosachharides. This study also provides insight into the mode of generating diversity in interactions, using a common scaffold provided by the structure of individual lectin molecules containing multiple binding sites in them.

P#41

Mucin binding proteins from *Streptococcus pneumoniae*

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Streptococcus pneumoniae is the most common bacterial cause of acute respiratory infections and otitis media. During the past decade, an increase in penicillin-resistant strains has emphasized the need for vaccines.

Since commercially available vaccines do not offer protection from all serotypes, we investigated other strategies to develop a vaccine with broader application. For infection to occur, *S. pneumoniae* must first attach to epithelial cells. Since mucins are thought to modulate bacterial colonization, we examined the interaction between human nasopharyngeal mucin (HNM) and surface proteins of *S. pneumoniae*. Utilizing an overlay assay, we have identified the presence of at least three mucin-binding proteins (MBPs) in the phosphate-buffered saline extract of *S. pneumoniae*. They were isolated by ion-exchange chromatography in DEAE-Sepharose CL-6B. Following Western transfer to PVDF membrane, the protein bands were excised and N-terminal amino acid sequences determined. By comparison of the sequences to the publicly available *S. pneumoniae* genome sequence (TIGR), PCR primers were designed and products were obtained for two of the MBPs (12 and 14kDa). The sequences were recombinantly expressed and purified. These proteins have homology to ribosomally associated proteins in *S. pneumoniae* and other bacteria. Mice were vaccinated intranasally with r12 kDa MBP and challenged with *S. pneumoniae*. A statistically significant decrease in the level of colonization by *S. pneumoniae* was observed. This demonstrates that mucosal immunization with r12 kDa MBP can reduce pneumococcal colonization in the nasopharynx of mice.

P#42

Partial amino acid sequence of the mannose 6-phosphate receptor (MPR 300) protein from the invertebrate *Unio*

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A putative mannose 6-phosphate receptor protein (MPR 300) has been affinity purified on phosphomannan gel from the invertebrate mollusc, *unio* (Udaya lakshmi *et al*., 1999). In order to make a structural comparison of this receptor to the other known MPR 300 proteins (mammals, chicken), purified *Unio* receptor was subjected to reductive carboxymethylation. This protein was digested with TPCK trypsin, and the peptides obtained were separated by HPLC. For three peptides, the purity and the molecular mass was determined by mass spectrometry. The amino acid sequence of these peptides by Edman degradation revealed that the mollusc receptor shows greater then 90% amino acid sequence homology to the known vertebrate MPR 300 protein sequences suggesting evolutionary conservation of this protein. The results of these will be presented and discussed.

1. Udaya lakshmi, Y *et al* (1999) *Biosci Reports* **19**: 403-409

P#43

Analysis of carbohydrate binding site in proteins

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Whole-genome sequencing efforts have generated a large number of orphan open reading frames whose function(s) has to be determined. Several computational and experimental tools are available to bridge this gap between sequence and function. Study of binding site architecture is one such computational tool wherein local conformational features of ligand binding site in terms of 3D pattern are analyzed, since microenvironment is more critical than the overall topology. The aim of the present study is to search for the presence of local 3-D patterns among sugar-binding proteins. The mechanism by which these proteins achieve ligand-specificity is not yet completely understood. Using a knowledge-based approach, the features of ligand-binding site have been studied by analyzing the 3-D structures of protein-sugar complexes. Results from such a study will be presented.

P#44

Thermodynamic analysis of the interaction of porphyrins with bitter gourd (*Momordica charantia*) seed lectin

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Owing to the use of porphyrins in photodynamic therapy (PDT) for the treatment of malignant tumors, and the preferential interaction of lectins with tumor cells, studies on lectin-porphyrin interaction are of significant interest [1-5]. In this study, the interaction of several free-base and metalloporphyrins with *Momordica charantia* lectin (MCL) has been investigated by absorption spectroscopy. Each subunit of MCL binds one porphyrin molecule and the stoichiometry was unaffected by the presence of lactose, clearly indicating that porphyrin and carbohydrate ligands bind at different sites. Both cationic and anionic porphyrins bind to the lectin with comparable affinities ($K_a = 10^3 - 10^5\ M^{-1}$), suggesting that hydrophobic interactions play an important role in the binding process. Thermodynamic parameters obtained for the interaction of *meso*-tetracarboxyphenyl porphyrinato copper(II) (CuTCPP) from the temperature dependence of its binding to MCL ($\Delta H = -99.6\ kJ.mol^{-1}$; $\Delta S = -248.9\ J.mol^{-1}.K^{-1}$) indicate that besides nonpolar interactions, hydrophilic forces may also play a significant role in the MCL-porphyrin interaction.

1. Bhanu K et al., *Curr. Sci.* **73**, 598 (1997).
2. Komath SS et al., *Biosci. Rep.* **20**, 265-76 (2000).
3. Komath SS et al., *J. Photochem. Photobiol. B:Biol.* **55**, 49-55 (2000).
4. Kenoth R et al., *Eur. J. Biochem.* **268**, 5541-49 (2001). [5] Goel M et al., *J. Biol. Chem.* **276**, 39277-81 (2001).

P#45

Isolation of a partial cDNA clone that encodes the goat mannose 6- phosphate receptor (MPR 300) protein

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Mannose 6-phosphate receptors (MPR 300 and 46) from goat and other non-mammalian vertebrates have been affinity purified from our laboratory employing phosphomannan Sepharose gels. In a recent study we also developed an ELISA method to quantify the receptor proteins from different tissues of goat and chicken. In the present study we employed RT-PCR strategy to obtain a partial cDNA clone that encodes for the goat MPR 300 protein in the amino terminal region. Using degenerate primers and RT-PCR, we were able to amplify a fragment of 804 bp, which was subcloned into a TA cloning vector. Bacteria were transformed

and the positive clones identified by blue/white selection. The cDNA sequence was determined from the plasmid DNA isolated. When the nucleotide sequence was converted to protein sequence and compared to the known MPR 300 sequences, there was striking homology of the sequences to the bovine, human, and mouse MPR 300 proteins. The results of these will be presented and the structural homology of the MPR 300 proteins discussed.

1. Siva Kumar, N *et al* (1997) *Comp. Biochem.Physiol.***1188**: 805-809
2. Suresh, K *et al* (2002) *J.Biochem.Biophs.Meth* **52/2**: 111-119

P#46

Physiological role of a novel TF antigen binding lectin from *Sclerotium rolfsii* a phytopathogenic fungus

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Sclerotium rolfsii a phytopathogenic fungus secretes a lectin at different stages of its growth which has fine sugar specificity towards Gal β 1-3 GalNAc-Ser/Thr an oncofetal antigen. Apart from the glycoconjugate moiety, peptide back bone is essential for the recognition. Because of this sugar recognition property it binds to malignant cells. Histochemistry of malignant colon tissues using FITC labeled *Sclerotium rolfsii* lectin showed different patterns compared to PNA, with strong binding to goblet cells.

We have positive indications for implicating this lectin for its functional role in the development and morphogenesis of the fungus. FITC labeled antibodies of the lectin binds discretely at the branching points of the developing hyphae, but uniformly on the sclerotial bodies indicating the distribution pattern of the lectin. Also the antibodies inhibit the germination of sclerotial bodies. Further FITC labeled lectin binds to few glycolipid fractions of the *Sclerotium rolfsii* fungus separated by HPTLC, indicating the presence of lectin receptor. Hitherto there are no reports on the functional role of phytopathogenic fungal lectins. However many workers speculated their role in host parasite interactions similar to bacterial adhesins. But our findings with this lectin implicate the lectin's role in the development and morphogenesis of the fungus. These studies answer hitherto argued role of phytopathogenic fungal lectins and is interesting to note the occurrence of Galβ1-3GalNAc containing glycoconjugates in this fungus.

P#47

Effect of banana lectin and potato lectin on mast cells

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The major factor in false food allergy is the direct effect of lectins on mast cells. They appear to cross-link the glycans of two IgE molecules on mast cells/basophils, thereby causing activation and release of histamine. Some lectins have been studied for their ability to release histamine from basophils *in vitro*. We selected lectins from widely consumed foods, namely, banana (*Musa paradisiaca*) and potato (*Solanum tuberosum*), to study their effect on mast cells. Banana lectin has a specificity for mannose, and potato lectin for $(GlcNAc)_2$. The effect of lectins (concentration: 100 µg/mL in 50% glycerinated PBS) on mast cells was assessed by skin prick test (SPT) on normal and allergic subjects. SPT response is a measure of histamine and other cell mediators liberated upon activation of mast cells *in vivo*. Of 66 allergic subjects tested, 22 gave a positive SPT (33.3%) for banana lectin. In the case of potato lectin, 23 gave a positive SPT (35.4%) out of 65 allergic subjects tested. However, all 20 normal subjects gave a negative SPT with both banana and potato lectins. The SPT data clearly indicates that banana and potato lectins activate the mast cells in about a third of the allergic subjects. It seems likely that a higher density of IgE on mast cells of some allergic subjects, or individual differences in the carbohydrate chains (on the cell surface or in IgE molecules) could account for susceptibility of some individuals to a particular lectin.

P#48

Structure-energetic basis of exquisite saccharide recognition selectivity by P75/AIRM1/Siglec-7

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Siglec-7, alternatively termed p75/AIRM1, is the first example of a siglec that is found predominantly on natural killer cells, an important component of the first line of host defenses against cancer cells and viral diseases. We demonstrate by x-ray crystallographic, calorimetric and spectroscopic analyses that recognition of sialylated oligosaccharide by siglec-7 involves favorable contributions to the binding free energy change emanating from both enthalpic and entropic factors, without dramatic conformational changes in secondary structural elements. This, together with negative heat capacity change for the recognition of Neu5Acα2,8Neu5Acα2,3Galβ1,4Glc by siglec-7 suggest that hydrogen-bonding as well as hydrophobic interactions determine the binding reaction. The source of the differential recognition of the sialylated trisaccharides Neu5Acα2,3Galβ1,4Glc, and Neu5Acα2,6Galβ1,4Glc is entropic in nature. Binding of either Neu5Ac or Galβ1,4Glc alone to Siglec-7 was undetectable. These results demonstrate unequivocally, a hitherto unreported energetic basis of the exquisite specificity of the sialic acid dependent ligand recognition by siglec-7 at moderate range of equilibrium association constants.

3. Glycans

P#49

Non-enzymatic browning of eye lens α-crystallin by methylglyoxal: effect on structure and function

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Non-enzymatic glycation of proteins leads to the formation of advanced glycation end products (AGE). AGE formation alter structural and functional properties of many proteins, which are implicated in the diabetic and uremic complications. Reactive dicarbonyl compounds, methylglyoxal (MG) and glyoxal (G) are formed from triose phosphates and auto-oxidation of reducing sugars. These compounds are highly reactive against proteins forming covalent crosslinks and fluorescent products similar to non-enzymic browning. As MG and G levels are known to increase several folds in cataract and diabetic conditions, it could be considerable importance to study the effect of MG on long-lived proteins such as eye lens crystallins. α-Crystallin, one of the major lens proteins, has been shown to function like a molecular chaperone by preventing the aggregation of other proteins. In the present study we demonstrate the formation of AGE (including argypyrimidine) by fluorescence spectroscopy upon incubation of α-crystallin with MG. MG treatment also leads to formation of high molecular weight aggregates (as shown by HPLC, SDS-PAGE and Western blot) and decreased stability of the protein (as assessed by DSC). Further, circular dichroism data indicate loss of secondary and tertiary structure of α-crystallin due to MG modification. These structural changes will be discussed with regard to its chaperone-like function.

P#50

Chemical synthesis of *Leishmania* lipophosphoglycan (LPG): efforts towards construction of the conserved glycan core

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The protozoan parasite *Leishmania donovani,* causing visceral leishmaniasis, has remarkable ability to survive and proliferate in extreme hydrolytic environments during its digenetic life cycle. *Leishmania* assembles a unique cell surface GPI-anchored phosphoglycan termed lipophosphoglycan (LPG), which is a multifunctional virulence factor essential for the infectivity and survival of the parasite [1].

LPG contains a conserved hexasaccharide core with an internal galactofuranose residue, which is absent in mammalian glycobiology. A parasite mutant (R2D2, *lpg1*) defective in Gal_f addition has been shown to be non-infective and is unable to synthesize LPG.

In our ongoing work on chemical synthesis and biosynthesis [2-7] of LPG and its structural domains, we have designed an efficient strategy for the synthesis of Gal_f containing glycan core of LPG, the progress in this synthetic work would be presented.

1. Turco SJ, Spath GF, Beverley SM (2001) *Trends Parasitol.* **17**: 223-6
2. Sahai P, Vishwakarma RA (1997) *J. Chem. Soc., Perkin Trans I* :1845-49
3. Tyagi AK, Vishwakarma RA (1998) *Tetrahedron Lett.* **39**: 6069-72
4. Upreti M, Vishwakarma RA (1999) *Tetrahedron Lett.* **40**: 2619-22
5. Upreti M, Ruhela D, Vishwakarma RA (2000) *Tetrahedron* **56**: 6577-84
6. Sahai P, Chawla M, Vishwakarma RA (2000) *J. Chem. Soc., Perkin Trans I*: 1283-90
7. Ruhela D, Vishwakarma RA (2001), *Chem. Commun.*: 2024-25

P#51

Multivalent peptidoglycan mimetics as modulators of bacterially-induced TNF-α production

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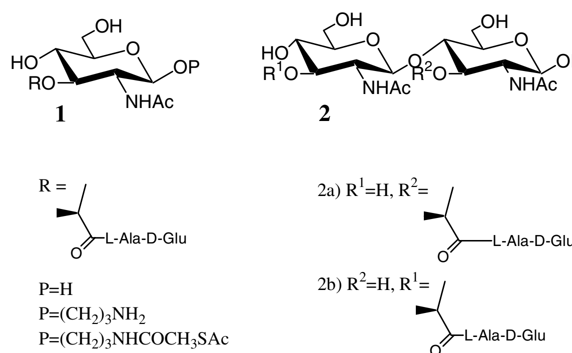
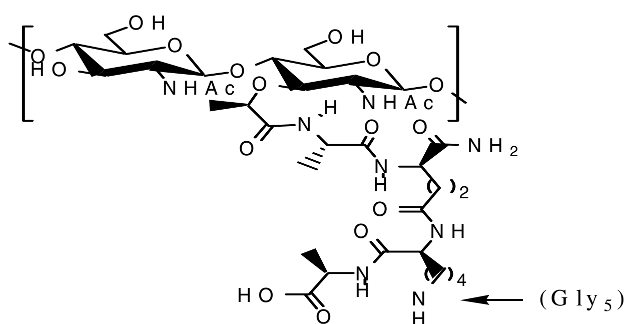
Peptidoglycan (PGN), a major gram-positive bacterial cell wall component, is known to induce multiple host-derived inflammatory inducers, most notably interleukin 1(IL-1), IL-6 and Tumor necrosis factor-α (TNF-α). Cell-signaling requires interaction of PGN with CD14, a cell-surface glycophosphatidylinositol receptor, and Toll-like receptor2 (TLR2), a trans-membrane receptor protein. Preliminary, studies have demonstrated that PGN mimetics are useful tools with which to study this complex pathway[1]. To further delineate the ligand requirements for binding to CD14 and TLR2 and also for induction of cell signaling, we have developed a convergent approach for the synthesis of a wide range of PGN part-structures. The synthetic derivatives include mono and disaccharide-derived repeating units which have been functionalized with an artificial spacer for attachment to poly[*N*-(acryloyloxy)succinimide] polymer to obtain a range of multivalent ligands. Synthesis of the novel analogs is here described and their ability to interact with CD14 and TLR2 and also to induce TNF-α production is outlined.



PGN part structures



Peptidoglycan (PGN)

1. Siriwerdena, A., Jorgensen, M., Wolfert, M., Vandenplas, M., Moore, J. N.; Boons, G.-J. *J. Am. Chem. Soc.*,**123** (33), 8145-8146 (2001).

P#52

Various heparin-binding growth factors specifically interact with oversulfated chondroitin sulfate E

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We have previously demonstrated that oversulfated chondroitin sulfate E (CS-E), derived from squid cartilage, specifically inhibited the neuronal cell adhesion mediated by midkine (MK), a heparin (Hep)-binding growth factor (GF) [1]. The neurite outgrowth promoting activities of CS-E towards rat embryonic day 18 hippocampal neurons [2] and the presence of oversulfated CS in chick and rat brains have been reported previously [1,3]. Hence the possibility of CS-E interacting with Hep-binding GFs during development was tested using CS-E. Filter binding assay and interaction analysis by the IAsys system was used to study the binding of [^{3}H]CS-E with Hep-binding GFs [MK, pleiotrophin (PTN), aFGF, bFGF, HB-EGF, FGF-10, FGF-16 and FGF-18]. The study demonstrated the direct binding of all GFs except aFGF to CS-E in a dose dependent manner. The kinetic constants k_a k_d and K_d showed that MK, PTN, FGF-16 and FGF-18 bound strongly to CS-E, with comparable affinity to that of Hep, while the affinity of binding of HB-EGF, bFGF and FGF-10 towards CS-E was lower than that for Hep. These findings suggest the possibility of CS-E being a binding partner or a receptor for various Hep-binding GFs.

1. Ueoka *et al* (2000) *J Biol Chem* **275**: 37407-37413
2. Clement *et al* (1999) *Neurosci Lett* **269**: 125-128
3. Kitagawa *et al* (1997) *J Biol Chem* **272**: 31337-31381

P#53

Role of polysialic acid and CD56 on human natural killer cells

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Cell surface expression of CD56 demarcates two subsets of human lymphocytes that have natural killing ability: natural killer cells (NK cells) defined as CD56+, CD3-; and killer T cells, defined as CD56+, CD3+. Although the function of this glycoprotein on immune cells is unknown, it is the lone scaffold for the addition of polysialic acid (PSA). CD56 is also expressed in the nervous system, where it is denoted as neural cell adhesion molecule (NCAM) for its role in forming stable cell-cell contacts. The addition of PSA, a bulky and negatively-charged glycan, to NCAM abrogates this molecule's ability to participate in homophilic or heterophilic binding. Thus, in the nervous system, presentation of PSA is regulated according to the need for stability or plasticity. In contrast, the biology of PSA on immune cells has been little studied to date. Here, we explore the regulation and role of PSA on NK and killer T cells.

P#54

Synthesis of novel triterpenoid trisaccharides

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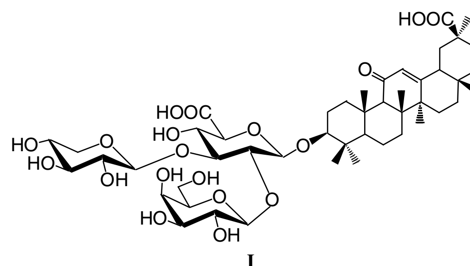
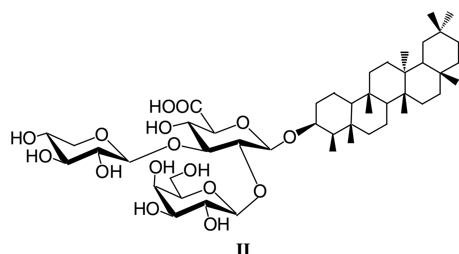
Many different classes of saponins have been isolated from terrestrial plants and from some marine animals and some of them have attracted considerable interest due to their biological activities. [1,2] We have been interested in the synthesis of glycyrrhetinic acid and friedelin trisaccharides and developed a feasible synthetic route, by first attaching the glucoside residue to the triterpene and then extending the sugar chain sequentially. The glycosylation procedures [3,4] were carried out using appropriate protecting group patterns on the glucoside residue, which were chosen depending on the triterpene to be used. Removal of 4,6-protecting groups followed by oxidation to the glucuronic acid and concomitant acetyl cleavage afforded the saponins **I** and **II**. Both structures were confirmed by means of NMR spectroscopy and mass spectrometry.





(1) S. B. Mahato and A. K. Nandy, *Phytochemistry*, 30 (1991) 1357-1390.
(2) T. Schöpke and K. Hiller, *Pharmazie*, 45 (1990) 313-342.
(3) Zi-Hua Jiang, *Dissertation*, Hartung-Gorre, Konstanz, 1995.
(4) PhD Thesis of Jorg Schimmel, Konstanz, 2001.

P#55

Glycotargeting : Glycocluster oligonucleotides conjugates

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In search of specific and highly selective sugar clusters for cell receptors such as membrane lectins, various di- and oligo-saccharides (Os) were coupled to small peptide cores (linkers) through an amide bond : Os-Glp-βAla (or Osl). These glycosynthons [Quétard *et al*., 1998, *Bioconj Chem* **9**, 268-76] were used to synthesize oligolysine-based glycoclusters : Osl-$[Lys(Osl)]_n$-Ala-Cys-NH_2, with n = 1 to 5. The glycoclusters used in this work derived from lactose, Lewis b (Fucα-2Galβ-(Fucα4-)-3GlcNAcβ-3(Galβ-4Glc), Lewis a (Galβ-(Fucα4-)-3GlcNAcβ-3Galβ-4Glc) and Lewis x (Galβ-(Fucα3-)-4GlcNAcβ-3Galβ-4Glc) isolated as glycosynthons upon HPLC from a mixture of both. The glycocluster specificity was assessed by flow cytometry and surface plasmon resonance.

The 3'-end of a phosphorothioate-type oligonucleotide (21-mer, 5'-triethoxydithiopyridinyl-3'-aminohexyl), [Aubert *et al*., 2000, *NAR* **28**, 818-25] was labelled with fluorescein isothiocyanate. This fluorescent oligonucleotide was then coupled to the thiol group of the cysteine residue of a glycocluster through a disulfide bridge. After gel filtration, the pure conjugate was shown to be free of the oligonucleotide monomer and dimer, by polyacrylamide gel electrophoresis.

The oligonucleotide glycoclusters conjugates containing fucose were recognized and taken up by cells expressing fucose-specific lectins, as shown by flow cytometry and confocal microscopy. Based on their high specificity and exquisite selectivity, these glycoconjugates have a therapeutic potential.

This work was supported by grants from Vaincre la mucoviscidose.

P#56

Systematic analysis of *N*-linked oligosaccharides in brains

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We have developed an automated system to isolate and analyze oligosaccharides of glycoproteins contained in crude tissue samples. Oligosaccharides were released from acetone precipitated tissues by hydrazinolysis and *N*-acetylated. These oligosaccharides are converted to pyridylamino (PA) derivatives by GlycoTag (TaKaRa) for fluorescence detection with high sensitivity. Excellent separation can be achieved by a combination of size-fractionation and reversed-phase HPLC. It is possible to collect and analyze oligosaccharides from the formalin-fixed specimen. This is an advantage in analyzing clinical tissues. We determined the structure of abundantly expressed N-linked oligosaccharides in the brain and quantified them. There were small differences in the amount of each N-linked oligosaccharides species among the disease-free brains.

Several brain tissues from neurodegenerative disease patients were then analyzed: total of 20 examples (70.9±7.4 years old), including brains from Alzheimer's disease (AD), corticobasal degeneration (CBD), and multiple system atrophy (MSA) patients. Several oligosaccharide structures were changed in these brains such as BA2, which is the brain specific oligosaccharide that we identified.

P#57

Heparin *N*-sulfonation affects its antiproliferative activity on bovine pulmonary artery smooth muscle cells

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Heparin (HP) *N*-sulfonation (S) affects its antiproliferative activity (APA) on bovine pulmonary artery smooth muscle cells (BPASMC) *in vitro*. This was demonstrated after determination of the total Upjohn HP sulfur content as sulfonate ($-SO_3^-$) before and after chemical *N*-deS.
Sulfonate content was quantified by using Fourier transform infrared spectroscopy (FT-IR), and selecting the band at 1260-1200 cm^{-1} that originates from S=O stretching vibrations of sulfate ($-O-SO_3^-$) and sulfonate ($-SO_3^-$) in polysaccharides containing these moieties. The amounts of $-SO_3^-$, determined on a standard curve of Pentosan Polysulfonate generated under the same conditions, were 21.87 and 16.51 % (w/w) before and after *N*-deS, respectively. BPASMC were grown in the presence of 1, 10 and 100 µg of HP/ml; their growth dropped to 79.12, 49.84 and 20.24 % respectively. When the cell media contained 1, 10, 100 µg of *N*-desulfonated HP/ml, cell growth was 92.55, 86.66, and 74.79 (w/w), respectively. Upon *N*-deS, HP lost about 2/3 of its AP potency. Because the sulfonate lost during *N*-deS is 24.49 % (w/w) of the total HP sulfonate content, these data show that *N*-S affects its APA on BPASMC.(Supp. by NIH Grant HL 39150)

P#58

Role of (1,6) branched *N*-oligosaccharides in lung metastasis

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Cancer cells show several surface modifications associated with the metastatic phenotypes which are believed to aid them to negotiating different steps in metastasis. The role of one such modification, viz., expression of (1,6) branched N-oligosaccharides in lung metastasis was investigated using low (B16F1) and high (B16F10) metastatic melanoma cells.

The results showed that the lungs were the most preferred organ for adhesion and the only organ for metastasis of these melanoma cells. The metastatic potential of the cells was found to correlate with their ability to adhere to the lungs, which in turn correlated with the surface expression of (1,6) branched N-oligosaccharides. Inhibition of expression of these oligosaccharides was found to inhibit both adhesion and metastasis to the lungs. Integrin and Lysosome Associated Membrane Proteins (LAMPs) were found to be amongst the major carrier of these oligosaccharides in melanoma cells. Of the LAMPs, only LAMP1 was found to get translocated to the surface, while LAMP2 remained cytoplasmic. The extent of translocation of LAMP1 to the surface and the amounts of (1,6) branched N-oligosaccharides and poly N-Acetyl lactosamine substitutions on LAMP1, correlated with the metastatic potential of the cells. We show here that the poly N-Acetyl lactosamine structures on the surface could be the major contributor towards the adhesion and possibly thus metastasis of melanoma cells to the lungs.

P#59

Affinity of *H. pylori* adhesion to Le^b-oligosaccharide depends on the carbohydrate carriers

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Objective: Helicobacter pylori have been shown to recognize the Le^b-oligosaccharide. In order to elucidate the possible role of carbohydrate carrier in adhesion of *H. pylori* to Le^b-oligosaccharides, the present study involved comparison of adhesion of *H. pylori* KH202 to immobilized Le^b-oligosaccharide carried on different carriers, i.e., Le^b-oligosaccharide conjugated with polyacrylamide, BSA, and dipalmitoylphosphatidylethanolamine (Le^b-PAA, Le^b-BSA, and Le^b-DPPE).
Results: All of the Le^b-oligosaccharide-carrying neoglycoconjugates served as ligands for *H. pylori*. However, the amount of Le^b-antigen on solid phase required for adhesion differed among them. *H. pylori* required 10-fold and 100-fold quantities of Le^b-antigen to adhere to Le^b-PAA and to Le^b-DPPE in comparison to the quantity of Le^b-antigen needed to adhere to Le^b-BSA, respectively. The adhesion of *H. pylori* to immobilized Le^b–PAA and Le^b-DPPE was strongly inhibited by 0.02 g/ml of Le^b-oligosaccharide, but the adhesion to immobilized Le^b-BSA was hardly inhibited by the oligosaccharide at the same concentration.
Conclusion: These results indicated that *H. pylori* preferentially recognized Le^b-oligosaccharide carried on BSA among three types of neoglycoconjugates. Therefore, the carbohydrate carriers affect the affinity of *H. pylori* to Le^b oligosaccharide.

P#60

Carbohydrate analysis of *Bacillus anthracis* spores and exosporium by high-PH anion-exchange chromatography

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Glycoconjugates are ubiquitous and important constituents of gram-positive and gram-negative bacteria. High-pH anion-exchange chromatography (HPAEC) with electrochemical pulsed amperometric detection is a very useful method for the analysis of carbohydrates of glycoconjugates. HPEAC is commonly used for the qualitative and quantitative determination of carbohydrates found in mammalian glycoproteins. Very few reports are available for the determination of carbohydrates in bacterial polysaccharides using this method. *Bacillus anthracis* is the etiological agent of anthrax. The spore is the infectious form and possesses a loose-fitting diffuse layer called exosporium. In the present study, we employed HPEAC using Dionex Carbo Pac PA10 column with electrochemical pulsed amperometric detection for the analysis of carbohydrates from *B. anthracis* spores, vegetative cells and the purified exosporium. Results indicate the presence of rhamnose exclusively in *B. anthracis* spores and exosporium while this sugar is absent in the vegetative cells.

P#61

Viral transcriptional regulation of synthesis of bioactive surface carbohydrates in virus infected cells

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Recent data indicate that some viruses are able to interfere with selectin-dependent inflammatory responses by inducing the Lewis substance, Lewis y (Le^y) and blood group antigen A as decoys. Fucosyltransferases, sialyltransferases, sulfotransferases and core2 GlcNAc transferases are all involved in the synthesis of selectin-binding carbohydrates. In the present study virus infected cells were compared to uninfected cells as to expression of these transferases, using Real Time TaqMan-dependent quantitative PCR. About 12 different PCR-systems were optimised and adapted for sensitive and accurate detection and quantification of glycosyl transferase gene transcripts, after reverse transcription to DNA. The levels of glycosyl transferase mRNA was normalized with housekeeping genes. The preliminary results from the screening of HSV-1 and HSV-2 infected cells show changes in the levels of sialyltransferase (ST3galIV) mRNA compared to corresponding levels in uninfected cells. This may constitute a molecular mechanism for the changed levels of bioactive surface sialyl-containing glycans in infected cells.

P#62

Clustering of water molecules during its adsorption on cellulose

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Clustering of water molecules during its adsorption on cellulose, derived from *Eucalyptus regnans* was studied using the Zimm-Lundberg theory. The average cluster size of water molecules was determined with the help of wood adsorption isotherms at different temperatures. The effects of relative humidity on average cluster size were studied. Average cluster size progressively increased with an increase in humidity. At humidities corresponding to fiber saturation, larger clusters were formed. Formation of large clusters at high humidities close to

saturation is attributed to increased fraction of weakly bonded water and capillary condensation in such conditions. A description of adsorption of water on cellulose was provided in terms of average cluster size.

P#63

A chemo-enzymatic approach for developing a library of oligosaccharides to assess the specificity of carbohydrate binding proteins

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The use of recombinant protein techniques in glycosyltransferase production has not only alleviated the most time-consuming aspects of carbohydrate synthesis but it has also made it possible to produce complex carbohydrates in a large scale. In this study we report the chemo-enzymatic strategies used for developing an oligosaccharide library in multi-gram scale preparations. This library exhibits a wide spectrum of compounds from simple monosaccharides to complex multiunit Sialylated/ Fucosylated glycosides of N- and O- linked glycoconjugates. A simplified production of N-glycolylneuraminic acid (Neu5Gc) and 3-deoxy-D-glycero-D- galacto-2-nonulosonic acid (KDN) structures will also be discussed in the course of this study. The library will be used for development of a glycoarray to conduct the specificity studies of carbohydrate binding proteins within the Consortium of Functional Glycomics. Both the glycoarray and the library of compounds will be made available through the Consortium for investigators both inside and outside the program (http://glycomics.scripps.edu).

P#64

Target-oriented design and synthesis of structurally defined small molecule glycomimetics

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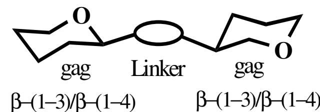
Small molecule therapeutics possessing distinct carbohydrate recognition domains are emerging as potential candidates in novel drug design and development involving anti-inflammatory, anticancer and antiviral agents. Moreover, their ability to recognize and interact with specific receptor proteins may have a profound influence in modulating various cellular responses. Hence, in order to understand and assess the exact nature of carbohydrate-protein interactions at a molecular level, synthesis of pure and well-defined small molecule carbohydrate derivatives is of prime importance. To this end, recognizing the key structural elements required, a series of low-molecular weight hyaluronan and heparin mimetic analogs separated by a short flexible spacer-arm were synthesized. Thus, using a combination of protection/deprotection protocols, *n*-pentenyl and imidate glycosidation, we have designed an efficient linear strategy for the construction of synthetic mimics related to the regular β-D-GlcA-(1−3)/(1−4)-β-D-GlcNHAc linked region of hyaluronan and heparin respectively. Furthermore, in order to achieve spatial control, these disaccharide molecules were used as efficient building blocks for the preparation of dimerized oligomers bridged by a flexible diamine linker. The present work will highlight our results and problems experienced during the synthesis along with the potential applications of these tailored mimics.



gag: glycosoaminoglycan

P#65

Iterative synthesis of *Leishmania* phosphoglycans in solution and on solid phase

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Leishmania, causative agent of leishmaniases, has a remarkable ability to proliferate in extreme environments during its digenetic life cycle. *Leishmania* assembles unique class of glycoconjugates named phosphoglycans, which include lipophosphoglycan (LPG) and secreted proteophosphoglycan. These are multifunctional virulence factors essential for infectivity and survival of the parasite [1].

Experiments to probe the function, biosynthesis and conformation of phosphoglycans requires efficient chemical synthesis. Since phosphoglycans are labile molecules due to presence of anomeric phosphodiester linkages, their synthesis is particularly challenging.

In our ongoing efforts towards chemical synthesis and biosynthesis [2-7] of LPG and it's structural domains, we report a new efficient synthesis of phosphoglycans, which does not involve any glycosylation steps, and the phosphoglycan chain can be extended either towards the non reducing (6′-OH) or reducing (1-OH) end in high yielding iterative steps. Our work on synthesis of phosphoglycans in solution and solid phase will be discussed.

1. Turco SJ, Spath GF, Beverley SM (2001) *Trends Parasitol.* **17**: 223-26.
2. Sahai P, Vishwakarma RA (1997) *J.Chem.Soc.,Perkin Trans 1*: 1845-49.
3. Tyagi AK, Vishwakarma RA (1998) *Tetrahedron Lett*. **39**: 6069-72.
4. Upreti M, Vishwakarma RA (1999) *Tetrahedron Lett*. **40**: 2619-22.
5. Upreti M, Ruhela D, Vishwakarma RA (2000) *Tetrahedron* **56**: 6577-84.
6. Sahai P, Chawla M, Vishwakarma RA (2000) *J.Chem.Soc.,Perkin Trans 1*: 1283-90.
7. Ruhela D, Vishwakarma RA (2001) *Chem. Commun*.: 2024-25.

P#66

Cytokeratin 13 peptides mimic *N*-Acetylglucosamine as a bacterial receptor

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Bacterial infections in humans are commonly initiated by the adherence of bacterial surface appendages, (pili, flagella) to host glycoconjugates. In some cases, peptides have been shown to mimic binding functions of carbohydrates as shown for cytokeratin 14 peptide which reacts with anti-GlcNAc antibodies (Shikhman et al. J. Immnol. 1994, 152:4375). The present study

suggests that a similar functional mimicry may be operative in the adherence of an opportunistic pathogen, *Burkholderia cepacia* complex (ET12 strain), to metaplastic respiratory epithelium of patients with cystic fibrosis.

In a series of experiments we have shown that the 22 kDa adhesin associated with cable pili of *B. cepacia* complex (ET12) binds to epithelial cell cytokeratin 13 (CK13), synthetic GlcNAc-rich oligosaccharides and hydrolysates of chitin (GlcNAc polymer). Lectins having affinity for GlcNAc (WGA and others) inhibited *B. cepacia* binding to the CK13 receptor. However, removal of GlcNAc from CK13 by treating with N-acetylglucosaminidase did not diminish CK13 receptor function. Overlapping 14 amino acid long peptides, (92 in total) spanning the length of the CK13 polypeptide were synthesized and used in microtiter plate binding assay as potential receptor structures for *B. cepacia*. To date seven peptides have been identified as containing potential binding epitopes for the *B. cepacia* adhesin. Since CK13 is highly expressed in the inflammatory respiratory mucosa of CF patients, our findings may explain the propensity of CF patients to chronic infection by *B. cepacia*.

Financial support from the Canadian Cystic Fibrosis Foundation

P#67

Changes in heparan sulfate during diabetes and its modulation by dietary fibre and butyric acid

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Diabetes is a disease of great concern to many. Among many complications, diabetic nephropathy is one of the serious consequences. During diabetes alterations in glomerular basement membrane involving heparan sulfate is known to take place. The present study is aimed at studying changes in heparan sulfate during diabetes and its modulation by dietary fibre and butyric acid – a product of dietary fibre fermentation.

Diabetes was induced in rats using streptozotocin maintained on diets containing starch or diets containing wheat bran (10%) or guar gum (5%) or butyric acid. Diabetic status and the status of diabetic nephropathy are ameliorated by the presence of dietary fibre and butyric acid. The changes in glycoconjugates during diabetes were studied by estimating the contents of sulfated glycosaminoglycans, total sugar, uronic acid, amino sugar, sulfate and protein. The activities of enzymes L-glutamine-fructose-6-phosphate aminotransferase (GFAT), N-acetyl-β-glucosaminidase and β-glucuronidase were studied in kidney. The glycosaminoglycans (GAG) were isolated from kidney and analyzed. Heparan sulfate was isolated, quantitated and separated by agarose gel electrophoresis. Diabetic condition resulted in decrease in heparan sulfate, which was ameliorated by the feeding of dietary fibre and butyric acid. The data will be discussed in relation to improvements in diabetic nephropathy state.

P#68

Inhibition of MMPs of synovial fluid by oligosaccharides

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Matrixmetalloproteinases (MMPs) are a group of zinc containing endopeptidases that degrades extracellular matrix (ECM) and are involved in normal and pathological processes. The increase in the activity of MMPs has been suggested to be one of the factors contributing to the cartilage destruction in arthritis. Therefore MMPs are considered as potential targets in the development of drugs. Assay of MMPs in the synovial fluid of osteoarthritic patients by zymography showed high activity of MMP-2 and MMP-9. Assay of MMPs in the synovial effusate of experimentally induced arthritic animals also showed high activity of MMP-9. Invitro studies by GAG, particularly chondroitin sulfate A showed inhibition of activity of these MMPs. An oligosaccharide fraction was produced by the fragmentation of the GAG, which showed marked MMP inhibitory activity. These results on the inhibitory effect of oligosaccharides derived from GAG on MMP activity is important in the light of the fact that absorption of oligosaccharides is better than that of intact GAGs.

P#69

The chemical structure of the O-specific polysaccharide of *Shewanella* O:22

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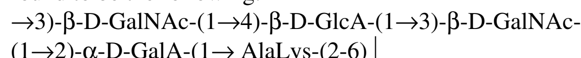
The lipopolysaccharide of the bacterium *Shewanella* O:22 was treated with 2% AcOH at 100°C for 2 h and the products were separated on Sephadex G-50 to yield the O-specific polysaccharide. The polysaccharide was examined by ^{1}H and ^{13}C NMR spectroscopy, including two-dimensional experiments COSY, TOCSY, ROESY, ^{1}H,^{13}C HMQC and HMBC, as well as one-dimensional NOE experiments in the difference mode.

The structure of the repeating unit of the polysaccharide was found to be the following:



→3)-β-D-GalNAc-(1→4)-β-D-GlcA-(1→3)-β-D-GalNAc-
(1→2)-α-D-GalA-(1→ AlaLys-(2-6)⌋

The unusual amino acid N^{ε}-(1-carboxyethyl)lysine (alaninolysine, AlaLys) present in the polysaccharide studied has previously been found in the O-specific polysaccharides of the bacteria *Providencia alcalifaciens* O23 [1] and *Proteus mirabilis* O13 [2].

1. Kocharova, N. A. *et al. Carbohydr. Res.*, **309**, 131-133 (1998).
2. Perepelov, A. V. *et al. Carbohydr. Res.*, **328**, 441-444 (2000).

P#70

Enzymatic synthesis of chondroitin with a novel chondroitin sulfate *N*-acetylgalactosaminyltransferase, CSGalNAc-T

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Medical University; [5]JGS Japan Genome Solutions, Inc.; [6]Mitsui Knowledge Industry Co., Ltd.; [7]New Energy and Industrial Technology Development Organization (NEDO); [8]Institute of Life Science, Soka University; [9]Mitsui Chemicals, Inc., Japan

We found a novel glycosyltransferase gene having a hypothetical β1,4-galactosyltransferase motif and cloned its open reading frame. The product of the gene transferred *N*-acetylgalactosamine to *para*-nitrophenyl-β-glucuronic acid. The *N*-acetylgalactosamine-glucuronic acid linkage has been identified only in chondroitin sulfate (CS), therefore, we examined its chondroitin elongation and initiation activities. *N*-acetylgalactosaminyltransferase activity was observed toward chondroitin poly- and oligosaccharides, CS oligosaccharides and linkage tetrasaccharide (GlcUA-Gal-Gal-Xyl-*O*-methoxyphenyl), and the chondroitin polysaccharide and linkage tetrasaccharide were better acceptor substrates than the others. These results suggest that this enzyme has *N*-acetylgalactosaminyltransferase activity in both the elongation and initiation of CS synthesis. Furthermore, we performed enzymatic synthesis of chondroitin pentasaccharide with four enzymes, β1,4-galactosyltransferase-VII, β1,3-galactosyltransferase-VI, glucuronyltransferase-I and this enzyme, and a synthetic xylose-peptide acceptor.

This work was performed as part of the R&D Project of Industrial Science and Technology Frontier Program (R&D for Establishment and Utilization of a Technical Infrastructure for Japanese Industry) supported by NEDO.

P#71

Gene discovery through expressed sequence tag (EST) in *Porphyridium* sp. : search for genes involved in sulfated polysaccharide biosynthesis

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The red microalgae have enormous potential as a source of valuable biochemicals, mainly sulfated polysaccharide, pigments and unsaturated fatty acids. Red microalgal polysaccharides may be used in a wide field of activities from engineering through cosmetics to health foods and anti-viral treatment. Thus, an understanding of the molecular genetics of the red algae constitutes the basis for exploitation of these organisms. However, progress in the study of the molecular genetics of these algae lags far behind that for green algae, plants and animals, mainly because of sparsity of physiology and biochemistry and lack of appropriate genetic tools. The main goal of this project is to discover genes involved in sulfated polysaccharide biosynthesis and other genes of scientific and industrial importance. We have sequenced about 6000 individual EST clones from various cDNA libraries of *Porphyridium* sp. Clustering and assembling of the sequencing data resulted in 1800 individual genes. About sixty percent of the genes have no similarity to known genes. Most of the genes showed a higher degree of homology to genes of lower organism, such as archaea, anaerobic bacteria, yeasts and other fungi. We have identified several genes involved in sugar, sulfur and fatty acid metabolism.

P#72

Synthesis and studies of azobenzene containing multivalent sugar ligands

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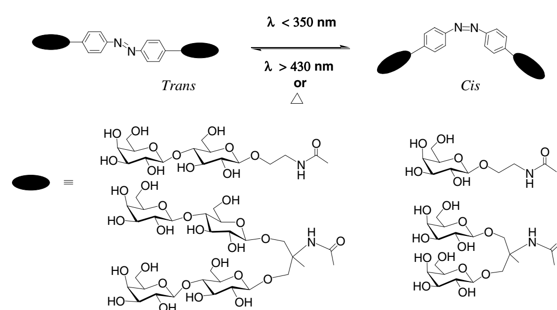
The phenomenon of 'glycoside cluster effect' has become a paradigm in the efforts towards studying complex carbohydrate – protein interactions [1]. One of the underlying design principles to observe this effect involves the clustering of constituent sugar ligands, which enhances the lectin binding avidities by several fold over and above the statistical increase in the number of individual sugar units. We have endeavored to synthesize glycoclusters incorporated with photoresponsive moiety that can serve as probes to study carbohydrate – protein recognition processes [2]. We have synthesized a series of azobenzene (AB) containing glycoconjugates bearing multivalent β–lacto- and galactopyranosides, some of which are shown in Figure 1, and investigated their reversible photoisomerisation behavior. An anomalous binding profile was observed from isothermal titration calorimetric studies that reveal the existence of a cooperativity in the binding of the lactose bearing bivalent azobenzene derivative towards its high affinity lectin *Peanut Agglutinin* (PNA). The synthesis of densely lactose-coated azobenzene derivatives, their isomerization behavior and lectin binding studies will also be presented.

Figure 1



Acknowledgements: This work was supported by CSIR (New Delhi). OS thanks UGC for Research Fellowship.

1. (a) Lee YC, Lee RT, *Acc. Chem. Res.* **28**, 321 (1995); (b) Dwek RA, *Chem. Rev.* **96**, 683 (1996).
2. Srinivas O, Mitra N, Surolia A, Jayaraman N, *J. Am. Chem. Soc*. **124**, 2124-2125 (2002).

P#73

Distinct chaperone-like functions of high-mannose type and complex-type *N*-glycans based on the conformations characteristic of each type

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It has recently become apparent that high-mannose type *N*-glycans directly promote protein folding [1-3], whereas complex-type ones play a crucial role in the stabilization of protein functional conformation [4]. Here an attempt was made to understand more deeply the mechanisms underlying these chaperone-like functions by the aid of the information obtained with the spacefill models of *N*-glycans. The promotive effect on protein folding was particularly remarkable for higher oligomannose *N*-glycans ($Man_{8\text{-}9}GlcNAc_2$), and seemed to be based on the unique *N*-glycan conformation having a hydrophobic concave surface. Further, it appears well established that complex-type *N*-glycans have a hydrophobic surface stretching over the whole side holding acetylamino groups, and

stabilize protein functional conformation through hydrophobic interactions with the protein hydrophobic surface unfavorable for protein stability. The *N*-glycan processing from the high-mannose type to the complex-type appears to be an ingenious device to enable *N*-glycans to play two chaperone-like functions.

1. Yamaguchi H and Uchida M (1996) *J. Biochem.* **120**: 464-477
2. Nishimura I *et al.* (1998) *J. Biochem.* **123**: 516-520
3. Kimura N *et al.* (1998) *J. Biochem.* **124:** 857-862
4. Toyoda T *et al.* (2000) *J. Biochem.* **128**: 731-737

4. Glycolipids

P#74

Preparation of neoglycolipids-coated magnetoliposomes and their application for drug delivery system

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We have shown that Fab`-conjugating magnetoliposomes (FMLs) can specifically bind to the carcinoma cells *in vitro* and *in vivo* [1]. In order to deliver magnetite particles into macrophages and dendritic cells (DC) based on carbohydrate-protein interaction, we prepared neoglycolipids-coated magnetoliposomes. Antigen-presenting cells such as macrophages and DC have been shown to express mannose-specific lectins or receptors on the cell surface. [2] The neoglycolipids-coated magnetoliposomes were used as specific ligands and applied them to hyperthermia therapy for tumor. We prepared magnetite particles by the nitrite oxidation methods. Next, optimization for the immobilization of the neoglycolipids was studied by a high-performance liquid chromatography system [3]. Moreover, their affinity to macrophages or dendritic cells *in vitro* experiment had been investigated.

References

(1) Le B et al (2001) *Jpn. J. Cancer Res* **92**. 1138-1145
(2) Nakata M et al (1998) *FEBS Let* **441**. 353-356
(3) Shimizu Y et al (2001) *J. Chromatogr. B* **754**. 127-133

P#75

Using anisotropy as a tool to understand nanometer scale organization of GPI-AP at the living cell membrane

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Membrane rafts are lateral inhomogenieties in the plasma membrane enriched in cholesterol, sphingolipids and lipid linked proteins like glyosylphosphatidyl inositol anchored proteins (GPI-AP) on the outer leaflet (ref 1) and palmitoylated and myristoylated proteins on the inner leaflet (ref 2). They are implicated in diverse cellular functions from endocytosis to signaling (ref 3). Experiments from our lab have shown that fluorescently labeled GPI anchored folate receptor (FR) as well as GFP-GPI exhibit density independent rate of energy transfer whereas transmembrane form doesn't. This organization is cholesterol dependant, since it is disrupted upon cholesterol depletion. Using anisotropy as a tool functional significance can be attributed to different structural groups of cholesterol. Structural features of a cholesterol molecule are an intact alicyclic chain, a free 3β hydroxyl group, a planar $\Delta^{5(6)}$ double bond angular methyl groups and a branched seven carbon alkyl chain at the 17 β position (ref 4). Whether these groups are also important for organization of GPI-AP is not known. I plan to use lipid analogues to replete cholesterol depleted cells and see if the analogue can functionally replace cholesterol.

If similar lateral heterogeneties are present on the inner leaflet of the membrane then it would be interesting to know if their size changes upon crosslinking GPI-AP on the outer leaflet i.e. do the rafts of outer membrane talk to each other. This can be achieved by looking at the intermolecular distance between the molecules.

1. Varma et. al*., Nature* 394, 798-801(1998).
2. Zacharias et. al*., Science* 296 913-916(2002)
3. Robinson, P. J., *Immunol Today* 12, 35-41(1991)
4. Schroeder, F. et. al*., Prog. Lipid Res.* 23, 97-113(1984)
5. Lackowicz, J. R., *Principles Of Fluorescence Spectroscopy*

P#76

Lipopolysaccharide kinases encoded in the genomes of gram-negative bacteria are related to the eukaryotic protein kinases

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Lipopolysaccharides [LPS], a major constituent of the outer membrane of Gram-negative bacteria, is important for bacterial viability and virulence. The phosphoryl substituents of the outer membrane core aids in stabilizing the membrane, in the absence of which membranes become leaky resulting in less virulent strains. Two key enzymes involved in the core phosphorylation namely WaaP gene product (heptose kinase) and Kdo kinase (Kdk) have been well characterized. The occurrence of the Kdk and its homologues, so far restricted to the Gram-negative pathogenic bacteria, is involved in virulence in *Haemophilus influenzae*. Using various profile-based sequence search and fold recognition methods, we show a significant similarity among WaaP gene products, Kdk, eukaryotic protein kinases (PK) and the kinase-like sequences encoded in the genomes of archea, bacteria and eukaryotes. Diverse substrate specificity of the eukaryotic PK superfamily is reiterated with the inclusion of LPS and an evolutionary relationship between the WaaP and Kdk is suggested from the study.

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P#77

Synthesis of NBD-labeled fluorescent glycosylphosphatidylinositol (GPI) anchor

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The GPI anchor is a post-translational modification that links certain specific proteins (GPI-APs) to outer leaflet of cell membranes [1], and is believed to be involved in clustering of such proteins in sphingolipid/cholesterol rich microdomains [2]. GPIs are expressed abundantly by protozoan parasites (*Leishmania*, *Trypanosoma* and Malaria) and are involved in their infectivity and survival. In our ongoing work on chemical synthesis of *Leishmania* GPIs [3-8], and to study their role in membrane organization using cytomimetic model giant-unilamellar-vesicles (GUV), we have designed and synthesized NBD labeled GPI anchor. For this the first key intermediate 1,2;4,5-di-O-cyclohexylidene-D-*myo*-inositol was coupled with 2-azido-3,4,6-tri-O-acetylβ-Dglucopyranosyltrichloroacetimidate and the product was deacetylated, benzylated, selectively deketalated and PMB protected at C-1 of inositol. This was followed by conversion of azido- to NH-BOC group. Further coupling with 1-[6-N-Cbz-aminohexanoyl]-2-stearoyl-3-O-[cyanoethyl - N, N - diisopropylphosphoramidite] – *sn* - glycerol, followed by cyanoethyl removal and hydrogenolysis exposed the free amino group in lipid tail at *sn*-1 ready for coupling with NBD probe. Results on this synthesis and application of fluorescent GPI probe in cytomimetic experiments will be presented.

1. McConville, MJ, Ferguson, MAJ (1993) *Biochem. J.*, **294**: 305-24
2. Simons K, Ikonen E (1997) *Nature*, **387**: 569-72
3. Sahai P, Vishwakarma RA (1997) *J. Chem. Soc., Perkin Trans I* :1845-49
4. Tyagi AK, Vishwakarma RA (1998) *Tetrahedron Lett.* **39**: 6069-72
5. Upreti M, Vishwakarma RA (1999) *Tetrahedron Lett.* **40**: 2619-22
6. Upreti M, Ruhela D, Vishwakarma RA (2000) *Tetrahedron* **56**: 6577-84
7. Sahai P, Chawla M, Vishwakarma RA (2000) *J. Chem. Soc., Perkin Trans I*: 1283-90
8. Ruhela D, Vishwakarma RA (2001), *Chem. Commun.*: 2024-25

P#78

A study into mechanism of endocytosis of folate receptor - a model GPI-anchored protein

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Glycosylphosphatidylinositol-anchored proteins (GPI-AP) are a diverse family of membrane proteins that are anchored via glycolipid moiety namely glycosylphosphatidylinositol. Work from our lab has shown that various GPI-AP are internalized via a mechanism that is independent of Clathrin, Dynamin or Caveolin and are delivered to Endocytic Recycling compartment (ERC). Using Folate receptor (FR)-as a model GPI-AP, Human Transferrin Receptor (TfR)-as a marker for Clathrin mediated endocytosis and FITC-Dextran - as a fluid phase probe, it is seen that the endocytic pathway of FR is also responsible for major fraction of Fluid uptake in CHO cells. While there is a extensive knowledge of the molecular players involved in clathrin mediated endocytosis, the mechanism of internalization of GPI-AP's is poorly understood. I have attempted to identify molecular players involved in GPI-AP endocytosis, using pharmacological agents, which are known to affect fluid phase uptake in other studies like growth factor stimulated fibroblasts. I have used Azithromycin, a macrolide antibiotic, Methyl isobutyl Amiloride, a Na+/H+ antiport blocker, Ly294002 and Wortmannin, both inhibitors of Phosphoinositide Kinases (PIK's) at low nM concentration. Azithromycin and Methyl isobutyl Amiloride had no affect on both dynamin dependent and independent-mechanisms of endocytosis. However, treatment of CHO cells with Wortmannin, a specific inhibitor of PI3Kinase affects FR, Fluid and TfR at different steps of endocytosis. Cdc42, a small molecule GTPase, was shown to be involved in internalization of GPI-AP and fluid phase uptake. Since Cdc42 and PIP's can both recruit actin polymerizing machinery, like WASP, Arp2/3, etc. identifying specific PIP lipid involved in the endocytosis of FR could throw light on the mechanism of internalization of GPI-AP's.

1. Shefali Sabharanjak , Pranav Sharma , RG Parton , Satyajit Mayor;*Dev Cell*, 2002 Apr;2(4):411-23.

P#79

Interactions between gangliosides and proteins in the exoplasmic leaflet of neuronal plasma membranes

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The biological roles of gangliosides are in part due to their ability to interact with membrane proteins modulating their function. Gangliosides closely associate with single or multiple signal transducer molecules within specialized sphingolipid-enriched membrane domains (SEMD), that provide a microenvironment within the plasma membrane for reciprocal interactions between lipid and proteins involved in the control of signal transduction. Specific protein-ganglioside interactions would be due to strong interactions between the oligosaccharide moiety of the ganglioside and a specific binding site in the protein. Thus, they likely involve protein molecules characterized by a extracitoplasmic domain. In order to study specific interactions between SEMD proteins and gangliosides in primary cultured cerebellar neurons, we combined the use of different analytical procedures. Membrane photolabeling with different radioactive photoactivable ganglioside derivatives was coupled with the labeling of cell surface proteins with biotin and total cell protein metabolic radiolabeling. Immunoseparation techniques were applied for the isolation of specific proteins or protein classes. This approach allowed to identify a strong interaction between gangliosides and the GPI-anchored neural cell adhesion protein TAG-1 within a complex lipid-protein microenvironment in SEMD.

5. Carbohydrates in cell cell interactions, cancer and defense

P#80

Carbohydrate-mediated recognition of anti-Fas antibody-induced apoptotic cells by macrophages

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Apoptotic cells are recognized and removed by macrophages. Although phosphatidylserine (PS) exposed on the apoptotic cells is known to mediate the recognition as an 'eat me signal', involvement of other cell-surface components has also been suggested. We have previously demonstrated that induction of apoptosis in Jurkat T cells using low concentrations of etoposide resulted in the carbohydrate-mediated recognition at an early stage of apoptosis [Beppu M *et al* (2001) *Glycoconjugate J* **18**:85]. Here, we show that similar carbohydrate-mediated recognition is inducible in Fas (CD95) –mediated apoptosis. Jurkat cells treated with a low concentration of anti-Fas antibody were susceptible to the recognition by THP-1 macrophages at 2 h and later stage of apoptosis (12-24 h). The recognition at 2 h was not observed when Jurkat cells had been treated with endo-β-galactosidase or neuraminidase. Treatment of Jurkat cells with anti-CD43 antibody blocked the recognition. This early recognition was not inhibited by annexin V. In contrast, the later stage recognition was inhibited by annexin V, but not by the glycosidase treatment. These results indicate that anti-Fas-induced apoptotic Jurkat cells are recognized by the macrophages through the cell-surface carbohydrate chains, most likely those of CD43, at the early apoptotic stage, and through the exposed PS at the later stage.

P#81

Preparation of conjugate composed of lipopolysaccharide from *Vibrio cholerae 01* bound to immunostimulatory glucan matrix

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The aim of the study was to prepare sandwich type immunoconjugate using detoxified surface lipopolysaccharide (LPS) from *Vibrio cholerae 01* bound multiply to β-glucan matrix and then to protein, human serum albumin. As a matrix, the carboxymethylated (1→3)-β-D-glucan, a soluble derivative of *Saccharomyces cerevisiae* cell-wall β-glucan that exhibits immunostimulating and radioprotective effects, was chosen. At the same time, the conjugate was constructed using an immuno-inert polysaccharide matrix - amylose, that was taken as a reference to evaluate immunomodulatory effect of the yeast β-glucan. Reaction conditions were optimized to receive desirable degree of substitution and water soluble products. Prepared constructs, in which yeast (1→3)-β-D-glucan or amylose served as matrices for binding of low molecular detoxified *V.cholerae* lipopolysaccharide, formed precipitation zones with antibody against *V. cholerae 01* in immunodifusion tests.

We suppose that repetitiveness of antigen epitope in conjugate allows cross-linking of B-cell receptors and will render stronger signals for antibody productions. The newly designed construct can serve as a prospective candidate for effective subcellular cholera vaccine.

P#82

Specificity of human anti-α-galactosyl antibodies involved in the NOR polyagglutination

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The antigens of NOR red cells responsible for their polyagglutination are two neutral glycosphingolipids, which are elongated forms of globoside, containing additional Galα1-4 or Galα1-4GalNAcβ1-3Galα1-4 residues. To characterize the specificity of anti-NOR anti-bodies, two oligosaccharides, Galα1-4GalNAc and Galα1-4GalNAcβ1-3Gal, were synthesized. The syntheses were based on a common (1-4)-β-GalNAc precursor and utilized benzyl glycoside and benzyl ether functions for persistent blocking of hydroxyls. The NOR-related oligosaccharides and other commercial α-galactosylated saccharides and saccharide-polyacrylamide(PAA) conjugates (kindly supplied by Dr. N.V. Bovin, Moscow) were tested for reactivity with anti-NOR and anti-Galα1,3Gal antibodies from human sera, using ELISA and hemagglutination-inhibition. Anti-NOR antibodies were inhibited weakly by galactose, over 10 times stronger by the disaccharides, Galα1-4GalNAc and Galα1-4Gal, and over 1000 times stronger by the trisaccharide Galα1-4GalNAcβ1-3Gal. Anti-NOR were not inhibited by the disaccharide Galα1-3Gal, a strong inhibitor of anti-Galα1,3Gal antibodies which, in turn, were not inhibited by the NOR-related trisaccharide. Inhibiton of anti-NOR antibodies by Galα1-4Gal and binding to Galα1-4Galβ1-4GlcNAcβ1-PAA showed their crossreactivity with blood group P1 antigen. In conclusion, anti-NOR represent a distinct species of anti-α-galactosyl antibodies whose combining site accomodates at least the Galα1-4GalNAcβ1-3Gal trisaccharide epitope.

P#83

Improving of L-asparaginase anti-cancer immunity

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Objectives: L-asparaginase of *Erwinia carotovora* 268 M; immunomodulating polysaccharide levan from *Z. mobilis* bacteria; its covalent conjugate. ***Methods:*** Periodate oxidation of levan with binding of its aldehyde form to the enzyme via α-amino and lysine-γ-aminogroups and subsequent reduction with $NaBH_4$. ***Results:*** Therapeutic use of anti-leukemic enzyme L-asparaginase is limited by immunosuppressive action, development of hypersensitivity and immunotoxicity. We turned out that levan's oxidized and subsequently reduced form is more active immunomodulator than unmodified levan. It raises the phagocytic activity of peripheral blood polymorphonuclear leukocytes, suppressed by disease: active leukocytes percentage - up to 89 %; their phagocytic index - to 9,3. The immobilized enzyme is shown to retain 98% of enzymatic activity after incubation in physiological saline at 37°C during 10 h in comparision with only 10% for free enzyme. Immobilization protects L-asparaginase against proteolysis by trypsin and α-chymotrypsin. High degree of L-asparaginase modification

reduces both the conjugate ability of binding the antibodies to free enzyme and the immunosuppressive effect of L-asparaginase *in vivo*, the last - for 80 %. ***Conclusion:*** Improvement of L-asparaginase stability and decrease of its undesirable immunosuppressive effect in the organism are the main acquisitions of conjugation of anti-cancer enzyme L-asparaginase with immunomodulating polysaccharide levan.

P#84

pp-GalNAc-T2 is an essential enzyme for *O*-linked glycosylation of IgA1 hinge region

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The hinge region of human immunoglobulin A1 (IgA1) possesses multiple *O*-glycans initiated by UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferases (pp-GalNAc-Ts). We found that six pp-GalNAc-Ts were expressed in B cells. The GalNAc-T activities were examined for a synthetic IgA1 hinge peptide that has nine possible *O*-glycosylation sites. pp-GalNAc-T2 showed the strongest activity and other pp-GalNAc-Ts possessed different substrate specificities from pp-GalNAc-T2, however, their activities were extremely weak. It was previously reported that IgA1 hinge region possesses a maximum of five *O*-glycans and their positions have been determined. We found that pp-GalNAc-T2 selectively transferred GalNAc residues to the same positions as reported. These results indicated that pp-GalNAc-T2 is an essential enzyme for *O*-linked glycosylation of IgA1 hinge region.

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P#85

Macromolecular conjugates of polysaccharides with antileukemic enzymes

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Objectives*:* L-Asparaginase from two different bacteria (*Escherichia coli* and *Erwinia carotovora*) was conjugated with polysaccharides possessing anticancer and immunomodulatory activities. ***Methods:*** *E.coli* L-asparaginase was coupled ionically to dextran sulfate. *Erwinia* L-asparaginase was bound covalently to fructose polymer levan of different molmasses (75 and 2,000 kDa) by method of periodate oxidation of carrier followed by reductive alkylation. **Results*:*** The resulting conjugates retained 25-90% of their enzyme activity. K_m of *Erwinia* L-asparaginase conjugated with levan was higher than the K_m of free enzyme, and increased with the higher extent of coupling and molmass of levan. Both *E.coli* and *Erwinia* L-asparaginases showed relatively high stability after conjugation with polysaccharides in conditions of increased temperature (up to 50^0C). Conjugation of *Erwinia* L-asparaginase with levan widened the range of enzymes pH stability, decreased its plasma clearance rate in non-immunized rabbits, and stabilized enzyme activity in the presence of rabbit antiserum raised against native enzyme. **Conclusion*:*** These results show a real possibility to improve the features of antileukemic enzyme L-asparaginase and may serve as a basis for further detailed biomedical investigations of polysaccharide conjugated L-asparaginase as anti-cancer agent.

P#86

Induction of cyclooxygenase-2 in monocyte/macrophage by mucins secreted from colon cancer cells

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Up-regulation of cyclooxygenase-2 (COX-2) and overproduction of prostaglandins have been implicated in the initiation and/or progression of colon cancer. However, it is uncertain in which cells and how COX-2 is induced initially in the tumor microenvironment. We found that a conditioned medium of the colon cancer cell line, LS 180, contained a factor to induce COX-2 in human peripheral blood mononuclear cells. This factor was purified biochemically and revealed to be mucins. A small amount of mucins (~100 ng protein/ml) could elevate PGE2 production by monocytes. The mucins induced COX-2 mRNA and protein levels of monocytes in a dose- and time-dependent manner, indicating a COX-2-mediated pathway. We have also examined immunohistochemically the localization of COX-2 protein and mucins in human colorectal cancer tissues. It is noteworthy that COX-2-expressing macrophages were located around the region where mucins were detectable, suggesting that COX-2 was also induced by mucins *in vivo*.

These results suggest that mucins produced by colon cancer cells play a critical role in the initial induction of COX-2 in the tumor microenvironment.

P#87

Pathological significance of the inverse correlation between expression level of syndecan-2 and metastatic potential of Lewis lung carcinoma cells

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We have found an inverse correlation between syndecan-2 expression and metastatic potential using Lewis lung carcinoma-derived different metastatic clones (P29, LM12-3, LM66-H11 clones with low, medium, highly metastatic potentials, respectively). P29 cells with stroma-inducing capacity exhibit tumorigenesis dependent on the fibronectin (FN)-rich interstitial type matrix formed by the induced stromal cells, whereas LM66-H11 cells show tumorigenesis dependent on the basement membrane formed by themselves. Reflecting this difference, the results obtained by *in vitro* analyses of cellular responses on adhesion to various FN-recombinant polypeptides and monospecific antibodies against syndecans showed that P29 cells formed stress fibers through the collaboration of syndecan-2 and -4 with integrin α5β1, whereas LM66-H11 cells formed cortical

actin structure under the action of integrin α5β1 alone. We demonstrated further that P29 cells formed filopodia under the action of syndecan-2 alone. A stable transfectant, H11-SN2, established after transfection of syndecan-2 cDNA into LM66-H11 cells, came to resemble P29 cells in several *in vitro* phenotypes including cell morphology, doubling time, saturation density and the FN-induced actin cytoskeletal organization, and acquired the capacity to elicit stromal induction *in vivo.* Conversely, *in vitro* treatment of P29 cells with antisense oligonucleotides of syndecan-2 mRNA resulted in their resemblance to the phenotypes of LM66-H11 cells. Moreover, when injected intravenously and subcutaneously, H11-SN2 cells surprisingly showed a marked decrease in pulmonary metastasis.

P#88

Fibronectin dependent upregulation of MMPs in hepatic stellate cells

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Immuno cytochemical and cell culture studies showed that hepatic stellate cells (HSCs) play a crucial role in the production of extra cellular matrix (ECM) components in normal and fibrotic conditions. In vitro studies using isolated HSCs maintained on matrix protein substrata viz; Col I and Fn also revealed that matrix proteins influence HSC specific activity on a selective basis. Intra cellular events that follow cell matrix interactions were studied by assessing the production of matrix metalloproteinases in vitro by cells maintained on matrix protein substrata. ELISA and zymography showed a Fn dependant upregulation of MMP 2 and MMP 9. Pretreatment of cells with antibodies to integrins decreased the expression of MMPs indicating that the upregulation of MMPs is mediated through integrins. On blocking tyrosine phosphorylation events, characteristic to integrin signaling by using inhibitors, the matrix dependant expression of MMPs was also affected. These results indicate that the intra cellular events following integrin mediated adhesion of hepatic stellate cells to matrix proteins involve tyrosine phosphorylation dependant signaling pathways.

P#89

Role of (1,6) branched *N*-oligosaccharides cancer cell invasion

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Increased expression of (1,6) branched *N*-linked oligosaccharides on the surface glycoproteins has been shown to be associated with malignant cells and the cells with invasive phenotype like trophoblast and activated endothelial cells. The role of these structures in invasion process is being studied using high metastatic B16F10 and its highly invasive variant, B16BL6.
Initial studies with cell lines showed that both cell lines form lung metastases when put in circulation, where as only B16BL6 cells shows lung metastases when injected subcutaneously. Flow cytometric analysis revealed that B16BL6 cells show-more cell surface (1,6) branched structures and sialylation compared to B16F10. Upregulation of GnT-V appear to be responsible for increased (1,6) branching as seen by RT-PCR, LAMP1 and (1 integrin were found to be amongst the major carrier of these oligosaccharides on melanoma cells.

The expression of these oligosaccharides appeared to regulate the adhesion on different extra cellular matrix components and invasion of these cells on the reconstituted basement membrane (matrigel). B16BL6 cells also showed upregulation in the expression of membrane tethered - matrix mellato-protease (MT-MMP) on the surface. The role of these oligosaccharides in adhesion motility and invasion and in surface localisation of MT-MMP is being investigated.

P#90

Protection of *Leishmania major* infection by immunization with oligomannose-coated liposome

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Liposomes coated with neoglycolipids constructed with mannopentaose and dipalmitoylphosphatidylethanolamine (Man5-DPPE) have been shown to induce cellular immunity against antigens encapsulated in the liposome. To address whether these neoglycolipid-coated liposomes can elicit a protective immune response against challenge infection, effects of immunization with soluble leishmanial antigens from *Leishmania major* encapsulated in the liposome were evaluated using *L. major* infection in susceptible BALB/c mice. Intraperitoneal immunization of mice with antigens in the Man5-DPPE-coated liposomes significantly suppressed footpad swelling in comparison to the control, non-immunized mice. In addition, the number of parasites decreased substantially in local lymph nodes of mice immunized with the antigen in Man5-DPPE-coated liposomes. Protection ageinst *L. major* infection in the immunized mice also coincided with an elevated level of IgG2a antibodies, which is a marker for Th1-like immune response. These results indicate the possibility of Man5-DPPE-coated liposome-encapsulated antigens as a vaccine that triggers cell-mediated immune response.

P#91

Preparation of a conjugate vaccine for cholera from synthetic fragments of the O-PS of *Vibrio cholerae* O:1

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Within our efforts to develop a conjugate vaccine for cholera, we have prepared oligosaccharides that mimic the structure of the O-PS of *Vibrio cholerae* O:1, serotype Ogawa. These chemically synthesized materials were conjugated to the carrier protein, BSA, using squaric acid chemistry. The progress of conjugation was monitored by Surface Enhanced Laser-Desorption Ionization-Time of Flight Mass Spectroscopy (SELDI-TOF MS) in conjunction with the ProteinChip® System. This technique provided nearly real-time information about the increasing molecular mass of the conjugate being formed. Consequently, the process of conjugation could be controlled and the conjugation reaction could be terminated when the desired molar hapten/BSA ratio was reached. The conjugation was carried out at a high molar hapten/BSA ratio. Synthetic oligosaccharides are expensive, labor-intensive commodities and, therefore, we have explored possibilities to recover the hapten used in excess at the onset of the conjugation. We have developed a protocol, which

allows isolation of this precious material, which can then be used it in similar conjugations. The protocol is generally applicable provided the conjugation chemistry leaves the unused hapten unchanged. This increases substantially the overall economy of conjugation of synthetic carbohydrates to carrier proteins.

P#92

Clinical usefulness of glycosylation of M-protein component in patents with multiple myeloma

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Multiple myeloma is characterized by clonal proliferation and accumulation of terminally differentiated B cells. High resolution agarose gel electrophoresis was performed in cases of multiple myeloma to delineate serum proteins into different zones to confirm monoclonal gammopathy. After establishing monoclonal gammopathy, immunoglobulin sub-typing was confirmmed by radial immunodiffusion technique. M-protein component was isolated by affiniity chromotography using sepharose protein-G columns. Glycoprotein constituents e.g. Sialic acid and hexoses were analysed from the isolated M-protein fraction. The level of glycosylation was correlated with clinical findings e.g. stage, response to treatment.

IgG type monoclonal gammonpathy was more prevalent. Glycosylation of M-protein component was found to be elevated and associated wiith disease aggressiveness and treatment response. Follow-up analysis showed decrease in M-protein component in responders as compared to their pretreatment level.

Our results indicated that analysis of glycosylation patterns of M-component have significant role in diagnosis and management of patients with multiple myeloma

P#93

A glycoprotein from *Mucuna pruriens* shares antigenic determinants with a protein from *Echis carinatus* venom

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In a recent paper of Guerranti et al. (2002) it has been demonstrated that extract of *M. pruriens*, a medicinal plant widely used in Nigeria against snakebite in traditional medicine, 1) protects mice against the letal effect of *Echis carinatus* venom; 2) the antivenin activity has an immune mechanism; and 3) proteins of *E. carinatus* venom and of *M. pruriens* extract have epitopes in common.

We purified from the water extract of the *M. pruriens* seeds, by affinity chromatograhy using ConA bound to Sepharose, a glycoprotein with an apparent MW of 28kDa which contains N- and O-linked oligosaccharides and we raised polyclonal antibodies against it in rabbit. By preliminary experiments of immunoblot analysis associated with enzymatic and chemical removal of oligosaccharides we found that the epitope of the anti-gp28 antibodies contains O-linked oligosaccharides. The importance of this seed protein in its anti-venom function resulted from preliminary experiments using the anti-gp28 serum or the purified IgG in immunoblot analysis of the *E. carenatus* venom proteins. In fact the results showed that one venom protein was strongly reactive to the anti-gp28 IgG.

1. Guerranti R *et al* (2002) *J Biol Chem* **277**: 17072-17078

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