CARBOHYDRATE LIGANDS AND THEIR PROTEIN RECEPTORS: BIOLOGICAL FUNCTION AND MOLECULAR INTERACTION

Organizer: Roger A. Laine

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Physical Probes of Protein-Glycoconjugate Interaction

CZ 001 ATOMIC INTERACTIONS BETWEEN CARBOHYDRATES AND PROTEINS. Florante A. Quiocho, Howard Hughes Medical Institutes and Baylor College of Medicine, Houston, TX 77030.

In recent years our laboratory has been engaged in the structure-function studies of five proteins that bind carbohydrates -- three bacterial periplasmic receptors for the active transport of and *chemotaxis* toward carbohydrates (monosaccharides and linear and cyclic oligosaccharides), one antibody against bacterial cell surface polysaccharide O-antigen and aldose reductase. While high resolution x-ray crystallography is our primary experimental approach in these studies, we have also utilized site-directed mutagenesis, rapid kinetics, calorimetry, low angle x-ray scattering, and theoretical techniques. Common recurring features of the atomic interactions between proteins and carbohydrates will be *presented in light of the* crystallographic analysis of these and other proteins. We also discovered that the active site aldose reductase has features that least favor binding of hexoses. Time permitting, other features of protein-carbohydrate interactions obtained by way of the other techniques will also be presented.

CZ 002 THE STRUCTURE OF A Ca^{2+}-DEPENDENT ANIMAL LECTIN COMPLEXED WITH AN OLIGOSACCHARIDE. William I. Weis*†, Wayne A. Hendrickson† & Kurt Drickamer†. *Dept. of Cell Biology, Stanford University School of Medicine, Fairchild Building, Stanford, CA 94305 & †Dept. of Biochemistry, Columbia University, 630 W. 168 St. New York, NY 10032

C-type (Ca^{2+}-dependent) animal lectins are a family of proteins that mediate protein-carbohydrate interactions at cell surfaces. This family includes endocytic receptors of hepatocytes and macrophages, the selectin cell adhesion molecules, proteoglycan core proteins, and serum mannose-binding proteins (MBPs) that function in immunoglobulin-independent host defense against pathogens. Each of these proteins contains one or more carbohydrate-recognition domains (CRDs) combined with domains responsible for the physiological function of the molecule. The C-type CRD is approximately 120 amino acids long, and contains 14 invariant amino acids and another 18 that are conserved in character. The 3-dimensional structure of the CRD from a rat MBP displays an unusual fold that is is stabilized by two Ca^{2+}. The positions of the conserved amino acids of the CRD motif indicate that this fold is shared among the members of this family. A 1.7 Å resolution structure of the MBP CRD complexed with a high-mannose type glycopeptide reveals an unusual mode of sugar binding in which the 3- and 4-OH groups of terminal mannose residues form direct coordination bonds with the required Ca^{2+}. Carbohydrate specificity is determined by a network of coordination and hydrogen bonds that stabilizes the ternary complex of protein, Ca^{2+}, and sugar. The binding of two of the three non-reducing branch termini of the oligosaccharide to neighboring CRDs in the crystal lattice suggests structural features likely to govern multivalent binding of proteins to carbohydrates.

CZ 003 SPECIFITY OF SIALIC ACID BINDING IN A MULTIVALENT WHEAT GERM AGGLUTININ - CELL SURFACE SIALOGLYCOPEPTIDE (GPA-T5) COMPLEX. Christine S. Wright, Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond, VA 23298

Sialic acid (N-acetylneuraminic acid), commonly found at exposed terminal positions of cell surface glycoconjugates, is widely recognized as the target sugar for viral attachment during infection. The major erythrocyte sialoglycoprotein glycophorin A (GPA) carries the receptor for both the influenza haemagglutinin and wheat germ agglutinin (WGA), a 4-domain dimeric lectin specific for NeuNAc. The crystal structures of these proteins and their complexes with simple sialosides have provided some insights into the stereospecific requirements for sialic acid binding. However, to obtain a better understanding of WGA-receptor interaction, involving larger oligosaccharide structures, a tryptic fragment of GPA (T-5), which bears the common O-linked tetrasaccharide NeuNAc-α2,3-Gal-ß1,3-(α2,6-NeuNAc)GalNAc-α1-, was co-crystallized with WGA(isolectin 1). Interaction of WGA with GPA *in vivo* is highly specific leading to protection of erythrocyte morphologies (1). As generally observed for lectin/cell binding, the binding isotherm exhibits positive cooperativity.

The crystal structure of the WGA-T5 complex was determined by x-ray diffraction techniques and refined at 2.0Å resolution (R=17.1%) (2). The bivalent tetrasaccharide is observed to interact with three non-equivalent WGA binding sites in two independent lattice environments. Two of these binding sites have not previously been observed. In the dominant binding mode WGA dimers are crosslinked by the tetrasaccharide in a non-covalent fashion through interactions of the two terminal NeuNAc residues: The α2,6NeuNAc residue occupies the well-characterized B-domain site in monomer I of one molecule, and the α2,3NeuNAc binds to the corresponding site in domain C (monomer II) of the opposing dimer molecule. This asymmetric selection of binding sites leads to open-ended infinite arrays of interlinked WGA molecules, a lattice structure that could explain the abnormal cell binding behavior and morphology controlling effect of WGA on erythrocytes. In the second less well-defined binding mode the tetrasaccharide interacts though its α2,6NeuNAc with the binding site of domain A in monomer II only, and the adjacent GalNAc forms a lattice self-contact with a neighboring twofold related tetrasaccharide molecule. Thus, this crystal complex is stabilized both by protein/sugar and sugar/sugar lattice contacts. Binding specificity in all three sites is conferred through hydrogen bonding and van der Waals' stacking interactions with three aromatic amino acids. Although the three sites are related by internal structural symmetry, binding affinities are expected to differ as some of the amino acids are not conserved. The biological relevance of this structure will be addressed with regard to the observed structural asymmetry, unequal binding site occupancies and sugar affinities, and modes of lectin self-aggregation.

(1) Lovrien, R. E. and Anderson, R. A. (1980), J. Cell. Biol. 85, 534-548.
(2) Wright, C. S. (1992) J. Biol. Chem. 267, 14345 - 14352.

Microbial Adhesion to Complex Carbohydrates

CZ 004 BACTERIAL INTERACTIONS WITH SALIVARY GLYCOPROTEINS, Susan J. Fisher[1], Alma Burlingame[1], Beth Gillece-Castro[2], Hakon Leffler[1] and Akraporn Prakobphol[1], [1]UCSF, San Francisco, CA 94143-0512 and [2]Genentech Inc. South San Francisco, CA 04080.

Bacteria express lectin-like proteins (adhesins) that interact with host mucosal glycoconjugates. Surface-associated receptors probably play an important role in promoting adherence. In contrast, receptors present on soluble glycoproteins may act as competitive inhibitors, promoting clearance rather than adherence. Microbial colonization of the oral cavity is similarly the result of an interplay of forces that promote either adherence or clearance. Salivary receptors present on teeth and mucosal surfaces are likely to enhance colonization. Soluble salivary components could prevent colonization by competition and/or promoting agglutination.

To begin to understand this complex biological situation it is necessary to identify, at the molecular level, the adhesive specificities that govern the interactions between bacteria that colonize the oral cavity and their salivary receptors. As an initial screening technique for detecting carbohydrate-mediated interactions we have developed an overlay assay in which parotid and submandibular/sublingual salivas are separated by SDS-PAGE, transferred to nitrocellulose membranes and overlaid with [^{35}S]methionine-labeled bacteria. Potential salivary receptors are detected by autoradiography. Once an interaction is detected, then the structures of the specific carbohydrate sequences that serve as receptors are determined.

Using this overlay technique we showed that the gram-negative periodontal pathogen *Fusobacterium nucleatum* interacts with a highly glycosylated proline-rich glycoprotein (PRG) that is a component of parotid saliva. Deglycosylation of the protein abolishes receptor activity, suggesting that adherence is mediated by the carbohydrate portion of the glycoprotein. We analyzed the PRG oligosaccharides by a combination of mass spectrometry techniques and nuclear magnetic resonance spectroscopy. The most abundant structure was an asialo-, biantennary saccharide that contained two fucose residues on one antenna. To understand the structural basis of *F. nucleatum* binding, we screened glycolipids and neoglycolipids carrying carbohydrate structures related to those of the PRG for receptor activity; components with unsubstituted terminal lactosamine residues best supported adherence. Neoglycolipids constructed from PRG oligosaccharides were also receptors. Treatment with ß-galactosidase, but not α -fucosidase, abolished binding, suggesting that unsubstituted lactosamine units, including the 6-antenna of the major oligosaccharide, mediate *F. nucleatum* adherence.

We used the same experimental strategy to characterize the interaction of several species and strains of streptococci with salivary glycoproteins. In 3 cases (*Streptococcus sanguis* 72-40 and 804, *S. sobrinus* OMZ 176) highly specific interactions with a single salivary component were detected. Removal of sialic acid residues from the low molecular weight salivary mucin prevented adherence of one of these strains (*S. sanguis* 72-40), suggesting that this saccharide either mediates binding or is a critical component of the receptor site. In 5 cases (*S. gordonii* G9B and 10558, *S. sanguis* 10556, and *S. oralis* 10557 and 72-41) interactions with multiple salivary components were detected. Taken together, our results suggest that the experimental strategy we have devised is useful for understanding the structural basis of the complex interactions between bacteria and their salivary receptors.

CZ 005 GLYCOLIPID EPITOPES AS MEMBRANE-CLOSE BINDING SITES FOR BACTERIA AND VIRUSES

Karl-Anders Karlsson[1], Maan Abul Milh[1], Jörgen Bergström[1], Hamid Dezfoolian[1], Erling Norrby[2], Nicklas Strömberg[1], Susann Teneberg[1], Göran Wadell[3], [1]Department of Medical Biochemistry, University of Göteborg, Sweden, [2]Department of Virology, Karolinska Institute, Stockholm, Sweden, [3]Department of Virology, University of Umeå, Sweden

Glycosphingolipids are mainly associated with the surface membranes of cells. There are several saccharide sequences which have not been detected in peptide-linked form but are exclusively lipid-linked and thus are potential true membrane attachment sites. Such sites may also create membrane proximity for penetration. Several microbes have selected for binding to glycolipids of this kind, including lactosylceramide for several bacteria (1), GM1 ganglioside for cholera toxin, Galα4Gal-containing isoreceptors in case of *E. coli* (1,2) and other bacteria, and HexßCer in case of viruses (3). Recent data support the idea that membrane proximity is essential for the GM1 pentasaccharide to mediate cholera toxin penetration and action (4). In case of *E. coli* there is evidence that variant binders of Galα4Gal have different selectivities for cells (human versus dog urinary tract) depending in part on membrane-induced steric effects on the accessibility of the second to fourth sugar from ceramide (5,6). A series of viruses (adeno-, herpes-, myxo-, rhabdo-, reo- and retro-viruses including HIV-1) bind to one-sugar glycolipids on TLC plates (3,5,7,8) and on cells (7,8), and antibodies against such glycolipids may block the infection with the virus (7,8). In this case, the closeness to the membrane bilayer may induce penetration into the cell (3). For lactosylceramide-binding bacteria the meaning may be to establish a true cell adhesion as a second-step after selection of host with a cell- or tissue-specific attachment site at further distance from the bilayer (1).

Thus a binding to a membrane-close epitope of a glycolipid may improve the selectivity, induce penetration or assure cell adhesion.

1. K.-A. Karlsson, Annu. Rev. Biochem. 58(1989)309
2. N. Strömberg et al, EMBO J. 9(1990)2001
3. K.-A. Karlsson et al, Antiviral agents, US Patent no. 4,980,462(1990)
4. T. Pacuszka et al, Biochemistry 30(1991)2563
5. K.-A. Karlsson et al, In "Molecular Recognition in Host-Parasite Interactions", eds. T.K. Korhonen et al, Plenum Press, in press (1992)
6. Strömberg et al, Proc. Natl. Acad. Sci. USA 88(1991)9340
7. Bhat et al, Proc. Natl. Acad. Sci. USA 88(1991)7131
8. N. Yahi et al, J. Virol. 66(1992)4848

CZ 006 ROLE OF GLYCOLIPID BINDING IN B CELL DIFFERENTIATION, Clifford A. Lingwood, The Hospital for Sick Children, Department of Microbiology, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

The glycolipid Gb_3 (gal α 1 - 4 gal α 1 - 4 glucosylceramide) is a differentiation antigen in human B cell ontogeny (CD77). This glycolipid is a receptor for the E. coli elaborated verotoxin (VT) and VT has been shown to be selectively cytotoxic for IgG as opposed to IgM committed human B cells *in vitro*. Cells *in vitro* are co-sensitive to VT and growth inhibition by α 2 interferon and Gb_3 deficient cells are resistant to α 2 interferon. Treatment of Daudi cells *in vitro* with α 2 interferon has been shown to induce differentiation. The structural basis of a proposed interaction between the VT receptor, Gb_3 and the human α 2 interferon receptor was recognized with the identification of homologous sequences in the α 2 interferon receptor and the receptor binding B subunit of VT. Evidence will be presented to support a functional interaction between Gb_3 and the interferon receptor to modulate α 2 interferon signal transduction in B cells.

Using the homology between the α 2 interferon receptor and the verotoxin B subunit as a model, amino acid sequence data banks were searched for other proteins which shared these regions of homology. CD19 is the earliest B cell differentiation antigen thus far determined. This member of the IgG super family is involved in the regulation of antigenic stimulation of B cells. CD19 shows similar regions of homology to the verotoxin B subunit as are shared by the α 2 interferon receptor. We have, therefore, begun a series of studies to determine whether CD19 is also associated with Gb_3 in the plane of the B cell plasma membrane. Evidence in support of this concept will be presented and discussed.

Physical Attributes of Carbohydrate-Protein Binding Systems

CZ 007 OLIGOSACCHARIDE–ANTIBODY COMPLEXES; OBSERVATIONS ON COMPLEX STABILITY AND DESIGN OF IMPROVED INHIBITORS. D R. Bundle, E. Eichler, F.–I. Auzanneau, H. Baumann, B. W. Sigurskjold.
Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada.

Three solved crystal structures of distinct antibody Fabs each complexed with oligosaccharide fragments of bacterial O–antigens (*Shigella flexneri*, *Salmonella typhimurium*, and *Brucella abortus*) revealed in each case well resolved electron density that defined the orientation and conformation of the antigenic determinant in both "pocket" and "groove" type binding sites. The position of no more than 3 hexose residues could be "seen" in each structure, although dodecasaccharide and pentasaccharide ligands were used in co–crystallization with Fab. This suggests that for antibodies, complementary carbohydrate epitopes are smaller than the 6 hexose units previously envisaged. Calorimetry experiments point to weak interactions in the immediate vicinity of the binding site but no electron density is observed for sugar residues in this region.

Functional group replacement for the *Salmonella* trisaccharide epitope shows that a cluster of hydroxyl groups located at one surface of the oligosaccharide form the crucial hydrogen bonds of the ligand–antibody complex (a feature first proposed by Lemieux[1] and subsequently seen in several antibody and lectin complexes). Structural modification of the ligand at those points positioned adjacent to the periphery of the protein site exhibit generally small activity changes, probably related to solvation effects. Indirect evidence for this interpretation can be found in linear enthalpy–entropy compensation plots seen not only for ligand structural modifications, but also for protein engineered Fab mutants.

Calorimetry and crystal structure data show that the trisaccharide epitope, α–D–Gal(1→2)[α–D–Abe(1→3)]α–D–Man fills the binding site[2]. Partial acid hydrolysis removed single abequose residues from the phage derived octasaccharide [→3)α–D–Gal(1→2)[α–D–Abe(1→3)]α–D–Man(1→4)α–L–Rha(1–]$_2$ to yield two heptasaccharide A and B. These univalent ligands showed dramatically different association constants that varied 50 fold. The lower binding energy of one heptasaccharide results from entropy loses due to conformational restrictions imposed by the protein.

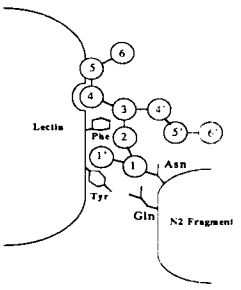
During mapping studies with modified ligands it was observed that substitutions of specific hydroxyl groups by H or Cl provided inhibitors with increased inhibitory power. For the *Shigella* groove type binding site we have increased the inhibitory power of a trisaccharide 100 fold by combining at least two distinct substitutions. In this way it was possible to obtain small carbohydrate inhibitors with activities in the nanamolar range.

References

1. R. U. Lemieux, *Chem. Soc. Rev. 18*, 347–374 (1989).
2. M. Cygler, D. R. Rose and D. R. Bundle, *Science, 253*, 442–445 (1991).

CZ 008 X-RAY STRUCTURES OF LATHYRUS OCHRUS LECTIN WITH SACCHARIDES AND GLYCOCONJUGATES , Yves Bourne[1], Marie-Pascale Nésa[1], Pierre Rougé [2], Joel Mazurier [3], Dominique Legrand [3], Jean Montreuil [3] , Geneviève Spik [3] and Christian Cambillau [1], [1] Laboratoire de Cristallographie et de Cristallisation des Macromolécules Biologiques, Faculté de Médecine Secteur-Nord, Bd Pierre Dramard, 13 326 Marseille Cedex 15, France, [2]Laboratoire de Biologie Cellulaire, Faculté des Sciences Pharmaceutiques, Université Paul Sabatier, 35 chemin des Maraichers, 31 062 Toulouse, France, [3] Laboratoire de Chimie Biologique, UMR111-CNRS, Université des Sciences et Techniques de Lille, 59655 Villeneuve d'ascq, France.

The aim of our work is to determine the parameters which allow protein saccharides (or glycans) recognition. We used two mannose/glucose specific isolectins from *Lathyrus ochrus* (LOL-1 and LOL-2, 50kDa) , and crystallized them in the presence of various saccharides and Asn-linked glycoconjugates. LOL-1 could form well diffracting crystals (2.0Å) with monosaccharides - mannose and glucose muramic acid - and with a trisaccharide . Structures of a LOL1-biantennary octosaccharide complex and of a LOL1- triantennary polysaccharide complex have been also analyzed at 2.3 Å resolution. LOL1 could also crystallize with a murNAc-dipeptide (MDP) and the structure determined at 2.0Å. Crystals of LOL-2 with a full biantennary glycan from the human lactotransferrin, with 3 amino-acids attached, diffracted to a lower resolution (2.8 Å), wich nevertheless allowed to trace the polysaccharidic chain. Finally, crystals of LOL-2 with a *glycoprotein* (the N2 fragment of human lactotransferrin, 18kDa) could be generated and analyzed at a resolution of 3.0 Å. In From the complexes with monosaccharides, we could analyze the "monosaccharide binding site" (mbs) and its net of hydrogen bonds and hydrophobic contacts. Upon saccharide binding, a loop of the lectin moves toward the saccharide by *ca* 1 Å. The position of the mannose in the "mbs"of all the other complexes described is kept constant. The complexes with the trisaccharide as well as the octosaccharide showed the importance of water bridging molecules, between the lectin and the saccharide, to help establishing the saccharide conformation. In the latter structure, the role of hydrophobic contacts has been confirmed, involving three lectin aromatic rings. The mannose binding site is occupied by Man 4 from the antenna 1-3 linked. This could be confirmed by the fact that crystals of a Man-4' branched triantennary saccharide could be obtained, leading to a 3D structure, whereas the Man-4 branched triantennary saccharide did not crystallize. The two antennae interact with the lectin, whereas GlcNAc-2 faces the bulk water. In the lactotransferrin glycopeptide and glycoproteic fragment N2, the saccharidic biantenna has a different conformation (see figure). Man 4 is still in the "mbs" and the 1-3 linked antenna has a similar conformation. The 1-6 linked antenna is now pointing in the water; Fuc 1' interacts with the lectin as well as GlcNAc 1 and GlcNAc 2. Therefore, the saccharidic moiety of the biantenna can adopt severall very different conformations.



CZ 009 CONFORMATIONS OF ELAM-SPECIFIC CARBOHYDRATES, B.N. Narasinga Rao[1], Mina Nashed[1], Falguni Dasgupta[1], Saeed Abbas[1], David Tyrrell[1], Carrol Foxall[1], Paul James[1], Makoto Kiso[2] and Akira Hasegawa[2], [1]Glycomed, Inc., Alameda, CA 94501, [2] Gifu University, Gifu, Japan

One of the early steps in the homing of leukocytes to sites of inflammation or injury involves the adhesion of leukocytes to vascular endothelium. This intercellular adhesion is mediated by a family of three cell-adhesion molecules, termed *selectins*, to specific carbohydrate structures. The carbohydrate ligand for E-selectin has been characterized by several research groups to be the sialyl-Lewis^x (NeuNAc(α2,3)Gal(1,4)[Fucα1,3]GlcNAc) epitope of sialylated-fucosylated-lactosamine structures.

As a basis for understanding the relationship between activity of sialyl-Le^x (SLe^x) and its three-dimensional structure, solution conformations of sLe^x-Lac-OSE hexasaccharide have been determined using 500 MHz NMR, empirical energy computations and molecular dynamics simulations. The nuclear Overhauser enhancement (NOE) measurements suggest that the Le^x moiety adopts a restricted conformation. The sialic acid shows some flexibility around its glycosidic linkage.

Several SLe^x analogs have been screened for their activity using ELISA and cell-based assays. Structure-activity correlations suggest that the sialyl-Le^x moiety binds along a surface to the E-selectin. Inhibitory activities of several sialyl-Lewis^x derivatives have been rationalized on the basis of their conformational similarities to SLe^x.

Virus Protein/Carbohydrate Adhesion

CZ 010 O-GLYCOSYLATION AND ROTAVIRUS BINDING TO CELLS: ARYL-α-D-GALACTOSAMINIDES AS INHIBITORS OF ROTAVIRUS INFECTION OF SUSCEPTIBLE CELLS. James H. Gilbert and Mary E. Schaefer. Glycomed, Inc., Alameda, CA 94501.

The interaction of rotavirus with the carbohydrates of macromolecules and cell surfaces has been well documented. The oligosaccharrides on mucin are required for inhibition of infection *in vitro* and *in vivo*. Sialic acid is important for the binding of SA11 rotavirus to tissue culture cells. The binding of rotavirus to glycolipids resolved by thin layer chromatography has been shown to depend upon the oligosaccharide. We have utilized inhibitors of the N- and O-linked glycosylation pathways to further examine the role of carbohydrate in rotavirus infection of cells. Aryl-α-D-N-acetyl-galactosaminides were used as specific inhibitors of O-glycosylation. MA104 cells treated with these compounds showed reduced ability to bind ^{35}S-methionine labelled SA11 rotavirus as well as reduced infectability as measured by plaque assay. Tunicamycin treated cells were unchanged in their ability to bind virus. The inhibition is dose dependent and specific for the α-anomer of galactosamine. The nature of the aryl group is apparently unimportant. Partially purified MA104 cell membranes were resolved by SDS-PAGE and probed with SA11. Several bands were seen in untreated cells which were markedly reduced in lanes containing membranes from treated cells. Finally, cell free supernates from the mucin producing human colonic carcinoma cell line, LS174T, were shown to be inhibitory to rotavirus infection of MA104 cells. These cells were treated with benzyl-α-D-galactosaminide which inhibits the mucin glycosylation and results in secretion of benzyl-oligosaccharides into the medium (1). Cell free supernatants from treated cells show a marked increase in the inhibition of rotavirus infectivity of MA104 cells. ^{35}S-methionine labelled SA11 rotavirus binds to components of the media with low electrophoretic mobility when analyzed by SDS-PAGE and transfer to nitrocellulose. Our experiments suggest the oligosaccharide receptor for rotavirus on MA104 cells is on an O-linked glycoconjugate, and it may be a mucin or mucin-like molecule.

1. Kuan, *et al*, Jour. Biol. Chem. **264**:19271-19277. 1989.

Carbohydrate Recognition: Selectins and Their Ligands (A) (Joint)

CZ 011 LEUKOCYTE INTERACTIONS WITH P-SELECTIN, Rodger P. McEver, W.K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, and Oklahoma Medical Research Foundation, Oklahoma City, OK.

P-selectin is an adhesion receptor for myeloid cells and lymphocyte subsets that is expressed by megakaryocytes/platelets and endothelial cells. A sorting signal in the cytoplasmic tail directs P-selectin to secretory granules in both platelets and endothelial cells; it is then rapidly redistributed to the cell surface in response to agonists such as thrombin. An alternatively spliced soluble form of P-selectin is secreted constitutively because its C-terminus no longer faces the cytoplasm where it can serve as a sorting signal. Membrane P-selectin and recombinant soluble P-selectin bind with apparent high affinity to a saturable number of protease-sensitive sites on myeloid cells. Cell recognition requires a conformational change in the lectin domain resulting from occupancy of two Ca^{2+}-binding sites. Peptides from discontinuous regions of the lectin domains of selectins block neutrophil adhesion to both P- and E-selectin. Ca^{2+} binds to peptides from two regions and enhances their ability to support myeloid cell adhesion. Although P-selectin, like the other selectins, binds to multivalent forms of the tetrasaccharides sialyl Lewis x and sialyl Lewis a, the carbohydrate structures of high affinity ligands on cell surfaces have not been defined. P-selectin binds preferentially to a single glycoprotein in extracts of myeloid cells that may correspond to the high affinity binding sites on intact neutrophils. The glycoprotein ligand appears to be a disulfide-linked homodimer with subunits of Mr 120,000. It is heavily sialylated, expresses the sialyl Lewis x epitope, and appears to contain only 1 or 2 one N-linked glycans but many clustered O-linked oligosaccharides. Further studies of this and other high affinity ligands for selectins may help clarify how selectins mediate rapid cell adhesion under flow conditions in the circulation.

Carbohydrate Recognition: Selectins and Their Ligands (B) (Joint)

CZ 012 **SELECTIN LIGANDS AND ANTAGONISTS - BEYOND SIALYL LEWIS X**, Brian K. Brandley, Mina Nashed, Falguni Dasgupta, Saeed Abbas, Darwin Asa, Carrol Foxall, and John Musser. Glycomed, Inc., Alameda, CA 94501.

The discovery of the selectins and the importance in leukocyte-endothelium adhesion has recently refocused attention on lectin mediated cell adhesion. Several potential carbohydrate ligands have been identified for the selectins. These can be broadly divided into two groups: 1) Sialyl Lewis X (sLe^x) and related oligosaccharides, and 2) sulfated carbohydrates. All three functional groups on sLe^x oligosaccharides, although with differing avidities. We have identified the functional groups on sLe^x analogs that replace sialic acid and fucose residues with simpler and more stable substituents. Although the process is ongoing, we have been successful at replacing the sialic acid residue with acetic or lactic acid groups. The second group of ligands all contain sulfate on a carbohydrate support, and bind to the selectins with characteristics that differ from sLe^x binding. Such compounds are recognized by L-Selectin and P-Selectin, but in general, not E-Selectin. The most recently discovered member of this class of ligands is an HNK-1 reactive epitope, the SulfoGlucuronlyNeoLacto (SGNL) structure. While the biological implication of this second group of potential ligands is unclear, our data may indicate the L-Selectin and P-Selectin can bind via a mechanism independent of, or in conjunction with, sLe^x like structures.

CZ 013 LEUKOCYTE ADHESION DEFICIENCY (LAD II) - DEFICIENCY IN SIALYL LEWIS X, A LIGAND FOR SELECTIN - DUE TO GENERAL FUCOSE DEFICIENCY, Amos Etzioni[1,2], John M. Harlan[3], M. Laurie Phillips[4], Shimon Pollack[1,2], Ruth Gershoni-Baruch[1,2] and James C. Paulson[4]. Rambam Medical Center and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [3]University of Washington, Seattle, and [4]Cytel Corporation, San Diego, CA.

"It is in her moments of abnormality that nature reveals her secrets." (Goethe) The occurrence of recurrent bacterial infections, neutrophil dysfunction and normal expression of CD18 integrins in two unrelated children suggested an as yet undescribed adhesion deficiency. The fact that both children exhibited the rare Bombay blood group and were Lewis negative, each involving carbohydrates with different fucose linkages, a possible defect in the fucose containing ligand of E- and P-selectin, sialyl-Lewis-X (SLe^x), was considered. Indeed, using a monoclonal SLe^x antibody, no expression of SLe^x on the patients' neutrophils was detected. Adhesion to IL-1β or histamine activated endothelial cells was markedly decreased (<5% of control). The observation that the neutrophils did not bind to recombinant E- and purified P-selectins confirmed the SLe^x deficiency as the basis for the adhesion deficiency. Low binding of fucose specific lectins to EBV virus transformed patient β-lymphocytes was observed, while the binding of mannose specific lectins was normal, providing further evidence for a general fucose deficiency as the primary defect. The existence of the patients and their deficiency emphasizes the essential role of the endothelial cell selectin and the SLe^x ligands on the neutrophils in an early step of neutrophil recruitment to sites of infection. We would like to designate this syndrome as LAD II and the previously described CD18 deficiency as LAD I.

CZ 014 SELECTINS AS THERAPEUTIC TARGETS FOR INFLAMMATORY DISEASE, James C. Paulson[1], M. Laurie Phillips[1], Amos Etzioni[2], Hiroshi Asako[3], D. Neil Granger[3], John M. Harlan[4], Michael S. Mulligan[5], Peter A. Ward[5] and Robert K. Winn[4], [1]Cytel Corporation, San Diego, CA, [2]Rambam Medical Center and The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [3]Louisiana State University Medical Center, Shreveport, LA, [4]University of Washington, Seattle, WA, [5]University of Michigan, Ann Arbor, MI.

Recruitment of neutrophils to sites of inflammation in tissue injury involves the combined action of multiple adhesion molecules, cytokines, and chemoattractants. Three members of the selectin family, L-selectin, E-selectin and P-selectin, participate in the initial rolling of neutrophils on the activated endothelium of the blood vessel wall. The endothelial cell selectins, E-selectin and P-selectin, mediate adhesion by recognition of a carbohydrate ligand, sialyl Lewis X (SLe^x), expressed on the carbohydrate groups of glycoprotein receptors on the resting neutrophil. Based on 1) the discovery of a new human leukocyte adhesion deficiency (LAD II) involving the absence of SLe^x expression on patient neutrophils and 2) inhibition of neutrophil recruitment in response to E- and P-selectin antagonists, the interaction of the endothelial cell selectins with their carbohydrate ligand on neutrophils appears to be a prerequisite step for efficient neutrophil recruitment. These findings suggest that E- and P-selectin are suitable targets for development of therapeutic agents to prevent inflammatory disease mediated by neutrophils and other leukocytes recognized by these adhesion molecules.

Pharmaceutical Development in the Carbohydrate Ligand Arena

CZ 015 DEVELOPMENT OF BUTYRYL CASTANOSPERMINE (MDL 28,574) AS AN ANTIVIRAL DRUG, Mohinder S. Kang[1], Debbie L. Taylor[2], Paul S. Liu[3], Prasad S. Sunkara[1], and A. Stanley Tyms[2], [1]Marion Merrell Dow Inc., Cincinnati, Ohio 45215, [2]Medical Research Council Collaborative Centre, Mill Hill, London, and [3]FDA, Kensington, MD.

The identification of human immunodeficiency virus (HIV) as the etiologic agent of AIDS and the finding that HIV replication is involved in the initiation and progression of the disease have prompted the targeting of viral replicative process for therapeutic intervention. Several steps of the replicative cycle have been the subject of intensive investigation. The nucleoside analogs (AZT, ddC, and ddI) that have been approved for treatment of AIDS patients inhibit viral reverse transcriptase, but are associated with severe toxicity and the long term efficacy is compromised by development of resistant viral strains. We have investigated the effect of modification of viral glycoproteins by inhibitors of glycoprotein processing on viral infectivity. Two compounds, castanospermine (Cast) and deoxynojirimycin (DNJ) which are inhibitors of α-glucosidase I, have shown antiviral activities against murine leukemia virus (MOLV) and HIV. We have synthesized a number of derivatives of Cast and evaluated them against α-glucosidase I, MOLV and HIV in cell culture. From these studies, BuCast (castanospermine 6-butyrate) emerged as the most potent inhibitor of α-glucosidase I and antiviral agent. BuCast showed moderate inhibitory activity against purified glucosidase I (IC_{50}:1.3μM) compared to Cast (0.1μM) and DNJ (3.6μM). However, it is more potent against the enzyme in mammalian cells (IC_{50}:2.7μM) compared to Cast (IC_{50}:53μM) as measured by accumulation of glucose containing oligosaccharides suggesting the enhanced uptake of BuCast by mammalian cells. BuCast was also the most effective compound against growth of HIV (IC_{50}=1.1μM) when compared to Cast (39 μM), DNJ (560 μM), and N-BuDNJ (IC_{50}=56μM) (Searle, currently undergoing clinical trials). BuCast treatment of HIV chronically infected cells resulted in inhibition of the cleavage of the precursor glycoprotein gp160 which was characterized by an increase in Mwt of 6-7KD. However, no quantitative differences were observed in gp120 expression on the infected cell surface or cell free gp120 but virions were unable to bind efficiently to the host T-cell and might account for the reduced infectivity after BuCast treatment. BuCast reacted synergistically when used in combination with the current therapies for HIV infection, AZT, ddC, or ddI. Bucast is well absorbed by oral administration and relatively nontoxic with an acute LD_{50} of more than 2 g/kg in mice and rats. Based on these studies, BuCast appears to be a promising compound that warrants clinical evaluation in AIDS patients.

CZ 016 ACTIVE SPECIFIC IMMUNOTHERAPY (ASI) OF CANCER USING SYNTHETIC CANCER-ASSOCIATED CARBOHYDRATE ANTIGENS, B. Michael Longenecker[1,2], and Grant D. MacLean[3], [1]Department of Immunology, University of Alberta, [2]Biomira Inc., [3]Department of Medicine, Cross Cancer Institute, Edmonton, Alberta, Canada.

Our goal is to develop chemically-defined, synthetic "vaccines" for the ASI of human cancer. We have focused our efforts on cancer associated epitopes expressed on carcinoma cell surface mucin molecules. We have synthesized carbohydrate, peptide, and glycopeptide epitopes expressed on carcinoma cell surface mucin molecules encoded by the human MUC1 gene, formulated them into immunotherapeutic "vaccines" and tested them in animal models and in some cases, in human cancer patients.

The Thomsen-Friedenreich (TF) antigen (=βGal1→3αGalNAc) and the sialyl-Tn antigen (=αNANA2→6αGalNAc) are strongly expressed on a variety of human carcinomas with little expression on most normal tissues. Mice immunized with synthetic TF conjugated to KLH (TF-KLH) plus RIBI adjuvant produce TF specific antibody, TF specific DTH reactions and increased survival following challenge with a highly aggressive mouse mammary adenocarcinoma (TA3-Ha) expressing a mucin (epiglycanin) with multiple TF epitopes. Low dose cyclophosphamide, which inhibits mucin-induced immune suppression, increases the effectiveness of the TF-KLH "vaccine".

Mice immunized with STn-KLH plus RIBI adjuvant produced IgG MAbs which showed specific binding to αNANA(2→6)αGalNAc (but not βNANA(2→6)αGalNAc), to ovine submaxillary mucin (which has multiple STn epitopes) and human tumor cells expressing STn epitopes.

Two phase I clinical trials have been completed using TF-KLH plus DETOX™ in 12 ovarian cancer patients and sialyl-Tn plus DETOX™ in 12 breast cancer patients. Specific humoral responses were noted including a vigorous hapten-specific IgG response. Complement-mediated cytotoxic antibodies against appropriate tumor cell targets developed in most of the patients following immunization. All of the ovarian cancer patients had extensive metastatic disease on entry into the trial. A significant correlation between CD4:CD8 ratios at trial entry and survival was noted in these patients. One of the ovarian cancer patients who was immunized with the STn-KLH formulation after having completed her full course of TF-KLH immunizations demonstrated a specific sequential humoral immune response to each hapten.

Two of the breast cancer patients entered the trial with widespread disease but the remaining 10 patients entered with minimal metastatic disease with measurable metastatic lesions in all cases. Three of the 12 patients showed progressive disease despite ASI, including the two patients who had widespread disease on trial entry. Four patients demonstrated stable disease for 7-12 months after trial entry and one patient showed progressive disease after 8 months of stable disease. Two patients showed a mixed response, both subsequently having disease progression. One patient had a good partial response for approximately 7 months then showed progressive disease several months following her ASI. One additional patient had a partial response which has been maintained for approximately 6 months. Phase II/III studies will be required to confirm the efficacy of our synthetic ASI formulations.

CZ 017 HEPARIN MIMETICS AS INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS-HOST CELL INTERACTIONS. Stanley Tyms[1], Debra Taylor[1], Richard L. Jackson[2], and Alan D. Cardin[2], Marion Merrell Dow Research Institute, [1]MRC Collaborative Centre Laboratory Mill Hill, London and [2]Cincinnati, Ohio, USA.

The infection of monocytes and T-lymphocytes of the immune system by the Type-1 Human Immune Deficiency Virus (HIV-1) is mediated by a specific, high affinity interaction between the viral envelope glycoprotein gp120 and CD4 antigen on the target cell surface. Entry involves virus cell membrane fusion, uncoating and translocation of the nucleocapsid core. Heparin inhibits HIV-1 infection during the absorption/entry phase but is limited as a therapy by its anticoagulant action. A series of chemically-defined polydisperse sulfonic acid polymers which consist of biphenyl disulfonic acid monomers (n) joined by urea linkages were designed as mimetics of heparin. MDL 101,028 showed limited anticoagulant activity and potent anti-HIV effect in the T-cell line JM as measured by the formation of syncytia, p24 antigen synthesis and RT activity (IC50 values 0.02-0.1 μM). MDL 101,028 blocked the growth of various HIV strains, clinical isolates and AZT-resistant mutants of HIV-1. The antiviral activity was observed in different T-cell lines, human PBMCs and in monocytic cells. When MDL 101,028 was added to JM cells with established infections (e.g. 36-48 h p.i.), the compounds cleared cultures of syncytia and altered further progression of infection with an increase in the number of protected T-cells. Time-lapse video recordings indicated that MDL 101,028 destabilized the membranes of syncytia with their consequent degradation. Pretreatment of uninfected $CD4^+$C8166 cells blocked their fusion with chronically-infected H9 cells but also protected JM cells from acute infection of HIV-1. These findings suggest a direct interaction between CD4 and MDL 101,028. Structural studies on soluble rCD4 identified the drug interaction site within the variable-1 domain of the receptor. The rCD4-gp120 interaction and the acute infection of JM cells by HIV-1 showed similar potencies of inhibition (nM) by MDL 101,028 with polymer chain lengths of n=6 to n=9 being optimal. The novel mode of action, antiviral potency and limited anticoagulant effects may lend MDL 101,028 to the treatment of HIV-1 infection.

Lectins and Their Interface with Carbohydrate Ligands

CZ 018 DISCOVERY OF A METAL-INDEPENDENT β-GALACTOSIDE-BINDING LECTIN FROM A NEMATODE *C. ELEGANCE*. Ken-ichi Kasai, and Jun Hirabayashi, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, JAPAN

Two groups of animal lectins have so far been well studied. Lectins belonging to one of them, metal-independent β-galactoside-binding lectins consisting of 14-kDa- and 30-kDa-type subfamilies, have been found only in vertebrates, and therefore, are supposed to have functions needed for only higher animals, in contrast to Ca-dependent lectins which seemed to play more general and fundamental roles in carbohydrate-mediated biological phenomena because they are distributed from vertebrates to invertebrates. However, it turned out that the metal-independent lectins are also distributed much more widely than expected and consequently should have fundamental roles which are not restricted to vertebrates but are common to almost all animals, because we found that a β-galactoside-binding protein isolated from a nematode *Caenorhabditis elegance* is homologous to vertebrate metal-independent lectins.

We isolated proteins having affinity for asialofetuin-agarose from *C. elegance* [1], and cloned a full-length cDNA for one of them having relative molecular mass of 32 kDa (32-kDa GBP)[2]. The clone contained a single open reading frame encoding 279 amino acids, including the initiator methionine. Significant sequence homology to the vertebrate metal-independent lectins (25-30% identities) was observed. Moreover, the nematode 32-kDa GBP proved to have a unique polypeptide architecture; that is, it is composed of two tandemly repeated homologous domains, each consisting of about 140 amino acids. Thus, this protein is constructed with a duplicated fundamental unit which is similar to the vertebrate 14-kDa lectins. In spite of the extreme phylogenic distance between nematodes and vertebrates (divergence > 6 x 10^8 years ago), both of the two repeated domains of the nematode 32-kDa GBP retained most of the amino acid residues conserved in vertebrate lectins.

The nematode 32-kDa GBP is distinct from mammalian 30-kDa metal-independent lectins because the latter are chimeras of a 14-kDa lectin domain (C-terminal half) and a domain of unknown function (N-terminal half). At present, we can classify animal metal-independent lectins into three categories: (1) "proto type" represented by vertebrate 14-kDa lectins; (2) "tandem-repeat type" represented by the nematode 32-kDa GBP: (3) "chimera type" represented by mammalian 30-kDa lectins. They have been evolved from a common ancestor protein which should have existed in the Precambrian era. We were also informed recently that a lectin isolated from a sponge species was found to have homology to this family (personal communication).

The finding of the nematode GBP is not only emphasized the importance of metal-independent lectins in the animal kingdom but also provided an extremely useful experimental system for elucidating biological regulation in which recognition of carbohydrates plays crucial roles.

[1] Hirabayashi, J., et al., (1992) *J. Biochem.* 111, 553
[2] Hirabayashi, J., et al., (1992) *J. Biol. Chem.* 267, 15485

CZ 019 STRUCTURE AND FUNCTIONAL PROPERTIES OF FOUR S-LAC LECTINS,
Hakon Leffler, Robert Atchison, Michael Gitt, Margret Huflejt, Ragnar Lindstedt, Steve Massa, and Samuel Barondes, Department of Psychiatry, University of California, San Francisco, CA 94143-0984.

We have compared the structure and localization of four soluble lactose binding (S-Lac) lectins (Fig. 1).

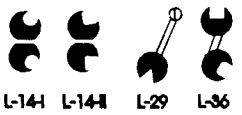


Fig. 1. Domain structure of S-Lac lectins. Carbohydrate-binding domains are filled and other domains open or hatched.

L-14-I, also known as L-14, BHL and galaptin, is a non-covalent dimer of two identical carbohydrate-binding domains. L-29, also known as Mac-2, εBP and L-34, consists of a monomer that contains a C-terminal carbohydrate-binding-domain and an N-terminal Pro/Gly rich domain. L-14-II, a new S-Lac lectin which we recently cloned has a similar structure and is about 40% identical in sequence to L-14-I. L-36 is a new lectin having two carbohydrate-binding domains in the same peptide chain. Each domain is about 40% identical to the other and to L-29 but less related to L-14-I and II. L-29 and L-14-I are widely distributed in the body and L-29 is most abundant in epithelial cells and macrophages while L-14-I is found in non-epithelial cells such as muscle. In contrast L-36 and L-14-II are restricted to the gastrointestinal tract. L-36 expression was found in all parts but most abundant in the large intestine. L-14-II expression was strong in the lower small intestine but weak in other parts.

As a model system for function of S-Lac lectins in polarized epithelial cells we have analyzed Madin Darby Canine Kidney (MDCK) cells cultured on porous membranes. L-29 was the only S-lac lectin found in these cells and was about 100 fold more abundant than in fibroblasts. The sequence of the dog L-29 was very similar to that of other species but was about 20 amino acids longer, due to additional repeating units in the Pro/Gly rich domain. The N-terminus was blocked by acetylation and 15% of the lectin was phosphorylated at serine residues within the first 15 amino acids. Although most of the lectin is cytosolic a fraction is slowly secreted at the apical (luminal) side.

In a study of the physical properties of human recombinant L-29 the lectin bound with apparent cooperativity to an immobilized glycoconjugate probably by forming aggregates. This phenomenon occurred at concentrations observed inside and outside of MDCK cells suggesting that it might occur *in vivo*. The aggregation required the N-terminal part, suggesting that the phosphorylation we found there might play a role in regulation of this behaviour. Supported by grant # 279 from TRDRP, California to H.L and from NIH to S B..

Banquet Address (Joint)

CZ 020 **TOYS FOR GLYCOBIOLOGY**, Ole Hindsgaul, Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada

The field of Glycobiology is blowing wide open with ever increasing reports that specific sugar sequences on complex carbohydrates can act as ligands to initiate important recognition events. The active structures are often complex, tetra- to hexasaccharides, and are difficult to isolate from natural sources in quantities sufficient for systematic investigation. As a result, enormous pressure is being exerted on synthetic carbohydrate chemists by glycobiologists to "make me this and make me that", and make them quickly! The requested compounds are usually predicted by the glycobiologist to have an activity, for example: to assay an enzyme activity, to inhibit a selectin or to target a glycoprotein or cell to a carboydrate-recognizing receptor. The importance of the requested compounds is emphasized to the carbohydrate chemist by refering to them as invaluable "**Tools for Glycobiology**".

Both chemical and enzymatic synthesis are used to produce the required synthetic probes but, most often, the compounds require many "post-doc months" to prepare. When they are finally finished the products are sent off to the glycobiologist by courier. Unfortunately, the compounds often end up either in a black hole or in the back of a fridge since by the time they are finished the original problem has either been solved, reformulated, or has lost its importance. The research director or group leader must then explain to the post-docs and students that they did very nice work, but I'm sorry no one is interested in these compounds anymore.

After a recent post-doc and student rebellion in my group over such matters, I was convinced that sometimes the synthetic chemical efforts were not well appreciated and that it was the glycobiologists who were having all the fun. Certainly, organic chemists know very little about experiments with cells or animals and they should not be the ones to design such experiments. However, they can make novel molecules that do not naturally exist (we call these "**Toys for Glycobiology**") and perhaps some component of the research in glycobiology should be guided by this ability, i.e. very long-shot chemical ideas. The trials and tribulations of generating such toys, and convincing people to use them, form the topic of this lecture.

Glycosaminoglycans and Specific Binding Proteins

CZ 021 **MOLECULAR RECOGNITION OF PROTEIN-GLYCOSAMINOGLYCAN INTERACTIONS AND THEIR POTENTIAL PHARMACEUTICAL APPLICATIONS.** Alan D. Cardin and Richard L. Jackson. Marion Merrell Dow Research Institute, Cincinnati, Ohio 45215.

The chemical heterogeneity of heparin accounts for its diverse biological activity, including its anticoagulant, antiviral and antiproliferative properties. Presumably, different heparins interact with particular protein targets in mediating these biological actions. To understand the molecular recognition of protein-heparin interactions, forty-nine regions in 21 proteins were identified as potential heparin-binding sites based on the sequence organization of their basic and nonbasic residues. Twelve well-known heparin-binding sequences in vitronectin, apolipoprotein E and B-100, and platelet factor 4 were used to search for potential heparin-binding regions in other proteins. Consensus sequences for glycosaminoglycan recognition were determined as [-X-B-B-X-B-X-] and [X-B-B-B-X-X-B-X] where B is the probability of a basic residue and X is a hydropathic residue. In 1987, we predicted the heparin-binding domains in endothelial cell growth factor, pupurin, antithrombin-III, fibronectin, heparin cofactor II, von Willabrand's Factor, N-CAM, basic FGF and other proteins. In this presentation, these predictions are analyzed with respect to recent experimental results now available in the literature. These findings, along with our earlier predictions, suggest that these consensus sequence elements represent nucleation sites in the heparin-binding regions of proteins for the recognition of polyanions. Many of the sequences conforming to these consensus motifs show prominent amphipathic periodicities, having both α-helical and β-strand conformations as determined by predictive algorithms and circular dichroism studies. Molecular dynamics simulations of the interaction of heparin with apoliproprotein E suggests that heparin may act as a conformational lock to stabilize protein solution conformation. Three dimensional model representations of the interaction of heparin with antithrombin III, the envelope glycoprotein gp120 of HIV-1, and basic fibroblast growth factor help illustrate this point. These molecular modeling approaches have been useful in the rational design of heparin mimetics towards the treatment of viral diseases and atherosclerosis.

CZ 022 MOLECULAR INTERACTION BETWEEN HEPARIN AND PEPTIDE DOMAINS OF SELECTED HEPARIN BINDING PROTEINS.

Robert B. Harris[1], Ruth Tyler-Cross[1], Dulce F. Soler[2], and Michael Sobel[3]. Depts. [1] Biochemistry and Molecular Biophysics and [3] Surgery, Virginia Commonwealth Univ./Medical College Virginia, Richmond, VA 23298. [2] Harvard Univ. Med. School, Boston, MA 02115.

The ability of proteins to bind heparin, a highly sulfated heterogeneous glycosaminoglycan, likely depends on the conformational uniqueness of specific binding domains. Based on the motif of a consensus heparin- binding synthetic peptide, a 23-residue sequence (Tyr^{565}-Ala^{587}) of human von Willebrand factor (vWF) was identified that binds heparin with affinity comparable to the native protein. The peptide undergoes a conformational change upon binding heparin and the binding event can be quantitated by titration calorimetry. Furthermore, when immobilized on Sepharose so as not to compromise essential residues, the vWF domain peptide is an effective affinity ligand; a high affinity heparin has been prepared which is 30-fold more potent in inhibiting vWF-mediated platelet agglutination compared with unfractionated heparin. This high affinity heparin retains its anti-Xa activity. In other experiments, a heparin was chemically modified to destroy its antithrombin binding properties. This heparin possessed greater potency for binding vWF than the starting material and it was also bound and released from the vWF peptide affinity column. Thus, the antithrombin-binding and vWF inhibiting properties of heparin can be clearly dissociated and a subfraction of heparin refined which possesses enhanced potency for inhibition of platelet-vWF interactions. A heparin with these properties may be useful as a regional or systemic antithrombotic agent at sites of high platelet reactivity in the arterial circulation. Although the heparin binding domain of vWF is devoid of α-helix character, heparin binding domains of other proteins are postulated to be helix structures which present a surface of high positive charge density. Thus, a synthetic 19-residue peptide designed to be α-helix in character was synthesized and its interaction with heparin was studied. Binding heparin increases the helix content of the peptide from 75 to 100% and increases both the thermal stability of the heparin-peptide complex (by 1.3 kcal/mol to 4.5 kcal/mol) and the melting point of the helix/coil transition (from 25 to 50°C). Thus, heparin stabilizes the conformation of this peptide. As recently shown, heparin also appears to stabilize the native conformation of acidic fibroblast growth factor and may thus serve to facilitate the interaction of this and numerous other proteins with their cellular receptors.

CZ 023 ACTIVATION OF HEPARIN COFACTOR II BY GLYCOSAMINOGLYCANS, Douglas M. Tollefsen, Washington University School of Medicine, St. Louis, MO 63110.

Heparin cofactor II (HCII) is a member of the serpin family of protease inhibitors which serve as pseudosubstrates for their target proteases. Among the proteases involved in coagulation and fibrinolysis, HCII is specific for thrombin. Thrombin attacks the reactive site Leu444-Ser445 peptide bond in HCII and becomes trapped in a stable 1:1 complex. Dermatan sulfate and heparin bind to HCII and increase the rate of inhibition of thrombin >1000-fold. Site-directed mutagenesis of the HCII cDNA expressed in bacteria reveals that the binding sites for dermatan sulfate and heparin overlap but are not identical. HCII binds non-specifically to heparin oligosaccharides containing 4 or more monosaccharide units. By contrast, HCII preferentially binds to a minor dermatan sulfate hexasaccharide composed of 3 IdoA(2-SO4)-GalNAc(4-SO4) disaccharide units. HCII contains a unique hirudin-like domain near the N-terminus which includes 2 sulfated tyrosine residues surrounded by aspartate and glutamate residues. Analysis of deletion mutations of HCII suggests that this acidic domain interacts with the glycosaminoglycan-binding site in native HCII. Displacement of the acidic domain by glycosaminoglycans may allow this region to bind thrombin and thereby facilitate the inhibitory reaction.

Late Abstract

LIGAND-LECTIN INTERACTIONS, Irwin J. Goldstein, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-0606.

Plant lectins classically have been described as multivalent carbohydate binding proteins. Recent reports indicate the existence of monovalent species and the discovery that many of these interesting proteins also appear to bind hydrophobic ligands. Additionally, some lectins have recently been shown to bind small peptides at their carbohydrate binding sites. [PNAS 89, 5393, 5398 (1992)].

Examples of the above properties of lectins will be presented. These include peptides derived from a hexapeptide epitope library that mimic the binding of specific carbohydrates to lectins; the unique carbohydrate binding properties of several mannose-specific lectins, and the sialic acid-binding lectin of the slug (*Limax flavus*), and their application in the isolation and separation of biopolymers. The synthesis of glycosides containing hydrophobic p-nitrobenzyl groups at the 2-O- and 3-O- positions of D-glucose and D-mannose and their binding to the pea, lentil, jack bean and *Vicia faba* lectins will be described. A series of 2-O- and 3-O- spin-labelled derivatives of D-glucose and D-mannose were also prepared and tested for their inhibitory potency.

Supported by NIH grant GM 29470.

Physical Probes of Carbohydrate Structure and Binding

CZ 100 STRUCTURE AND ACTIVITY OF A SYNTHETIC PEPTIDE FROM THE GLYCOSAMINOGLYCAN (GAG) BINDING SITE OF HEPARIN COFACTOR II, Frank C. Church and Herbert C. Whinna, Center for Thrombosis & Hemostasis, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7035

We synthesized a peptide from the GAG-binding site of the blood plasma serine proteinase inhibitor (serpin) heparin cofactor II (HC): $HC^{165-195}$, KDFVNASSKYEITTIHNLFRKLTHRLFRRNF (Whinna, H. C., Blinder, M. A., Szewczyk, M., Tollefsen, D. M. and Church, F. C. (1991) J. Biol. Chem. **266**, 8129-8135). Increasing amounts of $HC^{165-195}$ peptide (concentration to block 50% of inhibition activity is in parentheses) negated the accelerated HC/thrombin inhibition reaction by heparin (~250 nM) and dermatan sulfate (~500 nM). Circular dichroism spectra were obtained for the $HC^{165-195}$ peptide alone and peptide with low molecular weight heparin (LMWH), heparin and dermatan sulfate. GAGs increased the α-helical content of the peptide in the following order (% α-helix of peptide or peptide/GAG complex is in parentheses): no addition (7%) < LMWH (32%) < heparin (42%) < dermatan sulfate (55%). Secondary structure analysis by the Garnier-Osguthorpe-Robson method predicts this region is 55% α-helix. Molecular modeling of reactive site re-annealed HC, based on the crystal structure of its serpin homologue α_1-proteinase inhibitor, shows this region is 48% α-helix. Our results suggest that the $HC^{165-195}$ peptide binds to GAGs, an α-helical conformation is preferable in the presence of GAGs, and that GAGs may stabilize an unstable α-helical conformation in the GAG-binding site of HC for serpin "activation".

CZ 101 ^{1}H NMR ON A $MAN_3GLCNAc_2$ GLYCODECAPEPTIDE: STRUCTURE AT THE N-GLYCOSYLATION SITE

JefferyT.Davis[†*], Shirish Hirani[‡], Catherine Bartlett[‡], Shan-Ho Chou[§] and Brian R. Reid[†], [†] Departments of Chemistry and Biochemistry and [§] Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195; [‡] Genzyme Corporation, One Kendall Square, Cambridge, MA 02139.

A glycodecapeptide SerIleGluPheGlyThrAsnIleSerLys, 1, containing the pentasaccharide Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ1-NH attached to the γ-carboxamide of Asn7 was purified from hen ovomucoid. 2D NMR was used to assign nonexchangeable proton and exchangeable amide NH proton resonances of both the pentasaccharide and decapeptide domains. A NOESY experiment in H_2O showed that the orientation of the sidechain acetamido group about the C2-N bond (τ) was different for the two internal GlcNAc sugars. For GlcNAc-1, the NH-H3 NOE crosspeak was the strongest intraresidue NH-sugar H NOE, constraining τ to the 60-90 degree range, while for GlcNAc-2, the sidechain NH was closer to H1 and H2, indicating a τ of 120-140 degrees. The GlcNAc-1 carboxamide carbonyl is oriented towards the peptide in a conformation which could be stabilized by hydrogen bonds between the sidechain carbonyl and the NH and OH of the bidentate Ser9 in the adjacent N-glycosylation site. Such a chelated structure may be important for N-glycoprotein biosynthesis.

Sequential (i, i+1) NOEs provided evidence that the Asn-Ile-Ser tripeptide sequence adopts a gamma-type turn conformation. Sequential NOEs between Ile8 NH and Ser9 NH, Ile8 NH and Asn7 NH, and between Ile8 NH and an Asn7 βH are consistent with a mainchain (Ile8 NH) to sidechain (Asn7 γ-CO) H bond. An identical NOE pattern, observed in both H_2O and DMSO, implied that the N-glycosylation site in the nonglycosylated decapeptide adopts a similar nonrandom solution structure. Five octapeptides with analogous sequences to the ovomucoid decapeptide were probed for sequence-specific stabilization of the proposed turn conformation. While NH exchange in aqueous solution was found to be dominated by the hydrophobicity of local amino acid sidechains, both the lowered NH temperature coefficients and the decreased NH exchange rates in DMSO for the Xaa (n+1) NH residue of the N-glycosylation site were consistent with the gamma-type turn conformer existing in DMSO. Our results indicate that the N-glycosylation site in glycodecapeptide 1 is stabilized by peptide mainchain-sidechain H bonds and by peptide-sugar interactions. This structure may be important in protein folding.

CZ 102 HEAVY CHAIN CONSTANT DOMAINS INFLUENCE APPARENT SPECIFICITY OF IgG ANTIBODIES FOR NEO-GLYCOCONJUGATES OF VARYING EPITOPE DENSITY, Neil S. Greenspan, Laurence J. N. Cooper, Alexander R. Shikhman*, and Madeleine W. Cunningham*, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, and *Department of Microbiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

We have previously shown that variable domain-identical mouse IgG monoclonal antibodies (mAb) specific for the N-acetyl-glucosamine (GlcNAc) residues of streptococcal group A carbohydrate bind with different functional affinities to three strains of group A streptococci of varying epitope density. In this study, we show that IgG3, IgG1, and IgG2b mAb exhibit substantial differences in binding to GlcNAc-bovine serum albumin (GlcNAc-BSA) conjugates, and the relative binding patterns are substantially influenced by the molar ratio of GlcNAc to BSA. The IgG3 mAb binds better than the IgG1 and IgG2b mAb to $GlcNAc_{50}$-BSA. However, the IgG2b mAb binds slightly more effectively to $GlcNAc_{20}$-BSA than the IgG3 mAb. Using GlcNAc:BSA ratios of 10:1, 5:1, and 1:1, the IgG2b mAb clearly binds better than the IgG3 mAb. The IgG1 mAb remains the weakest binder at all GlcNAc:BSA ratios. Since these mAb differ only in the heavy chain constant domains, these functional differences must ultimately be attributed to structural differences in the C_H domains. The ability of IgG3 mAb to bind cooperatively to multivalent antigens, through a mechanism dependent on intact Fc regions, and IgG subclass-associated differences in segmental flexibility are likely to be involved in these IgG subclass-associated differences in binding to multivalent antigen.

CZ 103 THE METAL AND SACCHARIDE BINDING SITES OF THE LENTIL LECTIN : AN X-RAY STUDY. Remy Loris, Jan Steyaert and Lode Wyns. Laboratorium voor Ultrastructuur, Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium.

The X-ray structure of the orthorhombic crystal form of the lentil lectin was determined by molecular replacement using the 1.7 Å resolution pea lectin model and refined at 2.3 Å resolution. The final R-value for all data between 7.0 and 2.3 Å resolution is 16.02 % and deviations from ideal bond distances and angles are 0.018 Å and 2.5° respectively. The C-terminus of the ß-chain proved to be some 25 amino acids larger than previously anticipated. This together with several inconsistencies between the previously determined amino acid sequence and the observed electron density forced a redetermination of the amino acid sequence of the protein. The overall structure is very similar to that of pea lectin and isolectin I of *Lathyrus ochrus*, the most prominent deviations being confined to loop regions and the regions of intermolecular contact. The largest difference between the pea and lentil lectins is situated in the region of amino acids 73 to 79 of the ß-chain. As the other legume lectins, each lentil lectin monomer contains one calcium ion in a highly conserved environment. The transition metal binding site is different from the other legume lectin structere as the manganese is penta- and not hexacoordinated. The puttative monosaccharide binding cleft apparently contains an acetate molecule.

CZ 104 ARE THEY RIGID OR FLEXIBLE? CONFORMATIONAL ANALYSIS OF A BRANCHED TRISACCHARIDE AND A (1→6) LINKED DISACCHARIDE, Thomas Peters and Thomas Weimar, Institute for Biophysical Chemistry, University of Frankfurt, 6000 Frankfurt/M. 70, Germany

It is a much debated question to what extent carbohydrate chains may, or even must, adapt to protein binding pockets. First, it is important to define the inherent flexibility of the saccharide epitopes in question. From conformational analysis studies performed during the past ten years a picture emerged indicating that although certain oligosaccharide fragments such as the blood group antigens are rather rigid (1), others exhibit considerable flexibility (2). Here, we would like to present the conformational analysis of two saccharides one of which belonging to the rigid and the other to the flexible "class". The emphasis will be on a method which allows to "detect" flexibility or rigidity on the basis of NMR experimental data. One dimensional transient NOE buildup curves have been obtained for the N-glycoprotein type disaccharide α-L-Fuc(1→6)-β-D-GlcNAc-OMe **1** and for the branched trisaccharide β-D-ManNAc(1→4)[α-D-Glc(1→3)]-L-Rha **2** which represents the repeating unit of the *O*-antigen of *Aeromonas salmonicida*. Experimental buildup curves were compared to theoretical curves from Metropolis Monte Carlo (MMC) simulations (3) and from minimum energy calculations performed with the program GEGOP (4). It turned out that the use of different temperature parameters for the MMC simulations allowed the identification of NOEs that are sensitive to varying conformation distributions at glycosidic linkages, which gives a handle to potentially discriminate between different conformational models that include flexibility at glycosidic linkages. Implications for potential binding reactions with receptor proteins will be discussed.

(1) H. Thøgersen, R. U. Lemieux, K. Bock, and B. Meyer, Can. J. Chem., 60 (1982) 44-57. (2) D. A. Cumming, and J. P. Carver, Biochemistry, 26 (1987) 6664-6676. (3) T. Peters, B. Meyer, R. Stuike-Prill, R. Somorjai, and J.-R. Brisson, Carbohydr. Res., in press. (4) R. Stuike-Prill, and B. Meyer, Eur. J. Biochem., 194 (1990) 903-919.

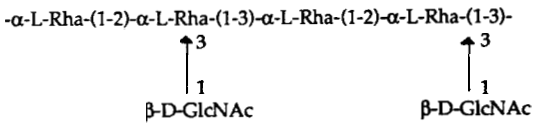
CZ 105 MOLECULAR INTERACTION OF SYNTHETIC OLIGOSACCHARIDES WITH ANTITHROMBIN III, Maurice Petitou*, Lionel Mourey**, Jean P. Samama** and Marc Pascal*. *Department of Carbohydrate Chemistry, Sanofi Recherche, 94256 Gentilly, France. **Laboratoire de Cristallographie Biologique, IBMC/CNRS, 15, rue René Descartes, 67084 Strasbourg, France.

Antithrombin III (AT III) is a member of the serine protease inhibitors superfamily (serpins) which circulates in plasma in an inactive form. AT III can be activated by heparin, and a specific pentasaccharide sequence in this polysaccharide is the key for this activation. We have synthesized the corresponding pentasaccharide and several analogs, in order to precisely assess the role of the different structural features in the interaction with AT III (1). We have also studied the conformation of the pentasaccharide (2) and we concluded that the molecule can be divided into two rigid parts with a "flexible" iduronic acid residue acting as a hinge between them. We now have at our disposal the 3-D structure of AT III deduced from crystallography studies (3). Preliminary analysis supports the idea that the interaction of AT III and the heparin pentasaccharide takes place in two steps: the first involves interaction of the non reducing end trisaccharide with basic amino acid residues on the D helix of the protein; the second is a conformational change, both at the protein and the pentasaccharide sides, which reinforces the binding and exposes the reactive site loop to cleavage by target proteinases (factor Xa, plasmine). This model is also compatible with enzyme kinetics data (4).

References:
1. Petitou M. and van Boeckel C.A.A., Progr. Nat. Prod. Chem., 60(1992)143-210. **2.** Ferro D.R. *et al*, J. Am. Chem. Soc. 108(1986)6773-6778. **3.** Mourey L. *et al*, submitted for publication. **4.** Olson S.T. *et al*, J. Biol. Chem., 267(1992)12528-12538.

CZ 106 STRUCTURE OF THE STREPTOCOCCI GROUP A POLYSACCHARIDE DETERMINED FROM MOLECULAR DYNAMICS CALCULATIONS, J. Bruce Pitner, Molecular Biology Department, Becton Dickinson Research Center, P.O. Box 12016, Research Triangle Park, NC 27709

The *Streptococci* Group A polysaccharide is composed of a repeating L-rhamnose backbone with alternating 1-3 and 1-2 linkages and a branched N-acetyl glucosamine residue linked 1-3 to each second rhamnose (see below). Molecular dynamics simulations were performed on representative fragments of the polysaccharide to determine the preferred torsional angles between units. An AMBER-type potential function was used for the simulations with Homans carbohydrate parameters. A helical geometry was obtained for the rhamnose backbone with the N-acetyl glucosamines positioned perpendicular to the helix axis. The influence of temperature, interval length, and other dynamics variables on the calculations will be discussed.

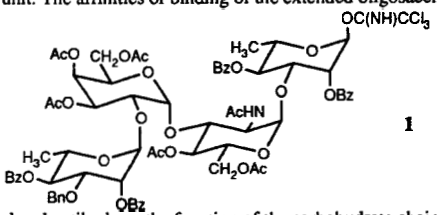


CZ 107 SYNTHESIS OF EXTENDED FRAGMENTS OF THE O-POLYSACCHARIDE OF *Shigella dysenteriae* TYPE 1 AND THEIR INTERACTIONS WITH A MONOCLONAL ANTIBODY Vince Pozsgay, Cornelis P.J. Glaudemans, John B. Robbins, Rachel Schneerson *National Institute of Diabetes, Digestive and Kidney Diseases and National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892*

Shigella dysenteriae type 1 is a Gram-negative human pathogen causing dysentery with a high incidence of mortality. There is evidence that antibodies to the native, O-specific polysaccharide (O-SP), which is composed of the tetrasaccharide repeating unit **A**, confer protection on

2)-a-D-Gal*p*-(1→3)-a-D-Glc*p*NAc-(1→3)-a-L-Rha*p*-(1→3)-a-L-Rha*p*-(1→ **A**

the host and it is possible that such protection can also be achieved by a synthetic vaccine containing structurally well-defined, synthetic fragments of the O-SP. In order to relate the immunogenic characteristics to the size of the carbohydrate chain we are currently preparing extended fragments related to **A**. These haptens will be covalently linked to an immunogenic protein for use as synthetic antigens. The synthesis of carbohydrates of such complexity is not without difficulty and requires the determination of a set of optimized conditions for each step to achieve acceptable overall yields. The synthetic strategy adopted involves the preparation of four heterofunctional monosaccharide intermediates which were combined in a highly stereoselective, stepwise manner to provide the tetrasaccharide repeating unit donor/acceptor molecule **1**. Sequential combinations of compound **1** afforded oligomers of the repeating unit. The affinities of binding of the extended oligosaccharides



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to a monoclonal antibody as the function of the carbohydrate chain-length will be reported.

CZ 108 **ANALYSIS OF SEQUENCE-FUNCTION RELATIONSHIPS IN OLIGOSACCHARIDES USING ARTIFICIAL INTELLIGENCE TECHNIQUES.** Robert A. Rastall, Simon J. E. Taylor and Christopher Bucke, Division of Biotechnology, and the Centre for Parallel Computing, University of Westminster, London W1M8JS.

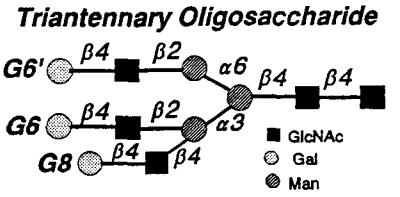
An expert system to perform sequence analysis of oligosaccharides is being developed. Information on the biological activities of oligosaccharides, for instance receptor and antigenic activities, *in vitro* bioactivities, pathological associations and biological sources, is being collated. To enable use of knowledge-based systems, biological information has been encoded into frames and a semantic network.

In addition, sequence comparison is facilitated by use of a directed acyclic graph representation and similarity matrices. Initially, 2D pattern recognition techniques are being applied to prove the viability of the sequence comparison approach, this will later be extended to 3D and 4D pattern recognition.

The system is capable of explaining its reasoning process and thus qualifying any identification of sequence-function relationships. To date the system contains a sample knowledge base of sequences and associated biological information pertaining to the gangliosides.

CZ 109 PRECISE MODE OF BINDING BETWEEN TRIANTENNARY GLYCOPEPTIDE AND THE RAT HEPATIC LECTIN, Kevin G. Rice and Yuan C. Lee, College of Pharmacy, Ohio State University, Columbus, OH 43210, and the Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

The rat hepatic lectin (*RHL*) functions in clearance of desialylated serum glycoproteins. Previous studies established that a single type of N-linked triantennary oligosaccharide (shown below) binds to the lectin with optimal affinity. We constructed photoaffinity labeling probes from this triantennary glycopeptide by attaching a photolyzable group individually to the C6 position of each terminal galactose residue. Photoaffinity labeling isolated rat hepatocytes with these probes demonstrated that galactose residue **G6** and **G6'** bind to sites on *RHL1*, whereas galactose residue **G8** binds to a site on *RHL2/3*. Thus, the three dimensional conformation of galactose residues on triantennary oligosaccharide apparently reflect the arrangement of complementary binding sites on the receptor.



Therefore, we have examined the conformation of the triantennary glycopeptide by attaching a donor fluorophore to the N-terminus of the glycopeptide and an acceptor fluorophore individually to each terminal galactose residue. Time-resolved fluorescence energy transfer experiments revealed flexibility in isomers containing the acceptor attached to galactose **G6** or **G6'** by the presence of two populations of donor/acceptor distances representing the antennas in extended and folded conformations. Conversely, the antenna containing galactose **G8** was conformationally rigid. This unexpected correlation between antenna flexibility and specificity of binding of triantennary to the rat hepatic lectin allows us to propose that antenna flexibility may promote proper docking of target residues to the lectin.

CZ 110 CARBOHYDRATE-LECTIN INTERACTIONS AS STUDIED BY ^{1}H-NMR SPECTROSCOPY

Herman van Halbeek and Leszek Poppe, Complex Carbohydrate Research Center and Department of Biochemistry, The University of Georgia, Athens, GA 30602-4712

Determining the three-dimensional structure of carbohydrates in solution is critical to understanding their biological function. Although most carbohydrates are highly flexible and adopt multiple conformations when free in solution, the conformation and dynamics of free oligosaccharides in aqueous solution can be probed by *(i)* measurement of scalar $^3J_{HH}$ couplings, *(ii)* measurement of inter-glycosidic scalar $^3J_{CH}$ couplings, and *(iii)* detection of interglycosidic {^{1}H,^{1}H} NOEs, at various magnetic field strengths and sample temperatures [1]. Appropriate experimental conditions (solvent system, sample temperature) enable us to use OH and NH protons in the NOE measurements [1,2]. NMR spectroscopy is even more powerful in the comparison of oligosaccharides in their free state versus bound to a lectin of relatively large molecular weight. The conformation in the bound state, as well as the changed dynamics of the oligosaccharide can be probed, *e.g.*, by transfer-NOE measurements. We will illustrate various aspects of analyzing the conformation and internal dynamics of sialyl-α(2→6)-lactose [2] and sialyl-α(2→3)-lactose, both in free state and bound to lectins (*Sambucus nigra* agglutinin and *Maackia amurensis* leukoagglutinin, respectively).

[This research is supported by NIH grant P41-RR-05351]

[1] H. van Halbeek and L. Poppe (1992) *Magn. Reson. Chem.* **30**: in press.
[2] L. Poppe, R. Stuike-Prill, B. Meyer and H. van Halbeek (1992) *J. Biomol. NMR* **2**: 109-136.

CZ 111 PURIFICATION, CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES ON THE RHIZOME LECTIN FROM STINGING NETTLE AND ITS COMPLEX WITH NN'N"TRIACETYLCHITOTRIOSE. Lode Wyns, Minh Hoa Dao Thi and Remy Loris. Laboratorium voor Ultrastructuur, Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium.

Single crystals were grown from affinity-purified stinging nettle lectin and from its complex with the specific trisaccharide NN'N"triacetylchitotriose by vapour diffusion at room temperature. The lectin crystallizes in space group $P2_12_12_1$ with unit cell dimensions a = 54.3 (1) Å, b = 62.2 (1) Å and c = 92.4 (2) Å. and diffracts to 3.0 Å resolution. The assymmetric unit contains three lectin monomers. The crystals of the lectin-trisaccharide complex have space group $P2_12_12_1$ with cell constants a = 37.69 (4) Å, b = 48.97 (6) Å and c = 57.32 (4) Å. These crystals diffract to at least 2.0 Å resolution and the asymmetric unit contains one lectin monomer. The two domains of the stinging nettle lectin show homology with the four domains from the dimeric wheat germ agglutinin, which also recognizes oligomers of N-acetylglucosamine. In WGA, the carbohydrate recognition sites are formed by the combining sites of two hevein domains from the two monomers. It would therefore be interesting to see how a monomeric protein consisting of only two such domains can possess more than one carbohydrate recognition site. Also the molecular interaction between the two domains themselves would be of interest, since in WGA only few direct interactions between domains belonging to the same polypeptide are observed. A three-dimensional X-ray structure determination is on its way.

Selectins and Related Adhesive Anomena

CZ 200A CARBOHYDRATE BINDING PROTEIN (CBP-35) IS REGULATED BY MACROPHAGE COLONY STIMULATING FACTOR CSF-1, Gary Balian, Prema Ramakrishna and Peter Quesenberry. University of Virginia School of Medicine, Charlottesville, VA 22908

A 35 kilodalton polypeptide which is synthesized by bone marrow cells is sensitive to degradation with bacterial collagenase. This protein does not contain hydroxyproline, synthesis is not stimulated by the addition of ascorbate or transforming growth factor-β and it is therefore not a collagenous component of the extracellular matrix. Synthesis of 35k is elevated in bone marrow macrophages, and in the cell line P388D_1. Chemical cleavage of 35k prepared from these cells and amino acid sequencing of its fragments revealed the identity of 35k as carbohydrate binding protein-35 which is a galactose-specific lectin and forms part of the heterogeneous nuclear ribonucleoprotein complex that binds RNA. CBP-35 mRNA produced by macrophages in response to colony stimulating factor-1 was inhibited at 50 units per ml CSF-1 and stimulated at 500 and 2000 units per ml. CBP-35 is immunologically indistinguishable from MAC-2, a macrophage cell surface protein which is a non-integrin receptor for laminin. Whereas antibodies to MAC-2 localized to the surface of macrophages and P388D_1, cells from the bone marrow stroma showed only intracellular localization with anti-MAC-2 and no cell surface staining. The effects on CBP-35 mRNA particularly with respect to the various locations of the 35k protein in bone marrow stromal cells and in macrophages may be important to the process of differentiation of bone marrow cells. The recognition by MAC-2 of carbohydrates that are present on extracellular matrix proteins may be a crucial event.

CZ 201CARBOHYDRATE MEDIATED ADHESION OF HUMAN T CELLS TO THYMIC EPITHELIUM. Linda Baum[1], Mabel Pang[1], Terry Wu[1], Nancy Perillo[1], Angelo Delageane[2] and Jeff Seilhamer[2], Department of Pathology, UCLA School of Medicine, Los Angeles CA 90024[1], and Incyte Pharmaceuticals Inc., Palo Alto, CA 94304[2].

Interactions between thymic epithelium and immature thymocytes are crucial for proper development of immunocompetent T cells. We have previously identified a soluble galactose-specific lectin in human thymic epithelial cells. In vitro binding assays, in which primary cultures of thymic epithelial (TE) cells bind radiolabelled T-lymphoblastoid cell lines (T-LCL), demonstrate that binding of T-LCL to TE cells is inhibited by the presence of lactose and thiodigalactoside, indicating that the TE cell galctose-specific lectin may mediate T-cell binding. In the presence of the glycoprotein inhibitors fetuin and bovine submaxillary mucin, decreased binding was observed; however, no inhibition of binding was seen with alpha 1-acid glycoprotein, transferrin and thyroglobulin, suggesting that O-linked oligosaccharides contain the ligand(s) recognized by the TE cell lectin.

To identify possible ligands on the surface of T-LCL, lectin blots were performed on extracts of T-LCL membrane proteins, using recombinant lectin conjugated to biotin. These experiments indicate that two major O-glycosylated T cell glycoproteins bind the lectin. Further studies are aimed at defining the role of these T cell glycoproteins in mediating the observed adhesion of T-LCL and TE cells, and determining the role of lectin-ligand interactions in thymocyte development.

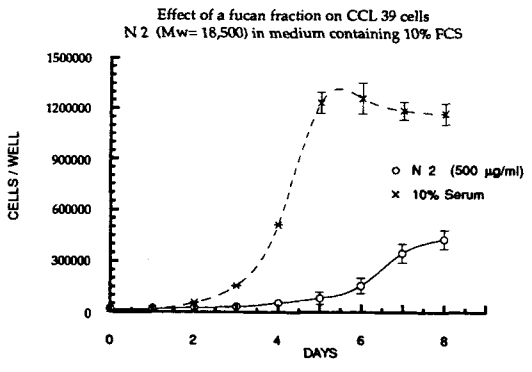
CZ 202 COMPLEXITY OF CARBOHYDRATE STRUCTURES ASSOCIATED WITH THE PERIPHERAL LYMPH NODE ADDRESSIN. Ellen L. Berg, Jodi Goldberg, David P. Andrew, and Eugene C. Butcher, Department of Pathology, Stanford University Medical School, Stanford, CA 94305 and the Center for Molecular Biology in Medicine, Veterans Administration Medical Center, Palo Alto, CA 94304.

Minimal carbohydrate structures have been described as ligands for the selectins, L-, P-, and E-selectin; however, it is likely that the natural ligands for these cell adhesion molecules are much more complex. Our initial indication of this was the finding that although L-selectin weakly recognizes Sialyl Lewis a- and Sialyl Lewis x-containing neoglycoconjugates, these structures are not expressed by high endothelial venules (HEV) and are not recognized by mAb MECA-79. On the other hand, MECA-79 recognizes most or all components of the peripheral lymph node vascular addressin, PNAd, a naturally occurring ligand(s) for L-selectin expressed on HEV in mouse and man. More recent experiments suggest that even more complexity of L-selectin ligands exists. We have developed a panel of mAbs against purified PNAd, immunoisolated from human tonsil. Although these mAbs appear similar to MECA-79, as their reactivity by immunohistochemistry on human tonsil is restricted to HEV and on Western blot they recognize the same pattern of glycoprotein species as does MECA-79, these mAbs appear to recognize distinct epitopes. The determinants recognized by many of these mAbs are sensitive to neuraminidase, unlike the MECA-79 epitope, and strikingly, while MECA-79 recognizes a subset of venules in appendix, several of these mAbs do not. These findings point to significant diversity in the carbohydrate determinants expressed by HEV; demonstrate that these are differentially represented in different sites *in vivo*; and underscore the remarkable ability of monoclonal antibodies to detect differences in carbohydrate structure. It is interesting to speculate that differences in the structure of carbohydrate L-selectin ligands may be associated with functional differences in the capacity of specific leukocyte subsets to be recruited into lymphoid and inflammatory tissues. *(Supported by grants from the NIH and by the Veterans Administration. E.L.B. is a Special Fellow of the Leukemia Society of America, D.P.A. is a fellow of the A.H.A. and E.C.B. is an Established Investigator of the A.H.A.)*

CZ 203 EFFECT ON TUMOR CELLS OF FUCANS, SULFATED POLYSACCHARIDES EXTRACTED FROM BROWN SEAWEEDS, M. Ellouali, C. Boisson-Vidal and J. Jozefonvicz, LRM, University Paris-XIII, 93430 Villetaneuse , France.

Glycosaminoglycans are common constituents of the extracellular matrix and cell membranes. They serve as specific information-bearing components involved in several biological functions such as cell adhesion, regulation of receptor functions, cell growth and cell migration. Sulfated polysaccharides such as heparin and fucans extracted from brown seaweeds could exhibit biological activities with potential interests in pharmacology and medicine. Studies have shown that fucans inhibit tumoral cell growth *in vitro* and/or *in vivo*.

To understand the fucan action mechanism as well as to establish a possible relationship between anticoagulant and antitumoral activities, we have tested *in-vitro* the purified fractions extracted from Ascophyllum nodosum on several tumor cell lines. The inhibitory effect of these fractions on tumor cells is related to the sulfate group content and the serum concentration. In the various phases of the cell cycle, no modification of the cell distribution was observed.



CZ 204 UNRAVELLING THE BINDING SPECIFICITIES OF E-SELECTIN USING OLIGOSACCHARIDE PROBES (NEOGLYCOLIPIDS) DERIVED FROM O- AND N-GLYCOSYLATED PROTEINS, T. Feizi[1], C-T. Yuen[1], M.S. Stoll[1], A.M. Lawson[2], W. Chai[2], M. Larkin[1], J. O'Brien[1], F.X. Sullivan[3], A.C. Stuart[3], T.J. Ahern[3] and D. Cumming[3], [1]Glycoconjugates and [2]Clinical Mass Spectrometry Sections, MRC Clinical Research Centre, Harrow, Middx HA1 3UJ, UK, and [3]Genetics Institute, Inc, Cambridge MA 01240.

The inducible endothelial adhesion molecule E-selectin which has a crucial role in the initial stages of leucocyte recruitment to sites of inflammation, may also be involved in the blood-borne spread of tumour cells. Using recombinant full length E-selectin expressed as a transmembrane protein in transfected Chinese hamster ovary cells (E-CHO), and neoglycolipids as immobilized oligosaccharide probes, we have been making two approaches to elucidating the carbohydrate-binding specificities of this cell adhesion molecule. In the first approach (J. Biol. Chem. 267, 13661-13668, 1992), the binding of the ^{3}H-labelled E-CHO was evaluated to neoglycolipids derived from a series of structurally defined human milk oligosaccharides whose sequences are homologous to those on membrane glycolipids. Results showed a density-dependent binding of the membrane-associated selectin not only to 3'-sialyl-Le^{a}- and 3'-sialyl-Le^{x}/SSEA-1-type sequences, but also to their asialo analogues. In the second approach, the subject of the present communication, binding of E-CHO to neoglycolipids derived from the sugar chains of O- and N-glycosylated proteins is being investigated. Results are providing further insights into the combining specificities of E-selectin. In particular, a novel class of oligosaccharide ligand: sulphate-containing, has been revealed among the O-linked oligosaccharides on a human tumour-derived glycoprotein. This binding activity is substantially greater than those of lipid-linked Le^{a} and Le^{x}/SSEA-1 sequences and is at least equal to that of the 3'-sialyl-Le^{x}/SSEA-1 analogue synthesised by A. Hasegawa, M. Kiso and associates.

CZ 205 MONOMERIC, MONOVALENT DERIVATIVES OF SIALIC ACID SPECIFIC LECTINS: PREPARATION AND APPLICATION FOR THE STUDY OF CELL SURFACE GLYCO-CONJUGATES BY FLOW CYTOMETRY

Hanae Kaku[1)], Yasuyuki Mori[2)], Irwin J. Goldstein[3)] and Naoto Shibuya[1)], 1) *Dept. Cell Biology, Natl. Inst. Agrobiol. Resources, Tsukuba, Ibaraki, Japan, 2) Dept. Biol. Product, Natl. Inst. Animal health, Tsukuba, Ibaraki, Japan, 3) Dept. Biol. Chem., Univ. Michigan, Ann Anbor, Michigan.*

Lectins are multivalent protein/glycoprotein having ability to bind carbohydrates. Therefore, they are able to agglutinate cells and precipitate polysaccharides and glycoproteins. However, the multivalent nature of lectins sometimes causes problems for their application, e.g., to flow cytometry/cell sorting.

We have established the stable subunits of *Sambucus sieboldiana* bark lectin (MSSA; specific to Neu5Acα2-6Gal/GalNAc sequence) and *Maackia amurensis* leukoagglutinin (MMAL; specific to Neu5Acα2-3Galβ1-4GlcNAc/Glc sequence) by the selective reduction of disulfide bridges between the subunits and following alkylation with 4-vinylpyridine[1)]. Both MSSA and MMAL failed to agglutinate rabbit erythrocytes or CHO cells and also failed to precipitate fetuin. Analysis of their interaction with fetuin using ELISA which does not require the presence of multiple binding sites, however, revealed that MSSA and MMAL still retained the ability to bind carbohydrates including the original specificity towards the sialylated oligosaccharides.

Flow cytometric analysis showed that human histocytic lymphoma U937 cells were clearly stained with FITC-labeled MSSA (FITC-MSSA) without any detectable agglutination. Similarly, FITC-MMAL stained CHO cells too. This staining was inhibited with the oligosaccharides/glycoconjugates corresponding to the binding specificity of these lectins. Both of these monomeric derivatives could be used in a concentration range which gave enough bright staining for the detection whereas the native one suffered from the agglutination to get enough bright staining. Using these two fluorescent probes, differential expression of sialylated glycoconjugates on the cell surface of several cell lines could be shown.

These observations suggest that these monomeric lectin derivatives can be invaluable tools for the study of the expression of cell surface glycoconjugates containing α2-6 and α2-3 linked sialic acid sequences by flow cytometry.

1) Kaku, H. and Shibuya, N., *FEBS Lett.*, **306**, 176-180 (1992).

CZ 206 VARIANT SIALYL LEWIS X ANTIGENS EXPRESSED ON HUMAN MEMORY T CELLS AS DEFINED BY A MONOCLONAL ANTIBODY: **Reiji Kannagi,** Laboratory of Experimental Pathology, Research Institute, Aichi Cancer Center, Chikusa-ku, Nagoya, 464, JAPAN.

A murine monoclonal antibody (2F3, IgM), raised against a chemically synthesized sialyl Lewis X glycolipid, was found to react preferentially with a subset of CD4^{+} CD45RA^{-} memory T cells among peripheral lymphocytes in healthy individuals. The specificity of the antibody was in clear contrast to that of typical hitherto reported anti-sialyl Lewis X antibodies, such as SNH-3, CSLEX-1 and FH-6. These classical anti-sialyl Lewis X antibodies reacted with human NK cells, but did not detect any particular subset of resting T cells in the peripheral blood of healthy individuals, if the cells were not activated. 2F3 was reactive to exactly the same series of glycolipid antigens as SNH-3 and other typical anti-sialyl Lewis X antibodies, but recognized a distinct set of carbohydrate side chains of glycoproteins, which were quite different from those recognized by the typical anti-sialyl Lewis X antibodies. A subset of memory T cells is known to adhere to E-selectin, but the problem has been that these cells do not appear to express the sialyl Lewis X antigen, the ligand for E-selectin, at least when the typical anti-sialyl Lewis X antibodies were used for detection. Our results suggest that various molecular species of sialyl Lewis X antigens are present on carbohydrate side chains of cellular glycoproteins, and also that human memory T cells preferentially express variant type sialyl Lewis X antigens, which are reactive with 2F3, but are not detected by the typical anti-sialyl Lewis X antibodies.

CZ 207 GANGLIOSIDES AS LIGANDS FOR SIALOADHESIN, A CELL ADHESION RECEPTOR ON MACROPHAGES

S. Kelm[a], C. Dubois[b], J. Müthing[c], R. Schauer[a], P.R. Crocker[d]
[a]Biochemisches Institut, University of Kiel, Kiel, Germany
[b]Institut Pasteur, Paris, France; [c]Institut für Zellkulturtechnik, University of Bielefeld, Bielefeld, Germany; [d]ICRF, Institute for Molecular Medicine, University of Oxford, Oxford, England;

Sialoadhesin is a macrophage restricted receptor which mediates specific adhesion of cells through recognition of suitable glycoconjugate ligands. Especially, high binding of sialoadhesin was observed to bone marrow cells, some leukocyte populations and lymphoma cell lines. A function of sialoadhesin in leukocyte maturation and trafficking had been proposed, based on its specificity and on its localization in bone marrow and at specific sites in lymph nodes and spleen. In a previous study[1] it has been shown that sialoadhesin specifically binds to glycoconjugates carrying the terminal structure Neu5Acα2→3Galβ1→3GalNAc.

To study potential ligands for sialoadhesin, glycosphingolipids were extracted from cells of interest and analyzed in a TLC overlay assay using labelled sialoadhesin. In addition, glycolipids purified from such extracts with defined structure were used to verify the bands to which sialoadhesin bound in the TLC overlay assays. With sialoadhesin, series of bands were identified in extracts from bone marrow cells, neutrophils, and murine lymphoma MDAY-D2, a cell line which binds sialoadhesin very well. In contrast, no bands could be detected in lymphoma cell lines D33-W25 or YAC-1, which express similar glycosphingolipids as MDAY-D2. However, whereas glycolipids from MDAY-D2 mainly contain Neu5Ac, for the other cell lines Neu5Gc is the major sialic acid.

Since the data from cell binding assays correlate well with the occurrence of glycolipids which bind sialoadhesin, we can assume that the expression pattern of glycolipids on cells can modulate interactions of these cells with macrophages which are mediated by sialoadhesin.

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

CZ 208 **SELECTIN-LIKE ACTIVITY OF MEMBRANE-ASSOCIATED C-REACTIVE PROTEIN ON RAT AND HUMAN MACROPHAGES PLAYS A KEY ROLE IN UPTAKE OF TOXIC SILICA PARTICLES.**
Victoria Kolb-Bachofen and Christine Egenhofer, Institute for Immunobiology, Fac. of Medicine, Heinrich-Heine-University, Moorenstr.5, D-4000 DÜSSELDORF, FRG.
Quarz dust particles are known to excert rapid and selective killing of macrophages after their uptake into the phagocytes. Killing is dependent on uptake of the particles and leads to destruction of phagolysosomes as the earliest event.
Experiments were made to describe the opsonization mechanism(s) and the macrophage receptor(s) involved in silica uptake. Freshly isolated rat liver macrophages (Kupffer cells), rat alveolar macrophages or human monocytes were incubated at 37°C with silica particles in the presence or absence of autologous plasma or purified plasma fibronectin and cell viability assessed by trypan blue exclusion at various times.
During cocincubation with silica particles >80% of macrophages were lysed in the presence of plasma or purified fibronectin but not in their absence (viability >90%).
Macrophages could be protected from lysis by addition of the monosaccharide GalNAc but not by GlcNAc. Galactosylated albumin exerted full protection from lysis but not mannosylated albumin nor native albumin. The pentapeptide GRGDS also prevented macrophage lysis in synergy with N-acetyl-galactosamine. Enzymatic deglycosylation of fibronectin reduced lysis significantly. Binding experiments (at 4°C) revealed initial binding as primarily galactose-inhibitable, suggesting integrin-mediated binding as a later event necessary for effective uptake. Data indicate a prime opsonizing activity for fibronectin and dual recognition via the lectin-like galactose-specific binding activity of membrane associated C-reactive protein on the liver macrophages and by integrin receptor(s).

CZ 209 **USE OF A FUCOSYLTRANSFERASE FROM HUMAN MILK IN THE SYNTHESIS OF SIALYL LEWIS^a AND SIALYL LEWIS^x TETRASACCHARIDES MODIFIED AT THE C-2 POSITION OF THE REDUCING UNIT,**
P.V. Nikrad, M.A. Kashem[1], K.B. Wlasichuk[1], G. Alton[2] and A.P. Venot[1]. [1]Alberta Research Council, Carbohydrate Research Program, Box 8330, Stn. F., Edmonton, Alberta, CANADA, T6H 5X2. [2]Department of Food Sciences, University of Alberta, Edmonton, Alberta, CANADA, T2G 2E1.

The fucosyltransferase preparation from human milk contains the co-purified Gal(ß1-3/4)GlcNAc α1,3/4-FT (Lewis enzyme) and the Gal(ß1-4)GlcNAc α1,3-FT (serum enzyme). This enzyme transfers L-fucose to type I (Gal(ß1-3)GlcNAc and type II (Gal(ß1-4)GlcNAc) acceptor analogues modified at the C-2 position of the reducing unit. L-fucose was transferred to the synthetic Neu5Ac(α2,3)Gal(ß1-3/4)GlcX-OR trisaccharides (X=N_3, NH_2, NH-CO-CH_2-CH_3) and R=$(CH_2)_8CO_2CH_3$). The sialyl Lewis^a and sialyl Lewis^x analogues were obtained and characterized by ^{1}H-n.m.r.

CZ 210 **IDENTIFICATION OF NEW α(1,3)FUCOSYLTRANSFERASE ACTIVITIES OF CHO CELLS,**
Barry Potvin, Diana Yoon and Pamela Stanley, Department of Cell Biology, Albert Einstein College of Medicine, New York, NY 10461

The critical role of α(1,3)fucosyltransferases (α(1,3)Fuc-T) in synthesizing carbohydrate ligand(s) recognized by the selectin group of cellular adhesion molecules has motivated attempts to differentiate between the numerous α(1,3)Fuc-Ts that exist in mammalian cells. A major goal of these efforts is to determine which of the enzymes may be required for particular cell recognition events.

Chinese hamster ovary (CHO) cells do not express α(1,3)Fuc-T activity. However, following mutagenesis and selection, mutants that synthesize different α(1,3)Fuc-T activities have been isolated. Four mutants that express α(1,3)Fuc-T with distinct properties that are expected to be the products of different genes have been termed LEC11, LEC12, LEC29 AND LEC30. Since the number of mammalian α(1,3)Fuc-T activities that might participate in the synthesis of selectin ligands is greater than four, additional CHO mutants expressing novel α(1,3)Fuc-T activities were sought. Three new cell lines have been isolated from screens for α(1,3)Fuc-T - expressing cells from populations of CHO cells subjected to different mutagenic treatments. The properties of the α(1,3)Fuc-T activities of these lines will be compared with those of the previously isolated CHO mutants. Comparisons of substrate specificity, metal ion requirements, inhibitor sensitivities, kinetic properties, and pH requirements are in progress. One of the new mutants (LEC31) has an α(1,3)Fuc-T with properties similar to those of LEC12 and LEC30 but is distinguished by altered kinetic properties. The other two new mutants have an α(1,3)Fuc-T with properties similar to LEC11 CHO cells but can be distinguished by an increased requirement for $MnCl_2$.

A panel of monoclonal antibodies that recognize α(1,3)Fuc-T residues in different contexts and can be used to assay the cellular products of the different α(1,3)Fuc-T's provide further evidence that the LEC31 a(1,3)Fuc-T is a novel activity compared to those previously described in CHO cells. The combined data indicate that at least one and up to three new α(1,3)Fuc-T activities have been identified. Two of the new mutants synthesize sialyl-Le^x, a ligand recognized by the selectin group of molecules.

CZ 211 **EXPRESSION OF AN ENDOGENOUS SOLUBLE GALACTOSE SPECIFIC LECTIN IN HUMAN LYMPHOID TISSUES.**
Jeff Seilhamer[1], Mabel Pang[2], Nancy Perillo[2], and Linda Baum[2], Incyte Pharmaceuticals, Inc., Palo Alto, CA 94304[1], and Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024[2].

Administration of exogenous soluble galactose-specific lectins are known to modulate autoimmune processes in a number of animal disease models. However, it is not clear what role the endogenous homologues of these lectins play in regulating the immune system. To address this, we examined the distribution of a galactose specific lectin in human lymphoid tissue by immunohistochemical analysis. A striking pattern of lectin production was observed in lymph nodes. Reactive cells were found in germinal centers, the site of antibody-producing B cell proliferation, as well as in interfollicular zones, which contain primarily T cells. Staining was also seen in the vessel walls. However, mantle zones, which contain quiescent B cells, showed no reactivity. These findings suggest a possible function for this galactose-specific lectin in homing or adhesion of peripheral lymphocytes to discrete anatomic sites within lymphoid tissues.

CZ 212 CELL SURFACE GLYCOCONJUGATES AS POSSIBLE TARGET STRUCTURES FOR HUMAN NATURAL KILLER CELLS, Hans Voshol[1], Hub F.J. Dullens[2], Willem Den Otter[2] and Johannes F.G. Vliegenthart[1], Utrecht University, [1]Bijvoet Center, Department of Bio-Organic Chemistry, PO Box 80075, 3508 TB Utrecht, The Netherlands and [2]Department of Pathology, PO Box 85500, 3508 GA Utrecht, The Netherlands.

Natural killer (NK) cells are $CD3^-$, $CD16/56^+$ lymphocytes, which display spontaneous *in vitro* cytotoxicity against a variety of tumor cells and virus-infected cells, without apparent MHC-restriction or prior antibody-sensitization. As yet, specific NK-receptors nor target structures have been defined. The purpose of our investigation is to explore whether cell surface glycoconjugates and their carbohydrate chains are involved in the interaction between NK cells and target cells.

It was established that the recognition of K562 target cells by NK cells depends on the presence of protein-bound target structures. Subsequent investigations showed that the NK-susceptibility of various N-glycosylation variants of K562 cells, obtained by enzymatic deglycosylation and treatment with glycosylation inhibitors, was comparable to that of untreated cells. Therefore, our attention is currently focused on target cell surface O-glycans and on the receptor molecules on NK cells and the clinically more relevant LAK cells (interleukin-2 stimulated NK cells).

This investigation was supported by the Dutch Cancer Society (grant UUKC 88-14).

Malignancy and Carbohydrate Recognition

CZ 213 **IDENTIFICATION OF O-GLYCOSYLATION SITES ON MUC1 TANDEM REPEAT PEPTIDES GLYCOSYLATED BY PANCREATIC AND BREAST ADENOCARCINOMA CELL LINES.** Isao Nishimori, Fulvio Perini, Kim Mountjoy, Tom Caffrey, and Michael Hollingsworth, Eppley Institute, University of Nebraska Medical Center, Omaha NE 68198-6805

The MUC1 mucin is produced by many different epithelial tissues in the body. Previous work from several laboratories suggests that different tissues and tumors derived from them perform distinct post-translational modifications (glycosylation) to this protein. We have recently undertaken a project designed to determine if there are differences among tumor cell lines derived from different organs in the activity and specificity of the UDP-GalNAc: polypeptidyl N-acetyl-galactosaminyltransferase (Gal-NAc transferase) with the MUC1 protein. We have employed an assay slightly modified from one developed in Dr. Robert Hill's laboratory, in which peptides are used as acceptor substrates for Gal-NAc transferase in cell lysates. The glycopeptides are purified and sequenced to determine positions of attachment of carbohydrate to the peptides. Optimized concentrations of UDP-[^{3}H]GalNAc and a 30 residue synthetic peptide based on the MUC1 tandem repeat were reacted in an imidazole buffer with Mn with different cell lysates for four hours. Gal-NAc transferase activity in two pancreatic adenocarcinoma cell lines (HPAF, PANC1) and two breast adenocarcinoma cell lines (BT20, MCF7) were, respectively, 680, 162, 1964, and 5578 pmol/mg protein/hour. Glycopeptides from these assays have been purified by chromatography on Dowex 1 and P-10 columns, and then HPLC on a C-18 column. Purified glycopeptides were sequenced on a Porton 2090E microsequencer and glycosylated positions determined by measuring the incorporated radioactivity in fractions collected following each round of Edman degradation and amino acid analysis. It has been possible thus far to analyze only up to 12 residues for glycosylation. A single glycopeptide species was identified by HPLC analysis in HPAF lysates and was found to be glycosylated at the underlined positions: GVTSAPDTRPAP. Two glycopeptide species were identified by HPLC analysis in MCF7 lysates, however both showed glycosylation at the following positions: GVTSAPDTRPAP. These results suggest that these two tumor cell lines may glycosylate the same peptide at distinct positions. The evaluation of additional peptide glycosylation sites within the tandem repeat, and of glycopeptides generated from other cell lysates is in progress.

CZ 214 **INCREASED EXPRESSION OF A LYSOSOMAL MEMBRANE GLYCOPROTEIN IN THE CELL SURFACE OF POORLY METASTIC COLONIC CELLS RESULTS IN STRONGER ADHESION TO E-SELECTIN EXPRESSING CELLS**

[1]Ritsuko Sawada, [2]John B. Lowe, and [1]Minoru Fukuda

[1]La Jolla Cancer Research Foundation, La Jolla, California 92037, and [2]Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Lysosomal membrane glycoproteins, lamp-1 and lamp-2 are the major carriers for poly-*N*-acetyllactosamines (for review, see *J. Biol. Chem. 261*:21327-21330, 1991). We have shown that highly metastic colonic tumor cell lines express more lamp molecules on the cell surface than poorly metastic cell lines derived from the same human colon carcinoma (*J. Biol. Chem. 267*:5700-5711, 1992). Since lamp molecules likely carry carbohydrate ligands for E- and P-selectin, we then tested if the increased expression of lamp-1 on the cell surface results in stronger adhesion to E-selectin expressing cells. Poorly metastic SP cells were transfected with cDNA encoding wild-type lamp-1 or lamp-1 mutant in which the cytoplasmic tyrosine was substituted with histidine. As shown previously, mutation of cytoplasmic tyrosine abolishes lysosomal targeting of lamp-1 and mutated lamp-1 is transported to plasma membrane instead of going to lysosomes (*J. Cell. Biol. 111*:955-966, 1990). Several clones were isolated from each transfection and representative cell lines were cloned, which show different amounts of cell surface lamps by flow cytometric analysis. In parallel, Chinese hamster ovary (CHO) cells were transfected to stably express E-selectin. The adhesion of various SP cells expressing different amounts of cell surface lamp-1 was tested for the adhesion to E-selecting expressing CHO cells, as well as IL-1β activated endothelial cells.

The results clearly indicate that the extent of the cell adhesion is directly proportional to the amount of cell surface lamp. This adhesion can be inhibited by sialyl Le^x containing glycolipid, whereas control sialylparaglogoside had no effect. Such adhesion can be inhibited by the culture medium derived from A431 cells continuously expressing soluble lamp-1, and synthesizing sialyl Le^x structures.

These results indicate that the increased surface expression of lamp-1 results in better adhesion to E-selectin expressing cells. The results suggest that these tumor cells likely adhere to endothelial cells at metastic sites through the interaction between cell surface carbohydrates of tumor cells and E- and P-selectins on endothelial cells. Further studies are necessary to determine if the increased expression of lamp-1 on the cell surface results in tumor metastasis (supported by CA 48737).

CZ 215 MONOCLONAL ANTIBODIES THAT RECOGNIZE THE TRISACCHARIDE EPITOPE (Galα 1,3 Galβ1,4GlcNAc) PRESENT ON EHRLICH TUMOR CELL MEMBRANE GLYCOPROTEINS, Masaru Takagaki, James Varani*, and Irwin J. Goldstein. Dept. Biol. Chem., *Dept. Pathology, Univ. of Michigan, Ann Arbor, Michigan 48109.

The glycoconjugates of Ehrlich ascites tumor cell plasma membranes contain a high density of α-D-Galp end groups which generally are present as the trisaccharide units Gal α1,3 Gal β1,4 GlcNAc. We have prepared monoclonal antibodies against these membrane oligosaccharides. Among hybridomas generated, we selected two clones which produced monoclonal antibodies showing reactivity towards the Gal α-1,3 Gal β 1,4 GlcNAc trisaccharide sequence and expanded them in pristane-treated BALB/c mice. The monoclonal antibodies, purified on a column of Gal α 1,3 Gal β 1,4 GlcNAc-Synsorb, recognized the Gal α1,3 Galβ 1,4 GlcNAc sequence present in murine laminin and bovine thyroglobulin. Inhibition studies employing an ELISA revealed that two trisaccharides: Gal α1,3 Galβ 1,4 GlcNAc/Glc were the best inhibitors whereas Gal β1,4 GlcNAc LacNAc, Gal α1,3 Gal and Gal α1,6 Glc (melibiose) were poor inhibitors.

Previously, we reported the relationship between the content of terminal α-D-Galp groups on a series of murine tumor cell lines and their malignant potential. In the present study, we examined six cell lines and detected this trisaccharide residues on all of the highly malignant cells using this monoclonal antibody. Low-malignant cells showed little or no reactivity with this monoclonal antibody. The results suggest that α-D-Galp units on the malignant tumor cell surface are present as Gal α 1,3 Gal β1,4 GlcNAc- units. This monoclonal antibody is now available to detect this sugar sequence on glycoconjugates present on cells and tissue sections. Supported by NIH grant CA20424.

CZ 216 DIFFERENTIAL EXPRESSION OF GLYCOPROTEINS CONTAINING α-D-GALACTOSYL GROUPS ON CELLS OF HIGH- AND LOW-MALIGNANT POTENTIAL, J. Varani, J. Lowe and J. Petryniak, Departments of Pathology and Internal Medicine, The University of Michigan, Ann Arbor, MI 48109

Highly-metastatic murine tumor cells were reactive with *Griffonia simplicifolia* isolectin B4, (which recognizes α-D-galactosyl residues) by indirect immunofluorescence (FACS analysis) while low-metastatic tumor cells from the same tumor were much less reactive. In spite of the difference in lectin binding, the high- and low- metastatic cells expressed equal levels of steady-state mRNA for α 1-3 galactosyl transferase. Furthermore, the low-metastatic cells were reactive with *Erythrina cristagalli lectin.* This lectin recognizes N-acetyllactosamine residues, which serve as acceptors for α-D-galactose. Glycoproteins were isolated from lysates of 125-I-surface labeled cells by affinity chromatography on *Griffonia simplicifolia* I lectin and specific elution with methyl α-D-galactoside. SDS-PAGE analysis of the isolated glyoproteins revealed both quantitative and qualitative differences between the high- and low-metastatic cells. In order to understand how expression of α-D-galacosyl - containing glycoproteins might influence the malignant process, adhesion and motility studies were conducted. The cells expressing high levels of α-D-galactosyl residues on their glycoproteins were much more motile than the α-D-galactosyl-deficient cells and were more adherent to a variety of substrates including laminin and monolayers of endothelial cells. Antibodies reactive with α-D-galactosyl residues (i.e., antibodies to the blood group B antigen) interferred with both responses. Taken together, these data indicate that the expression of cell surface glycoproteins containing α-D-galactosyl residues may facilitate events that are important in the metastatic process.

CZ 217 MOUSE MACROPHAGE LECTIN RECOGNIZES Tn-ANTIGEN, Kazuo Yamamoto, Chizu Ishida, Yukiko Konami, Toshiaki Osawa and Tatsuro Irimura, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan

Macrophages are known to play important roles in the self defence mechanisms, such as endocytosis of foreign materials and destruction of tumor cells. Previously, we demonstrated that the mouse macrophage galactose and N-acetylgalactosamine-specific lectin (Mϕ lectin) may participate in the binding of the macrophages to tumor cells (1) and also reported the cloning of a cDNA encoding Mϕ lectin (2). Mϕ lectin consisits of 304 amino acid residues having a molecular weight 34,595. It belongs to a family of membrane-bound C-type aminal lectins, which contains a hydrophobic membrane-spanning sequence preceded by an N-terminal cytoplasmic region and C-terminal carbohydrate-recognition domain. Soluble Mϕ lectin was expressed in *E. coli* and the carbohydrate-binding specificity of the lectin was investigated by means of immobilized lectin affinity chromatography. Glycopeptides carrying multiple GalNAc-Ser/Thr (Tn- antigen) had strong affinity with the column, whereas the affinity of this lectin with sialylated or galactosylated ones were lower than Tn-antigen. In the case of Asn-linked oligosaccharides, increase in the number of Galβ1-4GlcNAc sequences also contribute to the affinity of the oligosaccharides for Mϕ lectin.

(1) Oda, S. et al. *J. Biochem.* 105, 1040-1043 (1989)
(2) Sato, M. et al. *J. Biochem.* 111, 331-336 (1992)

CZ 218 *Abstract Withdrawn*

Microbial Adhesion

CZ 300 LECTINS OF DIFFERENT SPECIFICITIES CHANGE THE GUT'S MICROBIAL POPULATION IN VIVO BY MODIFYING RECEPTOR-GLYCOSYLATION OR BY BLOCKING BACTERIAL ADHESION (CHEMICAL PROBIOSIS), Susan Bardocz, Stanley W.B. Ewen, George Grant, Arpad Pusztai, The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK. and Willy J. Peumans, Els J.M. Van Damme, Lab. Phytopathol. Plant Protection, Katholieke Univ. Leuven, B-3001 Heverlee, Belgium.

Methods for the use of dietary lectins which can prevent bacterial infection by changing the function, state of glycosylation and bacterial ecology of the gut have been systematically explored. For example, it has been shown that hyperplastic growth of the rat small intestine induced by kidney bean lectin, PHA, results in faster epithelial turnover. Because of their increased rate of migration, epithelial cells have less time to complete all the reaction steps in the process of conversion of polymannosyl- membrane glycans of juvenile cells to the complex glycosyl side chains characteristic of mature epithelial villus cells. The resulting increase in the number of terminal mannose residues on luminal membranes provides more receptors for the adhesion of mannose-fimbriated bacteria, including *E.coli* and salmonella spp., leading to overgrowth of the small intestine by these bacterial species. In contrast, the strictly mannose-specific lectins of Amaryllidaceae, particularly the agglutinin from *Galanthus nivalis* bulbs, GNA, by competing for the same adhesion sites, can displace E.coli from the small intestinal lumen, or when applied preventively, can stop small intestinal overgrowth by this bacteria. Similarly, plant lectins with specificity for neuraminic acid can displace or partially block the adhesion of bacterial species which use binding sites containing sialylated glycans. Furthermore, adhesion sites for these bacteria can also be reduced by overstimulating the secretion of mucinous glycans with the administration of muco-tractive lectins. Methods for the prevention of bacterial infection based on the principle of chemical probiosis are particularly attractive because this is achieved by using natural factors which are already part of our diet.

CZ 301 STRUCTURAL HETEROGENEITY OF THE GENE FOR FimH, THE MANNOSE-SENSITIVE LECTIN OF *Escherichia coli* TYPE 1 FIMBRIAE. D.L Hasty, E.V. Sokurenko, D.E. Ohman, P. Klemm and H.S Courtney. UT Memphis and the VA Med. Center, Memphis, TN and Tech. Univ. of Denmark, Lyngby.

E. coli and other *Enterobacteriaceae* express surface fibrillar structures, fimbriae, that are responsible for bacterial adhesion to host receptors. Type 1 fimbriae are polymers of a primary structural subunit, termed FimA, and minor amounts of a mannose-sensitive (MS) lectin-like component, FimH. It is this ancillary, lectin-like subunit that is commonly thought to cause binding to mannose-containing oligosaccharides of host receptors. FimH is thought to be present at intervals along the fimbrial length, but to be active only at the fimbrial tips. Previous studies have suggested that there is considerable conservation of FimH structure among *Enterobacteriaceae* at both protein and nucleotide levels, as compared to the structural heterogeneity of FimA. We have recently found, however, that there are at least two functional forms of type 1 fimbriae, one that is exclusively MS lectin-like (L fimbriae) and another that exhibits the lectin-like adhesive activity but also binds to non-glycosylated segments of proteins (L/P fimbriae). Interestingly, the protein-binding activity of the L/P form of type 1 fimbriae is also MS. We wished to determine the structural basis for the different activities. The *fim*H genes from strains expressing either L or L/P fimbriae were amplified by PCR technology and ligated into plasmid pACYC177. The resulting plasmids used to complement a strain of *E. coli* carrying a compatible plasmid having a *fim* gene cluster with an insertionally inactivated *fim*H gene. The *fim*H genes were sequenced and it was found that the functional heterogeneity results from a 12 base-pair deletion in the *fim*H gene of the L/P fimbriae, but it is not yet known precisely how such a sequence change affects the structure of this important bacterial lectin. It is clear that inhibition of *E. coli* adhesion by mannose-containing saccharides does not, in itself, indicate that the host receptor molecule to which these organisms adhere contains mannosylated oligosaccharide moieties. It will be important to determine the functional consequences of this novel form of type 1 fimbriae, especially since adhesion of bacteria to mucosal surfaces is an early and important step in microbial colonization and infection of host tissues.

CZ 302 CLONING OF TWO HIGHLY UNUSUAL AND NOVEL CYSTEINE PROTEINASES FROM *DICTYOSTELIUM*: IDENTIFICATION OF POTENTIAL SERINE PHOSPHOGLYCOSYLATION (GlcNAc-1-P) ADDITION SITES, John Hirai and Hudson Freeze, La Jolla Cancer Research Foundation, La Jolla, CA, 92037.

A previously characterized cysteine proteinase (Proteinase I) from *Dictyosteium discoidium* is present in vegetative cells but disappears early in development. This lysozomal enzyme is unusual in that it contains multiple clusters of GlcNAc-1-P directly bound to serine. This novel type of modification is called phosphoglycosylation. To study this modification further, we sought to clone the gene coding for this cysteine proteinase, and we identified two novel and unusual cysteine proteinases (clones 5 and 28). Both genes are expressed only during vegetative growth in the early stages of development. Each has several highly conserved regions expected for cysteine proteinases. However, unlike any other known cysteine proteinase, including two previously cloned from *Dictyostelium*, they contain several clusters of poly-serine residues. In addition, clone 28 also has an adjacent region containing nine contiguous Ser-Gly repeats. These serine-containing repeats may be the sites of GlcNAc-1-P addition. In support of this, a crude membrane preparation catalyzes the addition of GlcNAc-1-P to a small peptide containing two Ser-Gly repeats. To date, GlcNAc-1-P has not been found in other organisms. The function of these multiple GlcNAc-1-P residues is yet unknown, but it may direct trafficking of these proteinases, regulate their activity or both. (Supported by NIGMS32485)

CZ 303ANTIBODIES TO *TOXOPLASMA GONDII* MAJOR SURFACE PROTEIN(SAG-1,P30) INHIBIT INFECTION OF HOST CELLS. Lloyd H. Kasper and Jose R. Mineo. Department of Microbiology, Dartmouth Medical School, Hanover, NH 03756

Monoclonal and polyclonal, monospecific antibodies to the major surface antigen of *Toxoplasma gondii* (SAG1, P30) inhibit infection of human fibroblasts and murine enterocytes. Fabs prepared from polyclonal, monospecific antibody to P30 also have this inhibitory effect on invasion, which indicates that this antibody directly blocks parasite infection of host cells rather agglutinating the parasite. Antibodies to another surface protein (P22) did not alter *in vitro* infection. If the inhibitory effect of antibody to P30 antibody were due to steric hindrance or complexing of surface epitopes contiguous to P30, antibodies to other surface epitopes would also be inhibitory and they are not. Urea treatment of antibody (which permits discrimination of high and low avidity antibody) did not alter effect of anti-P30 antibody. This observation indicates that the effect of the antibody to P30 was not an artifact of differences in the avidity of the antibody to P22 and P30. Heat inactivated antisera from mice infected with either RH or PTg strain *T. gondii*, (P30+), inhibit infection of fibroblasts when challenged with autologous wild type parasites by 86 and 40% respectively. In contrast, these antisera have little inhibitory effect (0% and 19% respectively) against infection of human fibroblasts by a P30-deficient mutant (PTgB). Antisera raised to the P30-deficient mutant had no significant effect on infection of cells by wild type strains which have surface P30. The neoglycoprotein, BSA-glucosamide, competitively blocks infection of human fibroblasts by P30+ tachyzoites with surface P30 but not those without surface P30. This observation indicates that there is likely to be a glycosylated host cell receptor to which *T. gondii's* major surface antigen SAG-1 (P30) binds. Mice infected perorally develop intestinal IgA antibody to the major 30 kd epitope of *T. gondii*. Thus, the major surface epitope of *T. gondii*, SAG-1 (P30), has an important, functional role in infection of host cells by *T. gondii* and elicits an intestinal antibody response following peroral infection.

CZ 304 PROTEIC RECEPTORS-MANNANS INTERACTIONS ON THE YEAST CELLS SURFACE, M.M. Mestdagh, A. Henquinet, C.L. Masy, Unité de Chimie des Interfaces (C.I.F.A.), Université Catholique de Louvain, Place Croix du Sud, 2/18, B-1348 Louvain-la-Neuve (Belgium).

Flocculation is governed by the competition between electrostatic repulsions (non-specific interactions) and polysaccharide-protein bonds (specific interactions) (Kihn et al., 1988).
A more detailed study of these specific interactions,with different strains, has allowed the stereochemical distinction of two lectin types (cell wall proteic receptors).
The first type characterizes strains whose flocculation is partially inhibited by the mannopyranose (MS group). This lectin interacts with all the hydroxyl groups of the sugar. The second type is present on the cell wall of strains whose flocculation is completely inhibited by the mannopyranose, glucopyranose, maltose and sucrose (GMS group). This lectin has a lower specificity for the C1 and C2 hydroxyl groups of the same derivative.
Moreover, our results suggest the presence of a hydrophobic region near the mannopyranose-binding site of the lectins; such a hydrophobic cavity has been observed in many lectins (Lis and Sharon, 1986).
Finally, these two receptors interact more markedly with disaccharides than monomers if the two monosaccharides are α1-2 or α1-4 linked for the GMS group and α1-2, α1-4 or α1-4 for the MS group.
In conclusion, proteic receptors of the GMS group present an inhibitory pattern similar to concanavalin A lectin (So and Goldstein, 1968); those of the MS group, an inhibitory pattern similar to Escherichia Coli type I lectin (Lis and Sharon, 1986) except for the behaviour of the oligomers.

Bibliography

Kihn J.-C., Masy C.L.& Mestdagh M.M. (1988), Can. J. Microbiol., 34 : 773-778.
Lis H. & Sharon N., (1986), Ann. Rev. Biochem., 55 : 35-69.
So L.L. & Goldstein I., (1968), Biochem. Biophys. Acta, 165 : 398-404.

CZ 305 ANTIGENIC VARIATION OF PILIN REGULATES THE ADHESION OF NEISSERIA MENINGITIDIS TO HUMAN CELLS. X. Nassif[1,2], J. Lowy [2] and M. So[2]. [1] Dept. of bactériologie, Hôpital Necker-Enfants-Malades, 75730 Paris cedex 15, France, [2] Dept. of Microbiology & Immunology, Oregon Health Sciences University, Portland, OR 97201.

Neisseria meningitidis is an extracellular human pathogen which has the ability to cross the blood-brain barrier and produce meningitis. The adhesion of Neisseria meningitidis to human cells is of primary importance in the pathogenicity of this bacterium. *Pili*, filamentous surface protein structures mainly composed of repeating subunits called pilin, have been shown to play an essential role in this step. However, among piliated strains, the degree of adhesion to epithelial cells is subject to both inter- and intrastrain variability. This suggests that factors other than the presence of pili *per se* are involved in this process. The *N.meningitidis pilin subunit undergoes extensive intrastrain* antigenic variation. Using piliated low and high adhesive derivatives of the same N.meningitidis strain, we prove that the expression of certain antigenic variants of pilin is required for adhesion of this bacterium to a human epithelial cell line. Bacterial antigenic variation has been considered a means of evading the immune system. However, we *demonstrate for the first time* that bacterial pathogens can use antigenic variation to regulate the expression of virulence factors. Work is currently in progress to define precisely the pilin epitope and the cellular receptor involved in this process.

CZ 306 THE ROLE OF PENETRIN AND TRANS-SIALIDASE IN TRYPANOSOME INVASION OF HOST CELLS AND ADHERENCE TO THE EXTRACELLULAR MATRIX. Miercio E.A. Pereira, Marina Chuenkova, and Eduardo Ortega-Barria. Tufts-New England Medical Hospital, Department of Medicine, Boston, MA 02111

Trypanosoma cruzi causes Chagas, an insidious and deadly disease slowly killing millions of people in Latin America. Like some host cells, *T. cruzi* must traffic between various tissues of infected mammals via the bloodstream; in the tissues, it thrives inside many types of cells. T. cruzi causes cell destruction, in particular of neurons in the heart and the gastro-intestinal tract.

We have identified two sugar-binding proteins in the trypanosome that may mediate tissue invasion and penetration into host cells. One is penetrin, a surface-located lectin that binds heparin and heparan sulfate as well as collagen. Recombinant and endogenous penetrin blocks infection of host cells in vitro, and when coating a substratum, promotes attachment and spread of mammalian cells. Non-invasive *E. coli* K-12 can change their phenotype when expressing penetrin by adhering and entering cultured cells, and when inside the cells, by escaping from endocytic vesicles into the cytosol, thus behaving like *T. cruzi*.

The other protein is the trans-sialidase (TS), a unique enzyme that can hydrolyze and transfer α2,3-linked sialic acid from and to βGal acceptors. Endogenous or recombinant TS promotes adhesion of *T. cruzi* when coating a substratum. Adhesion is particularly effective when the parasite is free of sialyl residues. TS binds to the basement membrane Matrigel and to soft tissues such as those in the skin and intestine. Although *T. cruzi* binds to Matrigel, binding is greatly enhanced by TS. This enhancement is blocked by mAb and carbohydrates reactive with TS. These in vitro results suggest that TS promotes *T. cruzi* adhesion to the ECM, a conclusion consistent with experiments in vivo, in which the enzyme, when priming soft tissues of mice before infecton in the same locale, can dramatically turn an otherwise non-lethal dose of parasites into a mortal inoculum.

TS also functions as an adhesion molecule for host cells, as it can support attachment and spread of epithelial and neuronal cells. Most interestingly, TS promotes neurite outgrowth, and it is even more effective than host-derived laminin in inducing axonal extension. TS is the first protein of microbial origin, whether viral, bacterial or protozoan, known to exert such activity on neuronal cells. The effect of TS on *T. cruzi* and host cells suggest that the enzyme is a virulence factor for Chagas.

CZ 307 ***THE BINDING OF INFLUENZA A AND SENDAI VIRUS TO GANGLIOSIDES FROM HUMAN GRANULOCYTES***

Jasna Peter-Katalinic[1], Frank Unland[2], Franz-Georg Hanisch[3], Dagmar Heitmann[2], and Johannes Mühing[2]

[1]*Institute for Physiological Chemistry, University of Bonn*; [2]Institute for Animal Cell Cultivation Techniques, University of Bielefeld; [3]Institute for Immunobiology, University of Cologne, Germany.

The peripheral human blood granulocytes express on their cell surfaces predominantly linear gangliosides of neolacto-series containing 3- and 6-linked sialic acid (1). In a direct solid-phase binding assay (overlay) the receptor potency of these gangliosides toward influenza A and Sendai virus has been investigated. Eight main components were structurally characterized by FAB-MS and methylation analysis. The major gangliosides were II3NeuAc-LacCer (GM3), IV3NeuAc-nLcOse$_4$Cer, IV6NeuAc-nLcOse$_4$Cer, and VI3NeuAc-nLcOse$_6$Cer containing $C_{24:1}$ and $C_{16:0}$ fatty acids. The differences in the affinity toward gangliosides were observed in human influenza A strains H1N1, H3N2 and Sendai virus, previously metabolically labelled with 35S-methionine, by autoradiography. Only slight preference in the association of H1N1 influenza A to 3-linked over 6-linked NeuAc of gangliosides from granuylocytes was observed. The affinity of H3N2 influenza A was similar to those of the Sendai virus showing higher selectivity toward NeuAc linked to the position 3 of the Gal unit in nLcOse6Cer.

CZ 308 Cloning and Characterization of an *Haemophilus influenzae* Type b Adhesin. J.E. Samuel, D.L. Weinstein, and H.C. Krivan. MicroCarb Inc., Gaithersburg, MD.

Many pathogenic bacteria bind specifically to cell surface glycosphingolipids containing terminal or internal GalNacβ1-4Gal sequences such as asialo-GM_1 (GA_1). In order to identify and characterize the adhesin(s) for one of these organisms, *Haemophilus infuenzae* type b (Hib), monoclonal antibodies were generated which inhibit the binding of Hib membranes to GA_1. Several antibodies recognized a protein approximately 47 kDa in mass on Western blot. This protein was solubilized from membranes by Sarkosyl and was proteolytically degraded by treatment of intact Hib with proteinase K. These antibodies were used to isolate immunoreactive *Escherichia coli* clones from a lambda-ZapII library of Hib DNA. The clones made an ~47 kDa immunoreactive protein which was also proteinase K sensitive on intact cells. Like *Haemophilus*, *E. coli* expressing this 47 kDa protein bound strongly to GA_1, with a half maximum binding receptor concentration of 0.1 μg/well, while the *E. coli* host strain, XL-1, bound weakly to the receptor. Sequence analysis of this clone identified an open reading frame which encodes a predicted 49 kDa protein containing an approximate 2 kDa signal sequence. A survey of *H. influenza* type b and non-typable strains demonstated that this outer membrane protein is highly conserved and therefore is a potential vaccine candidate against non-typable disease. The role that this putative adhesin plays in virulence is currently being tested by constructing Hib adhesin deletion mutants.

CZ 309 RECEPTOR-ACTIVE GLYCOSPHINGOLIPIDS FOR ENTEROPATHOGENIC ESCHERICHIA COLI K99 IN EPITHELIAL CELLS OF CALF SMALL INTESTINE, Susann Teneberg, Peter T.J. Willemsen#, Frits K. de Graaf# and Karl-Anders Karlsson, Department of Medical Biochemistry, University of Göteborg, Medicinargatan 9, S-413 90 Göteborg Sweden and #Biological Laboratory, Department of Microbiology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands.

Glycolipids were prepared from epithelial cells of the small intestine of a newborn calf and tested for *Escherichia coli* K99 binding activity, using a chromatogram binding assay and a microtiter well assay. The bacteria did not bind to any of the non-acid glycolipids, while in the acid fraction several binding glycolipids were detected. The acid glycolipids were isolated and characterized by mass spectrometry, proton NMR spectroscopy and enzymatic digestion as NeuAcα2-3Galβ4GlcβCer (NeuAc-GM3), NeuGcα2-3Galβ4GlcβCer (NeuGc-GM3), GalNAcβ4(NeuGcα2-3)Galβ4GlcβCer (NeuGc-GM2), Galβ3GalNAcβ4(NeuGcα2-3)Galβ4GlcβCer (NeuGc-GM1) and NeuGcα2-3Galβ3GalNAcβ4(NeuGcα2-3)Galβ4GlcβCer (NeuGc-GD1a). The K99-fimbriated *E.coli* bound to NeuGc-GM3, NeuGc-GM2 and NeuGc-GD1a, while NeuAc-GM3 and NeuGc-GM1 were negative. The epitope for the binding of K99-fimbriae is discussed, in relation to the minimum energy conformations of binding and non-binding glycolipids from calf small intestine and from other sources.

CZ 310 *PLASMODIUM FALCIPARUM* MALARIA ERYTHROCYTE ROSETTING IS MEDIATED BY PROMISCUOUS LECTIN-LIKE INTERACTIONS, Mats Wahlgren, Johan Carlson and Helena Helmby, Department of Infectious Diseases, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge, Sweden.

That erythrocyte rosetting, the spontaneous binding of uninfected to infected erythrocytes, plays a role in the pathogenesis of *P. falciparum* malaria is a view today supported by experimental evidence.

We have recently established that strain-specific low molecular weight polypeptides derived from the parasite and located on the surface of the infected erythrocyte, sc. rosettins, mediate rosetting. The adhesins ($\approx M_r$ 22,000) are available for external-labelling using ^{125}I and specific antibodies both stain the surface of live infected red cells in indirect immunofluorescence as well as as break preformed rosettes.

The receptor structures involved on the uninfected red cell seem strain-specific as a preference of rosetting was observed for either bloodgroup A/AB or B/AB rbc for all parasites tested. The higher affinity of rosette binding of bloodgroup A,B or AB rbc was reflected in larger rosettes when a given parasite was grown in rbc of the preferred bloodgroup. Rosettes of a bloodgroup A preferring parasite could be completely disrupted by heparin only when grown in bloodgroup O or B rbc but not when grown in bloodgroup A rbc. Similarly the rosettes of a bloodgroup B preferring parasite could more easily be disrupted by heparin when grown in bloodgroup O or A rbc than when grown in bloodgroup B rbc. Several different saccharides inhibited rosetting of group O rbc including two monosaccharides which are basic components of heparin. The rosetting of the same parasites grown in bloodgroup A or B rbc was less sensitive to heparin and was specifically inhibited only by the terminal saccharides of the A and the B bloodgroup antigens. Our results suggest that rosetting is mediated by multiple lectin-like interactions the usage of which relies on wether the receptors are present on the host cell or not.

Lectins

CZ 311 CHARACTERIZATION OF PLANT BINDING PROTEIN(S) THAT SPECIFICALLY RECOGNIZE BIOLOGICALLY ACTIVE OLIGOGLUCOSIDES, François Côté, Jong-Joo Cheong, Rob Alba and Michael G. Hahn, Complex Carbohydrate Research Center and Departments of Botany and Biochemistry, University of Georgia, Athens, GA 30602-4712, USA

Oligosaccharins, oligosaccharides with regulatory activities, are a recently identified class of signal molecules active in plants. Oligosaccharins regulate processes in plants such as the induction of defense responses, hormonal effects and development. We are studying the cellular signaling pathway induced by one of these oligosaccharins, a branched hepta-β-glucoside originally isolated from the mycelial walls of the phytopathogenic fungus, *Phytophthora megasperma* f.sp. *glycinea*. The induction of pterocarpan phytoalexin biosynthesis in soybean is an important plant defense response elicited by this oligoglucoside. Our research has focussed on the first step in this signal pathway, namely the specific recognition of the hepta-β-glucoside elicitor by binding protein(s) in soybean cells. This elicitor induces half-maximal accumulation of phytoalexins in soybean cotyledons at concentrations of ~10 nM. Structuraly related oligoglucosides are only active at concentrations 10- to 10^5-fold higher. Proteinaceous binding sites with properties expected of physiological receptors for the hepta-β-glucoside elicitor have been identified in soybean root membranes. These elicitor binding protein(s) (EBPs) co-migrate with a plasma membrane marker (vanadate-sensitive ATPase) on linear sucrose density gradients. Binding of a radioiodinated tyramine conjugate of the elicitor-active hepta-β-glucoside was specific, reversible, saturable, and of high affinity (K_d = 0.75 nM). Competitive displacement of the radiolabeled hepta-β-glucoside elicitor with a number of elicitor-active and structurally related inactive oligoglucosides demonstrated a direct correlation between the ability to displace the labeled elicitor and the elicitor activity of these molecules. Thus, the EBPs recognize the same structural elements of the hepta-β-glucoside elicitor that are essential for its phytoalexin-inducing activity, suggesting that the EBPs are physiological receptors for the elicitor.

The EBPs have been solubilized using the non-ionic detergent, *n*-dodecylsucrose. The solubilized EBPs retained the binding affinity (K_d = 1.8 nM) for the radiolabeled elicitor and showed the same specificity for elicitor-active oligoglucosides determined previously for the membrane-localized binding protein(s). Current research is directed toward the purification and cloning of the hepta-β-glucoside EBPs. Techniques being utilized include anion exchange and ligand affinity chromatography, and cloning by functional expression in a heterologous cell type. Purification and characterization of the hepta-β-glucoside binding protein(s) or their corresponding cDNAs is a first step toward elucidating how the hepta-β-glucoside elicitor triggers the signal transduction pathway that ultimately leads to the synthesis of phytoalexins in soybean. [Supported by a NSF grant (DCB-8904574), a NSERC post-doctoral fellowship (F.C.), and in part by the DOE-funded Center for Plant and Microbial Complex Carbohydrates (DE-FG09-87ER13810).]

CZ 312 INVESTIGATION OF THE LECTIN-LIKE BINDING DOMAINS IN PERTUSSIS TOXIN USING SYNTHETIC PEPTIDE SEQUENCES. IDENTIFICATION OF A SIALIC ACID BINDING SITE IN THE S2 SUBUNIT OF THE TOXIN, Louis D. Heerze, Pele C.-S. Chong and Glen D. Armstrong, Dept. of Medical Microbiology, University of Alberta, Edmonton, Alberta, Canada T6G2H7 and Connaught Center for Biotechnology Research, Willowdale, Ontario, Canada M2R 3T4.

Synthetic peptides corresponding to selected sequences in the S2 and S3 subunits of Pertussis toxin were prepared and evaluated for their ability to inhibit the binding of biotinylated Pertussis toxin and three biotinylated sialic acid specific plant lectins to fetuin and asialofetuin. The screening results indicated that two regions in the S2 subunit inhibited Pertussis toxin binding to fetuin at submillimolar concentrations while S3 sequences inhibited Pertussis toxin-biotin binding to asialofetuin albeit with lower affinity. These results confirm earlier findings which suggest that the S2 subunit is responsible for binding sialylated glycoconjugates. This was further confirmed by the ability of S2 peptides to inhibit the binding of the lectins from *Maackia amurensis* and wheat germ to fetuin.

Two additional peptides from the S2 subunit of Pertussis toxin were found to contain within their sequences a six amino acid fragment which has strong homology with a sequence in wheat germ agglutinin that has been shown to be a component of the sialic acid binding site as determined by X-ray crystallography. One of these sequences from S2 was biotinylated and evaluated for its ability to bind to carbohydrate. Through a series of experiments using fetuin, asialofetuin, asialoagalactofetuin and simple saccharides, the biotinylated peptide was shown to bind with high affinity to sialic acid containing glycoconjugates indicating that these sequences within the S2 subunit of Pertussis toxin also play an important role in binding sialic acid.

CZ 313 **Purification and characterization of two bovine serum lectins, Cl-40 and Cl-28, containing collagenous domains.** Holmskov U., Teisner B., Thiel S., Holt P., Laursen S. B.,Willis A. C., Reid K.B.M. and Jensenius J.C.
Department of Medical Microbiology, University of Odense and Department of Immunology, University of Aarhus, Denmark; MRC Immunochemistry Unit, University of Oxford, UK.

Two bovine lectins (CL-28 and CL-40) distinct from conglutinin were identified in serum by their Ca^{2+}-dependent binding to mannan. The lectins were isolated using PEG precipitation, mannan affinity chromatography immunospecific affinity chromatography and ion-exchange chromatography. On gel chromatography both lectins were shown to have an M_r of approx 750 kDa. On SDS-PAGE the CL-40 gave a single band of 120 kDa and the CL-28 gave several high molecular weight bands at non-reducing conditions. At reducing conditions the CL-40 behaved as a double band of 40 kDa, and CL-28 gave a band of 28 kDa. High levels of glycine (CL-40 25% and CL-28 17.7%) and the presence of hydroxyproline and hydroxylysine in the amino acid composition indicated that both molecules contained collagen-like structures. This was supported by their sensitivity to collagenase digestion. Using mannan as a ligand in a solid phase inhibition assay CL-40 showed the following carbohydrate binding specificity: mannose = ManNAc > L-fucose > GlcNAc > maltose = glucose >> lactose >> GalNAc. CL-28 showed the following carbohydrate binding specificity: L-fucose > mannose > ManNAc > GlcNAc > glucose >> maltose >> galactose >> lactose >> galNAc. The N-terminal sequence of C-28 (26aa) showed 59% identity with human MBP while that of CL-40 (27aa) only showed 14% identity with this protein. CL-40 showed 48% identity both with bovine conglutinin and with human SP-D. We conclude that CL-28 is likely to be the bovine analogue to rat and human MBP and that CL-40 it a hitherto undescribed circulating C-type lectin containing collagen domains.

CZ 314 THE CLONING AND EXPRESSION OF THE LIMA BEAN LECTIN IN E. COLI, Elizabeth T. Jordan and Irwin J. Goldstein, Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109

The lima bean lectin (purified from seeds of Phaseolus lunatus) is an $\alpha 2\beta 2$-type tetramer which agglutinates human type A erythrocytes. The lectin requires Ca^{2+} and Mn^{2+} in order to bind carbohydrate. Known metal ion ligands include an essential cysteine sulfhydryl group, and several Asp and Glu residues which are conserved among the leguminous lectins. We have cloned a fragment of DNA which encodes the mature protein (without signal sequence) by PCR. Evidence from southern blot analysis indicates a lectin gene family, and the PCR sequence has subsequently been verified from a genomic library. The gene encodes a mature polypeptide of 254 amino acids, with a predicted pI of 5.86, and 1 potential site for N-linked glycosylation. We have used the T7 RNA Polymerase system to overexpress the lectin in E. coli. While most of the protein is present in inclusion bodies, about 5mg per L culture is routinely purified in an active soluble form. This recombinant lectin (rLBL) although unglycosylated, and homotetrameric, retains the ability to bind both carbohydrate and hydrophobic ligands. rLBL retained full activity after treatment at 65°C for 15 min. (as did the native lectin), although both were inactivated after 15 min. at 79°C, suggesting that the carbohydrate moiety is probably not crucial for thermostability. With such an expression system in hand, we are in the process of generating site-specific mutants which will aid us in determining both metal and carbohydrate ligands within the lectin. Preliminary studies of these mutants will be presented.
Supported by NIH grant GM 29470.

CZ 315 CARBOHYDRATE-BINDING DOMAINS OF SEVERAL LEGUMINOUS LECTINS : CARBOHYDRATE-BINDING SPECIFICITY AND PEPTIDE SEQUENCE, Yukiko Konami, Kazuo Yamamoto, Toshiaki Osawa and Tatsuro Irimura, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Lectins are widely used as tools for the study of the carbohydrate constituents of cell surfaces and glycoproteins. Leguminous lectins resemble each other in their physicochemical properties regardless of their carbohydrate-binding specificity. Several anti-H(O) lectins have already been isolated and characterized for their carbohydrate-binding specificity [1-4]. We have also determined the primary structures of *Lotus tetragonolobus* lectin (LTA)[4], *Ulex europeus* lectin I and II (UEA-I and II)[5], *Laburnum alpinum* lectin I (LAA-I)[6] and *Cytisus sessilifolius* lectin I (CSA-I)[7], and compared them with those of several other leguminous lectins. Extensive homology was found throughout the stretch of the peptides. Furthermore, carbohydrate-binding peptides have been isolated from LTA, UEA-I, UEA-II, LAA-I and CSA-I by affinity chromatography after treatment of the lectins with endoproteinase Asp-N or Lys-C [7, 8]. The peptides having an affinity for the specific sugars were retarded on the affinity columns of GlcNAc oligomer-Sepharose for UEA-II, LAA-I and CSA-I, and Fuc-gel for LTA and UEA-I. The homology found in the amino acid sequences among these retarded peptides indicates the presence of a carbohydrate-binding region of homologous leguminous lectins. The sequences of the carbohydrate-binding peptides from the di-*N*-acetylchitobiose-binding (*Cytisus*-type) lectins, UEA-II (DSYFGKTYNPW), LAA-I (DTYFGKAYNPW) and CSA-I (DTYFGKTYNPW), were almost identical. These results strongly suggest that there is a close relationship between the carbohydrate-binding specificity and the sequences of the carbohydrate-binding peptide of leguminous lectins.

1) Konami, Y., Tsuji, T., Matsumoto, I. and Osawa, T. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 983-989.
2) Konami, Y., Yamamoto, K., Tsuji, T., Matsumoto, I. and Osawa, T. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 397-407.
3) Konami, Y., Yamamoto, K. and Osawa, T. (1991) Biol. Chem. Hoppe-Seyler 372, 103-111.
4) Konami, Y., Yamamoto, K, and Osawa, T. (1990) FEBS Lett. 268, 281-286.
5) Konami, Y., Yamamoto, K. and Osawa, T. (1991) J. Biochem. 109, 650-658.
6) Konami, Y., Yamamoto, K., Toyoshima, S. and Osawa, T. (1991) FEBS Lett. 286, 33-38.
7) Yamamoto, K., Konami, Y., Osawa, T.and Irimura, T. (1992) J. Biochem. 111, 436-439.
8) Konami, K., Yamamoto, K., Osawa, T. and Irimura, T. (1992) FEBS Lett. 304, 129-135.

CZ 316 ISOLATION AND CHARACTERIZATION OF A MANNAN-BINDING PROTEIN FROM CHICKEN SERUM.

S. B. Laursen, J. Hedemand, S. Thiel, U. Holmskov & J. C. Jensenius. Institute of Medical Microbiology, Aarhus University, Aarhus, National Institute of Animal Science, Foulum and Institute of Medical Microbiology, Odense University, Odense, Denmark.

The existence of the serum lectin, Mannan-Binding Protein also called Mannose-Binding Protein (MBP) has been shown in several species including humans, cows, rabbits, rats and mice. Here we describe the isolation and characterization of a mannan-binding lectin present in chicken serum. The lectin was purified by a double affinity chromatography procedure on beads coupled with monosaccharides followed by ion exchange and size permeation chromatography. The protein eluted corresponding to a size of 800 kDa from the size chromatography column. On SDS-PAGE the reduced protein migrates as a number of 24-34 kDa polypeptide chains. Collagenase digestion of the lectin yielded a double band at 21 kDa and 23 kDa, indicating the presence of collagen-like domains. The binding of mannan was shown to be Ca++-dependent and could be inhibited by monosaccharides in the following order : L-Fuc = Man = ManNAc > GlcNAc = Maltose > D-Fuc = Galactose. Glucoseamine, Galactosamine and GalNAc were not inhibitory. The inhibition pattern is similar to those reported for MBP from other species. It was shown, that chicken mannan-binding protein was able to activate autologous as well as human complement.

CZ 317 **ENDOCYTOSIS AND TRANSCYTOSIS OF L-29, A LECTIN SECRETED BY MDCK CELLS.** Ragnar Lindstedt, Gerry Apodaca, Keith Mostov, Sam Barondes and Hakon Leffler. University of California, San Francisco, CA 94143.

L-29 (also known as Mac-2, CBP-35 and εBP) is a cytosolic protein present in columnar epithelia and activated macrophages. This lectin has affinity for β-galactoside-containing glycoconjugates and we have demonstrated that in MDCK cells it is secreted predominantly into the apical medium.

To gain more insight into the fate and biological roles of secreted L-29 we have studied its binding to the MDCK cell surface and subsequent internalization.. ^{125}I-labeled recombinant human L-29 (rL-29) was bound to the apical or basal side of filter grown MDCK cell and the amount bound, internalized and transcytosed was measured. Of the apically bound lectin, 24% was internalized during a 60 min. chase and of this amount was 59% transcytosed. Of the basal bound lectin about 35% was endocytosed and 38% of the internalized transcytosed. No evidence for degradation or processing of the endocytosed lectin was found since it had the same mobility on SDS-PAGE. The endocytosis was confirmed using FITC-labeled rL-29 which was found in vesicles by confocal microscopy.

To learn more about the molecular mechanism for the endocytosis we examined first which part of L-29 was responsible and then the nature of the cellular ligand. The binding and endocytosis of L-29 appear to be mediated by cell surface galactosides because it was inhibited by lactose and was much lower in a galactoside deficient mutant of MDCK cells (MDCK RCA^r). There are reports indicating that the proline-glycine rich N-terminal of the lectin may aggregate the lectin and increase the binding affinity to glycoconjugates. However, the FITC-labeled carbohydrate binding C-terminal domain was internalized to a similar extent suggesting that a polyvalent binding to the cell surface is not necessary for binding and internalization. Cell surface coimmunoprecipitation suggested one 95 kDa glycoprotein as the predominant ligand for L-29.

CZ 318 THE B-CELL DIFFERENTIATION ANTIGEN CD19 CONTAINS A PUTATIVE GLOBOTRIAOSYL CERAMIDE (CD77) BINDING SITE. Mark D. Maloney and Clifford A. Lingwood, Department of Microbiology, Hospital for Sick Children, Toronto, Ontario, M5G 1X8, CANADA

B-subunit proteins of the verotoxin (shiga and shiga-like toxin) family have been shown to bind galα1-4gal residues of globo-series glycolipids, in particular globotriaosyl ceramide (Gb_3 or CD77). Significant homology between the extracellular domain of the type I interferon receptor and the Gb_3-binding B-subunits of verotoxins has been reported and a correlation between verotoxin- and α-interferon-resistance and loss of Gb_3 from cell membranes has been documented in Daudi cells of Burkitt lymphoma origin. We report herein that CD19 also has a putative Gb_3-binding site as determined by homology to verotoxin and the α-interferon receptor extracellular domain. CD19 is a 95 kD B cell membrane protein which plays a role in the regulation of B cell proliferation and differentiation. It has been shown to physically associate with other B cell surface proteins but has not previously been shown to interact with either lipid or carbohydrate moieties. The verotoxin B-subunit-like sequences on CD19 appear to be in close proximity due to the organization of intervening amino acids into disulfide-linked domains. Co-capping of CD19 and Gb_3 by anti-CD19 antibodies indicates that they are associated on the B cell surface. Gb_3 also caps with IgM using anti-IgM monoclonal antibody. This co-capping of Ig and Gb_3 is likely due to the presence of CD19 in the cap since anti-IgM has been shown to cap CD19,and IgM itself and other proteins known to cap with IgM lack a Gb_3-binding site as defined by sequence homology to verotoxins. Binding of anti-CD19 to the cell surface is decreased on Gb_3-deficient Daudi cells which suggests that Gb_3 may function to promote translocation, stability or a specific conformation of CD19 necessary for antibody recognition. PH1, a monoclonal antibody raised against the B-subunit of VT1, and anti-CD19 both recognize a 95 kD band in detergent extracts of Burkitt lymphoma cells. This suggests that the anti-verotoxin antibody is cross-reacting with the putative Gb_3-binding site on CD19. While the α-interferon receptor also has been reported to blot at a molecular weight of 95 kD, the levels of this protein per cell are extremely low and would presumably not be detected by Western blot. Also, the 95 kD band was not detected by Western blot with PH1 in the T98G glioblastoma cell line which lacks CD19 but possesses both α-interferon receptors and Gb_3. These findings suggest that glycolipids modulate certain functions of membrane proteins during B cell differentiation.

CZ 319 L-14 IN *XENOPUS*: AN ADULT BUT NOT AN EMBRYONIC PROTEIN. Philippe Marschal, Samuel H. Barondes, and Douglas N.W. Cooper. University of California, San Francisco, CA 94143

All vertebrates examined express one or more lectins in the L-14 family. These lectins are closely related in peptide sequence, are not membrane bound, have subunit Mr of 14-16 kD, bind lactoside sugars, and are expressed with developmental regulation in a wide range of tissues. Because L-14 is prominent in basement membranes and has particular affinity for polylactosamine chains on laminin, the lectin has been proposed to regulate developmental cell interactions with laminin. This has been demonstrated for muscle, where L-14 is secreted during differentiation and inhibits myoblast adhesion to a laminin substrate. To further explore the role of L-14 in development, we have cloned a *Xenopus laevis* L-14 and studied its expression in embryonic and adult tissues. Whereas L-14 is expressed in both embryonic and extraembryonic tissues during development of chick and mouse, we could detect no expression in *Xenopus* embryos prior to metamorphosis, using sensitive immunoassays for the protein and a sensitive RNase protection assay for the message. Furthermore, using affinity chromatography on lactosyl-Sepharose, no other lectins in the L-14 size range could be detected prior to metamorphosis. In selected adult *Xenopus* tissues, levels of L-14 were in the same range as found for other vertebrates (0.9‰ of the soluble protein in skeletal muscle, 0.25‰ in heart, 1.1‰ in intestine, 0.06‰ in stomach, 0.003‰ in liver). However, adult *Xenopus* skin is exceptionally rich in L-14 (5% of soluble protein). *Xenopus* has been shown to secrete this lectin onto the skin surface along with a variety of defense peptides in response to stress. These results suggest that, at least for *Xenopus laevis*, major functions of L-14 are performed in adult tissues and might include some role in defense independent of interaction with laminin.

CZ 320 PURIFICATION AND CHARACTERIZATION OF A MANNOSE-SPECIFIC LECTIN FROM SHALLOT (*Allium ascalonicum*) BULBS, Hanqing Mo, Els J.M. Van Damme*, Willy J. Peumans*, and Irwin J. Goldstein. Dept. Biol. Chem., Univ. of Michigan, Ann Arbor, Michigan 48109, and *Laboratorium voor Plantenbiochemie, Katholieke Universiteit Leuven, Willem de Croylaan 42, B-3030 Leuven (Heverlee), Belgium.

A new mannose-binding lectin has been purified from shallot (*Allium ascalonicum*) bulbs by a combination of affinity chromatography, hydrophobic, and anion exchange chromatography. The lectin (*A. ascalonicum agglutinin, AAA*) appeared homogeneous by poly-acrylamide gel electrophoresis at pH 4.3 and by gel filtration on a Sephacryl S-200 HR column.

The carbohydrate binding properties of the purified AAA were investigated by quantitative precipitation and hapten inhibition assays. Purified AAA precipitated desialylated N-glycosylated glycoproteins as well as their asialoagalacto-derivatives, but did not precipitate either sialylated glycoproteins or mucins. AAA also reacted strongly with a highly branched yeast mannan obtained from *Saccharomyces cerevisiae* which possesses numerous terminal α1,3-linked mannobiosyl residues. Of the monosaccharides tested only D-mannose was a hapten inhibitor of the AAA-asialofetuin precipitation system, whereas D-glucose, D-altrose, D-talose, N-acetyl-D-mannosamine and derivatives of D-mannose including 2-deoxy, 2-fluoro, 3-deoxy, and 6-deoxy-D-mannose were non inhibitors. These results imply that the presence of equatorial hydroxyl groups at the C-3 and C-4 positions, an axial hydroxyl group at the C-2 position and a free hydroxyl group at the C-6 position of the pyranose ring are the most important loci for binding D-mannose to AAA. Of the oligosaccharides tested, the best inhibitors were oligosaccharides containing terminal Man α1,6 [Man α1,3] Man groups. Oligosac-charides containing either Man α1,3 Man or Man α1,6 Man units were also moderately good inhibitors of the AAA-asialofetuin precipitation system. These results indicate that AAA has an extended carbohydrate binding site, which is most complementary to a branched mannotriosyl residue, i.e. Man α1,6 [Man α1,3] Man.

Scatchard analysis of equilibrium dialysis data showed that AAA possesses one carbohydrate-binding site per subunit (Mr=11000) with an apparent association constant of $1.62 \times 10^4\ M^{-1}$.

A comparison is presented of the detailed carbohydrate binding properties and molecular structures of the AAA and several other mannose-binding lectins. Supported in part by NIH grant 29470.

CZ 321 **THE ONTOGENY OF HUMAN MANNAN-BINDING PROTEIN.** Steffen Thiel, Thorbjørn Bjerke, Steen B. Laursen, and Jens Chr. Jensenius. Institute for Medical Microbiology, Bartholinbygningen, Aarhus University, DK and Aarhus Community Hospital, Aarhus, DK.

Human serum mannan-binding protein is a collectin specific for mannose and GlcNAc terminated carbohydrate structures, which are found on several pathogenic microorganisms. After binding to the relevant polysaccharides MBP is an efficient activator of the $C1r_2C1s_2$ complex via a mechanism independent of antibody and C1q. MBP was isolated from human serum by published chromatography methods and an antisera were prepared by immunising rabbits with the pure protein. A sandwich-ELISA was developed using anti-MBP antibody from two different rabbits. When normal donors are analyzed a wide range of MBP serum concentration (≤1 ng/ml to ≤3000 ng/ml) with no apperant normal distribution is seen. The serum concentration of MBP was followed in consecutive samples from childrens. Blood samples were collected at birth and 3, 6, 9 and 12 month later. An increase in serum MBP concentration was observed in all children (an approximately 3-fold increase as compared to the sample taken at birth). A stabil level of MBP was reached after between 3 and 6 months.

CZ 322 **CARBOHYDRATE SPECIFICITY OF THE RECEPTOR SITES OF MISTLETOE TOXIC LECTIN-I**

Albert M. Wu [a], Lee-Kian Chin [a], Hartmut Franz [b], Uwe Pfüller [b] and Anthony Herp [a]

[a] *Glyco-Immunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, Chang-Gung Medical College, Kwei-San, Tao-yuan, Taiwan and* [b] *Universität Witten/Herdecke, Institute of Phytochemistry, Berlin, Germany.*

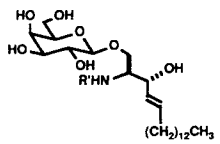
The carbohydrate specificity of mistletoe toxic lectin-I (ML-I) was studied by quantitative precipitin, precipitin-inhibition, and hemagglutination-inhibition assays. The results indicated that ML-I has a broad range of affinity for Gal α,β linked sequences. The galabiose (**E**, Galα1→4Gal) sequence, a receptor of the uropathogenic *E. coli* ligand, was one of the best disaccharide inhibitors tested. The lectin also exhibited affinity for **Lac** (Galβ1→4Glc), **T** (Galβ1→3GalNAc), **I/II** (Galβ1→3/4GlcNAc) and B (Galα1→3Gal) sequences. Galα1→4Gal and Galβ1→4Glc are frequently occurring sequences of many glycosphingolipids located at the mammalian cell membranes, such as intestinal and red blood cell surface membranes available, for ligand binding and toxin attachment. This finding provides important information concerning the possible mechanism of intoxication of cells by the mistletoe preparation.

This work aided by Grants from the Chang-Gung Medical Research plan (CMRP No. 293), Kwei-san, Tao-Yuan, Taiwan and National Science Council (NSC 81-0412-B-182-528, NSC 81-0412-B-182-41, and NSC 82-0418-B-182-009), Taipei, Taiwan.

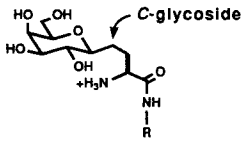
Development of Pharmaceuticals

CZ 400 CARBON-LINKED GALACTOSPHINGOLIPID ANALOGS BIND SPECIFICALLY TO HIV-1 gp120: APPLICATIONS FOR IMMUNOTARGETING, Carolyn R. Bertozzi, David G. Cook†, Francisco Gonzalez-Scarano† and Mark D. Bednarski; Department of Chemistry, University of California, Berkeley, CA 94720; †Department of Neurology, University of Pennsylvania Medical Center, Philadelphia, PA 19104.

The principal mode of infection by the human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) involves the interaction of the HIV envelope protein gp120 with CD4 on host lymphoid cells. The susceptibility of many CD4-negative cells to HIV infection, however, suggests the presence of an alternative entry pathway. Recent studies have implicated the glycolipid galactosyl ceramide (GalCer, **1**) as a cellular receptor for HIV-1 gp120 in both neural and colorectal-derived cell lines. We have designed a series of water-soluble, carbon-linked galactosphingolipid analogs that bind specifically to HIV-1 gp120 and block the interaction of gp120 with GalCer. These compounds (shown below, **2**) contain the essential galactose functionality of GalCer, but are resistant to both chemical and enzymatic deglycosylation *in vivo*. Examination of a series of derivatives with varying alkyl amide side chains (R) indicates that the hydrocarbon tail of sphingosine is a key structural element for gp120-GalCer recognition. Finally, we describe the use of DNP-conjugated derivatives as agents for antibody targeting to HIV-1.



1 (GalCer): R' = *hydroxystearoyl*



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CZ 401 STRUCTURE AND ANTIVIRAL MECHANISM OF MDL 101,028; A HEPARIN MIMETIC AND INHIBITOR OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 INFECTION. Alan D. Cardin,* Richard L. Jackson,* Debra Taylor,* Dale T. Blankenship,* Terry Bowlin,* and A. Stan Tyms+. Marion Merrell Dow Research Institute,* Cincinnati, OH 45215, The Medical Research Collaborative Center+, Mill Hill East, London.

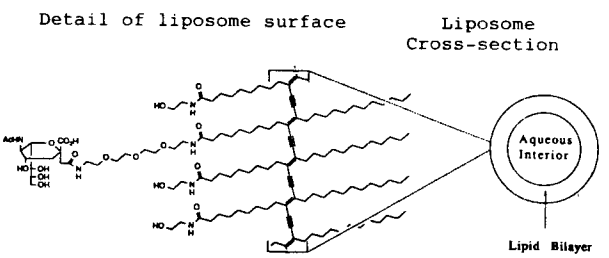
From molecular modeling predictions (Cardin and Weintraub, Arteriosclerosis, 9, 21-32, 1989), simple sulfonic acid polymers were designed to mimic heparin structure. We have examined the mechanism of inhibition of the Human Immunodeficiency Virus Type-1 (HIV-1) by MDL 101,028. MDL 101,028 consists of repeating biphenyl disulfonic acid block structures connected by urea linkages. MDL 101,028 protected the leukemic T-cell line JM against acute infection by HIV-1 particles when the cells were pretreated with drug and the drug washed out prior to virus challenge. This protection suggests a direct interaction between MDL 101,028 and the HIV-1 receptor, CD4. MDL 101,028 inhibited the binding of recombinant virus envelope glycoprotein gp120 (IC_{50} = 0.1 μM) and anti-CD4 monoclonal antibody OKT4A (IC_{50} = 0.3 μM) to membrane-associated CD4 on these cells and to recombinant soluble CD4. Under similar conditions of drug exposure, inhibition of virus growth in the JM system occurred with an IC_{50} = 0.05-0.2 μM. Preincubation of MDL 101,028 with $CD4^+$ C8166 cells blocked their fusion to H9 cells chronically infected with HIV-1 strain RF and III_B, a result consistent with CD4 blockade. Addition of MDL 101,028 to HIV-1 infected cultures containing syncytia resulted in the disappearance of these syncytia within 24 h, and the cultures continued to increase their cell numbers unlike the untreated, HIV-1 infected control cultures. In contrast, heparin neither blocked rgp120 binding to CD4 nor blocked the cofusion between uninfected $CD4^+$ C8166 cells and chronically-infected H9 cells. These results show a different mode of action for MDL 101,028 and heparin. A complex between MDL 101,028 and sCD4 was digested with ASP-N endoproteinase and the peptides sequenced by automated Edman degradation. The results showed that MDL 101,028 interacts with a specific region of the variable-1 immunoglobulin domain of the receptor known to be involved in the gp120-CD4 binding interaction. The novel antiviral mode of action and limited anticoagulant activity sets MDL 101,028 apart from heparin as a new class of antiviral agents.

CZ 402 EFFECT OF α-GLUCOSIDASE 1 INHIBITION ON HERPES SIMPLEX VIRUS (HSV) GLYCOPROTEIN SYNTHESIS AND VIRUS GROWTH *IN VITRO*, Mohinder Kang+,Parvin Ahmed*,Stanley Tyms*, Marion Merrell Dow Research Institute, +Cincinnati, Ohio, USA and *MRC Collaborative Centre Laboratory, Mill Hill, London

In general, all HSV glycoproteins are N-glycosylated with, in some cases, O-glycosylation present. The naturally occurring octohydroindolizine (castanospermine: CAST), a potent inhibitor of purified α-glucosidase 1, blocks the growth of HIV and HCMV (Taylor and Tyms, 1988. Antiviral Research 9, 152) but not HSV (unpublished). 6-O-butanoyl CAST (MDL 28574: BUCAST) and other acyl derivatives had improved antiviral effects which included good activity against HSV-2 by plaque reduction assay (IC50 30μM) but was less effective against wild-type HSV-1. Pre-exposure of HEF cells to BUCAST prior to infection increased the effect against HSV-2. At high multiplicity infections (0.1 to 1 pfu per cell), control of HSV-2 replication by BUCAST (100μM) was not immediately evident and required passage of BUCAST-exposed progeny virus in host-cells pre-treated with compound. The effects of BUCAST treatment on HSV-encoded glycoproteins was studied by Western-blot analysis with Mabs to gB, gD, gC all potentially important for attachment, penetration or virus neutralization. In all cases, the mature glycoproteins, as defined by size, were synthesized but with an increase in Mwt of the precursor molecules in the presence of Bucast: this was considered due to glucosylated high mannose oligosaccharides. This was confirmed by the susceptibility of the precursors to endo-H digestion and the relative insusceptibility of the mature form to treatment with this enzyme. The inhibition of HSV-2 growth after treatment with BUCAST however pointed to some critical change in HSV glycoprotein synthesis. A greater reliance on cell-to-cell fusion (syncytia) by HSV-2 over HSV-1 may be responsible for the effects. A plaque purified HSV-1 population of Syn^+ phenotype was inhibited by BUCAST treatment while the Syn^- phenotype was unaffected. Preliminary evidence suggests that an aberrant synthesis of gD may have a role in this susceptibility to BUCAST.

CZ 403 POLYMERIZED LIPOSOMES CONTAINING C-GLYCOSIDES OF SIALIC ACID ARE POTENT INHIBITORS OF INFLUENZA VIRUS HEMAGGLUTINATION AND IN VITRO INFECTIVITY, Jon O. Nagy, Wayne Spevak, Deborah H. Charych, Mary E. Schaefer, James H. Gilbert, Mark D. Bednarski, The Center for Advanced Materials, Lawrence Berkeley Labs, Berkeley, CA 94720 and Department of Microbiology and Immunology, Glycomed Inc., Alameda, CA 94501

The first example of a polymerized liposome containing carbon-glycosides of N-acetylneuraminic acid that can inhibit the influenza virus from binding to erythrocytes and inhibit in vitro infectivity is described. Liposomes prepared are 10,000 times more potent inhibitors of viral hemagglutination than monomeric sialosides. The most potent example tested contains a 0.017 mM concentration of the C-sialoside and can stop the infectivity of the virus as measured by a 97% reduction in plaque formation in vitro. Since liposomes are polimerized they are therefore well ordered structures and can serve as important models for studying multivalent interactions between pathogens and cells.



CZ 404 USE OF LECTINS FOR TARGETING DRUG-MICROPARTICLE COMPLEXES TO SELECTED SITES IN THE GASTROINTESTINAL TRACT AND FACILITATING THEIR TRANSPORT INTO THE SYSTEMIC CIRCULATION, Arpad Pusztai, George Kocsis, George Grant, Stanley W.B. Ewen, Susan Bardocz, The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK and Willy J. Peumans, Els J.M. Van Damme, Lab. Phytopath. Plant Protection, Kathol. Univ. Leuven, B-3001 Heverlee, Belgium.

The carbohydrate specificity and physiological properties of lectins for targeting microparticles to selected sites in the gastrointestinal tract have been systematically explored in rat model studies in vivo. Most orally administered lectins have been shown to be highly resistant to proteolytic breakdown during their passage through the entire gastrointestinal tract. Depending on their carbohydrate specificity, lectins can recognise and bind to glycans of luminal membrane receptors of small intestinal epithelial cells and this may be followed by their endocytosis. Most importantly, appreciable amounts of these lectins are then transported across the epithelium to the systemic circulation. With the kidney bean (*Phaseolus vulgaris*) lectin, PHA, this amount can reach 10 per cent of that administered orally, however, it is only slightly less with most other lectins which avidly bind to the brush border. As chemical coupling to microparticles (into which drugs are incorporated) does not abolish their interactions with the gut epithelium, lectins are well-suited for roles in targeting of orally administered drugs to selected intestinal sites and facilitating their transport across the gut epithelium. Furthermore, as the state of glycosylation of intestinal receptors can be engineered by dietary lectins in a way to substantially increase the number of desirable binding sites to suit any particular targeting lectin, the scope of their use in oral drug delivery is truly wide.

CZ 405 POTENT INHIBITION OF HSV INFECTION BY MDL 101,028 A BIPHENYL DISULFONIC ACID COPOLYMER, Stanley Tyms*, Parvin Ahmed*, Richard L. Jackson+ Alan D. Cardin+ Marion Merrell Dow Research Institute, *MRC Collaborative Centre Laboratory, Mill HIll, London NW7 1AD and +Cincinnati, Ohio, USA.

Cell surface Heparan sulphate is a key element of the receptor complex for herpes simplex virus (HSV) and human cytomegalovirus (CMV) (Ref). Both viruses are inhibited by the glycoaminoglycan, heparin. MDL 101,028 was selected from a series of polyanionic structures predicted to model heparin. MDL 101,028 is chemically homogeneous but polydisperse in chain length. By plaque reduction assay, MDL 101,028 potently inhibited HSV type-1 and type-2 growth with IC50 values of 0.1 μM. The minimal chain length with this potency consisted of 5-repeating units (MW- 2,000) with chain lengths of 9-10 repeating units being optimal. At high multiplicity of infection, MDL 101,028 inhibited both serotypes when the compound was included during virus adsorption but failed to influence replication once virus had penetrated. When compared to acyclovir (Zovirax$_R$), the currently preferred therapy for HSV, MDL 101,028 was about 10-fold more potent against HSV-2; the acylic nucleoside showed no antiviral activity when present only during virus adsorption. MDL 101,028 was >250-fold more potent against known acyclovir-resistant mutants of HSV-1 than acyclovir (IC50 acyclovir$_{Res}$ 26.4 μM vs. MDL 101,028, 0.1 μM). MDL 101,028 antagonized the host-cell interaction with a potency similar to that of heparin by inhibiting the attachment of [^{3}H] thymidine-labelled HSV type-1. Fusion of the virion envelope with the plasma membrane of the host cell is a key post-binding event for cell penetration. Penetration is mediated by certain virus-encoded surface glycoproteins which also play a role in cell:cell fusion, a dominant trait of syncytia$^+$ phenotypic viruses. As shown by time-lapse video recording of this phenotype, the process of snyctia enlargement (polykaryocytes) was totally independent of DNA synthesis (not blocked by acyclovir) and was partially blocked by heparin. MDL 101,028 abruptly inhibited this process. Thus, MDL 101,028 antagonises virus:cell interactions of this phenotype most probably by interfering with the fusion process. MDL 101,028 is currently being investigated in an animal model of HSV infection.

Glycosaminoglycans

CZ 406 SIMULTANEOUS BINDING OF TWO EXTRACELLULAR EFFECTOR MOLECULES TO SYNDECAN, AN INTEGRAL MEMBRANE, HEPARAN SULFATE-CONTAINING PROTEOGLYCAN. Markku Jalkanen, Jyrki Heino and Markku Salmivirta, Department of Medical Biochemistry and Centre for Biotechnology, University of Turku, P.O. Box 123, SF-20521 Turku, Finland.

Heparin/heparan sulfate interaction is required before, e.g. basic fibroblast growth factor (bFGF) can associate with its high affinity cell surface receptors and trigger signal transduction. On the other hand, promotion of cell growth and differentiation by growth factors during early development and organ formation are both temporally and spatially very precise. Syndecan is a well-characterized integral membrane proteoglycan that binds several extracellular matrix components via its heparan sulfate chains and is therefore suggested to participate in cell regulation. In addition, the expression of syndecan follows morphogenetic rather than histological boundaries during tissue formation. In this paper we show that syndecan, but not free heparan sulfate chains, can simultaneously bind both bFGF and extracellular matrix molecules, like fibrillar collagens and fibronectin. Moreover, increased DNA synthesis of 3T3 cells was observed when the 3T3 cells were exposed to beads coated with the fibronectin-syndecan-bFGF complex, indicating that bFGF remains biologically active even when immobilized to matrix via the heparan sulfate chains of syndecan. Finally, when bFGF was bound to the surface of another cell type (epithelial), co-culture with 3T3 cells stimulated 3T3 cell growth. Therefore, we suggest that syndecan-like molecules may determine sites of growth factor action at cell-matrix and cell-cell interfaces.

CZ 407 THE gp120 GLYCOPROTEIN OF HIV-1 BINDS TO SULFATED GLYCOCONJUGATES IN NEURAL TISSUE, Latov N, Dept of Neurology, Columbia University, New York, N.Y. 10032

The gp120 glycoprotein of HIV-1 was previously reported to bind to the sulfated oligosaccharides extran sulfate (DxS) and pentosamine sulfate (PS). We therefore tested its binding to sulfated glycoconjugates in neural tissue. We found that by ELISA, gp120 bound strongly to sulfatide (GalS) but not to cerebroside, GM1 ganglioside, or sulfated glucuronyl paragloboside. It also bound to the Myelin Associated Glycoprotein (MAG), and the binding to MAG was inhibited by antibodies to its sulfated carbohydrate epitope HNK-1, but not to peptide regions of MAG. Binding to GalS and MAG was seen at gp120 concentrations of less than 1 ug/ml and was inhibited by DxS and PS, but not by dextran, heparan sulfate or chondroitin sulfate, indicating that the reaction did not result from non-specific binding to sulfated determinants. Anti-GalS and anti-MAG antibodies, which bind to rat dorsal root ganglia neurons and to human peripheral nerve myelin respectively, are implicated in human neuropathies, and binding of gp120 to these glycoconjugates in human nerves may have a role in the development of HIV associated neuropathy. gp120 expessed on the surface of infected macrophages might mediate fusion with neural cells, or the neural cells could be damaged by gp120 itself or by anti-gp120 antibodies which recognize gp120 bound to the cell surface. Other sulfated glycoconjugates might serve as receptors for gp120 in other regions of the nervous system.

CZ 408 INTERACTION OF SULFATED POLYSACCHARIDES WITH ARTERIAL SMOOTH MUSCLE CELLS, Didier Letourneur, Delphine Logeart and J. Jozefonvicz, LRM, University Paris-XIII, 93430 Villetaneuse , France.

Proliferation and migration of vascular smooth muscle cells (SMC) are postulated to be one of the key events in the pathogenesis of atheriosclerosis, as well as restenosis that occurs after vascular surgery such as angioplasty. Although a large number of mitogens for SMC are known, relatively few compounds that inhibit SMC proliferation have been identified.

Heparin and heparan sulfates are known to inhibit *in-vitro* and *in-vivo* the proliferation of vascular smooth muscle cells [1]. Structure-function studies have demonstrated that heparin antiproliferative activity is correlated to oligosaccharide size and charge. Such macromolecules also interact with components of the extracellular matrix and with receptors on the cell surface.

We have shown that synthetic sulfated polysaccharides from dextran exhibit antiproliferative activities [2]. Indeed, we hypothesized that a suitable distribution of functional sites (carboxylic and sulfonate groups) on dextran chains confers specific interaction with smooth muscle cells. Moreover, another family of sulfated polysaccharides from marine algae possess the same property. Because these compounds have low anticoagulant activity and are no cytotoxic, they might have some interests in preventing for instance SMC hyperplasia.

This work studied the action mechanism of these polymers on rat arterial smooth muscle cells. These results are compared with studies described with heparin [3]. Radioactive and fluorescent labelings have been made to follow them *in-vitro* and to detect high affinity binding sites.

[1] Clowes A.W. and Clowes M.M., Circ. Res 58, 839-845 (1986).
[2] Avramoglou T. and Jozefonvicz, J. Biomater. Sci. Polymer Edn., 3, 149-154 (1991).
[3] Castellot J.J. et al. J. Cell. Physiol., **124**, 13-20 (1985).

CZ 409 SUBSTRATE RECOGNITION BY MAMMALIAN HEPARANASE, Jin-ping Li, Dagmar Sandbäck, Israel Vlodavsky* and Ulf Lindahl, *Department of Medical and Physiological Chemistry, Biomedical Centre, Uppsala University, Uppsala, Sweden; *Hadassah Medical Organization, Jerusalem, Israel 91120*

Heparin and heparin sulfate (HS) are glycosaminoglycans composed of alternating D-glucosamine and hexuronic (D-glucuronic and L-iduronic) acid units, with sulfate substitutes in various positions. Endo-glycosidases capable of degrading these polymers have been found in various mammalian cells and tissues. One such enzyme was previously identified as an endo-ß-glucuronidase (Ögren and Lindhal, 1975). Cleavage of HS by presumably similar enzyme(s) ('heparanase') have been implicated in various functional contexts, such as extravasation of leucocytes, regulation of growth factor activity and metastasis (Bashkin *et al.*, 1990).The present study was aimed at defining the substrate recognition properties of a heparanase isolated from a human hepatoma cell line (SK-hep-1).

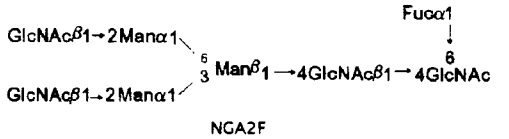
Capsular polysaccharide (PS) from *E. coli* K5 has the same -(ß1,4GlcA-1,4GlcNAc)$_n$-structure as the initial, nonsulfated PS precursor in heparin and HS biosynthesis (Vann *et al.*, 1981). The product obtained by N-deacetylation (hydrazinolysis) followed by chemical N-sulfation of this PS was not cleaved by the hepatoma heparanase, as indicated by gel chromatography. However, incubation of the N-sulfated K5 PS with the sulfate donor, PAPS, in the presence of solubilized microsomal enzymes (N-and O-sulfotransferases, GlcA C5-epimerase that converts D-glucuronic acid into L-iduronic acid units) from a heparin-producing mouse mastocytoma, yielded a product that served as a substrate for the heparanase. Moreover, chemically O-desulfated heparin which is similar to N-sulfated K5 PS but contains, in addition, L-iduronic acid units, was cleaved by the enzyme. These results suggest that the presence of such units may be essential for substrate recognition by the heparanase.

References: Ögren S. and Lindahl U., *J. Biol. Chem.* **250**, 2690 (1975); Bashkin P., Razin E., Eldor A. and Vlodavsky I., *Blood* 75, 2204 (1990); Vann, W.F., Schmidt, M.A., Jann,B and Jann K., *Eur. J. Biochem.* **116**, 359 (1981).

Late Abstract

SUBSTRATE SPECIFICITY AND SUBSTRATE MODELS OF α-L-FUCOSIDASE FROM *HALIOTIS RUFESCENS*,

ALLEN K. MURRAY, and JOHN H. VAN UDEN, GLYCOZYME, INC., 17935 Sky Park Circle, Suite E, Irvine, CA 92714-6321, USA. The substrate specificity of α-L-fucosidase from the marine mollusk, *Haliotis rufescens*, has been investigated. The goal of this study is to predict the potential for activity of the enzyme on various substrates. This predictive ability should eliminate, to some degree, the uncertainty involved when attempting to use glycosidases to modify the carbohydrate moiety of bioactive ligands. In an effort to understand the significant structural parameters which enable the enzyme to act on a substrate, using *Alchemy III*, we have constructed models of the substrates studied. The model studies have enabled us to identify significant characteristics of substrates and to correlate molecular distances and angles with the relative rates of activity of the enzyme. The α-L-fucosidase is active against the synthetic substrates *p*-nitrophenyl-α-L-fucopyranoside and 4-methylumbelliferyl-α-L-fucopyranoside. The enzyme has α-1,2, α-1,3 , α-1,4 and α-1,6 activities using 2'-fucosyllactose, 3-fucosyllactose, lacto-N-fucopentaose II (LNFP II), 6-α-fucosyl GlcNAc and (NGA2F) as substrates. The enzyme was also active against the fucose containing lipophosphoglycan and TF_1 Glycolipid from *Tritrichomonas foetus* although at much lower rates than the other substrates indicated. The enzyme is not active against fucoidan, an α-1,2-fucan which is sulfated in the 4 position.



NGA2F

It is interesting to note that the α-L-fucosidase released fucose from native horseradish peroxidase. Data on the relative activities against these and other glycoprotein and glycopeptide substrates will be presented along with the molecular structures and intramolecular distances determined from the computer models.