the 73 kDa protein exhibits typical features of a type II transmembrane protein anchored in the membrane by an uncleaved signal peptide at its *N*-terminus. No sequence homologies were found with other processing α-mannosidases except with ER-mannosidase from yeast, this homology being restricted, however, to peptide segments ranging in size from 10-25 amino acid residues.

S2.28

Mutations in the Arylsulfatase A Gene of Two Patients with Metachromatic Leukodystrophy

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disease with autosomal recessive inheritance caused by deficiency of the enzyme arylsulfatase A (ASA), which catalyzes the desulfation of galactose-3-sulfate present in sulfated glycolipids. We investigated the ASA gene of two MLD patients: one is juvenile type and the other is adult type. The mutation detected in the ASA gene in the juvenile type MLD was the same as reported by Kondo *et al.* (1991). On the other hand, the mutation observed in the ASA gene of the adult type MLD was a new one. In that mutation glycine at the position of 122, a residue that is highly conserved in the arylsulfatase gene family, was replaced by serine. In a transient expression study, the COS cells transfected with the mutant cDNA carrying ^{122}Gly→Ser did not show an increase of ASA activity and produced little immunoreactive material with anti-ASA antibody despite its normal mRNA level. These results are in agreement with the reduced immunoreactive material in the patient's liver and probably suggest that the substitution for the highly conserved glycine residue makes the ASA protein unstable.

S.3 TRANSMEMBRANE SIGNALLING

S3.1

The Role of Gangliosides and Sphingoglycolipids in Transmembrane Signalling

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BHK cell growth in chemically defined media requires fibroblast growth factor (FGF) as the sole growth factor. Exogenous addition of GM_3, but not other gangliosides, specifically arrest FGF-dependent BHK cell growth and inhibit FGF internalization despite the fact that FGF did not interact with GM_3. Results suggested that FGF signaling is specifically inhibited by GM_3 (Bremer, E. G. and Hakomori, S., *BBRC* 106:711 – 718, 1982). This initial observation a decade ago opened a series of subsequent studies by us and by others, focusing on whether or not specific gangliosides or sphingoglycolipids (SGLs) may involve modulation of key molecules involved in regulating transmembrane signal transduction, such as tyrosine kinases associated with various growth factor receptors, protein kinase C, etc. Subsequently, modified ganglioside and SGL breakdown products were studied as possible modulators of transmembrane signaling in analogy to phospholipid catabolite as modulator of transmembrane signaling.

All of these studies indicate that receptor-associated kinases such as EGF-, PDGF-, FGF-, and insulin-receptor kinases, as well as protein kinase C, *src* or *ras* kinases were positively or negatively modulated by specific gangliosides, SGLs and their catabolites. During this study we have detected novel structures such as lyso-GM_3, de-*N*-acyl-GM_3, plasmalo-psychosine, and *N*,*N*-dimethyl-sphingosine as minor but definitive cellular components as specific signaling molecule or modulator of transmembrane signaling. I shall present overall view of cooperative function of various gangliosides, SGLs and their derivatives in control of cell growth and cell adhesion. (Supported by NIH-NCI Outstanding Investigator Grant No. CA42505-08 and funds from The Biomembrane Institute)

S3.2

Ganglioside GM1 and its Metabolites in Cell Growth, Differentiation and Transmembrane Signalling

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Ganglioside GM1 is an important lipid constituent of eukaryotic plasma membranes that has been implicated in the regulation of cell growth and differentiation. However, its mechanism of action has remained poorly understood. Advances have been made by using the B subunit of cholera toxin, a protein which binds specifically and with high affinity to cell surface ganglioside GM1, affecting cellular proliferation and differentiation of a variety of cell types. Binding of the B subunit induced proliferation of quiescent 3T3 fibroblasts whereas it inhibited growth of neuroblastoma N18 cells with concomitant induction of neurite outgrowth. The binding of the B subunit to endogenous ganglioside GM1 did not elicit the classical intracellular second messenger systems, such as cAMP, diacylglycerol, or inositol trisphosphate. A sphingolipids cycle, comparable to the phosphoglycerolipids cycle, with its own messenger transducing products, sphingosine, sphingosine-1-phosphate, and ceramide, provides a provocative link between ganglioside GM1, and signal transduction. The involvement of these novel lipid metabolites in the cell growth responses mediated via ganglioside GM1 will be discussed. Recently, we observed that the B subunit mediated a large increase of intracellular free calcium resulting from a net influx from extracellular sources. The calcium influx was blocked by nickel, lanthanum, or cobalt ions, but was not altered when extracellular sodium was replaced with choline, ruling out the possible involvement of the sodium/proton exchange in the induction of this influx. No effect of B subunit could be observed in the presence of depolarization induced by potassium. Such occlusion suggests involvement of voltage-dependent calcium channels, of which there are at least three types, termed L, N, and T. ω-