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