sialidase reconstituted the 3C9 MoAb epitope in desialylated F3 but not that of Ch antiGal. Reactivity with F2 (chemoluminescent method) of sera from patients with active Ch infection showed 100% specificity and sensitivity.

S.13 EXPERIMENTALLY INDUCED CHANGES IN CELL GLYCOCONJUGATE PROFILES

S13.1
Manipulation of Glycolipids in the Cell and its Cell Biological Consequences

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One of the final goals of glycobiology is to understand why and how the extraordinary diversity of the carbohydrate structures of glycoconjugates is utilized for cell to cell (or cell to specific ligand) recognition in mammals, and what specific cellular responses are conducted as a consequence. To answer these questions, we have so far taken the following four types of approaches.

(1) Firstly, a tetrasialoganglioside, GQ1b, is capable of inducing and promoting neurite outgrowth of human neuroblastoma cells in a concentration of few pmoles per ml of the culture media (Nagai *et al.*, 1983). Several lines of evidence support that the activity is mediated initially by GQ1b-specific glycoreceptor of the cell surface membrane and subsequent phosphorylation at cell surface loci of membrane proteins in the presence of extracellular ATP (Nagai *et al.*, 1988). On the other hand, gangliosides like GM3 and the lacto series have a potential to inhibit cell growth or to guide differentiation of several cultured cell lines (Hakomori, S. *et al.*; Saito, M. *et al.*). Effective doses of these gangliosides usually lie in a range of μM concentrations, suggesting involvement of entirely different molecular mechanisms. It is likely that the activity may develop as a consequence of the formation of annular or boundary lipid structure with the protein components of signal transduction pathway in the mambrane.

(2) Monoclonal antibody to a disialoganglioside, GD3, specifically and reversibly inhibits growth of the human adenovirus E1a gene-transfected cells which express this ganglioside in the cell surface. The GM1 or GD1b ganglioside binding B subunit of cholera toxin enhances insulin and EGF inducible DNA synthesis in rat hepatocytes (Mitsui *et al.*, 1991). This may be somewhat analogous to cell growth responses to plant lectins. Interestingly, protein sequencing and DNA transfection study indicated that the transforming growth factor γ2 which is secreted from avian sarcoma virus-transformed rat cells is identical to galactose-binding protein (Yamaoka *et al.*, 1991). Galactose binding protein could be also involved in the control of cell to cell contact-dependent cell growth (Kajiyama *et al.*, in this symposium).

(3) Cell membrane glycoconjugates can be modified epigenetically under altered synthetic medium as seen in monkey kidney derived cells, JTC-12 P3 (Kawaguchi *et al.*, 1988). Increase in gangliosides GM3, GM2, and GD3 in the cells cultured in DM-170 (galactose + pyruvic acid but no glucose fed) were distinctly observed as compared with those cultured in DM-160 (glucose alone fed).

(4) Finally, it is likely that glyco-compounds may directly regulate gene expression as seen in the case of exogenously added synthetic sialylcholesterol which is promptly transported into cell nuclei to promote neurite outgrowth in a mouse neuroblastoma cell line, Neuro 2a (Yamashita *et al.*, 1991).

The above-mentioned four types of approaches should provide important clues for clarifying the roles of glycosphingolipids associated with particular cell functions.

S13.2
The Function of Glycosphingolipids as Revealed by Endoglycoceramidase

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Functions of Glycosphingolipids (GSLs) have mostly been studied by adding GSLs to responding cells, but the actual roles of GSLs on a cell surface *in situ* have yet to be determined. In order to elucidate cell surface GSL functions, we consider it necessary to develop a new enzymological method by which the oligosaccharides of GSLs can be liberated from living cells. The enzyme, endoglycoceramidase (EGCase), possessing a novel specificity for cleaving the linkage between the oliogosaccharide and ceramide of GSLs was discovered and characterization of it has been intensively carried out (1). EGCase, with the assistance of activator proteins (2), it has been made possible to cleave off the oligosaccharides from GSLs on the cell surface.

The most outstanding effect on cells upon treatment with EGCase has been shown to be the arrest of cell growth. In a study using A431 cells, treatment of the intact cell or the membrane fraction resulted in suppression of EGF receptor phosphorylation. Addition of ceramide or sphingosine to the membrane had no effects on the receptor phosphorylation, indicating that endogenous GSLs are present on the cell surface to assist EGF receptors to function properly, contrary to the report of Bremer *et al.* that GM3 exogenously added to cells suppresses cell growth and receptor phosphorylation (3). When B16 melanoma cells were treated with the enzyme, the rate of GSL synthesis was doubled in the cell treated with EGCase. Recovery of the amount of GSLs on the cell surface was achieved in 3 hr during incubation in the absence of the enzyme. The amount of the GSLs reached exactly the level of GSLs expected for the normal cell, suggesting that there is a subtle mechanism in the cell to maintain the amount and species of GSLs constant under given conditions (4). EGCase thus has become a powerful tool for describing the GSL functions.

1. Yamagata, T. and Ito, M. (1992) in "CRC Handbook of Endoglycosidases and Glycoamidases, CRC Press, pp. 133–182. 2. Ito, M. *et al.* (1991) *J. Biol. Chem.*, **266**, 7919–7926. 3. Ji, L. *et al.* (1991) *Seikagaku*, **63**, 790. 4. Ito, M. *et al.* (1992) *Seikagaku*, **64**, 822.