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Acceptor Specificity Studies of Fucosyl- and Sialyltransferases

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Academic dissertation

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ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by their roman numerals:

I Pykäri, M.*, Toivonen, S.*, Natunen, J.*, Niemelä, R., Salminen, H., Aitio, O., Ekström, M., Parmanne, P., Välimäki, M., Alais, J., Augé, C., Lowe, J. B., Renkonen, O., and Renkonen, R. (2000) The acceptor and site specificity of human α3-fucosyltransferase V. High reactivity of the proximal and low of the distal Galβ1-4GlcNAc unit in i-type polylactosamines *J. Biol. Chem.* 275, 40057-40063 (* equal contribution)[a]

II Toivonen, S., Nishihara, S., Narimatsu, H., Renkonen, O., and Renkonen, R. (2002) Fuc-TIX: a versatile α1,3-fucosyltransferase with a distinct acceptor- and site-specificity profile. *Glycobiology,* in press[b]

III Toivonen, S., Aitio, O., and Renkonen, O. (2001) α2,3-Sialylation of terminal GalNAcβ1-3Gal determinants by ST3Gal II reveals the multifunctionality of the enzyme. The resulting Neu5Acα2-3GalNAc linkage is resistant to sialidases from Newcastle Disease Virus and *Streptococcus pneumoniae. J. Biol. Chem.* 276, 37141-37148[a]

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ABBREVIATIONS

bFGF	basic fibroblast growth factor
CD	cluster of differentiation
Cer	ceramide
CHO	Chinese hamster ovary
DQFCOSY	double-quantum-filtered correlated spectroscopy
EGF	epidermal growth factor
ESL-1	E-selectin ligand 1
Fuc	L-fucose
Fuc-T	fucosyltransferase
Gal	D-galactose
GalNAc	N-acetyl-D-galactosamine
Glc	D-glucose
GlcNAc	N-acetyl-D-glucosamine
GlyCAM-1	glycosylated cell adhesion molecule 1
Hex	hexose
HexNAc	N-acetylhexosamine
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
Lac	lactose
LacdiNAc	GalNAcβ1-4GlcNAc
Lea	Lewis a, Galβ1-3(Fucα1-4)GlcNAc
Leb	Lewis b, Fucα1-2Galβ1-3(Fucα1-4)GlcNAc
Lex	Lewis x, Galβ1-4(Fucα1-3)GlcNAc
Ley	Lewis y, Fucα1-2Galβ1-4(Fucα1-3)GlcNAc
LN	N-acetyllactosamine (type 2), Galβ1-4GlcNAc
LNB	lacto-N-biose, type 1 N-acetyllactosamine, Galβ1-3GlcNAc
MAG	myelin associated glycoprotein
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
Man	D-mannose
Me	methyl
MHC	major histocompability complex
MOPS	3-(N-morpholino)propanesulfonic acid
NCAM	neuronal cell adhesion molecule
Neu5Ac	N-acetylneuraminic acid
NGF	nerve growth factor
NK	natural killer
NMR	nuclear magnetic resonance
PDGF	platelet-derived growth factor
PNA	peanut agglutinin
PSGL-1	P-selectin glycoprotein ligand 1
sLex	sialyl Lewis x, Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc
sLea	sialyl Lewis a, Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc
ST	sialyltransferase
TOCSY	total correlation spectroscopy
TrkA	tyrosine kinase A
VEGF	vascular endothelial growth factor
VIM-2	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc
WEFT	water-eliminated Fourier transformation
Xyl	D-xylose
X_2	GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc

1. INTRODUCTION

The surfaces of eukaryotic cells are covered with carbohydrates which show an enormous diversity of structure. The glycans mediate a wide variety of cellular recognition events, for example in the recruitment of leukocytes to sites of infection and their homing to lymph nodes, tumour metastasis, neural development, angiogenesis, fertilization, signal transduction and bacterial adhesion.

The biological functions of oligosaccharides are often carried out by the terminal elements of the glycans. Sialylation and fucosylation are typical terminal modifications that mediate specific functions. Both sialic acid and fucose contain structural features which distinguish them from the monosaccharides that make up the common core structures. These structural characteristics may be the reason why sialylated and fucosylated capping group structures are often specifically recognized by both endogenous and exogenous lectins that mediate their functions.

Glycan biosynthesis occurs primarily through the action of glycosyltransferases. Glycan structure is determined by the sequential action of glycosyltransferases, which add one monosaccharide at a time to a specific position on specific precursors. The precision of oligosaccharide biosynthesis is achieved by the strict acceptor substrate specificity of glycosyltransferases. "One glycosyltransferase - one glycosidic linkage" is an old paradigm in the field of oligosaccharide biosynthesis. However, more recently this view has been broadened to encompass the findings that many glycosyltransferases form redundant families, with more subtle differences in acceptor specificity between the members: some transferases can make two different linkages; and some have relaxed substrate specificities, being able to transfer two different monosaccharides or to two different monosaccharides. The clarification of the detailed specificities of the individual members of glycosyltransferase families will eventually help us understand the different biological roles of the various isoenzymes. The present study clarifies the detailed acceptor specificities of the α1,3-fucosyltransferases Fuc-TV and Fuc-TIX, and the α2,3-sialyltransferase ST3Gal II.

2. REVIEW OF THE LITERATURE

2.1 Glycan structure

Carbohydrate chains are attached to proteins and lipids on cell surfaces. Glycoproteins and free oligosaccharides are found in body fluids and secretions. A basic principle of glycan structure is that each class of glycans has a limited number of common core structures, to which a diversity of capping groups is attached to a more terminal location. The terminal capping groups are often responsible for the biological functions of carbohydrates. This review will deal mainly with mammalian glycosylation. For reviews of the structures described below, see (1,2).

2.1.1 Core structures

Glycoprotein glycans are N-linked to asparagine (N-glycans), or O-linked to serine or threonine (O-glycans). Mammalian N-glycans share a common pentasaccharide core Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn. On the basis of the structure that is assembled on the core they are divided into high-mannose, hybrid and complex types. The innermost GlcNAc of complex type N-glycans can be α1,6-fucosylated. O-glycans have four common core structures: core 1 (Galβ1-3GalNAcα1-Ser/Thr), core 2 (Galβ1-3(GlcNAcβ1-6)GalNAcα1-Ser/Thr), core 3 (GlcNAcβ1-3GalNAcα1-Ser/Thr) and core 4 (GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα1-Ser/Thr), and some less rare GalNAc-linked cores. Less common types of O-glycosylation include N-acetylglucosamine (3), fucose (4), or mannose (5) O-linked to serine or threonine. Of these, O-GlcNAc has only been characterized as a monosaccharide, but O-Fuc and O-Man can be further elongated. Heparan sulphate and chondroitin sulphate glycosaminoglycans are O-linked to their core proteins via a Galβ1-3Galβ1-4Xyl core. Lipid-linked glycans are divided into five families according to their core structures: ganglio- (GalNAcβ1-4Galβ1-4Glcβ1-Cer), globo- (Galα1-4Galβ1-4Glcβ1-Cer), isoglobo- (Galα1-3Galβ1-4Glcβ1-Cer), lacto- (Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer), and neolactoseries (Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer). In addition, the GPI-anchors of certain proteins contain a lipid-linked glycan as well.

2.1.2 Capping group structures

The core structures are often elongated by repeating Galβ1-4GlcNAcβ1-3 units called polylactosamines. Polylactosamine chains are either linear or branched at the C6 hydroxyl of the galactose residue. Polylactosamines on glycoproteins were first characterized in 1978 from human erythrocyte membranes by Roger Laine (6) and Heikki Rauvala (7) and their co-workers. Subsequently polylactosamines have emerged as common oligosaccharide structures on both glycoproteins and glycolipids (1,2). Polylactosamine backbones carry bioactive terminal epitopes, like the sialylated and fucosylated structures described here.

Fucosylated structures

Fucose is found α1,3-, α1,4- or α1,6-linked to N-acetylglucosamine and α1,2-linked to galactose in mammalian glycans (Figure 1). α1,3-linked fucose has been characterized on type 2 N-acetyllactosamine (Galβ1-4GlcNAc) forming the Lewis x structure (Galβ1-4(Fucα1-3)GlcNAc; Lex), and on the so-called LacdiNAc structure (GalNAcβ1-4GlcNAc). The Lewis a structure (Galβ1-3(Fucα1-4)GlcNAc) contains an α1,4-linked fucose on a type 1 N-acetyllactosamine. Both Lewis x and Lewis a also occur in sialylated and sulfated forms. Lewis x epitopes are also found as polyfucosylated sequences on polylactosamines. Lewis y (Fucα1-2Galβ1-4(Fucα1-3)GlcNAc) and Lewis b (Fucα1-2Galβ1-3(Fucα1-4)GlcNAc) are doubly fucosylated derivatives of Lewis x and Lewis a. In addition to Lewis y and Lewis b, α1,2-fucosylation occurs

in the ABO-blood group determinants A, B and H, and for example in the so called fucosyl-GM1 structure. Fucose also occurs α1,6-linked to the innermost core GlcNAc of complex N-glycans, and directly O-linked to Ser or Thr in certain proteins, where it can be elongated into an oligosaccharide such as Neu5Acα2-3Galβ1-4GlcNAcβ1-3Fucα1-Ser/Thr.

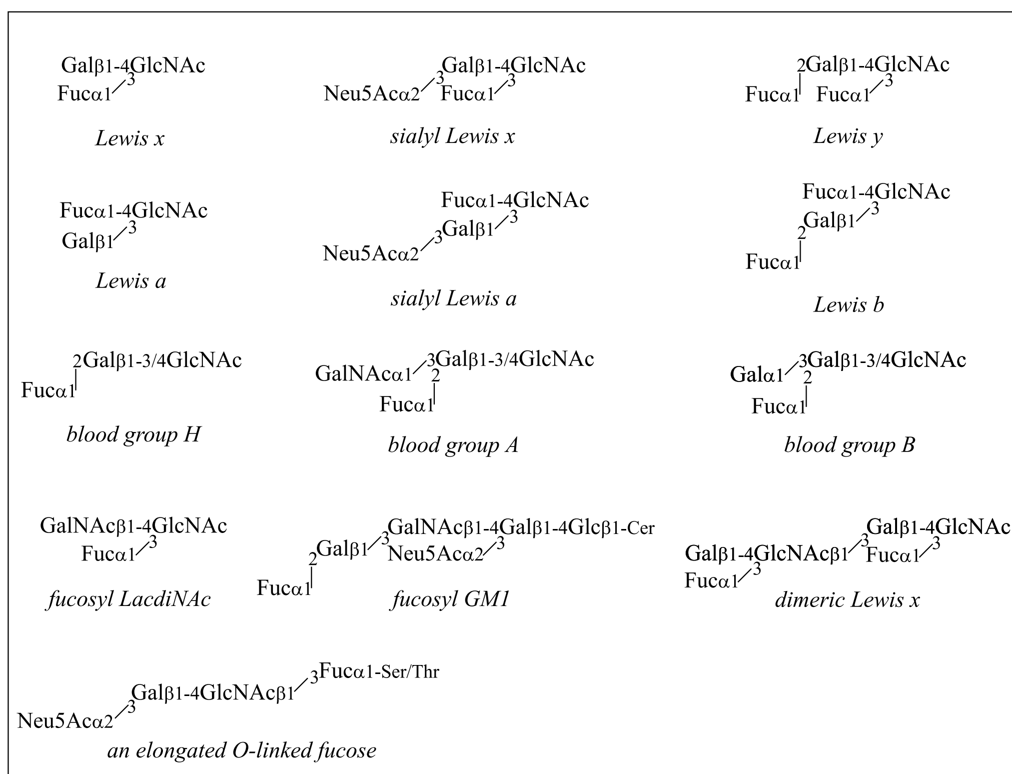


Figure 1. Examples of fucosylated glycan structures

Sialylated structures
Sialic acid is a common terminal monosaccharide in a variety of glycans. Due to its negative charge, it dramatically changes the physical and chemical properties of the glycan, which renders it capable of mediating a wide array of biological functions. Sialic acid occurs in mammalian systems mainly α2,3-linked or α2,6-linked to galactose or N-acetylgalactosamine, and α2,8-linked to another sialic acid. Sialylated glycolipids are called gangliosides. The Svennerholm nomenclature for glioseries gangliosides (8) is used throughout this text. Examples of sialylated glycan structures are presented in Figure 2.

2.2 Glycan biosynthesis

Glycan biosynthesis occurs primarily through the action of glycosyltransferases, although some glycosidases are involved as well. The precursor oligosaccharide of N-glycans is assembled on a dolichol lipid, and then transferred *en bloc* to the polypeptide by the oligosaccharyltransferase. O-glycan biosynthesis is initiated by a polypeptide GalNAc-transferase, and glycolipid biosynthesis by a glucosyl- or galactosyltransferase acting on ceramide. Glycan structure is determined by the sequential action of glycosyltransferases, which add one monosaccharide at a time to a specific position on specific precursors. Glycosyltransferases transfer monosaccharides from

activated donor sugars (e.g. CMP-Neu5Ac, GDP-Fuc) to the growing glycan. The precision of glycan biosynthesis is achieved through the strict acceptor substrate specificity of glycosyltransferases. This review will concentrate on the acceptor specificities of the cloned human glycosyltransferases that are responsible for the generation of fucosylated and sialylated capping groups on various backbone structures.

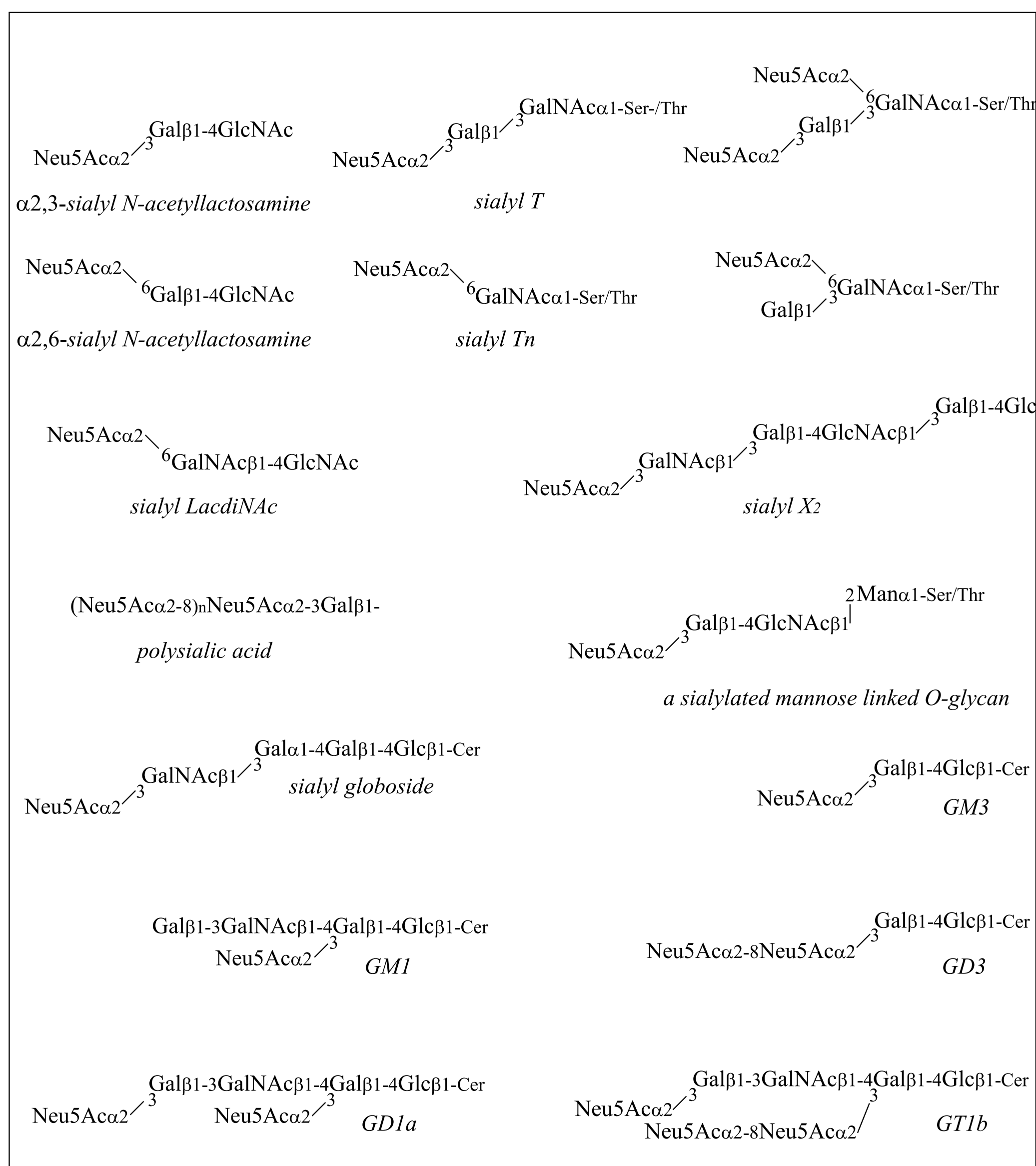


Figure 2. Examples of sialylated glycan structures

2.2.1 Fucosyltransferases

The cloned human fucosyltransferases have been designated Fuc-TI-IX. Fucosyltransferases I and II are α1,2-fucosyltransferases, III-VII and IX α1,3/4 fucosyltransferases, and Fuc-TVIII is an α1,6-fucosyltransferase that fucosylates the chitobiose core of N-glycans. In addition, the protein O-fucosyltransferase I, O-FucT-I, transfers fucose directly to serine or threonine in EGF-like repeats of certain proteins (9).

α1,3-Fucosyltransferases

Table I shows the relative reactivities of different acceptors with the cloned human α1,3-fucosyltransferases. The data have been compiled from a set of different studies that have been referred to in Table I. Some discrepancies exist between them. These differences may be due to differences in enzyme constructs, the exact structure of the acceptor (e.g. the nature of a possible aglycon), or reaction conditions such as the acceptor concentration, or the presence and nature of detergent. When experiments are performed with cell lysates rather than with purified enzymes, unexpected endogenous glycosyltransferase activities may occur (10,11).

Table I. The relative reactivities of different acceptor oligosaccharides with the α1,3-fucosyltransferases.

Acceptor	Fuc-TIII	Fuc-TIV	Fuc-TV	Fuc-TVI	Fuc-TVII	Fuc-TIX
LN	+	+++	+++	+++	-	+++
Neu5Acα2,3'LN	+	+	+++	+++	+++	-
Neu5Acα2,6'LN	-	-	-	-	-	
Fucα1,2'LN	++	+++	+++	+++	-	+++
Galα1,3'LN	+	+++	+++			
6-sulfo-LN	-	++	++			
6'-sulfo-LN	-	-	-			
3'-sulfo-LN	+	++	+++	+++	+	+
Lac	+	-	+	-	-	-
Fucα1,2'Lac	++	+	++	+	-	
Neu5Acα2,3'Lac	+	-	-	-		
LNB	+++	-	+	-	-	-
Neu5Acα2,3'LNB	++	-	+		-	
Fucα1,2'LNB	+++	+	++	-		-
Galα1,3'LNB	+++	-	++			
LacdiNAc	+	+++			-	++
Galβ1-3GalNAc	-	-	-	-	-	-
references	(12,14-19, 36,60)	(14-16,33, 36,60)	(14-16,36, 60)	(32,35,36, 38,60)	(33,50,51, 60)	(58-60)

+++: the best acceptor for the transferase in question, or having reactivity close to the best reactivity, ++: reactivity about half of that of the best acceptor, +: weak reactivity, -: no or very weak reactivity, blank: no information available

Fuc-TIII
Fuc-TIII was the first human α1,3-fucosyltransferase to be cloned in 1990 (12). Fuc-TIII is thought to correspond to the purified fucosyltransferase known as the "Lewis" enzyme (13), since it has both α1,3- and α1,4-fucosyltransferase activities, being able to fucosylate both Galβ1-4GlcNAc (type 2 lactosamine, LN) and Galβ1-3GlcNAc (type 1 lactosamine, LNB) (12). Fuc-TIII strongly prefers type 1 lactosamine acceptors to type 2 acceptors (12,14,15). In some systems Fuc-TIII has been reported to be completely inactive towards Galβ1-4GlcNAc (16-18).

α1,2-fucosylated lactosamines are better acceptors for Fuc-TIII than the corresponding unsubstituted ones, whereas α2,3-sialylated lactosamines are slightly less effective acceptors (14,15,17-19). Additionally, Fuc-TIII transfers to Glc in lactose (12,15,16), α1,2-fucosyllactose (15,16) and α2,3-sialyllactose (16), and to the reducing end glucose of Galβ1-4GlcNAcβ1-3Galβ1-4Glc and Galβ1-3GlcNAcβ1-3Galβ1-4Glc; a bifucosylated product is formed from the latter acceptor (19).

Analysis of glycolipids from Fuc-TIII transfected cells has shown that Fuc-TIII is able to make both Lex and sLex *in vivo* as well (20). Fuc-TIII synthesizes sLex and Lex in the ratio 14:1 on the N-glycans of β-trace protein *in vivo*, transferring preponderantly one fucose per diantennary N-glycan (21). Fuc-TIII has also been shown to be able to generate the VIM-2 epitope, that is to fucosylate the penultimate N-acetyllactosamine unit in a sialylated polylactosamine (22). When LNβ1-3'LNβ1-3'LN-2AB is used as the acceptor, Fuc-TIII strongly prefers the middle LN unit as the acceptor site (the reducing end GlcNAc is not likely to be available for fucosylation in this kind of acceptor, as it has been reductively aminated) (23).

Fuc-TIII is expressed at high levels in colon, stomach, small intestine, lung and kidney (24). Inactivating point mutations in the Fuc-TIII gene account for the Lewis negative (Le(a-b-)) phenotype on erythrocytes and secretions observed in 10% of Caucasians (25,26). An increased risk of atherosclerotic disease has been associated with Le(a-b-) phenotype, but the mechanism of this effect is unclear (27,28).

Fuc-TIV
Fuc-TIV has been independently cloned by three groups (29-31). It corresponds to the "myeloid" type of fucosyltransferase activity (13). Fuc-TIV fucosylates preferentially neutral type 2 N-acetyllactosamine (30,31). It does not react with type 1 acceptors (16,31,32), and reacts very weakly if at all with Neu5Acα2-3Galβ1-4GlcNAc (16,30,31,33). Fuc-TIV reacts very effectively with Fucα1-2Galβ1-4GlcNAc, generating the Lewis y epitope (14). Galα1-3Galβ1-4GlcNAc is a good acceptor, and N-acetyllactosamines sulfated at the 3-position of Gal or at the 6-position of GlcNAc show moderate reactivity (14). The analysis of glycolipids from Fuc-TIV transfected cells supports the view that Fuc-TIV synthesizes Lex rather than sLex (20). In the analysis of β-trace protein N-glycans, Fuc-TIV shows a sLex/Lex ratio 1:7 (21).

Like Fuc-TIII, Fuc-TIV fucosylates inner LN units within a polylactosamine chain. Fuc-TIV-transfected cells synthesize the VIM-2 epitope (Neu5Acα2-3'LNβ1-3'Lex) (30,31). It has been shown that Fuc-TIV prefers the inner LN units to the non-reducing end LN unit in both sialylated and non-sialylated polylactosamine acceptors (34). In sialylated polylactosamine acceptors the sialylated LN unit is virtually non-reactive, whereas in neutral polylactosamines the differences between the reactivities of the different LN units are less dramatic (34).

Fuc-TV

Fuc-TV is highly homologous to Fuc-TIII, but has a slightly different acceptor specificity profile (15). Fuc-TV reacts with both sialylated and non-sialylated type 2 N-acetyllactosamine, and weakly with type 1 N-acetyllactosamine and lactose (15). In *in vivo* studies Fuc-TV has been shown to synthesize both Lex and sLex on both glycolipids (20) and glycoproteins (21), with a slight preference towards sialylated structures. In addition, α1,2'-fucosylated, α1,3'-galactosylated, 3'-sulfated and 6-sulfated derivatives of LN serve as acceptors *in vitro* (14-16,35,36). (3' denotes modification at the 3-hydroxyl of the second monosaccharide residue from the reducing end, in this case Gal, whereas 3 would refer to modification at the reducing end monosaccharide residue, in this case GlcNAc.) Fuc-TV is able to react with chito-oligosaccharides, forming a terminal GlcNAcβ1-4(Fucα1-3)GlcNAc determinant (37). Like Fuc-TIII and Fuc-TIV, Fuc-TV preferentially fucosylates the inner LN unit in a polylactosamine chain (23). Fuc-TV expression is restricted to liver, colon and testicle (24). The detailed acceptor- and site-specificity of Fuc-TV has been studied in the present thesis (part I).

Fuc-TVI

The enzymatic properties of cloned Fuc-TVI correspond to the fucosyltransferase previously known as "plasma" type (13,35,38). Fuc-TVI reacts with sialylated and non-sialylated type 2 N-acetyllactosamine (32,35,36,38). The reactivities of type 1 lactosamine (32,36,38) and lactose (36,38) are very weak or non-existent. α1,2'-Fucosylated or 3'-sulfated type 2 lactosamines react efficiently (32,36). Additionally, Fuc-TVI has been shown to be able to fucosylate GlcNAcβ1-4GlcNAc (37).

Fuc-TVI makes both Lex and sLex on glycolipids *in vivo* (20). Fuc-TVI prefers longer glycolipids as acceptors than Fuc-TIII, -IV, -V and -VII, but the structures of the products have not been analyzed in detail (20). Fuc-TVI makes equal amounts of Lex and sLex on a glycoprotein substrate *in vivo*, and efficiently fucosylates both branches of a biantennary N-glycan, unlike Fuc-TIII, -IV, -V and -VII, which predominantly make monofucosylated N-glycans (21).

The site-specificity of Fuc-TVI seems to be different on sialylated and non-sialylated polylactosamines. Fuc-TVI preferentially fucosylates the inner LN unit in LNβ1-3'LNβ1-3'LN-2AB, and virtually no difucosylated product is formed (23). However, Fuc-TVI transfected CHO cells do not stain with anti-VIM-2 antibodies, but do stain with anti-difucosyl-sLex (Neu5Acα2-3'Lexβ1-3'Lex) antibodies (35). The clarification of the site-specificity of Fuc-TVI on sialylated and non-sialylated polylactosamines will require experiments where both types of acceptors are analyzed under the same experimental setup.

Fuc-TVI is expressed in liver, kidney, small intestine, colon, salivary gland, bladder, and uterus (24). Fuc-TVI in liver has been found to be essential for the fucosylation of acute phase proteins (39). Liver Fuc-TVI is also the major source of the α1,3-fucosyltransferase activity in plasma (39,40). In addition to the Golgi, Fuc-TVI has rather surprisingly been shown to be localized in the Weibel-Palade bodies of human endothelial cells (41). Individuals having a missense mutation in the Fuc-TVI gene show no obvious pathologies, indicating that Fuc-TVI function is dispensable in humans, or that other fucosyltransferases can compensate for the lack of Fuc-TVI (40).

Fuc-TIII, Fuc-TV and Fuc-TVI genes are highly homologous to each other (12,15,35,38). They form a cluster on human chromosome 19p13.3 (42). Fuc-TIII, -V and -VI are thought to have originated by duplication events in human evolution, since only one homologue has been found from the bovine genomic library (43). This bovine gene is thought to be an orthologous

homologue of the ancestor of the human Fuc-TIII, -V and -VI genes (43). The bovine α1,3-fucosyltransferase shows an acceptor specificity profile similar to human Fuc-TVI, consistent with the finding that bovine tissues do not express α1,4-fucosylated structures (43). Despite their high sequence homology, human Fuc-TIII, -V and -VI have different acceptor specificity profiles, as discussed above. Domain swapping experiments, sequence alignment of α1,3- and α1,3/4-fucosyltransferases, and site-directed mutagenesis experiments have demonstrated that only a few amino acids determine whether or not the fucosyltransferases can use type 1 lactosamines as substrates, and thus conform to their distinct acceptor specificity profiles (44-48).

Fuc-TVII

Fuc-TVII exhibits strict specificity towards α2,3-sialylated type 2 lactosamines (49,50). The reactivity of non-sialylated and type 1 acceptors is very weak or non-existent, depending on the assay conditions (33,50,51). Fuc-TVII prefers sialylated N-acetyllactosamines to non-sialylated ones also when studied *in vivo* with glycoprotein (21) and glycolipid (20) acceptors. Fuc-TVII is able to react with Neu5Acα2-3Galβ1-4(SO_3-6)-GlcNAc, generating the L-selectin ligand 6-sulfo sLex (52).

The site-specificity of Fuc-TVII is also strict. Fuc-TVII has been shown to fucosylate Neu5Acα2-3'LNβ1-3'LNβ1-3'Lacβ1-Cer glycolipid only at the non-reducing end, sialylated LN unit (53). Similar results have been obtained by using oligosaccharide acceptors: Fuc-TVII transfers preferentially to the non-reducing end, sialylated LN unit of both Neu5Acα2-3'LNβ1-3'LNβ1-3Galβ1-OMe and Neu5Acα2-3'LNβ1-3'LNβ1-3'LN (34). Fuc-TVII prefers the non-reducing end site also on prefucosylated polylactosamines: Neu5Acα2-3'LNβ1-3'Lexβ1-R type acceptors react efficiently, whereas Neu5Acα2-3'Lexβ1-3'LNβ1-R type acceptors react only weakly (34,51).

Fuc-TIV and Fuc-TVII are expressed on leukocytes, and they are thought to collaborate in the biosynthesis of selectin ligands (for discussion on selectins and selectin ligands, see section 2.3.1). They seem to be at least partially specialized in the way that Fuc-TVII directs the expression of P-selectin binding glycoforms of P-selectin glycoprotein ligand 1 (PSGL-1) and controls the rolling frequency of leukocytes, whereas Fuc-TIV directs the expression of E-selectin binding glycoforms of E-selectin ligand 1 (ESL-1) and dictates rolling velocity (54,55). Fuc-TVII-deficient mice show defects in their selectin ligand activity and leukocyte recruitment in inflammation (52). However, prominent selectin ligand activities remain in these mice, whereas in mice deficient in both Fuc-TIV and Fuc-TVII nearly all selectin ligand activity and leukocyte recruitment is absent (56). In the light of the complementary site-specificities of Fuc-TIV and Fuc-TVII (34), it seems likely that the biosynthesis of the complete repertoire of optimal ligands for all three of the selectins involves both internal and distal fucosylation of polylactosamine chains.

An individual having an inactivating missense mutation in his Fuc-TVII gene has been diagnosed with ulcer disease, non-insulin-dependent diabetes, osteoporosis, spondylarthrosis, and Sjögren's syndrome, but does not have a history of recurrent bacterial infections or leukocytosis (57).

Fuc-TIX

Fuc-TIX transfers fucose to non-sialylated type 2 lactosamine, but not to α2,3-sialylated type 2 lactosamine, or to type 1 lactosamine (58). 3'-sulfated type 2 lactosamine reacts weakly (59). α1,2'-fucosylated type 2 lactosamine is a good acceptor, but α1,2-fucosylated type 1 lactosamine

does not react (60). Lactose is non-reactive as well (60). LacdiNAc (GalNAcβ1-4GlcNAc) shows moderate reactivity (60). Fuc-TIX shows unique site-specificity on LNβ1-3'LNβ1-3'LN-2AB: it preferentially fucosylates the non-reducing end LN unit (23). The acceptor and site-specificity of Fuc-TIX has been studied in detail in this thesis (part II).

Fuc-TIX has a low degree of homology to the other α1,3/4 fucosyltransferases, and is phylogenetically quite distant from them (58). In contrast, the Fuc-TIX gene sequence is highly conserved between human, mouse, rat and hamster (58,61-63), indicating that it has been under strong selective pressure during evolution, and thus suggesting that it has an essential role in the organisms.

Fuc-TIX has a more restricted expression pattern than the other α1,3/4-fucosyltransferases. It is expressed as transcripts of different lengths in different tissues and developmental stages (58,60). Notably, Fuc-TIX is abundantly expressed in both the developing and mature brain (58,60). The expression of the Fuc-TIX product, the Lewis x epitope, is developmentally regulated in the brain, and it is thought to be involved in brain morphogenesis, as discussed in section 2.3.1. *Pax6*, a transcription factor involved in brain patterning and neurogenesis, controls the spatially and temporally restricted expression of the Lewis x epitope in the developing rat brain by regulating Fuc-TIX gene expression (64). A concomitantly occurring increase in the amounts of Lewis x bearing glycolipids and increased expression of Fuc-TIX has been observed during neural differentiation of PC19 embryonal carcinoma cells induced by retinoic acid (65).

Fuc-TIX has been shown to direct the synthesis of CD15 (non-sialylated Lewis x) on mature granulocytes (66), but its possible role in the selectin ligand biosynthesis remains to be elucidated. It is possible that Fuc-TIX accounts for the residual neutrophil infiltration in experimentally induced inflammation seen in mice that lack both Fuc-TIV and Fuc-TVII (56).

α1,2- and α1,6-Fucosyltransferases

Fuc-TI and Fuc-TII are α1,2-fucosyltransferases. Fuc-TI (H-enzyme) regulates the expression of the H antigen (Fucα1-2Gal) on erythrocyte membranes (67). Fuc-TII (Secretor enzyme) regulates the expression of the H antigen in the secretory fluids and digestive mucosa (68). Fuc-TVIII is an α1,6-fucosyltransferase, that fucosylates the chitobiose (GlcNAcβ1-4GlcNAc) core of N-glycans (69). Phylogenetic analysis indicates that the vertebrate α1,2-, α1,3/4- and α1,6-fucosyltransferase gene families have evolved by duplications, translocations, and divergent evolution from a single ancestral gene (70,71).

2.2.2 Sialyltransferases

Sialyltransferases transfer sialic acid from CPM-Neu5Ac to glycolipids and glycoproteins. To date, six α2,3-sialyltransferases, seven α2,6-sialyltransferases and five α2,8-sialyltransferases have been cloned. All sialyltransferases cloned to date contain a conserved region called a "sialylmotif", which is involved in binding the donor substrate CMP-Neu5Ac (72). Conserved cysteines in the sialylmotif form a disulfide linkage that seems to be essential for proper conformation and activity of the enzymes (73).

Nomenclature of sialyltransferases

The naming of sialyltransferases used to be rather confusing. Multiple names were given to the same enzyme, and even the same name was given to different enzymes. The names did not give sufficient information for distinguishing the different transferases. In 1996 a systematic nomenclature was proposed (74), and since then it has become universally used. This system comprises

four elements, for example the CMP-Neu5Ac: Galβ1-4GlcNAc α2,6-sialyltransferase is abbreviated ST6Gal I, where ST denotes sialyltransferase, 6 means that it is an α2,6-sialyltransferase, Gal is the acceptor sugar to which sialic acid is transferred, and I is the numeral assigned consecutively to each new gene in the subgroup. In the following sections the older names for the sialyltransferases are indicated in parenthesis following the current denotation. Unless otherwise stated, the data refer to human sialyltransferases.

α2,3-Sialyltransferases

ST3Gal I (ST3O, ST3GalA.1, SiaT-4a, SAT-4, ST-2, ST3Gal-Ia)
ST3Gal I transfers sialic acid to Galβ1-3GalNAc, but not to Galβ1-3GlcNAc or Galβ1-4GlcNAc (75-77). Mouse ST3Gal I has been shown to prefer glycoprotein acceptors (asialofetuin) to glycolipid acceptors (GM1 and asialo GM1) *in vitro* (78). The acceptor specificities of α2,3-sialyltransferases are summarized in Table II.

Table II. Relative reactivities of different acceptors with the α2,3-sialyltransferases

Acceptor	ST3Gal I	ST3Gal II	ST3Gal III	ST3Gal IV	ST3Gal V	ST3Gal VI
Galβ1-4GlcNAc	-	-	++	++		+++
Galβ1-3GlcNAc	-	-	+++	+		+
Galβ1-3GalNAc	+++	+++	+	+++		-
Galβ1-4Glc	-		+	+		
lactosylceramide	-		-	+	+++	-
asialo GM1	++	+++	-	+++	-	-
asialofetuin	+++	++	+++	+++	-	++
references	(76,77)	(83,84)	(77,87)	(77,92)	(98,101)	(100)

+++: the best acceptor for the transferase in question, or having reactivity close to the best reactivity, ++: reactivity about the half of that of the best acceptor, +: weak reactivity, -: no or very weak reactivity, blank: information not available

ST3Gal I is expressed in many tissues. High levels are found in heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen and peripheral blood leukocytes (76). Expression of ST3Gal I is elevated in breast cancer cells, associated with the synthesis of the cancer-associated epitope sialyl T (Neu5Acα2-3Galβ1-3GalNAcα1-Ser/Thr) (79,80).

ST3Gal I-deficient mice show increased apoptosis of $CD8^+$ T-cells (81). Activation of T-cells is associated with desialylation of core 1 O-glycans and concomitant increase in core 2 biosynthesis (82). The activated T-cells bearing core 2 O-glycans are destined for either apoptosis, or differentiation into memory cells, which is accompanied by the reappearance of sialylated core 1 O-glycans as the predominant O-glycan structure (81). Therefore it can be assumed that ST3Gal I is involved in the regulation of T-cell homeostasis.

ST3Gal II (ST3O-II, ST3GalA.2, SiaT-4b, ST3Gal-Ib)
Like ST3Gal I, ST3Gal II uses Galβ1-3GalNAc as an acceptor determinant (83,84). Galβ1-4GlcNAc and Galβ1-3GlcNAc are not sialylated by ST3Gal II (83). Mouse ST3Gal II prefers glycolipid acceptors to glycoprotein acceptors *in vitro* (78). Species-specific differences exist in the tissue expression patterns of ST3Gal II. Human ST3Gal II is expressed in heart, liver, skeletal muscle and lymphoid tissues, but not in brain or kidney (83), whereas in mouse and rat the expression of ST3Gal II is restricted to the brain and liver (85). ST3Gal II-deficient mice

accumulate ST3Gal II substrates GM1a and GD1b, but yet have significant amounts of the products GD1a and GT1b, possibly due to the action of ST3Gal I (86). The acceptor specificity of ST3Gal II was further studied in this thesis (part III).

ST3Gal III (ST3N, ST3GalB, ST-3, ST3Gal II)
ST3Gal III sialylates both Galβ1-3GlcNAc and Galβ1-4GlcNAc, the type 1 lactosamine being the preferred acceptor (87). Lactose and Galβ1-3GalNAc are poor acceptors (77,87). A commercial cloned α2,3-sialyltransferase, supposedly ST3Gal III, has been reported to be able to sialylate Galβ1-3GlcNAc where the 6-hydroxyl of GlcNAc has been substituted by sulphate or sialic acid (88). Rat ST3Gal III has been shown to prefer tri- and tetra-antennary N-glycans to biantennary N-glycans, and intact glycoproteins to glycopeptides (89), suggesting that it recognizes larger structures than the acceptor disaccharide determinant.

ST3Gal III is strongly expressed in liver, skeletal muscle, testis, and many fetal tissues (76,87). ST3Gal III expression level in the female rat pituitary gland has been shown to be regulated by oestrogens, and to be associated with the changes in the sialylation status of follicle-stimulating hormone, affecting its charge-isoform distribution and biological activity (90). An unexpected post-Golgi localization of ST3Gal III on the apical membrane of rat kidney tubule cells has been demonstrated (91).

ST3Gal IV (ST3O/N, ST3GalC, SiaT-4c, SAT-3, ST-4, STZ, ST3Gal III)
ST3Gal IV sialylates Galβ1-3GalNAc, Galβ1-4GlcNAc and it poorly sialylates Galβ1-3GlcNAc and lactose (77,92). ST3Gal IV has been shown to sialylate both glycolipids and glycoproteins presenting Galβ1-3GalNAc or Galβ1-4GlcNAc determinants (77). ST3Gal IV is strongly expressed in the placenta, testis and ovary (76). Cell type-specific transcriptional regulation has been described for ST3Gal IV in epithelial and leukemia cell lines (93,94). Transfection with ST3Gal IV has been shown to confer increased expression of sialyl Lewis x in Namalwa cells (92), suggesting a possible role in the selectin ligand biosynthesis. ST3Gal IV, together with Fuc-TVII, is rapidly upregulated upon activation of $CD4^+$ T-cells, resulting in the synthesis of P-selectin ligands and migration into inflamed tissue (95) (for selectins and selectin ligands see section 2.3.1). ST3Gal IV is upregulated in the hippocampus of the mouse following kindled seizures, suggesting that it may be involved in neural plasticity (96). ST3Gal IV-deficient mice have a markedly reduced number of platelets and have a significant increase in bleeding time, but the exact role of ST3Gal IV in hematopoiesis and hemostasis remains to be studied (97).

ST3Gal V (GM3 synthase)
GM3 synthase initiates the biosynthesis of the ganglio-series gangliosides by converting lactosylceramide into GM3 (Neu5Acα2-3Galβ1-4Glcβ1-Cer) (98). ST3Gal V is expressed tissue specifically, predominantly in the brain, skeletal muscle and testis (98). ST3Gal V has been shown to localize to axons in addition to the Golgi in mouse and rat neurons (99).

ST3Gal VI
ST3Gal VI shows a strict acceptor specificity towards type 2 lactosamines (100). Both glycoproteins and glycolipids containing Galβ1-4GlcNAc serve as acceptors for ST3Gal VI, but those containing Galβ1-3GlcNAc or Galβ1-3GalNAc do not. Free Galβ1-4GlcNAc oligosaccharide reacts readily with ST3Gal VI, Galβ1-3GlcNAc reacts weakly and Galβ1-3GalNAc not at all. Lactosylceramide is not an acceptor for ST3Gal VI (100).

α2,6-Sialyltransferases
ST6Gal I is the only known sialyltransferase that sialylates the 6-position of galactose (102,103). ST6GalNAc I and ST6GalNAc II sialylate the GalNAc in GalNAcα1-Ser/Thr, Galβ1-3GalNAcα1-Ser/Thr and Neu5Acα2-3Galβ1-3GalNAcα1-Ser/Thr (104-107). ST6GalNAc I and ST6GalNAc II require glycopeptide acceptors; they are inactive towards oligosaccharides and glycolipids containing the appropriate acceptor determinants (104,107). ST6GalNAc III has only been cloned from the mouse and rat so far (108,109). ST6GalNAc III and ST6GalNAc IV sialylate Neu5Acα2-3Galβ1-3GalNAc determinants in glycoprotein and glycolipid acceptors, but are inactive towards non-sialylated Galβ1-3GalNAc (108,110). ST6GalNAc V seems to be specific for GM1b (Neu5Acα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-Cer): it does not react with other glycolipids, or glycoproteins containing the Neu5Acα2-3Galβ1-3GalNAc determinant. ST6GalNAc V has only been cloned from the mouse so far, and it is expressed specifically in the brain (111,112). Mouse ST6GalNAc VI sialylates GM1b, GT1b and GD1a, but not glycoproteins (113).

α2,8-Sialyltransferases
ST8Sia I (GD3 synthase) is specific for GM3 (114-116). ST8Sia II (STX) and ST8Sia IV (PST) are α2,8-sialyltransferases capable of synthesizing polysialic acid (117,118). Polysialic acid is an α2,8-linked sialic acid polymer that occurs specifically on the embryonic form of the neural cell adhesion molecule and a limited number of other proteins, as discussed in section 2.3.2. ST8Sia III sialylates the sialic acid in Neu5Acα2-3Galβ1-4GlcNAc (119), and ST8Sia V sialylates the α2,3-sialylated gangliosides GM1b, GD1a, GT1b and GD3, but not GM3 (120).

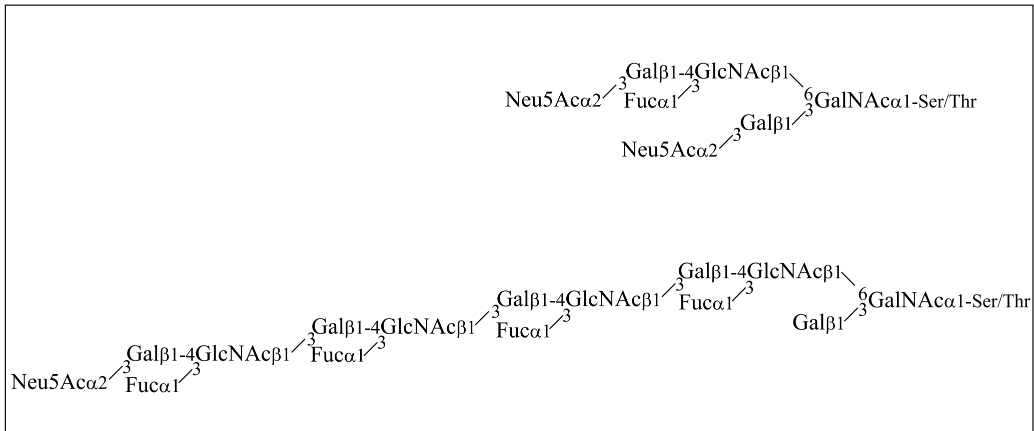
2.3. Glycan Function

Since sialylated and fucosylated epitopes are usually situated terminally in glycan structures, they are in a position which is easily accessible to lectins. Moreover, sialic acid and fucose have distinct structural characteristics that make them different from the monosaccharides that make up the common core structures. Therefore it is not surprising that a large proportion of the known glycan functions are associated with the sialylated and fucosylated structures. The next sections describe some examples of these, concentrating on the best-characterized phenomena and recent findings.

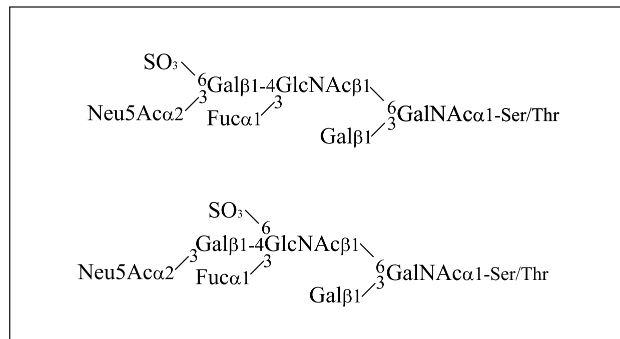
2.3.1. Functions of fucosylated glycans

The importance of fucosylated glycan structures is highlighted by the clinical manifestations of LAD II (leukocyte adhesion deficiency II; also known as CDG-IIc, congenital disorder of glycosylation IIc), a disorder where the GDP-fucose transporter, that provides the donor GDP-fucose for all fucosyltransferases, is defective (121). LAD II patients are deficient in all fucosylated glycans. Children suffering from LAD II have distorted facial features and frontal cerebral atrophy leading to severe psychomotor retardation. They suffer from growth retardation, general failure to thrive, and immunodeficiency leading to recurrent infections (122-124).

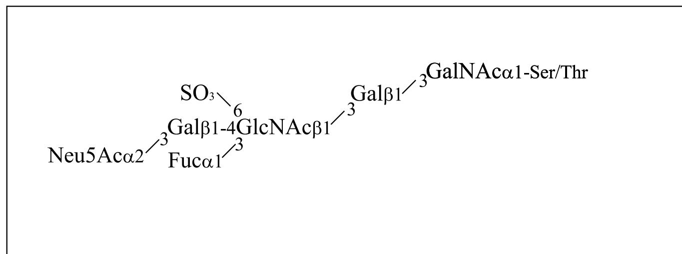
The selectins and their fucosylated ligands mediate vascular cell adhesion
The selectins mediate the primary adhesion and rolling step of the leukocyte extravasation cascade leading to leukocyte infiltration into inflamed tissue and homing to lymph nodes, reviewed in (125,126). Three selectins are known: L-selectin that is constitutively expressed by most leukocytes, P-selectin that is transported from storage vesicles to cell surface of endothelial cells and platelets as a response to inflammatory mediators, and E-selectin whose expression is induced on inflamed endothelium. All selectins bind to the sialyl Lewis x tetrasaccharide (127),



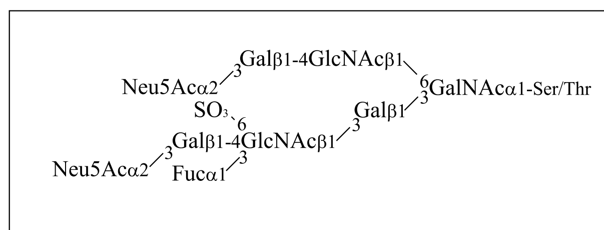
Oligosaccharide structures of PSGL-1.



Oligosaccharide structures of mouse GlyCAM-1.



The so called extended core 1 structure proposed to act as an L-selectin ligand.



A putative O-glycan structure from human tonsillar CD34.

Figure 3. Selectin ligand oligosaccharides

but their high-affinity biological ligands are more complex structures carried by specific proteins, and for a large part they are yet uncharacterized.

Several putative L-selectin glycoprotein ligands that carry sialylated and fucosylated oligosaccharides have been characterized (126,128). The best known oligosaccharide structures occurring on a L-selectin ligand are the O-glycans of mouse glycosylated cell adhesion molecule 1 (GlyCAM-1); the core 2 structures carrying 6- and 6'-sulfated sialyl Lewis x (Figure 3) (129). However, mice that lack a core 2 GlcNAc-transferase show normal lymphocyte homing (130). L-selectin ligands in these mice carry 6-sulfated sialyl Lewis x on an extended core 1 structure (Figure 3) (131). These observations indicate that the core 2 structure is not indispensable for L-selectin binding. CD34 is another L-selectin ligand glycoprotein. CD34 from human tonsillar high endothelial venules carries a sialylated, sulfated and fucosylated O-glycan, with a proposed structure where 6-sulfated sLex is β1,3-linked to the "core 1 galactose" (Figure 3) (132).

The best-characterized selectin-glycoprotein interaction is that between P-selectin and P-selectin glycoprotein ligand 1 (PSGL-1). PSGL-1 glycosylation has been characterized from the human promyelocytic cell line HL-60. PSGL-1 carries sialyl Lewis x on sialylated core 2, and a larger structure containing trivalent Lex (Figure 3) (133). Sulfation at specific tyrosines of the protein backbone is needed for binding to P-selectin (134). Binding studies and X-ray crystallography using a glycopeptide model containing the tyrosine sulfations and the O-glycan indicate that there is a stereospecific interaction between P-selectin and PSGL-1, to which each of the three tyrosine sulfates, the peptide backbone, and fucose/sialic acid on an optimally positioned core 2 O-glycan each contribute distinctly (135,136). E-selectin has been shown to bind sialylated, multiply fucosylated polylactosamines on glycoproteins such as ESL-1 (137,138), and glycolipids (139).

Many fucosylated glycans are tumour-associated antigens

Elevated expression of several fucosylated antigens is associated with malignant transformation. These include Lex (140), dimeric Lex (141), sLex (142), sialyl dimeric Lex (140,143), Ley (144), Lea (145), sLea (146), Leb (145), fucosyl GM1 (Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ1-Cer) (147) and globo H (Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer) (148). sLex, sialyl dimeric sLex and sLea have been proposed to have a role in hematogeneous metastasis of cancer, where malignant cells would adhere to the endothelial lining via E or P-selectin in a manner analogous to leukocyte adhesion (149,150). The expression of these antigens has been correlated with increased the metastatic potential of tumours and poor prognosis for cancer patients (151-153).

Fucosylated glycans play roles in fertilization and embryonic development

The molecular details of mammalian gamete adhesion remain for the large part unresolved. There is, however, substantial evidence that mammalian gamete adhesion is carbohydrate-mediated (154). Fucosylated glycans, such as Lewis x and sialyl Lewis x, have been proposed to be involved in high affinity sperm-egg adhesion (155,156).

The expression of many fucosylated oligosaccharide antigens is tightly regulated both spatially and temporally during development (157). The Lewis x epitope first appears at the 8-16 cell stage, thus correlating with the onset of compaction of the embryo (158). It has been proposed that homotypic interaction between Lewis x epitopes is involved in embryonal compaction (159,160).

The expression of Lewis x is thought to have a role in neural development. Lewis x is transiently expressed at defined regions of the developing brain, and often appears during critical stages of development (161-163). The amount of Lewis x bearing glycolipids has been shown to dramatically increase during the course of neural differentiation of PC19 embryonal carcinoma cells induced by retinoic acid (65). Lewis x has been implicated in glial-neuronal cell adhesion (164), and the segregation of cells to form different regions (165). It has not been established whether these adhesion events are mediated by homotypic Lex-Lex adhesion, or by a yet uncharacterized lectin with Lewis x binding activity. A glycoprotein bearing the Lewis x determinant is expressed in cell-cell contact- and the differentiation state-dependent fashion by corneal epithelial cells during development (166). The Lewis x epitope is also selectively expressed by cell populations undergoing important morphogenetic steps during the development of the pancreas (167), kidney (168) and lung (169).

Fuc-TI and its product the type 2 H-antigen define a unique expression pattern in the developing prostate and regulate epithelial cell proliferation during prostatic branching morphogenesis (170).

O-linked fucose is involved in the regulation of cell signalling

The EGF modules of certain proteins are glycosylated by fucose directly O-linked to serine or threonine (5). The Notch receptors function in cell-fate decisions, proliferation and apoptosis during development (171). The Fringe family of glycosyltransferases modify the ability of Notch to respond to its ligands by transferring a β1,3-linked GlcNAc to O-linked fucose on Notch (172). This structure is then elongated into Neu5Acα2-3Galβ1-4GlcNAcβ1-3Fucα1-O-Ser/Thr (172). O-linked fucose has also been shown to be essential for the growth factor activity of urokinase-type plasminogen activator (173) and signalling activity of Cripto (174).

Bacteria use fucosylated glycans on host cell surface for adhesion

Many bacteria, viruses and toxins adhere to host cell surface carbohydrates. It has been suggested that the evolutionary selection pressures of external origin, mediated by pathogens that recognize glycans, are an important factor driving the diversification of glycans (175). A Lewis b–binding adhesin has been characterized from *Helicobacter pylori*, a human pathogen that causes gastric ulcers and cancer (176). The intracellular parasite that causes granulocytic erlichiosis in humans specifically adheres to fucosylated PSGL-1 in a manner that mimics P-selectin binding (177). Fucosylated oligosaccharides of human milk inhibit the binding of various gastric pathogenic agents such as enteropathogenic *Escherichia coli* (EPEC), *Campylobacter jejuni*, and the heat stable enterotoxin of *E. coli* to their host cells (178). It has been postulated that the great diversity of free oligosaccharides found in human milk serves to protect the infant from bacterial infection.

Fucosylated glycans mediate angiogenesis

Fucosylated carbohydrate structures have been implicated to have a role in the induction of angiogenesis. Selectins and their carbohydrate ligands seem to have a versatile role in the multiple events that occur during inflammatory responses. Monoclonal antibodies against sialyl Lewis x and sialyl Lewis a inhibit capillary morphogenesis in a bovine *in vitro* model (179). Furthermore, soluble E-selectin has been shown to induce chemotaxis of human endothelial cells, and induce angiogenesis in rat cornea (180). On the basis of these observations it has been proposed that after the binding of leukocytes to endothelial cells, E-selectin expressed on the cell surface is cleaved and shed, and then recruits and activates endothelial cells (181). A selectin-independent carbohydrate-mediated signalling system may also contribute to angiogenesis. A carbohydrate structure that is recognized by an antibody against Lewis y and H-antigens is rapidly up-regulated on human endothelial cells by several cytokines and its amount is increased in

rheumatoid arthritis synovial fluid. Glucose analogues of Lewis y and H trisaccharides are chemotactic to human endothelial cells and induce angiogenesis in rat cornea (182).

2.3.2 Functions of sialylated glycans

The sialic acid-binding immunoglobulin superfamily lectins (Siglecs) mediate many different recognition and signalling phenomena

The Siglecs, sialic acid-binding immunoglobulin superfamily lectins, are a family of sialic-acid binding lectins that have sequence homology and share structural similarities (183). Ten Siglecs have been characterized to date (184). Their tissue expression, sialic acid recognition specificities and functions vary (Table III). The CD33-related Siglecs (Siglecs 3 and 5-10) contain conserved immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic tails, suggesting that they may be regulatory receptors in the immune system (184).

Siglecs 1-3 are expressed by different subsets of hematopoietic cells: Siglec 1 (sialoadhesin) by subsets of macrophages (185), Siglec 2 (CD22) by mature resting B-cells (186,187), and Siglec 3 by (CD33) by myeloid progenitors, monocytes and macrophages (188). Sialoadhesin binds to gangliosides and glycoproteins containing terminal α2,3-linked sialic acid (189), whereas CD22 binds specifically to Neu5Acα2-6Galβ1-4GlcNAc on N-glycans (190). Siglecs 1-3 are thought to be involved in the regulation of the immune system. Sialoadhesin is thought to mediate interactions of developing myeloid cells in the bone marrow (191) and lymphocyte trafficking (192). CD22 is proposed to have roles in the regulation of B-cell signalling (193), and B-cell homing to bone marrow (194). CD22-deficient mice show evidence of dysregulation of B-cell responses and expanded numbers of peritoneal B-cells (195,196). Mice that lack ST6Gal I, and therefore lack CD22 ligands, present a phenotype that partially overlaps with that of CD22 knock out mice, including a severe immunodeficiency (197). CD33 has been proposed to have a regulatory role in hematopoiesis and the myeloid cell maturation, since mAb-mediated crosslinking of CD33 inhibits the proliferation of myelomonocytic cell precursors (198) and generation of dendritic cells from monocytes (199).

Siglec 4, also called myelin associated glycoprotein (MAG), is expressed by myelin forming oligodendrocytes and Schwann cells (200,201). Various neuronal gangliosides bearing a terminal Neu5Acα2-3Galβ1-3GalNAc determinant have been proposed to be ligands for Siglec 4 (202). Additional sialic acid residues linked to the inner core, especially Neu5Acα2,6 on the GalNAc, enhance binding affinity (203). However, there is also evidence of glycoprotein ligands (204,205). Siglec 4 participates in myelin-axon interactions. Siglec 4 deficient mice have altered periaxonal architecture (206,207), and they exhibit demyelination and axonal degeneration when they get older than 8 months (208). In addition to its apparent role in myelin organization, Siglec 4 has been shown to promote neurite outgrowth from neonatal neurons, but inhibit axonal growth from adult neurons (209). The inhibition of neurite outgrowth is mediated by Siglec 4 binding to ganglioside GT1b with subsequent activation of Rho-kinase (210).

The newly cloned Siglecs 5-10 are less well characterized when it comes to their binding specificity and function. Siglec 5 is expressed by neutrophils and monocytes (211). Siglec 6 is expressed at high levels in placenta, and at moderate levels in B-cells, spleen and small intestine (212). Siglec 6 binds specifically to Neu5Acα2-6GalNAc (sialyl-Tn antigen) (212), whereas Siglec 5 exhibits the least specificity of the Siglecs for sialic acid linkage: it binds to α2,8-linked as well as to α2,3- and α2,6-linked Neu5Ac (189). Siglec 7 is a putative inhibitory receptor on NK-cells (213,214). It recognizes gangliosides containing the disialyl sequence Neu5Acα2-8Neu5Ac or an internal Neu5Acα2,6-branch (215,216). Siglecs 8-10 are specifically expressed

by different subsets of hematopoietic cells and bind to both α2,3- and α2,6-sialyllactose (217-219).

An eleventh Siglec has recently been cloned and named human Siglec-like molecule (Siglec-L1) (220) and S2V (221). Interestingly, in this Siglec a conserved arginine known to be essential for sialic acid binding has been mutated (220). The chimpanzee orthologue of Siglec-L1 retains the arginine and is fully functional, showing a binding preference toward N-glycolylneuraminic acid (220). It has been postulated that the mutation in human Siglec-L1 is evolutionarily related to the loss of N-glycolylneuraminic acid in human evolution (see the discussion about modified sialic acids below).

Table III. The Siglecs.

Siglec	other names	expression	binding specificity	function
Siglec 1	sialoadhesin	macrophages	Neu5Acα2,3	myeloid cell development?, lymphocyte traffic?
Siglec 2	CD22	B-cells	Neu5Acα2-3Galβ1-4 GlcNAc	regulation of B-cell signaling
Siglec 3	CD33	myeloid progenitors, monocytes, macrophages	Neu5Acα2-3/6Gal	hematopoeisis, myeloid cell maturation?
Siglec 4	MAG	myelin forming cells	Neu5Acα2-3Galβ1-3 (Neu5Acα2-6)GalNAc	myelin stability, regulation of neurite outgrowth
Siglec 5	OB-BP2	neutrophils, monocytes, macrophages, B-cells	Neu5Acα2,3/6/8	?
Siglec 6	OB-BP1, CD33L	placenta, B-cells	Neu5Acα2-6GalNAc	?
Siglec 7	p75/AIRM1	NK cells, monocytes, dendritic cells	Neu5Acα2-8Neu5Ac or internal Neu5Acα2,6	regulation of NK cell activation?
Siglec 8		eosinophils	Neu5Acα2,3/6	?
Siglec 9		neutrophils, monocytes, NK cells, B-cells	terminal Neu5Acα2,3/6	?
Siglec 10		eosinophils, dendritic cells, NK cells, B-cells	Neu5Acα2,3/6	?

Gangliosides modulate signal transduction
Gangliosides are thought to have roles in cell-cell recognition, cell-matrix interactions, and growth and differentiation of cells, especially neurons, but most of these events are poorly characterized at the molecular level, reviewed in (222,223). The functions of gangliosides as Siglec ligands were discussed in the previous section. An emerging theme of ganglioside function is their roles as receptor modulators. Gangliosides and tyrosine kinase receptors associate with each other in specialized microdomains of the cell membrane (224). Many of the experiments described below involve incubation of cells with exogenous gangliosides. The results obtained this way should be interpreted with caution, as culture conditions may affect the incorporation of gangliosides, and depending on the cell line, many exogenously added gangliosides become rapidly metabolized into others. Therefore it is not uncommon that conflicting results about the effect of gangliosides on growth factor receptors are obtained (225,226)

Exogenously added ganglioside GM1 has neurotrophic and neuritogenic effects both *in vivo* and *in vitro* (227-229). These effects are mediated by potentiation of the action of nerve growth factor (NGF): GM1 facilitates the dimerization and autophosphorylation of the NGF receptor tyrosine kinase A (TrkA) (230). Depletion of PC12 cells of glycosphingolipids inhibits the action NGF and abolishes TrkA autophosphorylation. These effects can be reversed by addition of GM1 to the culture medium, but not by other gangliosides (231). GM3 inhibits epidermal growth factor (EGF) -stimulated phosphorylation of the EGF receptor (232). The role of GM3 in the negative regulation of EGF signalling is supported by studies showing that cells with decreased amounts of gangliosides, especially GM3, either because of a metabolic defect in ganglioside biosynthesis (233) or overexpression of a specific sialidase (234) show increased EGF receptor autophosphorylation. Specific gangliosides have also been proposed to modulate signalling mediated by PDGF (235), interleukin-2 (236), bFGF (237), insulin (238), and VEGF (239).

Sialylation influences the activity and survival of T-cells
CD8 on immature T-cells binds the PNA lectin, which is specific for the core 1 (Galβ1-3GalNAc) structure (240). When T-cells mature, PNA-binding is lost due to sialylation of Galβ1-3GalNAc by ST3Gal I (82,241). Mature T-cells show a diminished ability to bind soluble class I MHC tetramers as compared to immature T-cells. This switch has been attributed to the Neu5Acα2-3Galβ1-3GalNAc structure on mature T-cells (242,243). Alteration in the domain-domain association or orientation of the CD8αβ coreceptor stalk by the carbohydrate have been proposed as a possible mechanism for the change in avidity (243). Activation of T-cells is associated with desialylation of core 1 O-glycans and concomitant increase in core 2 biosynthesis (82). The activated T-cells bearing core 2 O-glycans are destined for either apoptosis mediated by galectin-1 (244,245), or differentiation into memory cells, which is accompanied by the reappearance of sialylated core 1 O-glycans as the predominant O-glycan structure (81). ST3Gal I-deficient mice show increased apoptosis of $CD8^+$ T-cells (81). These observations show that the Neu5Acα2-3Galβ1-3GalNAc structure generated by ST3Gal I has an important role in the regulation of T-cell activity and homeostasis.

A sialylated glycan regulates cell adhesion mediated by α-dystroglycan binding to laminin
α-Dystroglycan carries an unusual mannose-linked O-glycan, which is elaborated by a sialylated N-acetyllactosamine: Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-Ser/Thr (246,247). This structure is essential for the binding of α-dystroglycan to laminin (246). Mutation of the β1,2-N-acetylglucosaminyltransferase involved in the biosynthesis of this structure causes muscular dystrophy and neuronal migration disorder (248).

Sialylated glycans and microbial pathogenesis

The attachment of bacteria, their toxins, and viruses to host tissue is considered to be essential for the pathogenic processes elicited by these agents. Many bacteria, toxins and viruses use sialylated glycans on host cell surface as receptors (249-251). Some examples are presented below.

Bacterial adhesins

In addition to binding to Lewis b, as mentioned in section 2.3.1, *Helicobacter pylori* has at least two different sialic acid-binding specificities, but the identity of the sialic acid-binding adhesins and their detailed specificities remain obscure. The first binding specificity is towards Neu5Acα2-3Gal and it is expressed when bacteria are grown on agar (252,253). The second binding specificity remains, when the bacteria are grown in broth and it is associated with Neu5Acα2-3Gal on complex polyglycosylceramides (254,255). The specific binding of *H. pylori* to sialylneolacto-hexaosylceramide and sialylneolacto-octaosylceramide isolated from human gastric adenocarcinoma has been demonstrated (256). S-fimbriated *E. coli* which cause meningitis in infants bind to glycoproteins carrying Neu5Acα2-3Gal on O-glycans (257). Interestingly, certain strains of *Streptococcus suis* which cause meningitis in piglets, similarly bind to terminal Neu5Acα2-3Gal, but in the context of a polylactosamine chain (258). Other sialic acid-binding bacteria include *Mycoplasma pneumoniae*, which binds to gangliosides (259), and *Haemophilus influenzae*, which binds to sialylated polylactosamines (260).

Bacterial toxins

Cholera toxin and the heat-labile enterotoxin from *Escherichia coli* bind to GM1 ganglioside, whereas tetanus toxin requires the internal disialyl sequence found in the GT1b series of gangliosides (261). *Clostridium botulinum* neurotoxins use gangliosides such as GT1b and GD1a as coreceptors together with synaptotagmin (262). Pertussis toxin from *Bordetella pertussis* binds to sialic acid on glycoproteins (263,264).

Viruses

The binding specificity of influenza virus hemagglutinin is dependent on the species of origin of the virus; most human influenza virus isolates preferentially bind to Neu5Acα2-6Gal (265,266). Influenza pandemics have been accompanied by the evolution of the receptor binding site from the narrower type found in avian viruses, that preferentially accommodates the α2,3-linkage, to the wider type with the preference to α2,6-linkage, found in human isolates (267). Specific recognition of larger ganglioside structures by influenza viruses has been implicated, although the detailed structure of the receptor remains unclear (268). Sendai virus binds to gangliosides containing the Neu5Acα2-3Galβ1-3GalNAc sequence, and shows highest affinity towards GQ1b (269). Mouse polyomavirus binds to α2,3-linked sialic acid (270), whereas the human polyomavirus JC binds to α2,6-linked sialic acid (271).

Molecular mimicry

Sialic acid is also used by pathogens as a means of evading host immune response. Some bacteria make polysaccharides that resemble or are identical to host glycoconjugates, which makes them poorly immunogenic. Examples include the polysialic acid containing capsule of certain strains of *Neisseria meningitidis* and *E. coli* (272), and the α2,3-sialylated lipo-oligosaccharide of *N. meningitidis* and *N. gonorrhoeae* (273).

Sialylated glycans as tumour-associated antigens

As mentioned earlier in the context of fucosylated oligosaccharides, aberrant glycosylation is a feature often associated with malignant transformation. A general increase in cell surface sialylation has been shown to correlate with metastatic potential (274,275).

An altered ganglioside profile is a common feature of many types of tumours. For example, GD3 expression is often enhanced in tumours as compared to normal tissue (276), and high level of GD3 has been associated with malignancy of the tumour (277) and poor survival of patients (278). Suppression of GD3 synthesis reduces cell migration, tumour growth, metastasis, angiogenesis, and vascular endothelial growth factor production of rat neuroblastoma cells (279,280). This indicates that gangliosides, specifically GD3, are involved in the regulation of tumour growth, possibly by stimulating angiogenesis. Other cancer-associated gangliosides include GD2 (281), GM2 (282,283), GT3 (282), and α2,6- and α2,3-sialylated lacto- and neolactoseries gangliosides (284-286). Tumour cells have been observed to shed gangliosides (287,288), which may have immunosuppressive activity (289-291).

In addition to gangliosides, the expression of many protein-associated carbohydrate antigens is altered in cancer. Enhanced expression of the sialyl T (292) and sialyl Tn (293,294) antigens, resulting from underprocessing of O-glycans (295), is a common feature of many cancer cells. Re-expression of polysialic acid on neuronal cell adhesion molecule (NCAM) has been reported in some tumours (296,297) (normally NCAM is polysialylated only in fetal tissues, as discussed below). Sialylated glycoconjugates bearing N-glycolylneuraminic acid are found on some human tumours (298). This is rather unexpected, since as discussed in the section "modified sialic acids", the human CMP-Neu5Ac hydroxylase gene has an exon deletion.

Polysialic acid modulates the function of the neuronal cell adhesion molecule

Polysialic acid, a linear homopolymer of α2,8-linked sialic acid, occurs in mammals almost exclusively on one protein, the embryonic form of NCAM (272). Polysialic acid modulates cell adhesion mediated by NCAM during cell migration, axon pathfinding and synaptogenesis, and thus has important functions in the development of the nervous system (299). NCAM is the major polysialic acid carrying protein in mammals, but this modification also occurs on the sodium channel α-subunit (300), on the polysialyltransferases that are responsible for its biosynthesis (301), and on integrin α_5 subunit, where it mediates binding to fibronectin (302).

Modified sialic acids

The term "sialic acid" is commonly used to refer to N-acetylneuraminic acid (Neu5Ac), although in fact the sialic acids are a family of 9-carbon acidic sugars (303). The most common sialic acids in mammals are N-acetylneuraminic acid (Neu5Ac), 9-O-acetylated N-acetylneuraminic acid (Neu5,9Ac) and N-glycolylneuraminic acid (Neu5Gc). Humans are exceptional, because a mutation in CMP-sialic hydroxylase has resulted in the loss of Neu5Gc, making Neu5Ac the predominant sialic acid in humans (304). The sialic acid modifications affect the biological functions of the molecules carrying them. For example, 9-O-acetylation is developmentally regulated, and also regarded as an onco-developmental antigen - it has been shown to reappear in cancer tissue (305,306). The presence of 9-O-acetylated N-acetylneuraminic acid or N-glycolylneuraminic acid also affects sialic acid recognition by pathogens (307,308) and endogenous lectins like Siglecs (189,220,309,310)

3. AIMS OF THE STUDY

The aim of this study was to study the function of fucosyl- and sialyltransferases by

1. Determining the detailed acceptor specificity profiles of Fuc-TV (I) and Fuc-TIX (II), as well as their site-specificities on polylactosamine acceptors.

2. Studying enzymatic synthesis of the Neu5Acα2-3GalNAc linkage (III).

and additionally to use the oligosaccharide containing Neu5Acα2-3GalNAc generated by ST3Gal II to study its susceptibility to sialidases (III).

4. MATERIALS AND METHODS

4.1 Acceptor oligosaccharides

LN (I,II), Galβ1-3GlcNAc (I,II), chitobiose (I,II) and Galβ1-4'LN (II) were from Sigma. Manβ1-4GlcNAc (I), Fucα1-2'LN (II) Neu5Acα2-6'LN (II) and GalNAcβ1-3Gal (III) were from Dextra (Reading, UK). Chitotriose (I) and chitotetraose (I) were from Seikagaku (Tokyo, Japan). Lactose (II) was from BDH Chemicals (Poole, UK). 2'-Fucosyllactose (II) was from Biocarb (Lund, Sweden). Neu5Acα2-3'LN (I,II) was from Oxford Glycosystems (Abingdon, UK). Globo-N-tetraose (III) was from Accurate Chemical and Scientific Corporation (Westbury, NY).

LNβ1-OMe (I), LNβ1-2Man (I,II), LNβ1-6Gal (I,II), LNβ1-3Galβ1-OMe (I,II), LNβ1-6Manα1-OMe (I, II), LNβ1-4GlcNAc (I) and 6-SO_3-LN (II) were synthesized by using bovine milk β1,4-galactosyltransferase (Sigma) from GlcNAcβ1-OMe (Sigma), GlcNAcβ1-2Man (Glyko, Novato, CA), GlcNAcβ1-6Gal (Sigma), GlcNAcβ1-3Galβ1-OMe (Sigma), GlcNAcβ1-6Manα1-OMe (Sigma), chitobiose and 6-SO_3-GlcNAc (Sigma), respectively, essentially as described in (311).

GalNAcβ1-4GlcNAc (I) and GalNAcβ1-4GlcNAcβ1-OMe (I, II) were synthesized from GlcNAc (Sigma) and GlcNAcβ1-OMe, respectively, by bovine milk β1,4-galactosyltransferase using UDP-GalNAc as a donor, as described in (312).

GlcNAcβ1-3'LN (I,II), LNβ1-3'LN (I,II), LNβ1-3'LNβ1-OMe (I), LNβ1-3'LNβ1-3Galβ1-OMe (I), GlcNAcβ1-3'LNβ1-3'LN (II) and LNβ1-3'LNβ1-3'LN (I) were synthesized by consecutive β1,4-galactosyltransferase and β1,3-GlcNAc-transferase (human serum) reactions as described in (313).

Galβ1-3(LNβ1-6)GalNAc (I,II) was synthesized from Galβ1-3GalNAc (Sigma) by using β1,6-GlcNAc-transferase (hog gastric mucosa) and bovine milk β1,4-galactosyltransferase as described in (314). LNβ1-3'(LNβ1-6')LN (I,II) was synthesized from LN by using human serum β1,3-GlcNAc transferase, hog gastric β1,6-GlcNAc transferase and bovine milk β1,4-galactosyltransferase as described in (315).

Galβ1-3GlcNAcβ1-3'LNβ1-3Galβ1-OMe (I) was prepared by galactosylating GlcNAcβ1-3LNβ1-3Galβ1-OMe with β1,3/4-galactosyltransferase activity present in Colo 205 cells, treating the resulting mixture with β1,4-galactosidase from *Diplococcus pneumoniae* to remove selectively the β1,4-linked galactose from LNβ1-3'LNβ1-Galβ1-OMe, and purifying the intact Galβ1-3GlcNAcβ1-3'LNβ1-3Galβ1-OMe from the mixture by gel filtration chromatography.

Lexβ1-3'LN (I) was obtained from LNβ1-3'LN by successive β1,6-GlcNAc-transferase (hog gastric mucosa), α1,3-fucosyltransferase (recombinant human Fuc-TVI, Calbiochem, La Jolla, CA) and β-N-acetylhexosaminidase (jack bean, Sigma) reactions, as described in (316). LNβ1-3'Lexβ1-3Galβ1-OMe (I) and LNβ1-3'LNβ1-3'Lex (I) were synthesized from GlcNAcβ1-3'LNβ1-3Galβ1-OMe and GlcNAcβ1-3'LN, respectively, by first fucosylating with α1,3-fucosyltransferase (recombinant human Fuc-TVI) and then elongating the chains to their full length with β1,4-galactosyltransferase (bovine milk) and β1,3-GlcNAc-transferase (human serum) reactions, essentially as described in (317,318). Lexβ1-3'LNβ1-3'LN (I) was synthesized by first "protecting" the two most reducing end LN units by β1,6-GlcNAc transferase present in rat serum, then fucosylating the free non-reducing end LN unit with recombinant human Fuc-TVI, and finally removing the β1,6-linked GlcNAc:s by β-N-acetylhexosaminidase (jack bean).

LNβ1-3'LNβ1-3'LNβ1-3'LN (I,II) was chemically synthesized as described in (319).

LNβ1-2Manα1-3(LNβ1-2Manα1-6)Manβ1-4GlcNAc (I,II) and LNβ1-2(LNβ1-4)Manα1-3[LNβ1-2(LNβ1-6)Manα1-6]Manβ1-4GlcNAc (I) were a gift from Prof. G. Strecker (Université des Sciences et Technologies de Lille, Villeneuve D'Ascq, France). LNβ1-2(LNβ1-4)Manα1-3(LNβ1-2Manα1-6)Manβ1-4GlcNAc (I) was prepared from the corresponding α2,6-sialylated oligosaccharide (also from Prof. Strecker) by desialylation with *Arthrobacter ureafaciens* sialidase.

All the α2,3-sialylated acceptors in parts I and II were synthesized from the corresponding neutral oligosaccharides by using recombinant rat ST3Gal III (Calbiochem). Neu5Acα2-3'Lexβ1-3'LN (II) was synthesized in the same way as Lexβ1-3'LN, but with a sialylation step preceding fucosylation, as described in (318).

GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc (III) was synthesized from Galβ1-4GlcNAcβ1-3Galβ1-4Glc (a gift from Prof. R. Cummings, University of Oklahoma) essentially as described in (320), by using ammonium sulphate precipitate of human serum as the enzyme source (321), and UDP-GalNAc as the donor.

4.2 Glycosyltransferase reactions

4.2.1 Fucosyltransferase assays (I, II)

GDP-[^{14}C]fucose (100 000 cpm, Amersham Pharmacia Biotech), GDP-fucose (1 nmol, Sigma) and the individual acceptors (50 nmol) were incubated for 1 h at 37 °C in 10 µl of 50 mM MOPS pH 7.2, 10 mM fucose, 5 mM ATP and 0.5% Triton X-100 and lysates of transfected cells (30-50 µg protein). Fuc-TV transfected CHO cells (I) were prepared as described in (15), and they contained 17-25 µU fucosyltransferase activity per mg lysate protein (1 U = 1 µmol/min). Fuc-TIX transfected Namalwa cells (II) were prepared as described in (58), and they contained 53-100 µU fucosyltransferase activity per mg lysate protein. The Fuc-TV reaction mixtures, but not the Fuc-TIX reaction mixtures, also contained 10 mM $MnCl_2$. The reactions were terminated by adding 10 µl of ethanol followed by 100 µl of ice cold water. To rule out possible activation of endogenous fucosyltransferase activity the substrate specificity of each batch of Fuc-TV transfected CHO cell lysates was tested with LN, LNB and Neu5Acα2-3'LN before the actual experiments to ascertain that the reactivity profile with these acceptors conformed to the unique specificity of Fuc-TV. Fuc-TIX experiments were carried out with a single batch of Namalwa cells lysates. The specificity profile was indicative of Fuc-TIX and no other known α1,3-fucosyltransferase. In both series of experiments the acceptors were monitored by gel filtration chromatography with UV-detection after incubation. No significant acceptor degradation by glycosidases was observed.

4.2.2 Sialyltransferase reactions (III)

ST3Gal II reactions. 600 nmol of acceptor oligosaccharide and 2.4 µmol of CMP-Neu5Ac were incubated with 40 mU of rat recombinant ST3Gal II (α2,3-(O)-sialyltransferase, Calbiochem) in 50 mM sodium cacodylate pH 6.0, 0.02% NaN_3, 0.05% BSA and 8 mM $MnCl_2$ in a reaction volume of 600 µl for 6 days at room temperature. 20 mU of fresh enzyme was added on day 3. The reactions were terminated by boiling for 3 minutes.

ST3Gal III reactions. 49 nmol of acceptor oligosaccharide and 100 nmol of CMP-Neu5Ac were incubated with 3.2 mU of rat recombinant ST3Gal III (α2,3-(N)-sialyltransferase, Calbiochem)

in 100 mM MOPS-NaOH pH 7.5, 0.02% NaN_3, and 8 mM $MnCl_2$ in a reaction volume of 12.5 μl for 6 days at room temperature.

4.3 Glycosidase reactions

4.3.1 β-Galactosidase reactions (I, II)

Jack bean β-galactosidase (Sigma or Seikagaku) reactions were carried out as described in (322).

4.3.2 β-N-Acetylhexosaminidase reactions

Jack bean β-N-acetylhexosaminidase (Sigma) reactions used in the structural analysis of α1,3-fucosyltransferase products (I, II) were carried out as described in (322). Jack bean β-N-acetylhexosaminidase reactions used to characterize the GalNAc-terminating oligosaccharides in part II were carried out in a similar manner but in the exhaustive conditions described in (323).

4.3.3 Endo-β-galactosidase reactions (I, III)

Endo-β-galactosidase digestions with *Bacteroides fragilis* (Roche Molecular Biochemicals, Basel Switzerland) and *Escherichia freundii* (Seikagaku) endo-β-galactosidases were performed as described in (324).

4.3.4 Sialidase reactions

Sialidase reactions in part III were performed in 40 μl reaction volume and 75 μM substrate concentration. The reactions were incubated for 20 h at 37°C and terminated by boiling for 3 minutes. *Clostridium perfringens* sialidase (New England Biolabs, Beverly, MA) reactions were carried out in 50 mM Na-phosphate buffer pH 4.5 with 167 mU of enzyme; Newcastle disease virus sialidase (Roche molecular biochemicals) reactions in 50 mM Na-phosphate buffer pH 5.5 with 8 mU of enzyme; and *Streptococcus pneumoniae* sialidase (Calbiochem) reactions in 50 mM Na-phosphate buffer pH 4.5 with 20 mU of enzyme.

Arthrobacter ureafaciens sialidase (Glyko) (I, II, III) reactions were carried out as described in (325).

4.4 Chromatographic methods

4.4.1. Paper chromatography (I, II)

Descending paper chromatography of radiolabelled oligosaccharides was carried out as described in (326), using the upper phase of n-butanol:acetic acid:water (4:1:5) (solvent A) as the eluant.

4.4.2 Gel filtration chromatography

Gel filtration in a Superdex Peptide HR 10/30 column (Amersham Pharmacia Biotech) was carried out as described in (34).

4.4.3 Ion exchange chromatography

The acceptors and reaction products of fucosyltransferase assays (I, II) were desalted and separated from unreacted donor in a mixed bed of ion exchange resins (Dowex AG-50 and Dowex AG-1 from Bio-Rad). The neutral oligosaccharides were eluted with water and the anionic oligosaccharides were eluted with 0.5 mM acetic acid as described in (38).

Anion exchange chromatography in a MonoQ (5/5) column (Amersham Pharmacia Biotech) (I, II, III) was carried out essentially as described in (314).

4.5 NMR spectroscopy (III)

Prior to the NMR experiments the saccharides (400-600 nmol) were lyophilized twice from D_2O and then dissolved in 40 µL of D_2O (99.996 atom %). The NMR experiments were carried out on a Varian Unity 500 spectrometer at 23°C using a gHX nano-NMR probe (Varian). A spinning rate of 2000 Hz was used. In recording 1D proton spectra a modification of the WEFT sequence (327) was used. The DQFCOSY and TOCSY experiments were carried out essentially as in (328).

For the gradient HMQC (329) and gradient HMBC experiments (330,331) (32 and 128 scans per t_1 value, respectively), matrices of 2k*256 and 2k*128 points were recorded and zero-filled to 2k*512 and 2k*256 points, respectively and a shifted sine-bell function was used. The average ^{1}H-^{13}C coupling constant was estimated to be 140 Hz and Δ_2 was 63.5 ms. The spectral widths F_1 and F_2 were 11250 Hz and 2400 Hz respectively.

4.6 Mass spectrometry

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of reaction products was performed with a BIFLEX™ mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany). The neutral oligosaccharides were analyzed essentially as in (318) and the sialylated oligosaccharides as in (332,333).

5. RESULTS

5.1 The acceptor specificity of Fuc-TV (I)

Lysates of CHO cells transfected with full-length human Fuc-TV reacted efficiently with N-acetyllactosamine (LN). The transfer rate was 1.0-1.5 nmol fucose per hour per mg lysate protein at 5 mM acceptor concentration (33-50 μg lysate protein, 0.55-1.3 μU fucosyltransferase activity). The relative Fuc-TV reactivities of various oligosaccharides, together with their Fuc-TIX reactivities, are shown in Table IV. It can be noted that Fuc-TV reacted efficiently with N-acetyllactosamine and GalNAcβ1-4GlcNAc (LacdiNAc). The reactivity of the type I lactosamine, Galβ1-3GlcNAc, with Fuc-TV was 13% of the reactivity of the type 2 lactosamine. Chitobiose and Manβ1-4GlcNAc were very poor acceptors.

The addition of a β1,3-linked GlcNAc on the non-reducing side of LN greatly enhanced its reactivity. Sialylation tended to enhance the reactivity as well: most α2,3-sialylated acceptors reacted better than the corresponding neutral ones.

The O-glycan analogue Galβ1-3(LNβ1-6)GalNAc was a good acceptor for Fuc-TV. LNβ1-2Man was quite an inefficient acceptor, whereas LNβ1-6Manα1-OMe reacted much more efficiently. The relative reactivities of LNβ1-6Gal and LNβ1-3Galβ1-OMe further supported the idea that LN units linked to the 6-position of the adjacent monosaccharide are favoured as acceptors. The Fuc-TV reactivities of the N-glycan analogs LNβ1-2(LNβ1-4)Manα1-3[LNβ1-2(LNβ1-6)Manα1-6]Manβ1-4GlcNAc and LNβ1-2(LNβ1-4)Manα1-3(LNβ1-2Manα1-6)Manβ1-4GlcNAc were 130% and 140% respectively of the reactivity of a single unconjugated LN.

5.2 The acceptor specificity of Fuc-TIX (II)

The relative reactivities of various oligosaccharide acceptors with Fuc-TIX are shown in Table IV. Lysates of Namalwa cells transfected with full-length human Fuc-TIX reacted efficiently with N-acetyllactosamine (LN). The transfer rate was 3.2-6.0 nmol fucose per hour per mg lysate protein at 5 mM acceptor concentration (28 μg lysate protein, 1.5-2.8 μU fucosyltransferase activity). In addition to LN, GalNAcβ1-4GlcNAcβ1-OMe, 6-SO_3-LN and Fucα1-2'LN reacted well, and chitobiose (GlcNAcβ1-4GlcNAc) appreciably well. Type 1 N-acetyllactosamine (Galβ1-3GlcNAc), lactose, Fucα1-2'Lac, Galβ1-4'LN, Neu5Acα2-6'LN and Neu5Acα2-3'LN were virtually unreactive.

The addition of β1,3-linked GlcNAc to the non-reducing end of the acceptor LN unit reduced its reactivity slightly, as did the addition of Gal to the reducing end as could be seen with LNβ1-6Gal and LNβ1-3Galβ1-OMe. In contrast to Fuc-TV, Fuc-TIX preferred LNβ1-3Galβ1-OMe to LNβ1-6Gal.

The N-glycan analogue LNβ1-6Manα1-OMe showed the highest reactivity of all the tested glycans. LNβ1-2Man and LNβ1-2Manα1-3(LNβ1-2Manα1-6)Manβ1-4GlcNAc reacted moderately well. LNβ1-2Man was a better acceptor for Fuc-TIX than for Fuc-TV. The O-glycan analogue Galβ1-3(LNβ1-6)GalNAc also had a high reactivity.

Table IV. Comparison of the relative reactivities of small oligosaccharide acceptors with Fuc-TV and Fuc-TIX.

Acceptor	Relative Fuc-TV reactivity	Relative Fuc-TIX reactivity
Galβ1-4GlcNAc (LN)	1.0	1.0
Galβ1-4GlcNAcβ1-OMe	1.0	n.d.
Galβ1-3GlcNAc (LNB)	0.13	0.04
Galβ1-4Glc	n.d.	0.01
GlcNAcβ1-4GlcNAc	0.04	0.20
GalNAcβ1-4GlcNAc	0.82	n.d.
GalNAcβ1-4GlcNAcβ1-OMe	1.2	0.84
Manβ1-4GlcNAc	0.03	n.d.
6-SO_3-LN	n.d.	0.40
Neu5Acα2-3'LN	1.7	0.05
Neu5Acα2-6'LN	n.d.	0.02
Fucα1-2'Lac	n.d.	0.03
Fucα1-2'LN	n.d.	0.51
GlcNAcβ1-3'LN	3.5	0.71
Galβ1-4'LN	n.d.	0.18
Galβ1-4GlcNAcβ1-4GlcNAc	0.9	n.d.
LNβ1-6Gal	1.4	0.50
LNβ1-3Galβ1-OMe	0.39	0.81
LNβ1-2Man	0.2	0.46
LNβ1-6Manα1-OMe	1.6	1.1
LNβ1-2Manα1-3(LNβ1-2Manα1-6) Manβ1-4GlcNAc	0.37	0.71
LNβ1-2(LNβ1-4)Manα1-3[LNβ1-2 (LNβ1-6)Manα1-6]Manβ1-4GlcNAc	1.3	n.d.
LNβ1-2(LNβ1-4)Manα1-3(LNβ1-2 Manα1-6)Manβ1-4GlcNAc	1.4	n.d.
Galβ1-3(LNβ1-6)GalNAc	3.0	0.87
Neu5Acα2-3'LNβ1-6Gal	3.8	n.d.
Neu5Acα2-3'LNβ1-3Galβ1-OMe	1.1	n.d.
Neu5Acα2-3'LNβ1-2Man	0.6	n.d.
Neu5Acα2-3'LNβ1-6Manα1-OMe	3.7	n.d.
Neu5Acα2-3Galβ1-3(LNβ1-6)GalNAc	2.5	n.d.

The figures represent the mean values of at least two experiments.
n.d. not determined

5.3 The site-specificity of Fuc-TV on polylactosamines (I)

The Fuc-TV products were degraded by sialidase (only the sialylated saccharides) and then by a mixture of β-galactosidase and β-N-acetylhexosaminidase to determine which GlcNAc residue was fucosylated in the multisite acceptors. The latter digestion removes all fucose-free LN units from the non-reducing end of the desialylated polylactosamines, but is unable to act on α1,3-fucosylated LN units. Thus, the desialylated chains were shortened in a way that established the

positions of the α1,3-fucosylated LN units. Products of these digestions were analyzed by paper chromatography to derive the site-specificity data shown in Figure 4.

Fuc-TV clearly preferred the most reducing end LN unit in all the polylactosamine acceptors. It is noteworthy that while the reactivities of the LN units at the non-reducing ends of the polylactosamine acceptors were significantly lower than that of free LN, the reactivity of the LNB unit (Galβ1-3GlcNAc) in LNBβ1-3LNβ1-3Galβ1-OMe was essentially the same as that of free LNB. As was the case with the smaller acceptors, α2,3-sialylated acceptors reacted better than corresponding neutral ones.

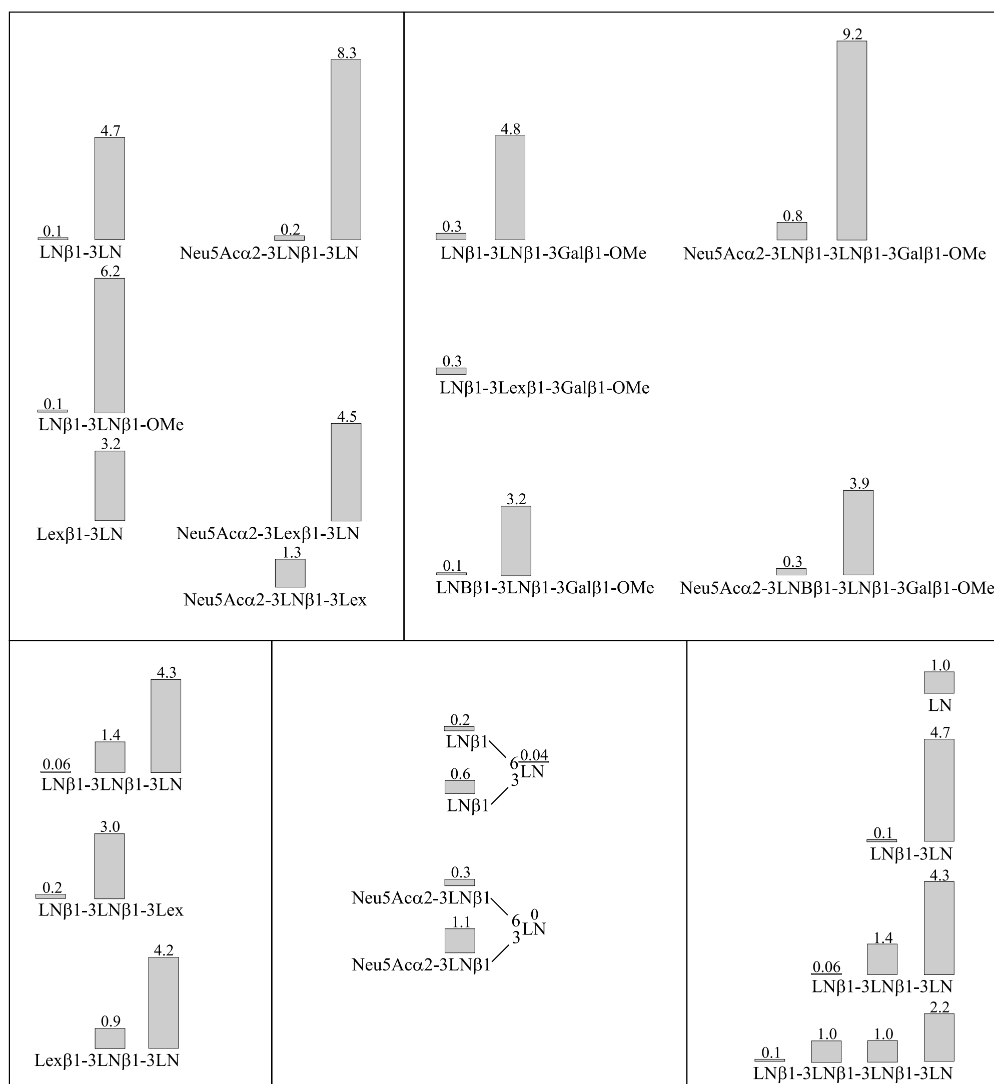


Figure 4. The site-specificity of Fuc-TV on polylactosamine acceptors. The bars and the values above them represent the relative reactivities of the individual acceptor sites as compared to that of free LN (1.0). The site-specificity analyses were carried out by treating the fucosyltransferase reaction products with β-N-acetylhexosaminidase and β-galactosidase, and analyzing the digests by paper chromatography as described in sections 4.3 and 4.4.

When prefucosylated polylactosamines were used as acceptors, it was noted that the Lewis x determinant reduces the reactivity of the LN unit adjacent to it on the reducing end side, but enhances the reactivity of the LN unit adjacent to it on the non-reducing end side. In the branched N-acetyllactosamines Fuc-TV showed a preference towards the β1,3-linked branch.

5.4 The site-specificity of Fuc-TIX on polylactosamines (II)

The site-specificity of Fuc-TIX was analyzed as described for Fuc-TV above (Figure 5). When multiple LN units were available, as in LNβ1-3'LN and LNβ1-3'LNβ1-3'LNβ1-3'LN, Fuc-TIX strongly preferred the terminal, non-reducing end site. Interestingly, both LN units of GlcNAcβ1-3'LNβ1-3'LN reacted equally well. Sialylation partially reversed the site-specificity of Fuc-TIX, as demonstrated by the good reactivity of the two most reducing end LN units in Neu5Acα2-3'LNβ1-3'LNβ1-3'LNβ1-3'LN, as compared to the poor reactivity of the two LN units adjacent to Neu5Ac. Fuc-TIX showed a preference for the β1,3-linked arm over the β1,6-linked arm of the branched acceptor. The LN unit at the branch point did not react at all.

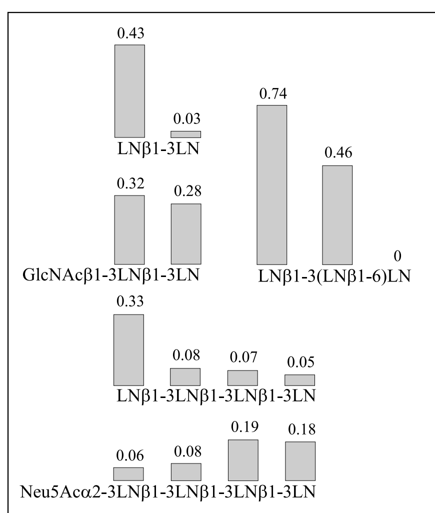


Figure 5. The site-specificity of Fuc-TIX on polylactosamine acceptors. The bars and the values above them represent the relative reactivities of the individual acceptor sites as compared to that of free LN (1.0). The site-specificity analyses were carried out by treating the fucosyltransferase reaction products with β-N-acetylhexosaminidase and β-galactosidase, and analyzing the digests by paper chromatography as described in sections 4.3 and 4.4.

5.5 Sialylation of GalNAcβ1-3Gal determinants by ST3Gal II (III)

The X_2 pentasaccharide (GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc), globo-N-tetraose (GalNAcβ1-3Galα1-4Galβ1-4Glc) and the disaccharide GalNAcβ1-3Gal were incubated individually with CMP-Neu5Ac and ST3Gal II. Purified sialylation products were initially analyzed by MALDI-TOF mass spectrometry, where they gave peaks at *m/z* 1200.44, 997.28 and 673.06, respectively, which were assigned to the $[M-H]^-$ of $Neu5Ac_1HexNAc_2Hex_3$, $Neu5Ac_1HexNAc_1Hex_3$ and $Neu5Ac_1HexNAc_1Hex_1$, respectively.

The sialylation product of GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc was not cleaved by β-N-acetylhexosaminidase, suggesting a non-reducing end location of the sialic acid. Endo-β-galactosidase cleaved the sialylated product of GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc into $Neu5Ac_1HexNAc_1Hex_1$ and $HexNAc_1Hex_1$, as analyzed by mass spectrometry.

The structures of the sialylated reaction products were analyzed in detail by NMR spectroscopy. The positions of the glycosidic linkages were identified by the correlations in the HMBC spectra, which were indicative of the structures Neu5Acα2-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Neu5Acα2-3GalNAcβ1-3Galα1-4Galβ1-4Glc and Neu5Acα2-3GalNAcβ1-3Gal.

ST3Gal III was inactive towards GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc.

5.6 The Neu5Acα2-3GalNAc linkage is resistant to Newcastle disease virus and *Streptococcus pneumoniae* sialidases (III)

Neu5Acα2-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc was incubated with different sialidases in conditions that completely cleaved off the sialic acid from Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc. The reaction products were analyzed by gel filtration chromatography and MALDI-TOF mass spectrometry. *Clostridium perfringens* and *Arthrobacter ureafaciens* sialidases completely desialylated Neu5Acα2-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc. By contrast, Newcastle disease virus and *Streptococcus pneumoniae* sialidases were able to desialylate only less than 10% of Neu5Acα2-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc.

6. DISCUSSION

6.1 The acceptor- and site-specificity profiles of the α1,3-fucosyltransferases

The α1,3-fucosyltransferases, like many other glycosyltransferases, form a redundant family. They all catalyze the same reaction, that is the transfer of fucose in α1,3-linkage to the GlcNAc in Galβ1-4GlcNAc. One reason for the existence of at least six human α1,3-fucosyltransferase isoenzymes may be the subtle differences in their acceptor specificities.

The differences in the acceptor profiles could reflect the various biological roles of the different α1,3-fucosyltransferases. For example, the leukocyte fucosyltransferases Fuc-TVII and Fuc-TIV have complementary acceptor- and site-specificity profiles: Fuc-TVII preferentially reacts with sialylated LN units at the non-reducing ends of polylactosamines, whereas Fuc-TIV preferentially fucosylates the inner LN units (34). Fuc-TIV and Fuc-TVII collaborate in the generation of functional selectin ligands (56). They seem to be at least partly specialized in the way that Fuc-TVII directs the expression of P-selectin binding glycoforms of PSGL-1 and controls the rolling frequency of leukocytes, whereas Fuc-TIV directs the expression of E-selectin binding glycoforms of ESL-1 and dictates rolling velocity (54,55).

6.1.1 The acceptor- and site-specificity Fuc-TV (I)

Fuc-TV is known to be quite flexible in its requirements for acceptor structure: it reacts with both sialylated and non-sialylated type 2 N-acetyllactosamine, and also weakly with type 1 N-acetyllactosamine and lactose (16). The data presented in paper I further expands the acceptor repertoire of Fuc-TV by showing that it can react with LacdiNAc (GalNAcβ1-4GlcNAc) nearly as efficiently as with LN, and also weakly with GlcNAcβ1-4GlcNAc and Manβ1-4GlcNAc.

Fuc-TV strongly preferred the most reducing end LN unit in all the polylactosamine acceptors tested. This is in accordance with earlier studies showing that Fuc-TV prefers the inner LN unit in LNβ1-3'LNβ1-3'LNβ1-R (23), where the reducing end LN unit is probably not available for fucosylation because of derivatisation. Fuc-TIII and Fuc-TVI similarly fucosylate the inner LN units of short neutral polylactosamines (23), but their site-specificity on longer and/or sialylated polylactosamines has not yet been studied.

While the reactivity of the non-reducing end LN unit in LNβ1-3R type structures with Fuc-TV was significantly lower than that of free LN, the reactivity of LNBβ1-3R was essentially the same as that of free LNB. These different reactivity patterns suggest that Fuc-TV may have two distinct adjacent acceptor substrate binding sites, one that preferably binds LN, and another that preferably binds LNB.

When prefucosylated polylactosamines were used as acceptors for Fuc-TV, it was noted that the Lewis x determinant enhances the reactivity of the LN unit adjacent to it on the non-reducing end side. This suggests that Fuc-TV may generate polyfucosylated lactosamines in a stepwise manner starting from the reducing end.

6.1.2 The acceptor- and site-specificity of Fuc-TIX (II)

Fuc-TIX reacted well with N-acetyllactosamine, Fucα1-2'LN, and GalNAcβ1-4GlcNAc confirming previous observations (60). Other N-acetyllactosamine analogues that were good acceptors to Fuc-TIX were LN sulfated at the 6-position of GlcNAc and chitobiose. Sulfated Lewis x

structures occur in L-selectin ligand glycoproteins (129), and on keratan sulfate (334,335), where they form polyfucosylated sequences. Very poor or nonexistent reactivity was observed for lacto-N-biose, lactose and Neu5Acα2-3'LN confirming earlier observations (58,60), and for Fucα1-2'Lac, Galβ1-4'LN, and Neu5Acα2-6'LN. The inability to use α2,3-sialylated N-acetyllactosamine as an acceptor is a property shared between Fuc-TIV (29,31) and Fuc-TIX, whereas none of the fucosyltransferases studied so far can react with α2,6-sialylated N-acetyllactosamine.

The present experiments confirmed the observation that Fuc-TIX preferentially fucosylates the non-reducing end LN unit of neutral polylactosamines (23). The modification of this LN unit at the 3-position of galactose with LN, Neu5Ac or sulphate significantly reduces the reactivity (23,59,62). However, the addition of a single β1,3-linked GlcNAc residue did not significantly reduce the reactivity of LN in the experiments described in part II. The high reactivities of GlcNAcβ1-3'LN and GlcNAcβ1-3'LNβ1-3'LN suggest that the enzyme can specifically recognize these type of structures. Therefore Fuc-TIX may participate in the biosynthesis of internally fucosylated polylactosamines, if the GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc product serves as an acceptor for subsequent galactosylation and elongation.

The site-specificity of Fuc-TIX was reversed on sialylated polylactosamines: the two most reducing end LN units of Neu5Acα2-3'LNβ1-3'LNβ1-3'LNβ1-3'LN were the most reactive. In fine detail the site-specificity of Fuc-TIX on sialylated polylactosamines seems to be intermediate between Fuc-TIV and Fuc-TV. Fuc-TIV prefers middle LN units (34), and Fuc-TV shows a strong preference towards the most reducing end LN unit as described above, whereas Fuc-TIX reacts equally well with the two most reducing end LN units. The site-specificities of α1,3-fucosyltransferases IV, V, VII and IX are summarized in Figure 6.

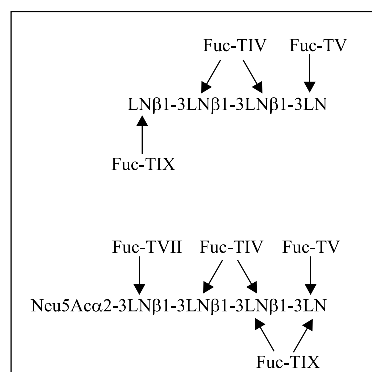


Figure 6. Comparison of the preferred acceptor sites within polylactosamine acceptors for α1,3-fucosyltransferases IV (34), V (present study), VII (34) and IX (present study).

Fuc-TIX efficiently fucosylated the N- and O-glycan analogues LNβ1-6Manα1-OMe, LNβ1-2Man, LNβ1-2Manα1-3(LNβ1-2Manα1-6)Manβ1-4GlcNAc and Galβ1-3(LNβ1-6)GalNAc. LNβ1-6Manα1-OMe was the best of all the studied acceptors for Fuc-TIX. LNβ1-2Man was a much better acceptor for Fuc-TIX than for Fuc-TV. In addition to being N-glycan branches, LNβ1-2Man and LNβ1-2(LNβ1-6)Man structures occur in the brain also O-linked to serine or threonine (246,247,336). α2,3-Sialylated LNβ1-2Manα1-Ser/Thr on dystroglycan is required for its binding to laminin (246). Disturbances in the synthesis of this structure lead to muscular dystrophy and neuronal migration disorder (248). Since sialylation and fucosylation of LNβ1-2Manα1-Ser/Thr seem to be mutually exclusive (246,247,336), it is possible that fucosylation regulates the binding of dystroglycan to laminin, and therefore cell adhesion. The brain expression and specificity pattern of Fuc-TIX make it a likely candidate for the synthesis of Lexβ1-2Manα1-Ser/Thr. The presentation of the Lewis x epitope on mannose-linked O-glycans, as

opposed to GalNAc-linked, may be a factor that affects its function and detectability. Fuc-TIX has been shown to synthesize the spatially and temporally regulated Lewis x epitope in the developing rat brain (64). However, the detailed structure that carries the epitope has not been solved.

The capability of Fuc-TV and Fuc-TIX to fucosylate the inner LN units of a polylactosamine chain suggests that they may have a role in the generation of the sialylated, multiply fucosylated polylactosamine selectin counterreceptors (133,139). However, further studies are needed to clarify the contribution of Fuc-TV and Fuc-TIX to the biosynthesis of multiply fucosylated polylactosamine chains. Mice that lack both Fuc-TIV and Fuc-TVII show some residual neutrophil infiltration in experimentally induced inflammation (56). This could be due to E- or P-selectin ligands generated by Fuc-TIX.

6.2 ST3Gal II is a multifunctional sialyltransferase (III)

The known mammalian α2,3-sialyltransferases, ST3Gal I-VI, transfer sialic acid to the galactose residue in Galβ1-4GlcNAc, Galβ1-3GlcNAc or Galβ1-3GalNAc, and show some promiscuity among the three acceptor types, as well as overlapping acceptor specificities with each other (77,100,101,337). Enzymatic α2,3-sialylation of GalNAc has not been described previously, although structures containing Neu5Acα2-3GalNAc have been reported (338-340).

ST3Gal II has been shown to act on glycoproteins and glycolipids containing terminal Galβ1-3GalNAcβ1-OR sequence (85). Results in part III show that ST3Gal II can also sialylate GalNAc in terminal GalNAcβ1-3Gal determinants in reaction conditions commonly used in enzymatic *in vitro* synthesis. This makes it one of the few glycosyltransferases reported to date which are capable of transferring to different acceptor monosaccharide residues. The clarification of the actual contribution of ST3Gal II to the biosynthesis of α2,3-sialylated GalNAc determinants will need further experiments such as comparison of the efficacy of GalNAcβ1-3Gal and Galβ1-3GalNAc as acceptor determinants, and the analysis of glycoconjugates from transgenic mice lacking ST3Gal II.

ST3Gal II emerges as yet another glycosyltransferase that challenges the dogma “one glycosyltransferase - one glycosidic linkage”. The best known of these is β1,4-galactosyltransferase that is induced by α-lactalbumin to transfer to glucose instead of N-acetylglucosamine (311). Glycosyltransferases that transfer to different monosaccharide residues without requiring an additional modifier molecule include the β1,3-galactosyltransferase β3GalT-V that transfers to both the terminal GalNAc of GalNAcβ1-3Galα1-4Galβ1-4Glc and the terminal GlcNAc of GlcNAcβ1-3Galβ1-4Glc (341), and the core 2/I β1,6-GlcNAc transferase that transfers to the GalNAc of Galβ1-3GalNAcα1-R and GlcNAcβ1-3GalNAcα1-R, as well as to the Gal of GlcNAcβ1-3Galβ1-R (342,343). Other examples include α1,3/4-fucosyltransferases III and V that transfer to the Glc of lactose as well as to the GlcNAc of N-acetyllactosamine, generating Galβ1-4(Fucα1-3)Glc and Galβ1-4(Fucα1-3)GlcNAc respectively (12,15). Finally, the bovine colostrum α2,6-sialyltransferase sialylates both the Gal of Galβ1-4GlcNAc-R and the GalNAc of GalNAcβ1-4GlcNAc-R (344), suggesting an acceptor recognition mechanism similar to that of ST3Gal II. Fuc-TIII and Fuc-TV are exceptional in their ability to make both Fucα1-3GlcNAc and Fucα1-4GlcNAc linkages (12,15).

6.3 Possible significance of α2,3-sialylation of GalNAc (III)

α2,3-sialylation of the X_2 structure (GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc) may play a role in bacteria-host interactions. It has been suggested that the X_2 epitope found on intestinal epithelium is the human receptor for *Clostridium difficile* toxin A. The X_2 structure shares structural features with Galα1-3Galβ1-4GlcNAc, which is considered to be the receptor for toxin A in animals, but does not occur in humans (345). The X_2 glycosphingolipid binds toxin A, but α2,3-sialylated X_2 does not bind (345). Therefore sialylation of X_2-like structures might be a protective measure against adhesion, and thus internalization and cytotoxic effects of *Clostridium difficile* toxin A.

The X_2 epitope is an example of molecular mimicry between human glycoconjugates and saccharides of pathogenic bacteria, a phenomenon that is proposed to have important roles in bacteria-host interactions, for example by camouflaging the bacterial surface from the host (272,346). The X_2 structure occurs in the lipo-oligosaccharide (LOS) of the *Neisseria gonorrhoeae* strain F62 (347). Sialylation of lipo-oligosaccharide converts gonococci into serum resistant organism, reviewed in (273). So far only the sialylation of the LOS structure Galβ1-4GlcNAcβ1-3Galβ1-4Glc by the neisserial sialyltransferase has been documented (273,348). However, *Neisseria gonorrhoeae* sialyltransferase has been shown to react with a GalNAc monosaccharide derivative (349). It seems possible that *Neisseria gonorrhoeae* can sialylate its LOS structure GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, as well as Galβ1-4GlcNAcβ1-3Galβ1-4Glc, since its sialyltransferase shows similar flexibility as the mammalian ST3Gal II described here.

6.4 Resistance towards Newcastle disease virus sialidase is not necessarily indicative of α2,6-linked Neu5Ac (III)

Newcastle disease virus is widely used in structural analysis of oligosaccharides to differentiate between α2,3- and α2,6-linked Neu5Ac, because it cleaves Neu5Acα2-3Gal bonds while leaving Neu5Acα2-6Gal and Neu5Acα2-6GalNAc bonds intact (350). However, the data presented in part III show that the Neu5Acα2-3GalNAc bond is resistant towards Newcastle disease virus sialidase in conditions that completely cleave Neu5Acα2-3Gal bonds. Resistance towards Newcastle disease virus sialidase has been used to identify Neu5Ac-GalNAc linkages as α2-6 (351-353). The results presented here show that when the sialic acid is linked to GalNAc, the sialidase specificities that have been characterized using substrates where the sialic acid is linked to galactose may not necessarily apply, and the results should be used with caution for structural analysis. This demonstrates that there are certain pitfalls in the use of glycosidases to characterize novel oligosaccharide structures.

7. SUMMARY

The carbohydrate structures that cover cell surfaces are involved in specific recognition events in a wide variety of biological phenomena. The structural diversity of glycans is achieved through the specific substrate requirements and a well defined order of action of the glycosyltransferases involved in their biosynthesis. The detailed acceptor specificities of the α1,3-fucosyltransferases Fuc-TV and Fuc-TIX, and α2,3-sialyltransferase ST3Gal II were examined in the present study.

The acceptor- and site-specificity of α1,3-fucosyltransferase V (Fuc-TV) (I)

α1,3-Fucosyltransferases form the Lewis x epitope (Galβ1-4(Fucα1-3)GlcNAc). Six human α1,3-fucosyltransferases have been cloned and characterized: Fuc-TIII-VII and Fuc-TIX. Distinct acceptor specificity patterns are emerging among them. Fuc-TV is known to react with both type 1 (Galβ1-3GlcNAc, LNB) and type 2 (Galβ1-4GlcNAc, LN) lactosamines, although much more efficiently with the latter. In the present study, Fuc-TV reacted very efficiently with N- and O-glycan analogues where the acceptor LN unit is carried on the 6-branch, namely LNβ1-6Manα1-OMe and Galβ1-3(LNβ1-6)GalNAc. Structures where the acceptor LN unit was β1,2- (LNβ1-2Man), β1,3- (LNβ1-3Galβ1-OMe) or β1,4-linked (LNβ1-4GlcNAc) were much poorer acceptors for Fuc-TV, suggesting that structures in which the LN unit is attached to the C6 hydroxyl fit better to the substrate binding site of Fuc-TV than those where the LN unit is linked to ring hydroxyl. All sialylated acceptors were more efficiently fucosylated than the corresponding neutral ones. When the site-specificity of Fuc-TV on polylactosamines containing multiple acceptor sites was studied, it was noticed that Fuc-TV strongly prefers the reducing end LN units. While the reactivity of the non-reducing end LN unit in LNβ1-3R type structures was significantly lower than that of free LN, the reactivity of the LNB unit in LNBβ1-3R was essentially the same as that of free LNB. These different reactivity patterns suggest that Fuc-TV may have two distinct acceptor substrate binding sites, one that preferably binds LN, and another that preferably binds LNB.

The acceptor- and site-specificity of α1,3-fucosyltransferase IX (Fuc-TIX) (II)

Fuc-TIX is the most recent member of the α1,3-fucosyltransferase family. In part II of this thesis the detailed acceptor specificity of Fuc-TIX, and its site-specificity on polylactosamines were determined. Fuc-TIX reacted efficiently with LN and Fucα1-2'LN, GalNAcβ1-4GlcNAc and 6-sulfated LN, but not with LNB, lactose, Neu5Acα2-3'LN or Neu5Acα2-6'LN. It was found that although Fuc-TIX preferentially fucosylates the distal, non-reducing end N-acetyllactosamine unit of a polylactosamine chain, the addition of a single GlcNAcβ1-3 residue on the distal side of the reacting unit does not significantly affect reactivity. Furthermore, on sialylated polylactosamines the site-specificity was reversed: Fuc-TIX preferentially fucosylated the two most reducing end lactosamine units of Neu5Acα2-3[Galβ1-4GlcNAcβ1-3]$_4$. Therefore it can be concluded that in addition to forming distal Lewis x epitopes, Fuc-TIX may have a role in the biosynthesis of internally fucosylated polylactosamines. The emerging acceptor- and site-specificity profile of Fuc-TIX is different from those of the other α1,3-fucosyltransferases studied so far.

α2,3-Sialylation of N-acetylgalactosamine by ST3Gal II (III)

Glycan structures containing α2,3-sialylated GalNAc-residues have been characterized from human tissues. However, enzymatic α2,3-sialylation of GalNAc has not been described previously. The known mammalian α2,3-sialyltransferases, ST3Gal I-VI, transfer sialic acid to the galactose in Galβ1-4GlcNAc, Galβ1-3GlcNAc or Galβ1-3GalNAc, and show some promiscuity among the three acceptor types, as well as overlapping acceptor specificities with each other. To elucidate the biosynthetic route to the Neu5Acα2-3GalNAc linkage, commercial recombi-

nant α2,3-sialyltransferases were tested for their ability to sialylate oligosaccharides containing a terminal GalNAcβ1-3Gal determinant. ST3Gal II efficiently sialylated the X_2 pentasaccharide (GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc), globo-N-tetraose (GalNAcβ1-3Galα1-4Galβ1-4Glc), and the disaccharide GalNAcβ1-3Gal. ST3Gal II has been thought to be specific for the Galβ1-3GalNAc determinant. The present results show that ST3Gal II is multifunctional, and could be renamed ST3Gal(NAc) II.

An understanding of the function, especially the substrate specificity, of glycosyltransferases is essential for studying the regulation of the biological recognition events mediated by glycans. The studies described above will help to understand the biosynthetic routes to biologically active terminal oligosaccharide epitopes.

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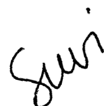

9. REFERENCES

1. Reuter, G., and Gabius, H.-J. (1999) Eukaryotic glycosylation: whim of nature or multipurpose tool *Cell. Mol. Life Sci.* **55,** 368-422

2. Varki, A., Cummings, R. D., Esko, J., Freeze, H., Hart, G., and Marth, J. D. eds. (1999) *Essentials of glycobiology*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

3. Hart, G. W. (1997) Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins *Annu. Rev. Biochem.* **66,** 315-335

4. Harris, R. J., and Spellman, M. W. (1993) O-Linked fucose and other post-translational modifications unique to EGF-modules *Glycobiology* **3,** 219-224

5. Endo, M. (1999) O-Mannosyl glycans in mammals *Biochim. Biophys. Acta* **1473,** 237-246

6. Järnefelt, J., Rush, J., Li, Y. T., Laine, R. A. (1978) Erythroglycan, a high molecular weight glycopeptide with the repeating structure (galactosyl-(1-4)-2-deoxy-2-acetamido-glucosyl(1-3)) comprising more than one-third of protein-bound carbohydrate of human erythrocyte stroma *J. Biol. Chem.* **253,** 8006-8009

7. Krusius, T., Finne, J., Rauvala, H. (1978) The poly(glycosyl) chains of glycoproteins. Characterisation of a novel type of glycoprotein saccharides from human erythrocyte membrane *Eur. J. Biochem.* **92,** 289-300

8. Svennerholm, L. (1963) Chromatographic separation of human brain gangliosides *J. Neurochem.* **10,** 613-623

9. Wang, Y., Shao, L., Shi, S., Harris, R. J., Spellman, M. W., Stanley, P., and Haltiwanger, R. S. (2001) Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase *J. Biol. Chem.* **276,**40338-40345

10. Clarke, J. L., and Watkins, W. M. (1999) Expression of human α-L-fucosyltransferase gene homologs in monkey kidney COS cells and modification of potential fucosyltransferase acceptor substrates by an endogenous glycosidase *Glycobiology* **9,** 191-202

11. Potvin, B., Kumar, R., Howard, D. R., and Stanley, P. (1990) Transfection of a human α-(1,3)fucosyltransferase gene into Chinese hamster ovary cells. Complications arise from activation of endogenous α-(1,3)fucosyltransferases *J. Biol. Chem.* **265,** 1615-1622

12. Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and the Lewis blood group α(1,3/1,4)fucosyltransferase *Genes Dev.* **4,** 1288-1303

13. Mollicone, R., Gibaud, A., Francois, A., Ratcliffe, M., and Oriol, R. (1990) Acceptor specificity and tissue distribution of three human α-3-fucosyltransferases *Eur. J. Biochem.* **191,** 169-176

14. Chandrasekaran, E. V., Jain, R. K., Larsen, R. D., Wlasichuk, K., DiCioccio, R. A., and Matta, K. L. (1996) Specificity analysis of three clonal and five non-clonal α1,3-L-fucosyltransferases with sulfated, sialylated, or fucosylated synthetic carbohydrates as acceptors in relation to the assembly of 3'-sialyl-6'-sulfo Lewis x (the L-selectin ligand) and related complex structures *Biochemistry* **35,** 8925-8933

15. Weston, B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) Isolation of a novel human α(1,3)fucosyltransferase gene and molecular comparison to the human Lewis blood group α(1,3/1,4)fucosyltransferase gene. Synthetic, homologous, nonallelic genes encoding enzymes with distinct acceptor substrate specificities *J. Biol. Chem.* **267,** 4152-4160

16. de Vries, T., Srnka, C. A., Palcic, M. M., Swiedler, S. J., van den Eijnden, D. H., and Macher, B. A. (1995) Acceptor specificity of different length constructs of human recombinant α1,3/4-fucosyltransferases. Replacement of the stem region and the transmembrane domain of fucosyltransferase V by protein A results in an enzyme with GDP-fucose hydrolyzing activity *J. Biol. Chem.* **270,** 8712-8722

17. Gallet, P. F., Vaujour, H., Petit, J. M., Maftah, A., Oulmouden, A., Oriol, R., Le Narvor, C., Guilloton, M., and Julien, R. (1998) Heterologous expression of an engineered truncated form of human Lewis fucosyltransferase (Fuc-TIII) by the methylotrophic yeast *Pichia pastoris Glycobiology* **8,** 919-925

18. Sousa, V. L., Costa, M. T., Palma, A. S., Enguita, F., and Costa, J. (2001) Localization, purification and specificity of the full-length membrane-bound form of human recombinant α1,3/4-fucosyltransferase from BHK-21B cells *Biochem. J.* **357,** 803-810

19. Costa, J., Grabenhorst, E., Nimtz, M., and Conradt, H. S. (1997) Stable expression of the Golgi form and secretory variants of human fucosyltransferase III from BHK-21 cells. Purification and characterization of an engineered truncated form from the culture medium *J. Biol. Chem.* **272,** 11613-11621

20. Kimura, H., Shinya, N., Nishihara, S., Kaneko, M., Irimura, T., and Narimatsu, H. (1997) Distinct substrate specificities of five human α-1,3-fucosyltransferases for *in vivo* synthesis of the sialyl Lewis x and Lewis x epitopes *Biochem. Biophys. Res. Commun.* **237,** 131-137

21. Grabenhorst, E., Nimtz, M., Costa, J., and Conradt, H. S. (1998) *In vivo* specificity of human α1,3/4-fucosyltransferases III-VII in the biosynthesis of Lewis^x and sialyl Lewis^x motifs on complex-type N-glycans. Coexpression studies from BHK-21 cells together with human β-trace protein *J. Biol. Chem.* **273,** 30985-30994

22. Sueyoshi, S., Tsuboi, S., Sawada-Hirai, R., Dang, U. N., Lowe, J. B., and Fukuda, M. (1994) Expression of distinct fucosylated oligosaccharides and carbohydrate-mediated adhesion efficiency directed by two different α1,3-fucosyltransferases. Comparison of E- and L-selectin-mediated adhesion *J. Biol. Chem.* **269,** 32342-32350

23. Nishihara, S., Iwasaki, H., Kaneko, M., Tawada, A., Ito, M., and Narimatsu, H. (1999) α1,3-Fucosyltransferase 9 (FUT9; Fuc-TIX) preferentially fucosylates the distal GlcNAc residue of polylactosamine chain while the other four α1,3FUT members preferentially fucosylate the inner GlcNAc residue *FEBS Lett.* **462,** 289-294

24. Cameron, H. S., Szczepaniak, D., and Weston, B. W. (1995) Expression of human chromosome 19p α(1,3)-fucosyltransferase genes in normal tissues. Alternative splicing, polyadenylation, and isoforms *J. Biol. Chem.* **270,** 20112-20122

25. Elmgren, A., Mollicone, R., Costache, M., Börjeson, C., Oriol, R., Harrington, J., and Larson, G. (1997) Significance of individual point mutations, T202C and C314T, in the human Lewis (*FUT3*) gene for expression of Lewis antigens by the human α(1,3/1,4)-fucosyltransferase Fuc-TIII *J. Biol. Chem.* **272,** 21994-21998

26. Mollicone, R., Reguine, I., Kelly, R. J., Fletcher, A., Watt, J., Chatfield, S., Aziz, A., Cameron, H. S., Weston, B. W., Lowe, J. B., and Oriol, R. (1994) Molecular basis for Lewis α(1,3/1,4)-fucosyltransferase gene deficiency (FUT3) found in Lewis negative Indonesian pedigrees *J. Biol. Chem.* **269,** 20987-20994

27. Ellison, R. C., Zhang, Y., Myers, R. H., Swanson, J. L., Higgins, M., and Eckfeldt, J. (1999) Lewis blood group phenotype as an independent risk factor for coronary heart disease (the NHLBI family heart study) sup *Am. J. Cardiol.* **83,** 345-348

28. Hein, H. O., Sorensen, H., Suadicani, P., and Gyntelberg, F. (1992) The Lewis blood group - a new genetic marker of ischaemic heart disease *J. Intern. Med.* **232,** 481-487

29. Goelz, S. E., Hession, C., Goff, D., Griffiths, B., Tizard, R., Newman, B., Chi-Rosso, G., and Lobb, R. (1990) ELFT: A gene that directs the expression of an ELAM-1 ligand *Cell* **63,** 1349-1356

30. Kumar, R., Potvin, B., Muller, W. A., and Stanley, P. (1991) Cloning of a human α(1,3)-fucosyltransferase gene that encodes ELFT but does not confer ELAM-1 recognition on Chinese hamster ovary cell transfectants *J. Biol. Chem.* **266,** 21777-21783

31. Lowe, J. B., Kukowska-Latallo, J. F., Nair, R. P., Larsen, R. D., Marks, R. M., Macher, B. A., Kelly, R. J., and Ernst, L. K. (1991) Molecular cloning of a human fucosyltransferase gene that determines expression of the Lewis x and VIM-2 epitopes but not ELAM-1-dependent cell adhesion *J. Biol. Chem.* **266,** 17467-17477

32. Sherwood, A. L., and Holmes, E. H. (1999) Analysis of the expression and enzymatic properties of α1-3fucosyltransferase from human lung carcinoma NCI-H69 and PC9 cells *Glycobiology* **9,** 637-643

33. Clarke, J. L., and Watkins, W. M. (1996) α1,3-L-fucosyltransferase expression in developing human myeloid cells. Antigenic, enzymatic, and mRNA analyses *J. Biol. Chem.* **271,** 10317-10328

34. Niemelä, R., Natunen, J., Majuri, M. L., Maaheimo, H., Helin, J., Lowe, J. B., Renkonen, O., and Renkonen, R. (1998) Complementary acceptor and site specificities of Fuc-TIV and Fuc-TVII allow effective biosynthesis of sialyl-triLex and related polylactosamines present on glycoprotein counterreceptors of selectins *J. Biol. Chem.* **273,** 4021-4026

35. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) Molecular cloning of a fourth member of a human α(1,3)fucosyltransferase gene family. Multiple homologous sequences that determine expression of the Lewis x, sialyl Lewis x, and difucosyl sialyl Lewis x epitopes *J. Biol. Chem.* **267,** 24575-24584

36. de Vries, T., Palcic, M. P., Schoenmakers, P. S., van den Eijnden, D. H., and Joziasse, D. H. (1997) Acceptor specificity of GDP-Fuc:Galβ1->4GlcNAc-R α3-fucosyltransferase VI (FucT VI) expressed in insect cells as soluble, secreted enzyme *Glycobiology* **7,** 921-927

37. Natunen, J., Aitio, O., Helin, J., Maaheimo, H., Niemelä, R., Heikkinen, S., and Renkonen, O. (2001) Human α3-fucosyltransferases convert chitin oligosaccharides to products containing a GlcNAcβ1-4(Fucα1-3)GlcNAcβ1-4R determinant at the nonreducing terminus *Glycobiology* **11,** 209-216

38. Koszdin, K. L., and Bowen, B. R. (1992) The cloning and expression of a human α-1,3fucosyltransferase capable of forming the E-selectin ligand *Biochem. Biophys. Res. Commun.* **187,** 152-157

39. Brinkman-Van der Linden, E. C. M., Mollicone, R., Oriol, R., Larson, G., Van den Eijnden, D. H., and Van Dijk, W. (1996) A missense mutation in the *FUT6* gene results in total absence of α3-fucosylation of human α1-acid glycoprotein *J. Biol. Chem.* **271,** 14492-14495

40. Mollicone, R., Reguine, I., Fletcher, A., Aziz, A., Rustam, M., Weston, B. W., Kelly, R. J., Lowe, J. B., and Oriol, R. (1994) Molecular basis for plasma α(1,3)-fucosyltransferase gene deficiency (FUT6) *J. Biol. Chem.* **269,** 12662-12671

41. Schnyder-Candrian, S., Borsig, L., Moser, R., and Berger, E. G. (2000) Localization of α1,3-fucosyltransferase VI in Weibel-Palade bodies of human endothelial cells *Proc. Natl. Acad. Sci. USA* **97,** 8369-8374

42. McCurley, R. S., Recinos, A., Olsen, A. S., Gingrich, J. C., Szczepaniak, D., Cameron, H. S., Krauss, R., and Weston, B. W. (1994) Physical maps of human α(1,3)fucosyltransferase genes FUT3-FUT6 on chromosomes 19p13.3 and 11q21 *Genomics* **26,** 142-146

43. Oulmouden, A., Wierinckx, A., Petit, J. M., Costache, M., Palcic, M. M., Mollicone, R., Oriol, R., and Julien, R. (1997) Molecular cloning and expression of a bovine α(1,3)-fucosyltransferase gene homologous to a putative ancestor gene of the human *FUT3-FUT5-FUT6* cluster *J. Biol. Chem.* **272,** 8764-8773

44. Dupuy, F., Petit, J. M., Mollicone, R., Oriol, R., Julien, R., and Maftah, A. (1999) A single amino acid in the hypervariable stem domain of vertebrate α1,3/1,4-fucosyltransferases determines the type 1/type 2 transfer. Characterization of acceptor substrate specificity of the Lewis enzyme by site-directed mutagenesis *J. Biol. Chem.* **274,** 12257-12262

45. Legault, D. J., Kelly, R. J., Natsuka, Y., and Lowe, J. B. (1995) Human α(1,3/1,4)-fucosyltransferases discriminate between different oligosaccharide acceptor substrates through a discrete peptide fragment *J. Biol. Chem.* **270,** 20987-20996

46. Nguyen, A. T., Holmes, E. H., Whitaker, J. M., Ho, S., Shetterly, S., and Macher, B. A. (1998) Human α1,3/4-fucosyltransferases. I. Identification of amino acids involved in acceptor substrate binding by site-directed mutagenesis *J. Biol. Chem.* **273,** 25244-25249

47. Vo, L., Lee, S., Marcinko, M. C., Holmes, E. H., and Macher, B. A. (1998) Human α1,3/4-fucosyltransferases. II. A single amino acid at the COOH terminus of FucT III and V alters their kinetic properties *J. Biol. Chem.* **273,** 25250-25255

48. Xu, Z., Vo, L., and Macher, B. A. (1996) Structure-function analysis of human α1,3-fucosyltransferase. Amino acids involved in acceptor substrate specificity *J. Biol. Chem.* **271,** 8818-8823

49. Natsuka, S., Gersten, K. M., Zenita, K., Kannagi, R., and Lowe, J. B. (1994) Molecular cloning of a cDNA encoding a novel human leukocyte α-1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant *J. Biol. Chem.* **269,** 16789-16794

50. Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanai, N., and Nishi, T. (1994) Expression cloning of a novel α1,3-fucosyltransferase that is involved in biosynthesis of the sialyl Lewis x carbohydrate determinants in leukocytes *J. Biol. Chem.* **269,** 14730-14737

51. Britten, C. J., van den Eijnden, D. H., McDowell, W., Kelly, V. A., Witham, S. J., Edbrooke, M. R., Bird, M. I., de Vries, T., and Smithers, N. (1998) Acceptor specificity of the human leukocyte α3 fucosyltransferase: role of FucT-VII in the generation of selectin ligands *Glycobiology* **8,** 321-327

52. Maly, P., Thall, A. D., Petryniak, B., Rogers, C. E., Smith, P. L., Marks, R. M., Kelly, R. J., Gersten, K. M., Cheng, G., Saunders, T. L., Camper, S. A., Camphausen, R. T., Sullivan, F. X., Isogai, Y., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (1996) The α(1,3)fucosyltrans-ferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis *Cell* **86,** 643-653

53. Stroud, M. R., and Holmes, E. H. (1997) Fucosylation of complex glycosphingolipids by recombinant fucosyltransferase-VII *Biochem. Biophys. Res. Commun.* **238,** 165-168

54. Huang, M. C., Zöllner, O., Moll, T., Maly, P., Thall, A. D., Lowe, J. B., and Vestweber, D. (2000) P-selectin Glycoprotein Ligand-1 and E-selectin Ligand-1 are differentially modified by fucosyltransferases Fuc-TIV and Fuc-TVII in mouse neutrophils *J. Biol. Chem.* **275,** 31353-31360

55. Weninger, W., Ulfman, L. H., Cheng, G., Souchkova, N., Quackenbush, E. J., Lowe, J. B., and von Andrian, U. H. (2000) Specialized contributions by α(1,3)-fucosyltransferase-IV and FucT-VII during leukocyte rolling in dermal microvessels *Immunity* **12,** 665-676

56. Homeister, J. W., Thall, A. D., Petryniak, B., Maly, P., Rogers, C. E., Smith, P. L., Kelly, R. J., Gersten, K. M., Askari, S. W., Cheng, G., Smithson, G., Marks, R. M., Misra, A. K., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (2001) The α(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing *Immunity* **15,** 115-126

57. Bengtson, P., Larson, C., Lundblad, A., Larson, G., and Påhlsson, P. (2001) Identification of a missense mutation (G329A; Arg^{110}-Gln) in the human *FUT7* gene *J. Biol. Chem.* **276,** 31575-31582

58. Kaneko, M., Kudo, T., Iwasaki, H., Ikehara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shiina, T., Inoko, H., Saitou, N., and Narimatsu, H. (1999) α1,3-Fucosyltransferase IX (Fuc-TIX) is very highly conserved between human and mouse; molecular cloning, characterization and tissue specific distribution of human Fuc-TIX *FEBS Lett.* **452,** 237-242

59. Ikeda, N., Eguchi, H., Nishihara, S., Narimatsu, H., Kannagi, R., Irimura, T., Ohta, M., Matsuda, H., Taniguchi, N., and Honke, K. (2001) A remodelling system of the 3'-sulfo-Lewis a and 3'-sulfo-Lewis x epitopes *J. Biol. Chem.* **276,** 38588-38594

60. Cailleau-Thomas, A., Coullin, P., Candelier, J. J., Balanzino, L., Mennesson, B., Oriol, R., and Mollicone, R. (2000) *FUT4* and *FUT9* genes are expressed early in human embryogenesis *Glycobiology* **10,** 789-802

61. Baboval, T., Henion, T., Kinally, E., and Smith, F. I. (2000) Molecular cloning of rat α1,3-fucosyltransferase IX (Fuc-TIX) and comparison of the expression of Fuc-TIV and Fuc-TIX genes during rat postnatal cerebellum development *J. Neurosci. Res.* **62,** 206-215

62. Kudo, T., Ikehara, Y., Togayachi, A., Kaneko, M., Hiraga, T., Sasaki, K., and Narimatsu, H. (1998) Expression cloning and characterization of a novel murine α1,3-fucosyltransferase, mFuc-TIX, that synthesizes the Lewis x (CD15) epitope in brain and kidney *J. Biol. Chem.* **273,** 26729-26738

63. Patnaik, S. K., Zhang, A., Shi, S., and Stanley, P. (2000) α(1,3)Fucosyltransferases expressed by the gain-of-function Chinese hamster ovary glycosylation mutants LEC12, LEC29, and LEC30 *Arch. Biochem. Biophys.* **375,** 322-332

64. Shimoda, Y., Tajima, Y., Osanai, T., Katsume, A., Kohara, M., Kudo, T., Narimatsu, H., Takashima, N., Ishii, Y., Nakamura, S., Osumi, N., and Sanai, Y. (2002) *Pax6* controls the expression of Lewis x epitope in the embryonic forebrain by regulating *α1,3-fucosyltransferase IX* expression *J. Biol. Chem.* **277,** 2033-2039

65. Osanai, T., Chai, W., Tajima, Y., Shimoda, Y., Sanai, Y., and Yuen, C.-T. (2001) Expression of glycoconjugates bearing the Lewis x epitope during neural differentiation of P19 EC cells *FEBS Lett.* **488,** 23-28

66. Nakayama, F., Nishihara, S., Iwasaki, H., Kudo, T., Okubo, R., Kaneko, M., Nakamura, M., Karube, M., Sasaki, K., and Narimatsu, H. (2001) CD15 expression in mature granulocytes is determined by α1,3-fucosyltransferase IX but in promyelocytes and monocytes by α1,3-fucosyltransferase IV *J. Biol. Chem.* **276,** 16100-16106

67. Larsen, R. D., Ernst, L. K., Nair, R. P., and Lowe, J. B. (1990) Molecular cloning, sequence, and expression of a human GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase cDNA that can form the H blood group antigen *Proc. Natl. Acad. Sci. USA* **87,** 6674-6678

68. Kelly, R. J., Rouquier, S., Giorgi, D., Lennon, G. G., and Lowe, J. B. (1995) Sequence and expression of a candidate for the human *Secretor* blood group α(1,2)fucosyltransferase gene (*FUT2)*. Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype *J. Biol. Chem.* **270,** 4640-4649

69. Yanagidani, S., Uozumi, N., Ihara, Y., Miyoshi, E., Yamaguchi, N., and Taniguchi, N. (1997) Purification and cDNA cloning of GDP-L-Fuc:N-acetyl-β-D-glucosaminide:α1-6 fucosyltransferase (α1-6 FucT) from human gastric cancer MKN45 cells *J. Biochem.* **121,** 626-632

70. Costache, M., Apoil, P. A., Cailleau, A., Elmgren, A., Larson, G., Henry, S., Blancher, A., Iordachescu, D., Oriol, R., and Mollicone, R. (1997) Evolution of fucosyltransferase genes in vertebrates *J. Biol. Chem.* **272,** 29721-29728

71. Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L., and Breton, C. (1999) Divergent evolution of fucosyltransferase genes from vertebrates, invertebrates, and bacteria *Glycobiology* **9,** 323-334

72. Datta, A. K., and Paulson, J. C. (1995) The sialyltransferase "sialylmotif" participates in binding the donor substrate CMP-NeuAc *J. Biol. Chem.* **270,** 1497-1500

73. Datta, A. K., Chammas, R., and Paulson, J. C. (2001) Conserved cysteines in the sialyltransferase sialylmotifs form an essential disulfide bond *J. Biol. Chem.* **276,** 15200-15207

74. Tsuji, S., Datta, A. K., and Paulson, J. C. (1996) Systematic nomenclature for sialyltransferases *Glycobiology* **6,** v-vii

75. Chang, M. L., Eddy, R. L., Shows, T. B., and Lau, J. T. Y. (1995) Three genes that encode human β-galactoside α2,3-sialyltransferases. Structural analysis and chromosomal mapping studies *Glycobiology* **5,** 319-325

76. Kitagawa, H., and Paulson, J. C. (1994) Differential expression of five sialyltransferase genes in human tissues *J. Biol. Chem.* **269,** 17872-17878

77. Kitagawa, H., and Paulson, J. C. (1994) Cloning of a novel α2,3-sialyltransferase that sialylates glycoprotein and glycolipid carbohydrate groups *J. Biol. Chem.* **269,** 1394-1401

78. Kojima, N., Lee, Y. C., Hamamoto, T., Kurosawa, N., and Tsuji, S. (1994) Kinetic properties and acceptor substrate preferences of two kinds of Galβ1,3GalNAc α2,3-sialyltransferases from mouse brain *Biochemistry* **33,** 5772-5776

79. Burchell, J., Poulsom, R., Hanby, A., Whitehouse, C., Cooper, L., Clausen, H., Miles, D., and Taylor-Papadimitriou, J. (1999) An α2,3 sialyltransferase (ST3Gal I) is elevated in primary breast carcinomas *Glycobiology* **9,** 1307-1311

80. Dalziel, M., Whitehouse, C., McFarlane, I., Brockhausen, I., Gschmeissner, S., Schwientek, T., Clausen, H., Burchell, J. M., and Taylor-Papadimitriou, J. (2001) The relative activities of the C2GnT1 and ST3Gal-I glycosyltransferases determine O-glycan structure and expression of a tumor-associated epitope on MUC1 *J. Biol. Chem.* **276,** 11007-11015

81. Priatel, J. J., Chui, D., Hiraoka, N., Simmons, C. J. T., Richardson, K. B., Page, D. M., Fukuda, M., Varki, N. M., and Marth, J. D. (2000) The ST3Gal-I sialyltransferase controls $CD8^+$ T lymphocyte homeostasis by modulating O-glycan biosynthesis *Immunity* **12,** 273-283

82. Piller, F., Piller, V., Fox, R. I., and Fukuda, M. (1988) Human T-lymphocyte activation is associated with changes in O-glycan biosynthesis *J. Biol. Chem.* **263,** 15146-15150

83. Giordanengo, V., Bannwarth, S., Laffont, C., Van Miegem, V., Harduin-Lepers, A., Delannoy, P., and Lefebre, J. C. (1997) Cloning and expression of cDNA for a human Gal(β1-3)GalNAc α2,3-sialyltransferase from the CEM T-cell line *Eur. J. Biochem.* **247,** 558-566

84. Kim, Y.-J., Kim, K.-S., Kim, H.-S., Kim, C.-H., Ko, J. H., Choe, I.-S., Tsuji, S., and Lee, Y.-C. (1996) Molecular cloning and expression of human Galβ1,3GalNAc α2,3-sialyltransferase (hST3Gal II) *Biochem. Biophys. Res. Commun.* **228,** 324-327

85. Lee, Y. C., Kojima, N., Wada, E., Kurosawa, N., Nakaoka, T., Hamamoto, T., and Tsuji, S. (1994) Cloning and expression of cDNA for a new type of Galβ1,3GalNAc α2,3-sialyltransferase *J. Biol. Chem.* **269,** 10028-10033

86. Chen, N., Soloway, P., and Lau, J. T. Y. (1998) Functional analysis of ST3Gal-2 by targeted mutagenesis *Glycobiology* **8,** A23

87. Kitagawa, H., and Paulson, J. C. (1993) Cloning and expression of human Galβ1,3(4)GlcNAc α2,3-sialyltransferase *Biochem. Biophys. Res. Commun.* **194,** 375-382

88. Chandrasekaran, E. V., Jain, R. K., Larsen, R. D., Wlasichuk, K., and Matta, K. L. (1995) Selectin ligands and tumor-associated carbohydrate structures: specificities of α2,3-sialyltransferases in the assembly of 3'-sialyl-6-sulfo/sialyl Lewis a and x, 3'-sialyl-6'-sulfo Lewis x and 3'-sialyl-6-sialyl/sulfo blood group T-hapten *Biochemistry* **34,** 2925-2936

89. Yeh, J. C. and Cummings, R. D. (1997) Differential recognition of glycoprotein acceptors by terminal glycosyltransferases *Glycobiology* **7,** 241-251

90. Damián-Matsumura, P., Zaga, V., Maldonado, A., Sánches-Hernándes, C., Timossi, C., and Ulloa-Aguirre, A. (1999) Oestrogens regulate pituitary α2,3-sialyltransferase messenger ribonucleic acid levels in the female rat *J. Mol. Endocrinol.* **23,** 153-165

91. Burger, P. C., Lötscher, M., Streiff, M., Kleene, R., Kaissling, B., and Berger, E. G. (1998) Immonocytochemical localization of α2,3(N)-sialyltransferase (ST3Gal III) in cell lines and rat kidney tissue sections: evidence for Golgi and post-Golgi localization *Glycobiology* **8,** 245-257

92. Sasaki, K., Watanabe, E., Kawashima, K., Sekine, S., Dohi, T., Oshima, M., Hanai, N., Nishi, T., and Hasegawa, M. (1993) Expression cloning of a novel Galβ(1-3/1-4)GlcNAc α2,3-sialyltransferase using lectin resistance selection *J. Biol. Chem.* **268,** 22782-22787

93. Taniguchi, A., Hasegawa, Y., Higai, K., and Matsumoto, K. (1999) Down-regulation of human Galβ1,3GalNAc/Galβ1,4GlcNAc α2,3-sialyltransferase (hST3Gal IV) gene during differentiation of the HL-60 cell line *Mol. Cell Biol. Res. Commun.* **2,** 197-201

94. Taniguchi, A., and Matsumoto, K. (1999) Epithelial-cell-specific transcriptional regulation of human Galβ1,4GlcNAc α2,3-sialyltransferase (hST3Gal IV) gene *Biochem. Biophys. Res. Commun.* **257,** 516-522

95. Blander, J. M., Visintin, I., Janeway, C. A., and Medzhitov, R. (1999) α(1,3)-Fucosyltransferase VII and α(2,3)-sialyltransferase IV are up-regulated in activated CD4 T-cells and maintained after their differentiation into Th1 and migration into inflammatory sites *J. Immunol.* **163,** 3746-3752

96. Okabe, A., Tawara, Y., Masa, T., Oka, T., Machida, A., Tanaka, T., Matsuhashi, H., Shiosaka, S., and Kato, K. (2001) Differential expression of mRNAs for sialyltransferase isoenzymes induced in the hippocampus of mouse following kindled seizures *J. Neurochem.* **77,** 1185-1197

97. Ellies, L. G., Ditto, D., Varki, N., Page, D., Le, D., and Marth, J. D. (1999) The ST3Gal-IV sialyltransferase function in hematopoiesis and hemostasis in vivo *Glycobiology* **9,** 1099

98. Ishii, K., Ohta, M., Watanabe, Y., Matsuda, K., Ishiyama, K., Sakoe, K., Nakamura, M., Inokuchi, J. I., Sanai, Y., and Saito, M. (1998) Expression cloning and functional characterization of human cDNA for ganglioside G_{M3} synthase *J. Biol. Chem.* **273,** 31652-31655

99. Stern, C. A., and Tiemeyer, M. (2001) A ganglioside-specific sialyltransferase localizes to axons and non-Golgi structures in neurons *J. Neurosci.* **21,** 1434-1443

100. Okajima, T., Fukumoto, S., Miyazaki, H., Ishida, H., Kiso, M., Furukawa, K., Urano, T., and Furukawa, K. (1999) Molecular cloning of a novel α2,3-sialyltransferase (ST3Gal VI) that sialylates type II lactosamine structures on glycoproteins and glycolipids *J. Biol. Chem.* **274,** 11479-11486

101. Fukumoto, S., Miyazaki, H., Goto, G., Urano, T., Furukawa, K., and Furukawa, K. (1999) Expression cloning of mouse cDNA of CMP-NeuAc:lactosylceramide α2,3-sialyltransferase, an enzyme that initiates the synthesis of gangliosides *J. Biol. Chem.* **274,** 9271-9276

102. Grundman, U., Nerlich, C., Rein, T., and Zettlmeissl, G. (1990) Complete cDNA sequence encoding human β-galactoside α2,6-sialyltransferase *Nucleic acids Res.* **18,** 667

103. Weinstein, J., Lee, E. U., McEntee, K., Lai, P.-H., and Paulson, J. C. (1987) Primary structure of β-galactoside α2,6-sialyltransferas. Conversion of membrane-bound enzyme to soluble forms by cleavage of the NH_2-terminal signal anchor *J. Biol. Chem.* **262,** 17735-17743

104. Ikehara, Y., Kojima, N., Kurosawa, N., Kudo, T., Kono, M., Nishihara, S., Issiki, S., Morozumi, K., Itzkowitz, S., Tsuda, T., Nishimura, S. I., Tsuji, S., and Narimatsu, H. (1999) Cloning and expression of a human gene encoding an N-acetylgalactosamine-α2,6-sialyltransferase (ST6GalNAc I): a candicate for synthesis of cancer-associated sialyl-Tn antigens *Glycobiology* **9,** 1213-1224

105. Kono, M., Tsuda, T., Ogata, S., Takashima, S., Liu, H., Hamamoto, T., Itzkowitz, S. H., Nishimura, S. I., and Tsuji, S. (2000) Redefined substrate specificity of ST6GalNAc II: A second candidate Sialyl-Tn synthase *Biochem. Biophys. Res. Commun.* **272,** 94-97

106. Kurosawa, N., Hamamoto, T., Lee, Y. C., Nakaoka, T., Kojima, N., and Tsuji, S. (1994) Molecular cloning and expression of GalNAc α2,6-sialyltransferase *J. Biol. Chem.* **269,** 1402-1409

107. Samyn-Petit, B., Krzewinski-Recchi, M. A., Steelant, W. F. A., Delannoy, P., and Harduin-Lepers, A. (2000) Molecular cloning and functional expression of human ST6GalNAc II. Molecular expression in various human cultured cells *Biochim. Biophys. Acta* **1474,** 201-211

108. Lee, Y.-C., Kaufmann, M., Kitazume-Kawaguchi, S., Kono, M., Takashima, S., Kurosawa, N., Liu, H., Pircher, H., and Tsuji, S. (1999) Molecular cloning and functional expression of two members of mouse NeuAcα2,3Galβ1,3GalNAc GalNAcα2,6-sialyltransferase family, ST6GalNAc III and IV *J. Biol. Chem.* **274,** 11958-11967

109. Sjoberg, E. R., Kitagawa, H., Glushka, J., van Halbeek, H., and Paulson, J. C. (1996) Molecular cloning of a developmentally regulated N-acetylgalactosamine α2,6-sialyltransferase specific for sialylated glycoconjugates *J. Biol. Chem.* **271,** 7450-7459

110. Harduin-Lepers, A., Stokes, D. C., Steelant, W. F. A., Samyn-Petit, B., Krzewinski-Recchi, M. A., Vallejo-Ruiz, V., Zanetta, J. P., Augé, C., and Delannoy, P. (2000) Cloning, expression and gene organization of a human Neu5Acα2-3Galβ1-3GalNAc α2,6-sialyltransferase: hST6GalNAc IV *Biochem. J.* **352,** 37-48

111. Ikehara, Y., Shimizu, N., Kono, M., Nishihara, S., Nakanishi, H., Kitamura, T., Narimatsu, H., Tsuji, S., and Tatematsu, M. (1999) A novel glycosyltransferase with a polyglutamine repeat; a new candidate for GD1α synthase (ST6GalNAc V) *FEBS Lett.* **463,** 92-96

112. Okajima, T., Fukumoto, S., Ito, H., Kiso, M., Hirabayashi, Y., Urano, T., Furukawa, K., and Furukawa, K. (1999) Molecular cloning of brain-specific GD1α synthase (ST6GalNAc V) containing CAG/glutamine repeats *J. Biol. Chem.* **274,** 30557-30562

113. Okajima, T., Chen, H. H., Ito, H., Kiso, M., Tai, T., Furukawa, K., Urano, T., and Furukawa, K. (2000) Molecular cloning and expression of mouse GD1α/GTaα/GQ1bα synthase (ST6GalNAc VI) gene *J. Biol. Chem.* **275,** 6717-6723

114. Haraguchi, M., Yamashiro, S., Yamamoto, A., Furukawa, K., Takamiya, K., Lloyd, K. O., Shiku, H., and Furukawa, K. (1994) Isolation of G_{D3} synthase gene by expression cloning of G_{M3} α-2,8-sialyltransferase cDNA using anti-G_{D2} monoclonal antibody *Proc. Natl. Acad. Sci. USA* **91,** 10455-10459

115. Nara, K., Watanabe, Y., Maruyama, K., Kasahara, K., Nagai, Y., and Sanai, Y. (1994) Expression cloning of a CMP-NeuAc:NeuAcα2-3Galβ1-4Glcβ1-1'Cer α2,8-sialyltransferase (GD3 synthase) from human melanoma cells *Proc. Natl. Acad. Sci. USA* **91,** 7952-7956

116. Sasaki, K., Kurata, K., Kojima, N., Kurosawa, N., Ohta, S., Hanai, N., Tsuji, S., and Nishi, T. (1994) Expression cloning of G_{M3}-specific α-2,8-sialyltransferase (G_{D3} synthase) *J. Biol. Chem.* **269,** 15950-15956

117. Nakayama, J., Fukuda, M. N., Fredette, B., Ranscht, B., and Fukuda, M. (1995) Expression cloning of a human polysialyltransferase that forms the polysialylated neural cell adhesion molecule present in embryonic brain *Proc. Natl. Acad. Sci. USA* **92,** 7031-7035

118. Scheidegger, E. P., Sternberg, L. R., Roth, J., and Lowe, J. B. (1995) A human STX cDNA confers polysialic acid expression in mammalian cells *J. Biol. Chem.* **270,** 22685-22688

119. Lee, Y.-C., Kim, Y.-J., Lee, K.-Y., Kim, K.-S., Kim, B.-U., Kim, H.-N., Kim, C.-H., and Do, S.-I. (1998) Cloning and expression of cDNA for a human Siaα2,3Galβ1,4GlcNA:α2,8-sialyltransferase (hST8Sia III) *Arch. Biochem. Biophys.* **360,** 41-46

120. Kim, Y.-J., Kim, K.-S., Do, S.-I., Kim, C.-H., Kim, S.-K., and Lee, Y.-C. (1997) Molecular cloning and expression of human α2,8-sialyltransferase (hST8Sia V) *Biochem. Biophys. Res. Commun.* **235,** 327-330

121. Lubke, T., Marquardt, T., Etzioni, A., Hartmann, E., von Figura, K., and Körner, C. (2001) Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency *Nat. Genet.* **28,** 73-76

122. Etzioni, A., Frydman, M., Pollack, S., Avidor, I., Phillips, M. L., Paulson, J. C., and Gershoni-Baruch, R. (1992) Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency *N. Engl. J. Med.* **327,** 1789-1792

123. Frydman, M., Etzioni, A., Eidlitz-Markus, T., Avidor, I., Varsano, I., Shechter, Y., Orlin, J. B., and Gershoni-Baruch, R. (1992) Ramba-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility and Bombay phenotype *Am. J. Med. Gen.* **44,** 297-302

124. Marquardt, T., Brune, T., Luhn, K., Zimmer, K.-P., Korner, C., Fabritz, L., van der Werft, N., Vormoor, J., Freeze, H. H., Louwen, F., Biermann, B., Harms, E., von Figura, K., Vestweber, D., and Koch, H. G. (1999) Leukocyte adhesion deficiency II syndrome, a generalized defect in fucose metabolism *J. Pediatr.* **134,** 681-688

125. Lasky, L. A. (1995) Selectin-carbohydrate interactions and the initiation of the inflammatory response *Annu. Rev. Biochem.* **64,** 113-139

126. Vestweber, D., and Blanks, J. E. (1999) Mechanisms that regulate the function of the selectins and their ligands *Physiol. Rev.* **79,** 181-213

127. Foxall, C., Watson, S. R., Dowbenko, D., Fennie, C., Lasky, L. A., Kiso, M., Hasegawa, A., Asa, D., and Brandley, B. K. (1992) The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis x oligosaccharide *J. Cell Biol.* **117,** 895-902

128. Varki, A. (1997) Selectin ligands: Will the real ones please stand up? *J. Clin. Invest.* **99,** 158-162

129. Hemmerich, S., Leffler, H., and Rosen, S. D. (1995) Structure of the O-glycans in GlyCAM-1, an endothelial-derived ligand for L-selectin *J. Biol. Chem.* **270,** 12035-12047

130. Ellies, L. G., Tsuboi, S., Petryniak, B., Lowe, J. B., Fukuda, M., and Marth, J. D. (1998) Core 2 oligosaccharide biosynthesis distinguishes between selectin ligands essential for leukocyte homing and inflammation *Immunity* **9,** 881-890

131. Yeh, J. C., Hiraoka, N., Petryniak, B., Nakayama, J., Ellies, L. G., Rabuka, D., Hindsgaul, O., Marth, J. D., Lowe, J. B., and Fukuda, M. (2001) Novel sulfated lymphocyte homing receptors and their control by a core1 extension β1,3-N-acetylglucosaminyltransferase *Cell* **105,** 957-969

132. Satomaa, T., Renkonen, O., Helin, J., Kirveskari, J., Mäkitie, A., and Renkonen, R. (2002) O-glycans on human high endothelial CD34 putatively participating in L-selectin recognition *Blood* **99,** 2609-2611

133. Wilkins, P. P., McEver, R. P., and Cummings, R. D. (1996) Structures of the O-glycans on P-selectin glycoprotein ligand-1 from HL-60 cells *J. Biol. Chem.* **271,** 18732-18742

134. Wilkins, P. P., Moore, K. L., McEver, R. P., and Cummings, R. D. (1995) Tyrosine sulfation of P-selectin glycoprotein ligand-1 is required for high affinity binding to P-selectin *J. Biol. Chem.* **270,** 22677-22680

135. Leppänen, A., White, S. P., Helin, J., McEver, R. P., and Cummings, R. D. (2000) Binding of glycosulfopeptides to P-selectin requires stereospecific contributions of individual tyrosine sulfate and sugar residues *J. Biol. Chem.* **275,** 39569-39578

136. Somers, W. S., Tang, J., Shaw, G. D., and Camphausen, R. T. (2000) Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe^x and PSGL-1 *Cell* **103,** 467-479

137. Patel, T. P., Goelz, S. E., Lobb, R. R., and Parekh, R. B. (1994) Isolation and characterization of natural protein-associated carbohydrate ligands for E-selectin *Biochemistry* **33,** 14815-14824

138. Steegmaier, M., Levinovitz, A., Isenmann, S., Borges, E., Lenter, M., Kocher, H. P., Kleuser, B., and Vestweber, D. (1995) The E-selectin-ligand ESL-1 is a variant of a receptor for fibroblast growth factor *Nature* **373,** 615-620

139. Handa, K., Stroud, M. R., and Hakomori, S.-I. (1997) Sialosyl-fucosyl poly-LacNac without the sialosyl-Le^x epitope as the physiological myeloid cell ligand in E-selectin-dependent adhesion: Studies under static and dynamic flow conditions *Biochemistry* **36,** 12412-12420

140. Kannagi, R., Fukushi, Y., Tachikawa, T., Noda, A., Shin, S., Shigeta, K., Hiraiwa, N., Fukuda, Y., Inamoto, T., Hakomori, S.-I., and Imura, H. (1986) Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing fucosyl or sialyl-fucosyl type 2 chain polylactosamine *Cancer Res.* **46,** 2619-2626

141. Hakomori, S. I., Nudelman, E., Levery, S. B., and Kannagi, R. (1984) Novel fucolipids accumulating in human adenocarcinoma 1. Glycolipids with di- or trifucosylated type 2 chain *J. Biol. Chem.* **259,** 4672-4680

142. Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, A., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E., and Hakomori, S.-I. (1984) Characterization of sialosylated $Lewis^x$ as a new tumor-associated antigen *Cancer Res.* **44,** 5279-5285

143. Fukushi, Y., Kannagi, R., Hakomori, S. I., Shepard, T., Kulander, B. G., and Singer, J. W. (1985) Location and distribution of difucoganglioside ($VI^3NeuAcV^3III^3Fuc_2nLc_6$) in normal and tumor tissues definied by its monoclonal antibody FH6 *Cancer Res.* **45,** 3711-3717

144. Abe, K., McKibbin, J. M., and Hakomori, S.-I. (1983) The monoclonal antibody directed to difucosylated type 2 chain (Fucα1-2Galβ1-4[Fucα1-3]GlcNAc; Y determinant) *J. Biol. Chem.* **258,** 11793-11797

145. Hakomori, S.-I., and Andrews, H. D. (1970) Sphingoglycolipids with Le^b activity, and the co-presence of Le^a, Le^b-glycolipids in human tumor tissue *Biochim. Biophys. Acta* **202,** 225-228

146. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, E., Steplewski, Z., Koprowski, H., and Ginsburg, V. (1982) A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II *J. Biol. Chem.* **257,** 14365-14369

147. Nilsson, O., Månsson, J.-E., Brezicka, T., Holmgren, J., Lindholm, L., Sörenson, S., Yngvason, F., and Svennerholm, L. (1984) Fucosyl-G_{M1} - a ganglioside associated with small cell lung carcinomas *Glycoconjugate J.* **1,** 43-49

148. Kannagi, R., Levery, S. B., Ishigami, F., Hakomori, S. I., Shevinsky, L. H., Knowles, B. B., and Solter, D. (1983) New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3 *J. Biol. Chem.* **258,** 8934-8942

149. Aigner, S., Ramos, C. L., Hafezi-Moghadam, A., Lawrence, M. B., Friederichs, J., Altevogt, P., and Ley, K. (1998) CD24 mediates rolling of breast carcinoma cells on P-selectin *FASEB J.* **12,** 1241-1251

150. Majuri, M.-L., Mattila, P., and Renkonen, R. (1992) Recombinant E-selectin-protein mediates tumor cell adhesion via sialyl-Lea and sialyl-Lex *Biochem. Biophys. Res. Commun.* **182,** 1376-1382

151. Matsushita, Y., Cleary, K. R., Ota, D. M., Hoff, S. D., and Irimura, T. (1990) Sialyl-dimeric Lewis-X antigen expressed on mucin-like glycoproteins in colorectal cancer metastases *Lab. Invest.* **63,** 780-791

152. Nakamori, S., Kameyama, M., Imaoka, S., Furukawa, H., Ishikawa, O., Sasaki, Y., Kabuto, T., Iwanaga, T., Matsushita, Y., and Irimura, T. (1993) Increased expression of sialyl $Lewis^x$ antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study *Cancer Res.* **53,** 3632-3637

153. Renkonen, J., Paavonen, T., and Renkonen, R. (1997) Endothelial and epithelial expression of sialyl $Lewis^x$ and sialyl $Lewis^a$ in lesions of breast carcinoma *Int. J. Cancer* **74,** 296-300

154. Wassarman, P. M. (1999) Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis and fusion *Cell* **96,** 175-183

155. Johnston, D. S., Wright, W. W., Shaper, J. H., Hokke, C. H., Van den Eijnden, D. H., and Joziasse, D. H. (1998) Murine sperm-zona binding, a fucosyl residue is required for a high affinity sperm-binding ligand. A second site on sperm binds a nonfucosylated, β-galactosyl-capped oligosaccharide *J. Biol. Chem.* **273,** 1888-1895

156. Oehninger, S., Patankar, M., Seppälä, M., and Clark, F. (1998) Involvement of selectin-like carbohydrate binding specificity in human gamete interaction *Andrologia* **30,** 269-274

157. Muramatsu, T. (1988) Developmentally regulated expression of cell surface carbohydrates during mouse embryogenesis *J. Cell. Biochem.* **36,** 1-14

158. Solter, D., and Knowles, B. B. (1978) Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1) *Proc. Natl. Acad. Sci. USA* **75,** 5565-5569

159. Eggens, I., Fenderson, B., Toyokuni, T., Dean, B., Stroud, M., and Hakomori, S.-I. (1989) Specific interaction between Le^x and Le^x determinants. A possible basis for cell recognition in preimplantation embryos and in embryonal carcinoma cells *J. Biol. Chem.* **264,** 9476-9484

160. Fenderson, B. A., Zehavi, U., and Hakomori, S.-I. (1984) A multivalent lacto-N-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective *J. Exp. Med.* **160,** 1591-1596

161. Allendoerfer, K. L., Durairaj, A., Matthews, G. A., and Patterson, P. H. (1999) Morphological domains of Lewis-X/FORSE-1 immunolabeling in the embryonic neural tube are due to developmental regulation of cell surface carbohydrate expression *Dev. Biol.* **211,** 208-219

162. Ashwell, K. W. S., and Mai, J. K. (1997) Developmental expression of the CD15 epitope in the hippocampus of the mouse *Cell Tissue Res.* **289,** 17-23

163. Mai, J. K., Andressen, C., and Ashwell, K. W. S. (1998) Demarcation of prosencephalic regions by CD15-positive radial glia *Eur. J. Neurosci.* **10,** 746-751

164. Sajdel-Sulkowska, E. M. (1998) Immunofluorescent detection of CD15-fucosylated glycoconjugates in primary cerebellar cultures and their function in glial-neuronal adhesion in the central nervous system *Acta Biochim. Pol.* **45,** 781-790

165. Götz, M., Wizenmann, A., Reinhardt, S., Lumsden, A., and Price, J. (1996) Selective adhesion of cells from different telencephalic regions *Neuron* **16,** 551-564

166. Cao, Z., Zhao, Z., Mohan, R., Alroy, J., Stanley, P., and Panjwani, N. (2001) Role of the Lewis^x glycan determinant in corneal epithelial cell adhesion and differentiation *J. Biol. Chem.* **276,** 21714-21723

167. Tuo, X.-H., Itai, S., Nishikata, J., Mori, T., Tanaka, O., and Kannagi, R. (1992) Stage-specific expression of cancer-associated type 1 and type 2 chain polylactosamine antigens in the developing pancreas of human embryos *Cancer Res.* **52,** 5744-5751

168. Candelier, J. J., Mollicone, R., Mennesson, B., Bergemer, A.-M., Henry, S., Coullin, P., and Oriol, R. (1993) α-3-Fucosyltransferases and their glycoconjugate antigen products in the developing human kidney *Lab. Invest.* **69,** 449-459

169. Miyake, M., Zenita, K., Tanaka, O., Okada, Y., and Kannagi, R. (1988) Stage-specific expression of SSEA-1-related antigens in the developing lung of human embryos and its relation to the distribution of these antigens in lung cancers *Cancer Res.* **48,** 7150-7158

170. Marker, P. C., Stephan, J. P., Lee, J., Bald, L., Mather, J. P., and Cunha, G. R. (2001) *fucosyltransferase1* and H-type complex carbohydrates modulate epithelial cell proliferation during prostate branching morphogenesis *Dev. Biol.* **233,** 95-108

171. Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999) Notch signalling: cell fate control and signal integration in development *Science* **284,** 770-776

172. Moloney, D. J., Panin, V. M., Johnston, S. H., J., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K. D., Haltiwanger, R. S., and Vogt, T. F. (2000) Fringe is a glycosyltransferase that modifies Notch *Nature* **406,** 369-375

173. Rabbani, S. A., Mazar, A. P., Bernier, S. M., Haq, M., Bolivar, I., Henkin, J., and Goltzman, D. (1992) Structural requirements for the growth factor activity of the amino terminal domain of urokinase *J. Biol. Chem.* **267,** 14151-14156

174. Schiffer, S. G., Foley, S., Kaffashan, A., Hronowski, X., Zichittella, A. E., Yeo, C. Y., Miatkowski, K., Adkins, H. B., Damon, B., Whitman, M., Salomon, D., Sanicola, M., and Williams, K. P. (2001) Fucosylation of Cripto is required for its ability to facilitate nodal signaling *J. Biol. Chem.* **276,** 37769-37778

175. Gagneux, P., and Varki, A. (1999) Evolutionary considerations in relating oligosaccharide diversity to biological function *Glycobiology* **9,** 747-755

176. Ilver, D., Arnqvist, A., Ögren, J., Frick, I.-M., Kersulyte, D., Incecik, E. T., Berg, D. E., Covacci, A., Engstrand, L., and Borén, T. (1998) *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging *Science* **279,** 373-377

177. Herron, M. J., Nelson, C. M., Larson, J., Snapp, K. R., Kansas, G. S., and Goodman, J. L. (2000) Intracellular parasitism by the human granulocytic erlichiosis bacterium through the P-selectin ligand, PSGL-1 *Science* **288,** 1653-1656

178. Newburg, D. S. (1997) Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? *J. Nutr.* **127,** 980S-948S

179. Nguyen, M., Strubel, N. A., and Bischoff, J. (1993) A role for sialyl Lewis-X/A glycoconjugates in capillary morphogenesis *Nature* **365,** 267-269

180. Koch, A. E., Halloran, M. M., Haskell, C. J., Shah, M. R., and Polverini, P. J. (1995) Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1 *Nature* **376,** 517-519

181. Ferrara, N. (1995) Leukocyte adhesion: missing link in angiogenesis *Nature* **376,** 467

182. Halloran, M. M., Carley, W. W., Polverini, P. J., Haskell, C. J., Phan, S., Anderson, B. J., Woods, J. M., Campbell, P. L., Volin, M. V., Bäcker, A. E., and Koch, A. E. (2000) Le^y/H: An endothelial-selective, cytokine-inducible, angiogenic mediator *J. Immunol.* **164,** 4868-4877

183. Crocker, P. R., Clark, E. A., Filbin, M., Gordon, S., Jones, Y., Kehrl, J. H., Kelm, S., Le Douarin, N., Powell, L., Roder, J., Schnaar, R. L., Sgroi, D. C., Stamenkovic, I., Schauer, R., Schachner, M., van den

Berg, T. K., van den Merwe, P. A., Watt, S. M., and Varki, A. (1998) Siglecs: a family of sialic-acid binding lectins *Glycobiology* **8,** v-vi

184. Crocker, P. R., and Varki, A. (2001) Siglecs, sialic acids and innate immunity *Trends Immunol.* **22,** 337-342

185. Crocker, P. R., Mucklow, S., Bouckson, V., McWilliam, A., Willis, A. C., Gordon, S., Milon, G., Kelm, S., and Bradfield, P. (1994) Sialoadhesin, a macrophage sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains *EMBO J.* **13,** 4490-4503

186. Erickson, L. D., Tygrett, L. T., Bhatia, S. K., Grabstein, K. H., and Waldschmidt, T. J. (1996) Differential expression of CD22 (Lyb8) on murine B cells *Int. Immunol.* **8,** 1121-1129

187. Sgroi, D., Varki, A., Braesch-Andersen, S., and Stamenkovic, I. (1993) CD22, a B cell specific immunoglobulin superfamily member, is a sialic acid-binding lectin *J. Biol. Chem.* **268,** 7011-7018

188. Simmons, D., and Seed, B. (1988) Isolation of a cDNA encoding CD33, a differentiation antigen of myeloid progenitor cells *J. Immunol.* **141,** 2797-2800

189. Brinkman-Van der Linden, E. C. M., and Varki, A. (2000) New aspects of Siglec binding specificities, including the significance of fucosylation and of the sialyl-Tn epitope *J. Biol. Chem.* **275,** 8625-8632

190. Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S., and Varki, A. (1995) Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22. Evidence for a minimum structural recognition motif and the potential importance of multisite binding *J. Biol. Chem.* **270,** 7523-7532

191. Schadee-Eestermans, I. L., Hoefsmit, E. C. M., van de Ende, M., Crocker, P. R., and van den Berg, T. K. (2000) Ultrastructural localisation of sialoadhesin (Siglec-1) on macrophages in rodent lymphoid tissues *Immunobiology* **202,** 309-325

192. van den Berg, T. K., Brevé, J. J. P., Damoiseaux, J. G. M. C., Döpp, E. A., Kelm, S., Crocker, P. R., Dijkstra, C. D., and Kraal, G. (1992) Sialoadhesin on macrophages: its identification as a lymphocyte adhesion molecule *J. Exp. Med.* **176,** 647-655

193. Tuscano, J., Engel, P., Tedder, T. F., and Kehrl, J. H. (1996) Engagement of the adhesion receptor CD22 triggers a potent stimulatory signal for B cells and blocking CD22/CD22L interactions impairs T-cell proliferation *Blood* **87,** 4723-4730

194. Nitschke, L., Floyd, H., Ferguson, D. J. P., and Crocker, P. R. (1999) Identification of CD22 ligands on bone marrow sinusoidal endothelium implicated in CD22-dependent homing of recirculating B cells *J. Exp. Med.* **189,** 1513-1518

195. O'Keefe, T. L., Williams, G. T., Davies, S. L., and Neuberger, M. S. (1996) Hyperresponsive B cells in CD22-deficient mice *Science* **274,** 798-801

196. Otipoby, K. L., Andersson, K. B., Draves, K. E., Klaus, S. J., Farr, A. G., Kerner, J. D., Perlmutter, R. M., Law, C.-L., and Clark, E. A. (1996) CD22 regulates thymus-independent responses and the lifespan of B cells *Nature* **384,** 634-637

197. Hennet, T., Chui, D., Paulson, J. C., and Marth, J. D. (1998) Immune regulation by the ST6Gal sialyltransferase *Proc. Natl. Acad. Sci. USA* **95,** 4504-4509

198. Vitale, C., Romagnani, C., Falco, M., Ponte, M., Vitale, M., Moretta, A., Bacigalupo, A., Moretta, L., and Mingari, M. C. (1999) Engagement of p75/AIRM1 or CD33 inhibits the proliferation of normal or leukemic myeloid cells *Proc. Natl. Acad. Sci. USA* **96,** 15091-15096

199. Ferlazzo, G., Spaggiari, G. M., Semino, C., Melioli, G., and Moretta, L. (2000) Engagement of CD33 surface molecules prevents the generation of dendritic cells from both monocytes and $CD34^+$ myeloid precursors *Eur. J. Immunol.* **30,** 827-833

200. Sternberger, N. H., Quarles, R. H., Itoyama, Y., and Webster, H. (1979) Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat *Proc. Natl. Acad. Sci. USA* **76,** 1510-1514

201. Trapp, B. D., Andrews, S. B., Cootauco, C., and Quarles, R. (1989) The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes *J. Cell Biol.* **109,** 2417-2426

202. Yang, L. J.-S., Zeller, C. B., Shaper, N. L., Kiso, M., Hasegawa, A., Shapiro, R. E., and Schnaar, R. L. (1996) Gangliosides are neuronal ligands for myelin-associated glycoprotein *Proc. Natl. Acad. Sci. USA* **93,** 814-818

203. Collins, B. E., Ito, H., Sawada, N., Ishida, H., Kiso, M., and Schnaar, R. L. (1999) Enhanced binding of the neural Siglecs, myelin-associated glycoprotein and Schwann cell myelin protein, to Chol-1 (α-series) gangliosides and novel sulfated Chol-1 analogs *J. Biol. Chem.* **274,** 37637-37643

204. DeBellard, M. E., Tang, S., Mukhopadhyay, G., Shen, Y. J., and Filbin, M. T. (1996) Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein *Mol. Cell. Neurosci.* **7,** 89-101

205. Strenge, K., Schauer, R., and Kelm, S. (1999) Binding partners for the myelin-associated glycoprotein of N_2A neuroblastoma cells *FEBS Lett.* **444,** 59-64

206. Li, C., Tropak, M. B., Gerlai, R., Clapoff, S., Abramow-Newerly, W., Trapp, B., Peterson, A., and Roder, J. (1994) Myelination in the absence of myelin-associated glycoprotein *Nature* **369,** 747-750

207. Montag, D., Giese, K. P., Bartsch, U., Martini, R., Lang, Y., Blüthmann, H., Karthigasan, J., Kirschner, D. A., Wintergerst, E. S., Nave, K.-A., Zielasek, J., Toyka, K. V., Lipp, H.-P., and Schachner, M. (1994) Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin *Neuron* **13,** 229-246

208. Fruttiger, M., Montag, D., Schachner, M., and Martini, R. (1995) Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity *Eur. J. Neurosci.* **7,** 511-515

209. Mukhopadhyay, G., Doherty, P., Walsh, F. S., Crocker, P. R., and Filbin, M. T. (1994) A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration *Neuron* **13,** 757-767

210. Vinson, M., Strijbos, P. J. L. M., Rowles, A., Facci, L., Moore, S. E., Simmons, D. L., and Walsh, F. S. (2001) Myelin-associated glycoprotein interacts with ganglioside GT1b. A mechanism for neurite outgrowth inhibition *J. Biol. Chem.* **276,** 20280-20285

211. Cornish, A. L., Freeman, S., Forbes, G., Ni, J., Zhang, M., Cepeda, M., Gentz, R., Augustus, M., Carter, K. C., and Crocker, P. R. (1998) Characterization of Siglec-5, a novel glycoprotein expressed on myeloid cells related to CD33 *Blood* **92,** 2123-2132

212. Patel, N., Brinkman-Van der Linden, E. C. M., Altmann, S. W., Gish, K., Balasubramanian, S., Timans, J. C., Peterson, D., Bell, M. P., Bazan, J. F., Varki, A., and Kastelein, R. A. (1999) OB-BP1/Siglec-6. A leptin- and sialic acid-binding protein of the immunoglobulin superfamily *J. Biol. Chem.* **274,** 22729-22738

213. Angata, T., and Varki, A. (2000) Siglec-7: a sialic acid-binding lectin of the immunoglobulin superfamily *Glycobiology* **10,** 431-438

214. Falco, M., Biassoni, R., Bottino, C., Vitale, M., Sivori, S., Augugliaro, R., Moretta, L., and Moretta, A. (1999) Identification and molecular cloning of p75/AIRM1, a novel member of the sialoadhesin family that functions as an inhibitory receptor in human natural killer cells *J. Exp. Med.* **190,** 793-801

215. Ito, A., Handa, K., Withers, D. A., Satoh, M., and Hakomori, S.-I. (2001) Binding specificity of siglec7 to disialogangliosides of renal cell carcinoma: possible role of disialogangliosides in tumor progression *FEBS Lett.* **498,** 116-120

216. Yamaji, T., Teranishi, T., Alphey, M. S., Crocker, P. R., and Hashimoto, Y. (2002) A small region of the natural killer cell receptor, Siglec-7, is responsible for its preferred binding to α2,8-disialyl and branched α2,6-sialyl residues. A comparison with Siglec-9 *J. Biol. Chem.* **277,** 6324-6332

217. Angata, T., and Varki, A. (2000) Cloning, characterization, and phylogenetic analysis of Siglec-9, a new member of the CD33-related group of Siglecs. Evidence for co-evolution with sialic acid synthesis pathways *J. Biol. Chem.* **275,** 22127-22135

218. Floyd, H., Ni, J., Cornish, A. L., Zeng, Z., Liu, D., Carter, K. C., Steel, J., and Crocker, P. R. (2000) Siglec 8. A novel eosinophil-specific member of the immunoglobulin superfamily *J. Biol. Chem.* **275,** 861-866

219. Munday, J., Kerr, S., Ni, J., Cornish, A. L., Zhang, J. Q., Nicoll, G., Floyd, H., Mattei, M. G., Moore, P., Liu, D., and Crocker, P. R. (2001) Identification, characterization and leucocyte expression of Siglec-10, a novel human sialic acid-binding receptor *Biochem. J.* **355,** 489-497

220. Angata, T., Varki, N. M., and Varki, A. (2001) A second uniquely human mutation affecting sialic acid biology *J. Biol. Chem.* **276,** 40282-40287

221. Yu, Z., Lai, C. M., Maoui, M., Banville, D., and Shen, S. H. (2001) Identification and characterization of S2V, a novel putative Siglec that contains two V set Ig-like domains and recruits protein-tyrosine phosphatases SHPs *J. Biol. Chem.* **276,** 23816-23824

222. Hakomori, S.-I. (1990) Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions *J. Biol. Chem.* **265,** 18713-18716

223. Lloyd, K. O., and Furukawa, K. (1998) Biosynthesis and functions of gangliosides: recent advances *Glycoconjugate J.* **15,** 627-636

224. Hakomori, S.-I. (2002) The glycosynapse *Proc. Natl. Acad. Sci. USA* **99,** 225-232

225. Li, R., Manela, J., Kong, Y., and Ladisch, S. (2000) Cellular gangliosides promote growth factor-induced proliferation of fibroblasts *J. Biol. Chem.* **275,** 34213-34223

226. Saqr, H. E., Lee, M. C., Burkman, A. M., and Yates, A. J. (1995) Gangliosides have a bimodal effect on DNA synthesis in U-1242 MG human glioma cells *J. Neurosci. Res.* **41,** 491-500

227. Cannella, M. S., Oderfeld-Nowak, B., Gradkowska, M., Skup, M., Garofalo, L., Cuello, A. C., and Ledeen, R. W. (1990) Derivatives of ganglioside GM1 as neuronotrophic agents: comparison of in vivo and in vitro effects *Brain Research* **513,** 286-294

228. Facci, L., Leon, A., Toffano, G., Sonnino, S., Ghidoni, R., and Tettamanti, G. (1984) Promotion of neuritogenesis in mouse neuroblastoma cells by exogenous gangliosides. Relationship between the effect and the cell association of ganglioside GM_1 *J. Neurochem.* **42,** 299-305

229. Sabel, B. A., Slavin, M. D., and Stein, D. G. (1984) Ganglioside treatment facilitates behavioral recovery from bilateral brain damage *Science* **225,** 340-341

230. Mutoh, T., Tokuda, A., Miyadai, T., Hamaguchi, M., and Fujiki, N. (1995) Ganglioside GM1 binds to the Trk protein and regulates receptor function *Proc. Natl. Acad. Sci. USA* **92,** 5087-5091

231. Mutoh, T., Tokuda, A., Inokuchi, J., and Kuriyama, M. (1998) Glucosylceramide synthase inhibitor inhibits the action of nerve growth factor in PC12 cells *J. Biol. Chem.* **273,** 26001-26007

232. Bremer, E. G., Schlessinger, J., and Hakomori, S.-I. (1986) Ganglioside-mediated modulation of cell growth. Specific effects of G_{M3} on tyrosine phosphorylation of the epidermal growth factor receptor *J. Biol. Chem.* **261,** 2434-2440

233. Weis, F. M. B., and Davis, R. J. (1990) Regulation of epidermal growth factor receptor signal transduction. Role of gangliosides *J. Biol. Chem.* **265,** 12059-12066

234. Meuillet, E. J., Kroes, R., Yamamoto, H., Warner, T. G., Ferrari, J., Mania-Farnell, B., George, D., Rebbaa, A., Moskal, J. R., and Bremer, E. G. (1999) Sialidase gene transfection enhances epidermal growth factor receptor activity in an epidermal carcinoma cell line, A431 *Cancer Res.* **59,** 234-240

235. Hynds, D. L., Summers, M., Van Brocklyn, J., O'Dorisio, M. S., and Yates, A. J. (1995) Gangliosides inhibit platelet-derived growth factor-stimulated growth, receptor phosphorylation, and dimerization in neuroblastoma SH-SY5Y cells *J. Neurochem.* **65,** 2251-2258

236. Zhao, J., Furukawa, K., Fukumoto, S., Okada, M., Furugen, R., Miyazaki, H., Takamiya, K., Aizawa, S., Shiku, H., Matsuyama, T., and Furukawa, K. (1999) Attenuation of interleukin 2 signal in the spleen cells of complex ganglioside-lacking mice *J. Biol. Chem.* **274,** 13744-13747

237. Rusnati, M., Tanghetti, E., Urbinati, C., Tulipano, G., Marchesini, S., Ziche, E., and Presta, M. (1999) Interaction of fibroblast growth factor-2 (FGF-2) with free gangliosides: biochemical characterization and biological consequences in endothelial cell cultures *Mol. Biol. Cell* **10,** 313-327

238. Nojiri, H., Stroud, M., and Hakomori, S.-I. (1991) A specific type of ganglioside as a modulator of insulin-dependent cell growth and insulin receptor tyrosine kinase activity. Possible association of ganglioside-induced inhibition of insulin receptor function and monocytic differentiation induction in HL-60 cells *J. Biol. Chem.* **266,** 4531-4537

239. Lang, Z., Guerrera, M., Li, R., and Ladisch, S. (2001) Ganglioside GD1a enhances VEGF-induced endothelial cell proliferation and migration *Biochem. Biophys. Res. Commun.* **282,** 1031-1037

240. Wu, W., Harley, P. H., Punt, J. A., Sharrow, S. O., and Kearse, K. P. (1996) Identification of CD8 as a peanut agglutinin (PNA) receptor molecule on immature thymocytes *J. Exp. Med.* **184,** 759-764

241. Gillespie, W., Paulson, J. C., Kelm, S., Pang, M., and Baum, L. G. (1993) Regulation of α2,3-sialyltransferase expression correlates with conversion of peanut agglutinin (PNA)$^+$ to PNA$^-$ phenotype in developing thymocytes *J. Biol. Chem.* **268,** 3801-3804

242. Daniels, M. A., Devine, L., Miller, J. D., Moser, J. M., Lukacher, A. E., Altman, J. D., Kavathas, P., Hogquist, K. A., and Jameson, S. C. (2001) CD8 binding to MCH class I molecules is influenced by T cell maturation and glycosylation *Immunity* **15,** 1051-1061

243. Moody, A. M., Chui, D., Reche, P. A., Priatel, J. J., Marth, J. D., and Reinherz, E. L. (2001) Developmentally regulated glycosylation of the CD8αβ coreceptor stalk modulates ligand binding *Cell* **107,** 501-512

244. Galvan, M., Tsuboi, S., Fukuda, M., and Baum, L. G. (2000) Expression of a specific glycosyltransferase enzyme regulates T cell death mediated by galectin-1 *J. Biol. Chem.* **275,** 16730-16737

245. Perillo, N. L., Uittenbogaart, C. H., Nguyen, J. T., and Baum, L. G. (1997) Galectin-1, an endogeneous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes *J. Exp. Med.* **185,** 1851-1858

246. Chiba, A., Matsumura, K., Yamada, H., Inazu, T., Shimizu, T., Kusunoki, S., Kanazawa, I., Kobata, A., and Endo, T. (1997) Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve α-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of α-dystroglycan with laminin *J. Biol. Chem.* **272,** 2156-2162

247. Smalheiser, N. R., Haslam, S. M., Sutton-Smith, M., Morris, H. R., and Dell, A. (1998) Structural analysis of sequences O-linked to mannose reveals novel Lewis X structure in cranin (dystroglycan) purified from sheep brain *J. Biol. Chem.* **273,** 23698-23703

248. Yoshida, A., Kobayashi, K., Manya, H., Taniguchi, K., Kano, H., Mizuno, M., Inazu, T., Mitsuhashi, H., Takahashi, S., Takeuchi, M., Herrmann, R., Straub, V., Talim, B., Voit, T., Topaloglu, H., Toda, T., and Endo, T. (2001) Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1 *Dev. Cell* **1,** 717-724

249. Eidels, L., Proia, R. L., and Hart, D. A. (1983) Membrane receptors for bacterial toxins *Microbiol. Rev.* **47,** 596-620

250. Karlsson, K. A. (1995) Microbial recognition of target-cell glycoconjugates *Curr. Opin. Struct. Biol.* **5,** 622-635

251. Varki, A. (1997) Sialic acids as ligands in recognition phenomena *FASEB J.* **11,** 248-255

252. Evans, D. G., Evans, D. J., Moulds, J. J., and Graham, D. Y. (1988) N-acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen *Infect. Immun.* **56,** 2896-2906

253. Johansson, L., and Karlsson, K. A. (1998) Selective binding by *Helicobacter pylori* of leucocyte gangliosides with 3-linked sialic acid, as identified by a new approach of linkage analysis *Glycoconjugate J.* **15,** 713-721

254. Johansson, L., Johansson, P., and Miller-Podraza, H. (1999) Neu5Acα3Gal is part of the *Helicobacter pylori* binding epitope in polyglycosylceramides of human erythrocytes *Eur. J. Biochem.* **266,** 559-565

255. Miller-Podraza, H., Bergström, J., Milh, M. A., and Karlsson, K. A. (1997) Recognition of glycoconjugates by *Helicobacter pylori.* Comparison of two sialic acid-dependent specificities based on hemagglutination and binding to human erythrocyte glycoconjugates *Glycoconjugate J.* **14,** 467-471

256. Roche, N., Larsson, T., Ångström, J., and Teneberg, S. (2001) *Helicobacter pylori*-binding gangliosides of human gastric adenocarcinoma *Glycobiology* **11,** 935-944

257. Parkkinen, J., Rogers, G. N., Korhonen, T., Dahr, W., and Finne, J. (1986) Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia coli* *Infect. Immun.* **54,** 37-42

258. Liukkonen, J., Haataja, S., Tikkanen, K., Kelm, S., and Finne, J. (1992) Identification of N-acetylneuraminyl α2-3 poly-N-acetyllactosamine glycans as the receptors of sialic acid-binding *Streptococcus suis* strains *J. Biol. Chem.* **267,** 21105-21111

259. Fakih, M. G., Murphy, T. F., Pattoli, M. A., and Berenson, C. S. (1997) Specific binding of *Haemophilus influenzae* to minor gangliosides of human respiratory epithelial cells *Infect. Immun.* **65,** 1695-1700

260. Loomes, L. M., Uemura, K., Childs, R. A., Paulson, J. C., Rogers, G. N., Scudder, P. R., Michalski, J. C., Hounsell, E. F., Taylor-Robinson, D., and Feizi, T. (1984) Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type *Nature* **307,** 560-563

261. Ångström, J., Teneberg, S., and Karlsson, K. A. (1994) Delineation and comparison of ganglioside-binding epitopes for the toxins of *Vibrio cholerae, Escherichia coli*, and *Clostridium tetani*: Evidence for overlapping epitopes *Proc. Natl. Acad. Sci. USA* **91,** 11859-11863

262. Nishiki, T., Tokuyama, Y., Kamata, Y., Nemoto, Y., Yoshida, A., Sato, K., Sekiguchi, M., Takahashi, M., and Kozaki, S. (1996) The high-affinity binding of *Clostridium botulinum* type B neurotoxin to synaptotagmin II associated with gangliosides G_{T1b}/G_{D1a} *FEBS Lett.* **378,** 253-257

263. Brennan, M. J., David, J. L., Kenimer, J. G., and Manclark, C. R. (1988) Lectin-like binding of pertussis toxin to a 165-kilodalton Chinese hamster ovary cell glycoprotein *J. Biol. Chem.* **263,** 4895-4899

264. Stein, P. E., Boodhoo, A., Armstrong, G. D., Heerze, L. D., Cockle, S. A., Klein, M. H., and Read, R. J. (1994) Structure of pertussis toxin-sugar complex as a model for receptor binding *Nat. Struct. Biol.* **1,** 591-596

265. Connor, R. J., Kawaoka, Y., Webster, R. G., and Paulson, J. C. (1994) Receptor specificity in human, avian and equine H2 and H3 influenza virus isolates *Virology* **205,** 17-23

266. Rogers, G. N., and Paulson, J. C. (1983) Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin *Virology* **127,** 361-373

267. Ha, Y., Stevens, D. J., Skehel, J. J., and Wiley, D. C. (2001) X-ray structures of H5 avian and H9 swine influenza virus hemagglutinins bound to avian and human receptor analogs *Proc. Natl. Acad. Sci. USA* **98,** 11181-11186

268. Miller-Podraza, H., Johansson, L., Johansson, P., Larsson, T., Matrosovich, M., and Karlsson, K. A. (2000) A strain of human influenza A virus binds to extended but not short gangliosides as assayed by thin-layer chromatography overlay *Glycobiology* **10,** 975-982

269. Markwell, M. A. K., Svennerholm, L., and Paulson, J. C. (1981) Specific gangliosides function as host cell receptors for Sendai virus *Proc. Natl. Acad. Sci. USA* **78,** 5406-5410

270. Fried, H., Cahan, L. D., and Paulson, J. C. (1981) Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells *Virology* **109,** 188-192

271. Liu, C. K., Wei, G., and Atwood, W. J. (1998) Infection of glial cells by human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal α(2-6)-linked sialic acids *J. Virol.* **72,** 4643-4749

272. Finne, J. (1985) Polysialic acid - a glycoprotein involved in neural adhesion and bacterial meningitis *Trends Biochem. Sci.* **10,** 129-133

273. Smith, H., Parsons, N. J., and Cole, J. A. (1995) Sialylation of neisserial lipopolysaccharide: a major influence on pathogenicity *Microb. Pathog.* **19,** 365-377

274. Pearlstein, E., Salk, P. L., Yogeeswaran, G., and Karpatkin, S. (1980) Correlation between spontaneous metastatic potential, platelet-aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derivatives of a rat renal sarcoma cell line *Proc. Natl. Acad. Sci. USA* **77,** 4336-4339

275. Yogeeswaran, G., and Salk, P. L. (1981) Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines *Science* **212,** 1514-1516

276. Nudelman, E., Hakomori, S.-I., Kannagi, R., Levery, S. B., Yeh, M. Y., Hellström, K. E., and Hellström, I. (1982) Characterization of a human melanoma-associated ganglioside antigen defined by a monoclonal antibody, 4.2 *J. Biol. Chem.* **257,** 12752-12756

277. Berra, B., Gaini, S. M., and Riboni, L. (1985) Correlation between ganglioside distribution and histological grading of human astrocytomas *Int. J. Cancer* **36,** 363-366

278. Ravindranath, M. H., Tsuchida, T., Morton, D. L., and Irie, R. F. (1991) Ganglioside GM3:GD3 ratio as an index for the management of melanoma *Cancer* **67,** 3029-3035

279. Zeng, G., Gao, L., Birklé, S., and Yu, R. K. (2000) Suppression of ganglioside GD3 expression in a rat F-11 tumor cell line reduces tumor growth, angiogenesis, and vascular endothelial growth factor production *Cancer Res.* **60,** 6670-6676

280. Zeng, G., Gao, L., and Yu, R. K. (2000) Reduced cell migration, tumor growth and experimental metastasis of rat F-11 cells whose expression of CD3-synthase is suppressed *Int. J. Cancer* **88,** 53-57

281. Watanabe, T., Pukel, C. S., Takeyama, H., Lloyd, K. O., Shiku, H., Li, L. T. C., Travassos, L. R., Oettgen, H. F., and Old, L. J. (1982) Human melanoma antigen AH is an autoantigenic ganglioside related to G_{D2} *J. Exp. Med.* **156,** 1884-1889

282. Hamilton, W. B., Helling, F., Lloyd, K. O., and Livingston, P. O. (1993) Ganglioside expression on human malignant melanoma assessed by quantitative immune thin-layer chromatography *Int. J. Cancer* **53,** 566-573

283. Tai, T., Paulson, J. C., Cahan, L. D., and Irie, R. F. (1983) Ganglioside GM3 as a human tumor antigen (OFA-I-1) *Proc. Natl. Acad. Sci. USA* **80,** 5392-5396

284. Fukuda, M. N., Bothner, B., Lloyd, K. O., Rettig, W. J., Tiller, P. R., and Dell, A. (1986) Structures of glycosphingolipids isolated from human embryonal carcinoma cells. The presence of mono- and disialosyl glycolipids with blood group type 1 sequence *J. Biol. Chem.* **261,** 5145-5153

285. Fukushi, Y., Nudelman, E., Levery, S. B., Higuchi, T., and Hakomori, S.-I. (1986) A novel disialoganglioside ($IV^3NeuAcIII^6NeuAcLc_4$) of human adenocarcinoma and the monoclonal antibody (FH9) defining this disialosyl structure *Biochemistry* **25,** 2859-2866

286. Ito, A., Levery, S. B., Saito, S., Satoh, M., and Hakomori, S.-I. (2001) A novel ganglioside isolated from renal cell carcinoma *J. Biol. Chem.* **276,** 16695-16703

287. Ladisch, S., Gillard, B., Wong, C., and Ulsh, L. (1983) Shedding and immunoregulatory activity of YAC-1 lymphoma cell gangliosides *Cancer Res.* **43,** 3808-3813

288. Portoukalian, J., David, M. J., Gain, P., and Richard, M. (1993) Shedding of GD2 ganglioside in patients with retinoblastoma *Int. J. Cancer* **53,** 948-951

289. Hachida, M., Uwabe, K., Nonoyama, M., Imamaki, M., Nemoto, S., Hoshi, H., Katsumata, T., Endo, M., and Koyanagi, H. (1993) The significant immunosuppressive effect of ganglioside GM_3 in heart transplantation *Transplantation* **56,** 479-482

290. Ladisch, S., Hasegawa, A., Li, R., and Kiso, M. (1995) Immunosuppressive activity of chemically synthesized gangliosides *Biochemistry* **34,** 1197-1202

291. Li, R., Villacreses, N., and Ladisch, S. (1995) Human tumor gangliosides inhibit murine immune responses *in vivo Cancer Res.* **55,** 211-214

292. Lloyd, K. O., Burchell, J., Kudryashov, V., Yin, B. W. T., and Taylor-Papadimitriou, J. (1996) Comparison of O-linked carbohydrate chains in MUC-1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. Demonstration of simpler and fewer glycan chains in tumor cells *J. Biol. Chem.* **271,** 33325-33334

293. Itzkowitz, S. H., Yuan, M., Montgomery, C. K., Kjeldsen, T., Takahashi, H. K., Bigbee, W. L., and Kim, Y. S. (1989) Expression of Tn, sialosyl-Tn, and T antigens in human colon cancer *Cancer Res.* **49,** 197-204

294. Terasawa, K., Furumoto, H., Kamada, M., and Aono, T. (1996) Expression of Tn and sialyl-Tn antigens in the neoplastic transformation of uterine cervical epithelial cells *Cancer Res.* **56,** 2229-2232

295. Brockhausen, I. (1999) Pathways of O-glycan biosynthesis in cancer cells *Biochim. Biophys. Acta* **1473,** 67-95

296. Takamatsu, K., Auerbach, B., Gerardy-Schahn, R., Eckhardt, M., Jaques, G., and Madry, N. (1994) Characterization of tumor-associated neural cell adhesion molecule in human serum *Cancer Res.* **54,** 2598-2603

297. Tanaka, F., Otake, Y., Nakagawa, T., Kawano, Y., Miyahara, R., Li, M., Yanagihara, K., Nakayama, J., Fujimoto, I., Ikenaka, K., and Wada, H. (2000) Expression of polysialic acid and *STX*, a human polysialyltransferase, is correlated with tumor progression in non-small cell lung cancer *Cancer Res.* **60,** 3072-3080

298. Malykh, Y. N., Schauer, R., and Shaw, L. (2001) N-Glycolylneuraminic acid in human tumours *Biochimie* **83,** 623-634

299. Rutishauser, U. (1996) Polysialic acid and the regulation of cell interactions *Curr. Opin. Cell Biol.* **8,** 679-684

300. Zuber, C., Lackie, P. M., Catterall, W. A., and Roth, J. (1992) Polysialic acid is associated with sodium channels and the neural cell adhesion molecule N-CAM in adult rat brain *J. Biol. Chem.* **267,** 9965-9971

301. Close, B. E., and Colley, K. J. (1998) *In vivo* autopolysialylation and localization of the polysialyltransferases PST and STX *J. Biol. Chem.* **273,** 34586-34593

302. Nadanaka, S., Sato, C., Kitajima, K., Katagiri, K., Irie, S., and Yamagata, T. (2001) Occurrence of oligosialic acids on integrin α_5 subunit and their involvement in cell adhesion to fibronectin. *J. Biol. Chem.* **276,** 33657-33664

303. Varki, A. (1992) Diversity in the sialic acids *Glycobiology* **2,** 25-40

304. Irie, A., Koyama, S., Kozutsumi, Y., Kawasaki, T., and Suzuki, A. (1998) The molecular basis for the absence of N-glycolylneuraminic acid in humans *J. Biol. Chem.* **273,** 15866-15871

305. Cheresh, D. A., Varki, A. P., Varki, N. M., Stallcup, W. B., Levine, J., and Reisfeld, R. A. (1984) A monoclonal antibody recognizes an O-acetylated sialic acid in a human melanoma-associated ganglioside *J. Biol. Chem.* **259,** 7453-7459

306. Toba, S., Tenno, M., and Kurosaka, A. (2000) An O-acetylated sialyl-Tn is involved in ovarian cancer-associated antigenicity *Biochem. Biophys. Res. Commun.* **271,** 281-286

307. Herrler, G., and Klenk, H. D. (1987) The surface receptor is a major determinant of the cell tropism of influenza C virus *Virology* **159,** 102-108

308. Higa, H. H., Rogers, G. N., and Paulson, J. C. (1985) Influenza virus hemagglutins differentiate between receptor determinants bearing N-acetyl-, N-glycolyl- and N,O-diacetylneuraminic acids *Virology* **144,** 279-282

309. Shi, W. X., Chammas, R., Varki, N. M., Powell, L., and Varki, A. (1996) Sialic acid 9-O-acetylation on murine erythroleukemia cells affects complement activation, binding to I-type lectins, and tissue homing *J. Biol. Chem.* **271,** 31526-31532

310. Sjoberg, E. R., Powell, L. D., Klein, A., and Varki, A. (1994) Natural ligands of the B cell adhesion molecule CD22β can be masked by 9-O-acetylation of sialic acids *J. Cell Biol.* **126,** 549-562

311. Brew, K., Vanaman, T. C., and Hill, R. L. (1968) The role of α-lactalbumin and the A protein in lactose synthetase: A unique mechanism for the control of a biological reaction *Proc. Natl. Acad. Sci. USA* **59,** 491-497

312. Palcic, M. M., and Hindsgaul, O. (1991) Flexibility in the donor substrate specificity of β1,4-galactosyltransferase: application in the synthesis of complex carbohydrates *Glycobiology* **1,** 205-209

313. Leppänen, A., Salminen, H., Zhu, Y., Maaheimo, H., Helin, J., Costello, C. E., and Renkonen, O. (1997) *In vitro* biosynthesis of a decasaccharide prototype of multiply branched polylactosaminoglycan backbones *Biochemistry* **36,** 7026-7036

314. Maaheimo, H., Renkonen, R., Turunen, J. P., Penttilä, L., and Renkonen, O. (1995) Synthesis of a divalent sialyl Lewis x O-glycan, a potent inhibitor of lymphocyte-endothelium adhesion. Evidence that multivalency enhances the saccharide binding to L-selectin *Eur. J. Biochem.* **234,** 616-625

315. Renkonen, O., Helin, J., Vainio, A., Niemelä, R., Penttilä, L., and Hilden, P. (1990) *Escherichia coli* β-galactosidase unexpectedly cleaves the hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc without branch specificity *Biochem. Cell Biol.* **68,** 1032-1036

316. Niemelä, R., Räbinä, J., Leppänen, A., Maaheimo, H., Costello, C. E., and Renkonen, O. (1995) Site-directed enzymatic α–(1,3)-L-fucosylation of the tetrasaccharide Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAc at the distal N-acetyllactosamine unit *Carbohydr. Res.* **279,** 331-338

317. Niemelä, R., Natunen, J., Penttilä, L., Salminen, H., Helin, J., Maaheimo, H., Costello, C. E., and Renkonen, O. (1999) Isolation and characterization of linear polylactosamines containing one and two site-specifically positioned Lewis x determinants: WGA agarose chromatography in fractionation of mixtures generated by random, partial enzymatic α3-fucosylation of pure polylactosamines *Glycobiology* **9,** 517-526

318. Räbinä, J., Natunen, J., Niemelä, R., Salminen, H., Ilves, K., Aitio, O., Maaheimo, H., Helin, J., and Renkonen, O. (1998) Enzymatic synthesis of site-specifically (α1-3)-fucosylated polylactosamines containing either a sialyl Lewis x, a VIM-2, or a sialylated and internally difucosylated sequence *Carbohydr. Res.* **305,** 491-499

319. Alais, J., and Veyrières, A. (1990) Syntheses of linear tetra-, hexa-, and octa-saccharide fragments of the i-blood group active poly-(N-acetyl-lactosamine) series. Blockwise methods for the synthesis of repetitive oligosaccharide sequences *Carbohydr. Res.* **207,** 11-31

320. Takeya, A., Hosomi, O., Shimoda, N., and Yazawa, S. (1992) Biosynthesis of the blood group P antigen-like GalNAcβ1-3Galβ1-4GlcNAc/Glc structure. A novel N-acetylgalactosaminyltransferase in human blood plasma *J. Biochem.* **112,** 389-395

321. Yates, A. D., and Watkins, W. M. (1983) Enzymes involved in the biosynthesis of glycoconjugates. A UDP-2-acetamido-2-deoxy-D-glucose: β-D-galactopyranosyl-(1-4)-saccharide (1-3)-2-acetamido-2-deoxy-β-D-glucopyranosyltransferase in human serum *Carbohydr. Res.* **120,** 251-268

322. Niemelä, R., Natunen, J., Brotherus, E., Saarikangas, A., and Renkonen, O. (1995) α1,3-Fucosylation of branched blood group I-type oligo-(N-acetyllactosamino)glycans by human milk transferases is restricted to distal N-acetyllactosamine units: The resulting isomers are separated by WGA-agarose chromatography *Glycoconjugate J.* **12,** 36-44

323. Natunen, J., Seppo, A., Helin, J., Reinhold, B. B., Räbinä, J., Costello, C. E., and Renkonen, O. (1997) Enzymatic transfer of a β1,6-linked N-acetylglucosamine to the α-galactose of globo-N-tetraose: *in vitro* synthesis of a novel hybrid pentasaccharide of lacto-globo type *Glycobiology* **7,** 711-718

324. Renkonen, O., Penttilä, L., Niemelä, R., and Leppänen, A. (1991) Single mid-chain GlcNAcβ1-6Galβ1-4R sequences of linear oligosaccharides are resistant to endo-β-galactosidase of *Bacteroides fragilis Glycoconjugate J.* **8,** 376-380

325. Seppo, A., Turunen, J. P., Penttilä, L., Keane, A., Renkonen, O., and Renkonen, R. (1996) Synthesis of a tetravalent sialyl Lewis x glycan, a high-affinity inhibitor of L-selectin-mediated lymphocyte binding to endothelium *Glycobiology* **6,** 65-71

326. Renkonen, O., Penttilä, L., Makkonen, A., Niemelä, R., Leppänen, A., Helin, J., and Vainio, A. (1989) The linear tetrasaccharide, Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc, isolated from radiolabeled teratocarcinoma poly-N-acetyllactosaminoglycan resists the action of *E. freundii* endo-β-galactosidase *Glycoconjugate J.* **6,** 129-140

327. Hård, K., van Zadelhoff, G., Moonen, P., Kamerling, J. P., and Vliegenthart, J. F. G. (1992) The Asn-linked carbohydrate chains of human Tamm-Horsfall Glycoprotein of one male. Novel sulfated and novel N-acetylgalactosamine-containing N-linked carbohydrate chains. *Eur. J. Biochem.* **209,** 895-915

328. Maaheimo, H., Räbinä, J., and Renkonen, O. (1997) ^{1}H and ^{13}C NMR analysis of the pentasaccharide Galβ(1-4)GlcNAcβ(1-3)[GlcNAcβ(1-6)]Galβ(1-4)GlcNAc synthesized by the mid-chain β-(1-6)-D-N-acetylglucosaminyltransferase of rat serum *Carbohydr. Res.* **297,** 145-151

329. Davis, A. L., Keeler, J., Laue, E. D., and Moskau, D. (1992) Experiments for recording pure-absorption heteronuclear correlation spectra using pulsed field gradients *J. Magn. Reson.* **98,** 207-216

330. Bax, A., and Summers, M. F. (1986) ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR *J. Am. Chem. Soc.* **108,** 2093-2094

331. Hurd, R. E., and John, B. K. (1991) Gradient-enhanced proton-detected heteronuclear multiple-quantum cohorence spectroscopy *J. Magn. Reson.* **91,** 648-653

332. Nyman, T. A., Kalkkinen, N., Tölö, H., and Helin, J. (1998) Structural characterization of N-linked and O-linked oligosaccharides derived from interferon-α2b and interferon-α14c produced by Sendai-virus induced human peripheral blood leukocytes *Eur. J. Biochem.* **253,** 485-493

333. Papac, D. I., Wong, A., and Jones, A. J. S. (1996) Analysis of acidic oligosaccharides and glycopeptides by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry *Anal. Chem.* **68,** 3215-3223

334. Brown, G. M., Huckerby, T. N., Morris, H. G., Abram, B. L., and Nieduszynski, I. A. (1994) Oligosaccharides derived from bovine articular cartilage keratan sulfates after keratanase II digestion: Implications for keratan sulfate structural fingerprinting *Biochemistry* **33,** 4836-4846

335. Tai, G. H., Huckerby, T. N., and Nieduszynski, I. A. (1993) N.m.r. spectroscopic studies of fucose-containing oligosaccharides derived from keratanase digestion of articular cartilage keratan sulfates. Influence of fucose residues on keratanase cleavage *Biochem. J.* **291,** 889-894

336. Chai, W., Yuen, C.-T., Kogelberg, H., Carruthers, R. A., Margolis, R. U., Feizi, T., and Lawson, A. M. (1999) High prevalence of 2-mono and 2,6-di-substituted Manol-terminating sequences among O-glycans released from brain glycopeptides by reductive alkaline hydrolysis *Eur. J. Biochem.* **263,** 879-888

337. Kono, M., Ohyama, Y., Lee, Y.-C., Hamamoto, T., Kojima, N., and Tsuji, S. (1997) Mouse β-galactoside α2,3-sialyltransferases: comparison of *in vitro* substrate specificities and tissue specific expression *Glycobiology* **7,** 469-479

338. Kundu, S. K., Harati, Y., and Misra, L. K. (1984) Sialosylglobotetraosylceramide: a marker for amyotropic lateral sclerosis *Biochem. Biophys. Res. Commun.* **118,** 82-89

339. Schwarting, G. A., Carroll, P. G., and DeWolf, W. C. (1983) Fucosyl-globoside and sialosyl-globoside are new glycolipids isolated from human teratocarcinoma cells *Biochem. Biophys. Res. Commun.* **112,** 935-940

340. Thorn, J. J., Levery, S. B., Salyan, M. E. K., Stroud, M. R., Cedergren, B., Nilsson, B., Hakomori, S.-I., and Clausen, H. (1992) Structural characterization of x, glycosphingolipid, its extended form, and its sialosyl derivatives: accumulation associated with the rare blood group p phenotype *Biochemistry* **31,** 6509-6517

341. Zhou, D., Henion, T. R., Jungalwala, F. B., Berger, E. G., and Hennet, T. (2000) The β1,3-galactosyltransferase β3GalT-V is a stage-specific embryonic antigen-3 (SSEA-3) synthase *J. Biol. Chem.* **275,** 22631-22634

342. Schwientek, T., Nomoto, M., Levery, S. B., Merkx, G., van Kessel, A. G., Bennett, E. P., Hollingsworth, M. A., and Clausen, H. (1999) Control of O-glycan branch formation. Molecular cloning of human cDNA encoding a novel β1,6-N-acetylglucosaminyltransferase forming core 2 and core 4 *J. Biol. Chem.* **274,** 4504-4512

343. Yeh, J. C., Ong, E., and Fukuda, M. (1999) Molecular cloning and expression of a novel β-1,6-N-acetylglucosaminyltransferase that forms core 2, core 4, and I branches *J. Biol. Chem.* **274,** 3215-3221

344. Nemansky, M., and van den Eijnden, D. H. (1992) Bovine colostrum CMP-NeuAc:Galβ(1-4)GlcNAc-R α(2-6)-sialyltransferase is involved in the synthesis of the terminal NeuAcα(2-6)GalNAcβ(1-4)GlcNAc sequence occuring on N-linked glycans of bovine milk glycoproteins *Biochem. J.* **287,** 311-316

345. Teneberg, S., Lönnroth, I., Torres Lopez, J. F., Galili, U., Ölwegård Halvarsson, M., Ångström, J., and Karlsson, K. A. (1996) Molecular mimicry in the recognition of glycosphingolipids by Galα3Galβ4GlcNAcβ-binding *Clostridium difficile* toxin A, human natural anti α-galactosyl IgG, and the monoclonal antibody Gal-13: characterization of a binding-active human glycosphingolipid, non-identical with the animal receptor *Glycobiology* **6,** 599-609

346. Moran, A. P., Prendergast, M. M., and Appelmelk, B. J. (1996) Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease *FEMS Immunol. Med. Microbiol.* **16,** 105-115

347. Yamasaki, R., Bacon, B. E., Nasholds, W., Schneider, H., and Griffiss, J. M. (1991) Structural determination of oligosaccharides derived from lipooligosaccharide of *Neisseria gonorrhoeae* F62 by chemical, enzymatic, and two-dimensional NMR methods *Biochemistry* **30,** 10566-10575

348. Gilbert, M., Watson, D. C., Cunningham, A., Jennings, M. P., Young, N. M., and Wakarchuk, W. W. (1996) Cloning of the lipooligosaccharide α-2,3-sialyltransferase from the bacterial pathogens *Neisseria meningitidis* and *Neisseria gonorrhoeae J. Biol. Chem.* **271,** 28271-28276

349. Izumi, M., Shen, G. J., Wacowich-Sgarbi, S., Nakatani, T., Plettenburg, O., and Wong, C. H. (2001) Microbial glycosyltransferases for carbohydrate synthesis: α-2,3-sialyltransferase from *Neisseria gonorrheae J. Am. Chem. Soc.* **123,** 10909-10918

350. Paulson, J. C., Weinstein, J., Dorland, L., van Halbeek, H., and Vliegenthart, J. F. G. (1982) Newcastle disease virus contains a linkage specific glycoprotein sialidase. Application to the localization of sialic acid residues in N-linked oligosaccharides of α_1-acid glycoprotein *J. Biol. Chem.* **257,** 12734-12738

351. Ervasti, J. M., Burwell, A. L., and Geissler, A. L. (1997) Tissue-specific heterogeneity in α-dystroglycan sialoglycosylation. Skeletal muscle α-dystroglycan is a latent receptor for *Vicia villosa* agglutinin B_4 masked by sialic acid modification *J. Biol. Chem.* **272,** 22315-22321

352. Green, E. D., and Baenziger, J. U. (1988) Asparagine-linked oligosaccharides on lutropin, follitropin, and thyrotropin. 1. Structural elucidation of the sulfated and sialylated oligosaccharides on bovine, ovine, and human pituitary glycoprotein hormones *J. Biol. Chem.* **263,** 25-35

353. Manzella, S. M., Dharmesh, S. M., Cohick, C. B., Soares, M. J., and Baenziger, J. U. (1997) Developmental regulation of a pregnancy-specific oligosaccharide structure, NeuAcα2,6GalNAcβ1,4GlcNAc, on select members of the rat placental prolactin family *J. Biol. Chem.* **272,** 4775-4782