Preface

Glycolipids are carbohydrate-attached lipids. Their role is to provide energy and also serve as markers for cellular recognition. They occur where a carbohydrate chain is associated with phospholipids on the exoplasmic surface of the cell membrane. The carbohydrates are found on the outer surface of all eukaryotic cell membranes. They extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues. This new book focuses on recent research results from around the world.

Short Communication A - Gaucher's disease, an inherited glycolipid storage disorder, is caused by a specific catabolic enzyme deficiency, this enzyme is called β-glucocerebrosidase (EC 3.2.1.45). Three clinical forms of Gaucher's disease have been described: type 1, or non-neuropathic; type 2, or acute neuropathic; and type 3, or subacute neuropathic.

Since 1980, scientists have had to answer several requests over the diagnosis of this metabolic disease. Requests emanating primarily from paediatrics departments. Fifteen cases were confirmed, within sight the intraleucocytic β-glucocerebrosidase enzyme activity measurement.

The authors report their experiment in the biochemical diagnosis of Gaucher disease by showing mainly the variability and the extreme heterogeneity of beta glucocerebrosidase activity during performed measurements. In addition, the authors expose the problems of etiologic diagnosis which certain patients raise, given the discordances between measured enzymatic activity and clinical signs of the disease in a person with Gaucher's disease. Moreover, the authors develop the biological parameters useful to proportion to the monitoring of the treatment, finally available.

Short Communication B - It is known that the concentration of sulfatide (SM4s), a major sulfoglycolipid in Madin-Darby canine kidney (MDCK) cells increases in a hyperosmotic medium due to the activation of the cerebroside sulfotransferase gene. The authors investigated the changes in molecular species of sulfoglycolipids in this cell line under osmotic stresses. Analysis by thin layer chromatography showed that the slower-moving band of SM4s specifically increased in hyperosmotic medium. Moreover, analysis by negative ion liquid secondary ion mass spectrometry demonstrated an increase in the ratios of hydroxy fatty acid species to those of corresponding non-hydroxy fatty acid ones of SM4s, under

hyperosmotic stress. Further, analysis of galactosylceramide, the precursor of SM4s, by negative ion LSIMS also showed a similar result, which suggests that the hydroxylation of fatty acids in sulfatide was specifically induced through the hydroxylation of its precursor glycolipid, under hyperosmotic stress. The elucidation of the mechanism that increases the hydrophilic molecular species in galactosphingolipids of renal cells under hyperosmotic stress may contribute to the *in vitro* study on the function of galactolipids containing hydroxy fatty acids.

Chapter I - Glycolipids—in particular gangliosides—are ubiquitous molecules on the surface of cells. They play roles in important cellular processes like differentiation and transformation. This chapter focuses on the role of gangliosides in the interaction of eukaryotic cells with microbes and microbe-derived elements, such as toxins, as well as the role of gangliosides in the activation of immune cells. Gangliosides can be considered cellular gateways for certain microbes and viruses that have the ability to interact and to invade eukaryotic cells. Most of these early interactions during infection initially take place in the lipid raft domains of the plasma membrane. Gangliosides also regulate the immune system and play a role in the efficacy of certain experimental mucosal adjuvants (i.e., substances that enhance the immune response against antigens). However, their mechanism of action is not yet well elucidated. Consequently, the outcome of some host-pathogen interactions may be manipulated at two distinct levels: at the initial host-pathogen interaction and through intervention at the specific immune response that is induced later.

Moreover, gangliosides serve as receptors for some host molecules, such as antibodies. In addition, their attachment can lead to the recruitment and activation of toll-like receptors and signaling transduction pathways that promote interactions and activate diverse type of cells. This chapter will explore the participation of gangliosides and lipid rafts in these diverse events. A better understanding of the molecular mechanism involved in these interactions will allow us to manipulate host-pathogen and host immune responses more effectively to prevent infectious diseases and to optimize vaccination and prophylaxis against certain diseases.

Chapter II - Gangliosides are complex glycosphingolipids present in all eukaryotic cells. They are composed of a hydrophobic ceramide backbone anchored in the lipid bilayer of plasma membrane and a polar head group with hydrophobic oligosaccharide chains and one or more residues of *N*-acetylneuraminic acid (sialic acid) at the termini that protrude into the extracellular space. Ganglioside species vary one from another in their ceramide composition and/or in their carbohydrate content. The kinds and amounts of gangliosides expressed in the plasma membrane are cell and tissue type dependent. Ganglioside composition may change during embryonic development, tissue differentiation, aging and degeneration or malignant transformation. Gangliosides have been recognized to play important roles in normal physiological functions as well as in pathological conditions, including tumor progression. They modulate many biologic processes, such as differentiation, proliferation, adhesion, migration, immune response, and act as receptors for viruses and bacterial toxins. Gangliosides also participate in apoptosis and signal transduction in several cell types. In mast cells, gangliosides may function in modulating secretory events as well as participating in mast cell development and recruitment. Mast cells have long been known to play a critical role in inflammatory and allergic reactions and contribute to host resistance. Following mast

cell activation by cross-linking of the high affinity IgE surface receptor (FcεRI) on the cell surface a number of inflammatory mediators are released. Previous studies using the monoclonal antibody (mAb) AA4, which recognizes two α-galactosyl derivatives of ganglioside GD_{1b} that are specific to rodent mast cells showed that these gangliosides are located in proximity to FcεRI. Binding of mAb AA4 to the gangliosides on the surface of mast cells produces morphological and biochemical changes similar to those seen with activation of FcεRI, but without histamine release. The expression of these gangliosides on the mast cell surface has also been shown to be related to mast cell maturation. Committed mast cell precursors do not express the gangliosides recognized by mAb AA4, but the gangliosides begin to be expressed on the cell surface jointly with FcεRI in very immature mast cells and continue to be expressed by mast cells in all stages of maturation. This chapter will present a short review on the biological functions of gangliosides and discuss some important evidence on the role of gangliosides in modulating FcεRI and in the maintenance of mast cell structure and function.

Chapter III - Glycolipids are, by definition, long carbohydrate and hydrophobic molecules that integrate into the plasma membrane and bind to diverse types of sugar residues at their extracellular domain. Their location on the external surface of the cell makes them excellent biomarkers for the prospective isolation of various subsets of cells from the central nervous system. In this chapter, the authors review glycolipids that have been used to identify diverse populations of neural stem and progenitor cells during development and in the adult. In particular, the authors focus our discussion on *stage specific embryonic antigen 1* and *4* (SSEA-1 and SSEA-4, respectively). These two glycolipids are widely used to monitor embryonic stem cell differentiation, but have more recently been employed in the identification and positive selection of two distinct subsets of neural stem and progenitor cells. The authors explore their possible biological functions and discuss differences between species. And finally, the authors speculate on their possible applications in the therapeutic correction of neurological damage.

Chapter IV - Mycobacterial diseases, including tuberculosis, leprosy, and disease due to nontuberculous mycobacteria, are the major cause of death from infectious diseases around the world. About one-third of the world population is latently infected with *Mycobacterium tuberculosis*. Over 8 million new cases and nearly 2 million deaths occur each year resulted from tuberculosis. Tuberculosis presents a significant health threat to the world. The pathogenicity of mycobacteria is related to their ability to escape killing by ingested macrophages, latent infection, and induce delayed-type hypersensitivity, such as granulomas. This has been attributed to several components of the mycobacterial cell wall, such as, complement activation factor, heat-shock protein, mycobacterial DNA binding protein, and glycolipids, including mycoloyl glycolipids (containing cord factor/trehalose dimycolate), phenolic glycolipid, sulfolipid, glycopeptidolipid, phosphatidylinositol mannoside, lipoarabinomannan, and lipomannan. Among them, glycolipids specific for mycobacteria play a key role in the pathogenesis, because mycobacteria are rich in lipids that possess pleiotropic activities. The complex interaction between a range of mycobacterial components and the host participate the pathogenesis. In this review the authors will focus on the pleiotropic activity of glycolipids in both the microbe and host. The better understanding of

mycobacterial pathogenicity may open the new avenue for the development of diagnostics, therapeutic and prophylactic interventions.

Chapter V - In autoimmune neuropathies such as Guillain-Barré syndrome (GBS) and IgM paraproteinemic neuropathy, serum antiglycolipid antibodies are frequently elevated in titer. Those antibodies are useful diagnostic markers and some of them may be directly involved in the pathogenetic mechanisms. In GBS, the carbohydrate structures of the microorganisms of the antecedent infections that mimic those of human glycolipids may induce production of the antiglycolipid antibodies. "Molecular mimicry" mechanism has been shown in the production of antiganglioside antibodies after *Campylobacter jejuni* infection and antigalactocerebroside antibodies after *Mycoplasma pneumoniae* infection. The antibodies may specifically bind to the regions where the respective antigens are located (for example, the antibodies against the ganglioside GQ1b may cause ophthalmoplegia by binding to the paranodal regions of the oculomotor, trochlear, and abducens nerves, where GQ1b is densely localized). The authors have proved this hypothesis by inducing sensory ataxic neuropathy in rabbits sensitized with the ganglioside GD1b, which is localized in the primary sensory neurons conveying deep sensation. The authors have recently found that the antibodies that specifically recognize a new conformational epitope formed by two gangliosides are sometimes present in the acute-phase sera of GBS patients. In particular, the antibodies against GD1a-GD1b complex are associated with severe GBS requiring artificial ventilation. Gangliosides are preferentially packaged with cholesterol to form lipid rafts, in which the carbohydrate portions of two different gangliosides may form a new conformational epitope. Within the rafts, gangliosides are considered to interact with important receptors or signal transducers. The antibodies against ganglioside complex may therefore directly induce nerve conduction failure and severe disability in GBS. More study is needed to elucidate the roles of antiglycolipid antibodies in the pathogenetic mechanisms of autoimmune neuropathies.

Chapter VI - The membrane raft hypothesis proposed that membrane lipid domains enriched in Chol and sphingolipids form dynamic sorting and signalling platforms for membrane proteins in cells. Cell membranes however cannot be accurately described with only the two categories of raft or non-raft; instead the authors should talk about different domains which should be characterized each in all its complexities. In this chapter the current picture on membrane domains is discussed, and the usefulness of model systems to understand molecular configuration is exemplified by monolayer isotherms, calorimetry and spectroscopy studies in model membranes containing ceramide. These results are interpreted with the aim of explaining the ability of ceramide to produce very large domains or "platforms" in cell membranes.

Chapter VII - Ceramide is an apoptosis-inducing signaling molecule in the sphingolipid metabolism which emerges as a potential target for anticancer therapy. Natural ceramide in solution has a uniquely folded butterfly conformer with strong internal hydrogen bonding between amide and two alcohols at C1 and C3 and with hydrophobic interaction between two long alkyl chain of sphingosine backbone and fatty acid. Its heterocyclic analogs as 3-alkanoyl or benzoyl-4-(1-hydroxyhexadec-2-enyl)-oxazolidin-2-ones were designed by binding of primary alcohol and amide in sphinogosine backbone as a carbamate. Those compounds without free primary alcohol have advantages not to perturb the ceramide

metabolism most of which involved in the primary alcohol of sphingosine backbone. This structurally constrained analog of ceramide may have better activity compared with the natural ceramide without pre-organization of substrate, if necessary. They were synthesized by addition of acyl halide to the common ring, 4-(1-*t*-butyldimethylsilyloxyhexadec-2-enyl)-oxazolidin-2-one, which was elaborated from chiral aziridine-2-carboxylate including stereoselective reduction and ring opening reactions. All compounds were tested as antileukemic drugs against human leukemia HL-60 cells. Many of them including propionyl, cyclopentanoyl and *p*-nitrobenzoyl-4-(1-hydroxyhexadec-2-enyl)-oxazolidin-2-ones showed better antileukemic activities than natural C2-ceramide with good correlation between cell death and DNA fragmentation. Cytotoxicity was induced by caspase activation without significant accumulation of endogenous ceramide concentration or any perturbation of ceramide metabolism.

Chapter VIII - Increasing lines of evidence suggest that cholesterol is a potent risk factor for the development of Alzheimer's disease (AD). An increase in the cholesterol level of neuronal membranes likely enhances both the generation and aggregation of the amyloid β-protein (Aβ). Regarding the enhancement of Aß aggregation, previous studies by the authors group and other groups suggest that cholesterol has both direct and indirect effects on such aggregation. The authors previously identified a novel Aβ species in the brain exhibiting early pathological changes of AD. On the basis of unique molecular characteristics, the authors hypothesized that GM1 ganglioside (GM1) plays a critical role in the pathological processes in AD. The mechanism underlying the binding of Aβ to GM1 ganglioside remains to be determined; however, their recent studies suggest that an increase in the cholesterol level in the membrane facilitates such binding. The distribution of cholesterol in neuronal membranes is likely to be altered by aging and the expression of apolipoprotein E4, both of which are strong risk factors for AD development. The putative roles of cholesterol and gangliosides in AD development are discussed.

Chapter IX - Basidiomycetous yeasts *Cryptococcus humicola* and *Pseudozyma fusiformata* secrete into the culture broth glycolipids which are a 16-(β-cellobiosyloxy)-hexadecanoic acid containing OH-groups at C-2 or C-2 and C-15 of fatty acid aglycon and also carrying O-acyl substituents in cellobiosyl fragment. In the case of *Cr. humicola,* 16-(tetra-O-acetyl-β-cellobiosyloxy)-2,15-dihydrohexadecanoic acid (1) and 16-(tetra-O-acetyl-β-cellobiosyloxy)-2-hydroxyhexadecanoic acid (2) were structurally defined as major products, while *P. fusiformata* secreted mainly 16-[6-O-acetyl-2'-O-(3-hydroxyhexanoyl)-β-cellobiosyloxy)-2,15-dihydroxyhexadecanoic acid (3). These compounds are lethal for many yeast and mycelial fungi. They exhibit higher activity at pH ~4.0 and in the temperature range of 20-30 °C, and had similar fungicidal activities against different yeasts including pathogenic *Cryptococcus* and *Candida* species. The cells of tremellaceous yeast *Filobasidiella neoformans* causing systemic cryptococcosis completely died after 30-min incubation with glycolipids 1-3 at 0.02 mg/ml concentrations. The same effect on ascomycetous yeast, including pathogenic *Candida* species, is achieved at five to eight times higher concentrations of glycolipids. Cellobiose lipid 3 of *P. fusiformata* inhibits the growth of phytopathogenic fungi *Sclerotinia sclerotiorum* and *Phomopsis helianthi* more efficiently than cellobiose lipids **1** and **2** from *Cr. humicola.*

Fully O-deacylated analogues of compounds 1-3, namely 16-(β-cellobiosyloxy)-2,15-dihydroxyhexadecanoic acid (4) and 16-(β-cellobiosyloxy)-2-hydroxyhexadecanoic acid (5), were studied together with a totally synthetic compound, 16-(β-cellobiosyloxy)-hexadecanoic acid (6), to assess the structural features determining their fungicidal activity. Compound 6 without OH-group and compound 5 with only one OH-group in the aglycon do not inhibit the growth of *F. neoformans* and *Saccharomyces cerevisiae*, while compound 4 double-hydroxylated in the fatty acid moiety inhibits the growth of both test cultures but at 5-10 times higher concentrations than compounds 1 and 2 from *Cr. humicola*. Thus, the structures of both the carbohydrate part and fatty acid aglycon moiety influence the fungicidal activity of cellobiose lipids under study. The treatment with cellobiose lipids causes the leakage of ATP and potassium ions from yeast cells. It suggests that the fungicidal activity of cellobiose lipids is due to their ability to enhance the nonspecific permeability of the cytoplasmic membrane. The effective concentrations of cellobiose lipids, which induce ATP release and cell death, were similar for the same test cultures. The results show that extracellular cellobiose lipids may be considered as promising fungicides for agriculture, medicine, and veterinary.