respectively. The branch positions were identified with endo-β-galactosidase. The following glycans carrying two GlcNAcβ1-6-branches were also formed in our experiments: Galα1-3'LactNAcβ1-3(GlcNAcβ1-6)'LactNAcβ1-3(GlcNAcβ1-6)'LactNAc and LactNAcβ1-3(GlcNAcβ1-6)'LactNAcβ1-3(GlcNAcβ1-6)'LactNAc.

S1.20

Control of Membrane Glycoprotein Fucosylation

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Human erythroleukemic (HEL) cells contain high activity for GDP-L-Fuc-*N*-acetyl-β-D-glucosaminide α1→3fucosyltransferase, although Fucα1→3GlcNAc residues are not found on the glycoproteins of HEL cells. To investigate these disparate results it was reasoned that differentiation of HEL cells may bring about glycosylation changes in the membrane glycoproteins. Treatment with phorbol 12-myristate 13-acetate (PMA) differentiates the HEL cells including the ability to adhere within a few hours whereas they normally grow in suspension culture. HEL cells were treated with 0.1 μM PMA, labeled with L-[^{3}H]fucose for two days as adherent cells and harvested. In contrast to HEL cells non PMA-treated, the glycopeptides derived from the PMA-treated cells contained a small amount of Fucα1→3GlcNAc residues as detected with almond α1→3(4)fucosidase. At the same time, α1→3fucosyltransferase activity in the cell extracts was similar with or without treatment. Therefore it is not the activity of α1→3fucosyltransferase *per se* which controls the cell surface expression of Fucα1 3GlcNAc. A study of the requirements of α1→3fucosyltransferase to fucosylate glycoproteins in HEL cells may provide information regarding the activation of ligands for Selectins as well as relate to the ability of other types of cells to form solid tumors at distal sites.

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S1.21

Characterization of a Rat Corpus Sulfotransferase for the 6-*O*-Sulfation of β-D-*N*-Acetylglucosamine Residues on Oligosaccharides

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The oligosaccharides of mucin in gastrointestinal tract are often sulfated. The functions of sulfate residues on the oligosaccharides have yet to be determined in detail but their strong acidic properties may importantly affect mucin molecules. In the rat corpus , at least two types of sulfation on mucin oligosaccharides have been demonstrated (Goso & Hotta (1989) *Biochem. J.* **264**, 805 – 812.). Their occurrence was suggested by the finding that oligosaccharides contained Galβ1-4GlcNAc(6-SO_4) or terminal GlcNAc(6-SO_4) sequences. To determine the molecular basis for production of these sulfated sequences, we have characterized sulfotransferases in the rat corpus microsome. Analysis using simple saccharide acceptors (GlcNAcβ-*O*-Me, GlcNAcα-*O*-Me, GlcNAcβ1-3Galβ-*O*-Me, Galβ1-4GlcNAcβ-*O*-Et, Galβ1-4GlcNAcβ1-6Gal, etc.) indicated an enzyme to transfer sulfate residues toward terminal but not internal β-D-GlcNAc residues of saccharides. Sulfate was not transferred toward α-D-GlcNAc residues even when located at the terminal position. Analysis of the products from saccharide acceptors bearing terminal GlcNAc residue showed the 6-*O*-position of GlcNAc residue to be sulfated by enzyme reaction. 6-*O*-Sulfated GlcNAc residues are present on mucin oligosaccharides and thus this enzyme would appear to transfer sulfate residues toward mucin oligosaccharides. The small degree of sulfate transfer toward internal GlcNAc residues in oligosaccharides by this enzyme may be indication that the Galβ1-4GlcNAc(6-SO_4) sequence is produced by the transfer of the Gal residue to the GlcNAc(6-SO_4) sequence. Other enzyme properties are as follows: (i) optimum pH of this enzyme is about 7.5, (ii) the enzyme does not require divalent cations and (iii) Triton X-100 stimulates enzyme activity.

S1.22

Sialyltransferase Activities in FR3T3 Cells Transformed with *ras* Oncogene : Decreased CMP-Neu5Ac : Galβ1-3GalNAc α-2,3-Sialyltransferase

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We have investigated the activity of CMP-Neu5Ac:Galβ1-3GalNAc α-2,3-sialyltransferase (EC 2.4.99.4) in FR3T3 cells transformed by the Ha-*ras* oncogene in which we had previously demonstrated the higher expression of the β-galactoside α-2,6-sialyltransferase (EC 2.4.99.1) (Le Marer *et al.*, 1992, *Glycobiology* **2**, 49 – 56). We clearly demonstrate using different acceptors that the presence of the activated *ras* gene decreased 4 fold the activity of α-2,3-sialyltransferase specific for *O*-glycan core 1. Based on the kinetic parameters and on mixing experiments, we can assume that this decreased enzymic activity reflects a decrease of the number of active *O*-glycan α-2,3-sialyltransferase polypeptide in *ras*-transformed cells rather than the occurrence of an inhibitor in these cells. However, no change in the binding of Peanut agglutinin was observed on the cell surface of *ras*-transformed FR3T3 suggesting that no change in the sialylation of *O*-glycan core 1 appeared in these cells. This suggests that, while the activity of the α-2,3-sialyltransferase was 4 fold decreased in *in vitro* assays, the remaining activity can be sufficient to maintain the same level of sialylation of endogenous acceptor.

S1.23

Investigation of the Sialyltransferases Present in Two Populations of Ehrlich Ascites Tumor Cells

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The sialyltransferase activities responsible for the synthesis of *O*-linked and *N*-linked sialoglycoproteins of two populations of Ehrlich ascites tumor cells (EAT-wt and EAT-c cells) were

studied. EAT-wt cells grow readily in peritoneum of mice; do not adhere to the extracellular matrix or grow in tissue culture. EAT-c cells adhere to extracellular matrix, grow readily in tissue culture, but poorly in mice.

Galβ3GalNAcα2, 3-sialyltransferase activity in EAT-wt cells is 5-fold greater than in EAT-c cells. Low levels of other sialyltransferase activities were expressed in EAT-wt cells assayed using *N*-acetyllactosamine, lacto-*N*-tetraose and benzyl α-GalNAc as acceptors. HPLC analyses of products synthesized from *N*-acetyl-lactosamine indicated that EAT-wt cells synthesize mostly Neu5Acα3 Gal β4GlcNAc *N*-linked chains, whereas EAT-c cells also produce measurable amounts of Neu5Acα6Gal β4GlcNAc *N*-linked oligosaccharides. There was evidence that some of the products synthesized by both α2,3- and α2,6-sialyltransferases are acylated.

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S1.24

Studies on Lacto-Series Glycosphingolipid Galactosyl- and Sialyltransferases Using Lac-PTDETN-Type Neoglycolipids as Acceptors

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Neoglycolipids of the 1-deoxy-1-phosphatidylethanolamino-lactitol (Lac-Ptd-Etn) type have recently been reported to substitute for glycosphingolipids (GSL) in glycosyltransferase assays (1). For investigating the biosynthesis of lacto-series GSL *N*-acetylated lacto-*N*-tetraosyl-PtdEtn (LNT-PtdEtn-(NAc)) and lacto-*N*-triaosyl-PtdEtn (LNTri-PtdEtn(NAc)) were synthesized. The structures of both neoglycolipids were corroborated by fast-atom-bombardment mass spectrometry (FAB MS). LNTri-PtdEtn(NAc) and LNT-PtdEtn(NAc) were then used as acceptors for rat liver Golgi galactosyltransferase and sialyltransferase, respectively, leading to the following results:

(a) LNTri-PtdEtn(NAc) was readily galactosylated. The reaction product co-migrated with LNT-PtdEtn(NAc) and terminal 1 – 4 galactosylation was proven by FAB MS. Competition experiments with GM2 and ovalbumin clearly demonstrated that neither ganglioside galactosyltransferase II nor glycoprotein galactosyltransferase were responsible for the galactosylation of the LNTri-neoglycolipid.

(b) Using LNT-PtdEtn(NAc) as acceptor for rat liver Golgi sialyltransferase three terminally sialylated products were obtained, whose structures were confirmed by FAB MS. The results of competition experiments with glycoproteins and gangliosides suggest the presence of additional sialyltransferases for lacto-series GSL.

The results of the above experiments once again showed that neoglycolipids of the 1-deoxy-1-phosphatidylethanolamino-lactitol type might be excellent substitutes for authentic GSL. Their use as glycolipid acceptors led to the detection of glycosyltransferases that seem to be specific for glycosylation of lacto-series GSL.

(1) Pohlentz, G. *et al.* (1992) Eur. J. Biochem, **203**, 387 – 392.

S1.25

Proposal for the Biosynthesis of Eubacterial Surface Layer (S-LAYER) Glycoproteins

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Two-dimensional crystalline surface layers (S-layers) have been observed as the outermost cell envelope component in many strains of walled eubacteria and archaebacteria. Recently it was demonstrated that not only archaebacteria but also eubacteria are able to glycosylate S-layer proteins. Up to now, the structures of 10 glycan chains of different eubacterial S-layer glycoproteins are elucidated [1]. Based on informations obtained on the biosynthesis of archaebacterial S-layer glycoproteins [2] we have started to analyze the biosynthesis of the glycan portions of eubacterial S-layer glycoproteins. After isolation of the putative glycoprotein precursors including nucleotide activated and lipid bound saccharides we propose a pathway for the biosynthesis of the S-layer glycoproteins of the eubacteria *Bacillus alvei* and *Clostridium thermosaccharo-lyticum*.

(1) Messner, P., Sleytr, U.B., (1991) Glycobiology **1**, 545 – 551.
(2) Hartmann, E., König, H., (1989) Arch Microbiol **151**, 274 – 281.

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S1.26

Sequential Activation of Glycosyltransferases During B Cell Differentiation

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In a previous study (1), we have shown that sequential shifts in the three major glycolipid series expression take place during B cell differentiation. Pre-B cells contained lacto-series type II chainbased glycolipids and GM3 ganglioside; mature/activated B cells did not express any more lactoseries compounds but had globo-series glycolipids (Gb3 and Gb4) and terminally differentiated B cells, in addition to these compounds, also contained GM2 ganglioside. In order to study the enzymatic basis of these coordinated changes, we analyzed the activities of the three glycosyltransferases involved in core structure synthesis of glycolipids, namely the β1→3GlcNactransferase (lacto-core, Lc3 synthetase), the α2→3 sialyltransferase (ganglio-core, GM3 synthetase) and the α1→4 Galtransferase (globo-core, Gb3 synthetase) as well as the activities of the two GalNactransferases responsible for the first steps in the synthesis of ganglio and globo-series glycolipids (GM2 and Gb4). A high specific activity of the Lc3 synthetase was detected in pro-B and pre-B cell lines whereas this enzyme was undetectable in the more differentiated cell lines. GM3 synthetase activity was moderate in the pre-B cells, high in lymphoblastoid cell lines (LCL) which represent mature/activated B lymphocytes and also elevated in two