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Visions & Reflections

The clinical potential of sphingolipid-based therapeutics

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Abstract. The era of sphingolipid-based therapeutics is upon us. A large body of work has been accumulating that demonstrates the distinct biological roles of sphingolipids in maintaining a homeostatic environment and in responding to environmental stimuli to regulate cellular processes. It is thus necessary to further investigate alterations in sphingolipid-metabolism in pathological conditions and, in turn, try to exploit altered sphingolipid-metabolizing enzymes and their metabolites as therapeutic targets. This review will examine how advances in the fields of drug delivery, drug discovery, synthetic chemistry, enzyme replacement therapy, immunobiology, infectious disease and nanotechnology have delivered the potential and promise of utilizing and/or targeting sphingolipid metabolites as therapies for diverse diseases.

Keywords. Sphingolipids, ceramide, therapeutics, inflammation, infectivity, immunity.

Introduction

Sphingolipids have routinely been implicated as mediators of cellular apoptosis, growth and differentiation [1, 2]. A compelling case can now be made for the emerging role of sphingolipids to regulate inflammation, immune response and infectivity. These additional roles for sphingolipids may be a function of both the biochemical and biophysical properties of these lipids. As an example, the pro-apoptotic sphingolipid ceramide exerts its effects via biochemical (second messenger and target proteins) [3, 4] as well as through biophysical (lipid microdomains, negative membrane curvature) properties [5–7]. Critical components linking these biochemical and biophysical mechanisms include the flux between sequential sphingolipid metabolites as well as the subcellular localization and compartmentalization of these sphingolipid metabolites [8].

A dynamic sphingolipid equilibrium has been described where pro-apoptotic sphingolipids exist in a balance with pro-survival sphingolipids (Fig. 1) [9]. The most common example of this equilibrium is the balance between ceramide and sphingosine-1-phosphate. When this balance shifts either way, it can lead to cellular death or growth arrest in the case of ceramide accumulation or alternatively to proliferative disorders (i.e. cancer, angiogenesis) in the case of formation of sphingosine-1-phosphate (Sph1P). The coordinate biochemical regulation of ceramidases and sphingosine kinases (SphKs) may serve as a critical control point regulating the dynamic flux between these metabolites. However, this is indeed an oversimplification, as other sphingolipid-based second messengers, including ceramide-1-phosphate (Cer1P) as well as the glycosphingolipids are also in dynamic flux with ceramide. Thus, a thorough understanding of the consequences of the regulation of these sphingolipid metabolites must include a detailed discussion of all of the enzymes responsible for these alterations in sphingolipid flux. Endogenous ceramide levels can also be reduced by activation of

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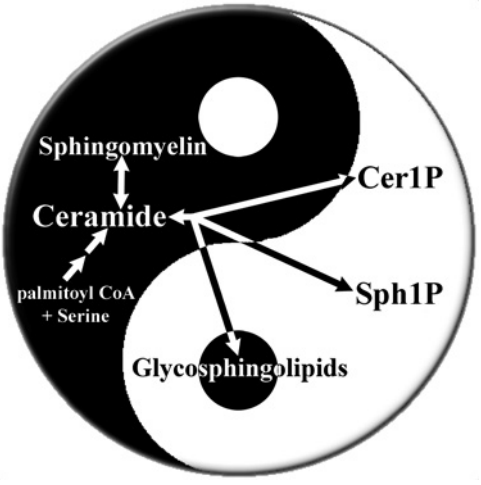

Figure 1. The Tao of sphingolipid metabolism. See text for further explanation.

ceramide kinase, glycoceramide synthases, or sphingomyelin synthase and enhanced via ceramide synthase or sphingomyelinases. In a similar scenario, activation of Sph1P lyase or Sph1P phosphatase could reduce endogenous Sph1P content and therefore increase the ratio of ceramide to prosurvival sphingolipids. Pharmacological or molecular manipulations of any of these enzymes have the potential to reset the critical balance between sphingolipid metabolites. Thus, understanding altered sphingolipid flux may identify new therapeutic targets for various diseases. With the National Institute of Health (NIGMS) funding of the Lipid MAPS Consortium to effectively utilize lipidomics to measure imbalances in lipid metabolism, this goal is now becoming a reality (http://www.lipidmaps.org).

Emerging roles of ceramide and ceramide metabolites in diseases

For over a decade now, enzyme replacement therapy (ERT) had been in use for lysosomal storage diseases, caused by accumulation of ceramide metabolites [10, 11]. Cerezyme and its predecessor, Ceredase (recombinant glucosylceramidase), has been the standard of care for Gaucher's disease. More recently, recombinant forms of agalsidase, Replagal and/or Fabrazyme have been approved for patients with Fabry's disease. Additionally, ERT for Niemann-Pick B disease (defective acid sphingomyelinase) shows promise as a future therapeutic [12]. Unfortunately, these drugs are only efficacious for visceral forms of these diseases, and are less effective against the neuropathic forms due to their inability to cross the blood-brain barrier. As an alternative strategy, substrate reduction therapy (SRT) with N-butyldeoxynojirimycin (NB-DNJ; Zavesca), an inhibitor of glucosylceramide synthase, can also be effective in patients with Gaucher's disease by restoring the balance between glucosylceramide synthase and glucosylceramidase. While this drug crosses the blood-brain barrier, there are still many side effects. These ERT and SRT adverse effects may be a reflection of less than optimal pharmacokinetic parameters, lack of drug specificity (SRT) and/or the lack of targeted drug delivery.

SRT is not just effective for diseases of accumulation of higher-order glycosphingolipids. Several recent studies demonstrate that the inhibition of de novo ceramide production itself is therapeutic for multiple diseases. First, inhibition of serine palmitoyltransferase, the first committed step of de novo synthesis, via the use of myriocin, was highly efficacious in an apolipoprotein E (apoE)-deficient mouse model of atherosclerosis. Inhibition of ceramide synthesis with myriocin decreased atherosclerotic plaque formation [13]. Inhibition of ceramide de novo synthesis is also therapeutic in an animal model of emphysema. Ceramide levels are increased in this emphysema model, which is created by treating with a vascular endothelial growth factor receptor inhibitor. Upon inhibition of the de novo pathway with either myriocin or fumonisin B_1 (ceramide synthase inhibitor), emphysema was inhibited [14]. Inhibiting the de novo pathway may also be a strategy for treating oral mucositis, which is a potential side effect of ionizing radiation used as treatment for head and neck cancer. Irradiated mice treated with fumonisin B_1 exhibited a less severe course of oral mucositis [15]. On the other hand, promising phase I oncological clinical trial results have been reported with agents that increase de novo synthesis of ceramide (fenretinide) combined with agents that inhibit ceramide catabolism (B13, safingol) [16–18].

Reduction of sphingolipid de novo flux may not be the only pharmacological methodologies to impact the actions of ceramide and ceramide metabolites. In fact, exogenous applications of pro-mitogenic sphingolipid mimetics have shown unusual applications in immunology. FTY720, a substrate for SphK, has shown tremendous promise as an immunosuppressant as evidenced by a recent phase 2A trial in renal transplants patients, in combination with cyclosporine A [19]. In addition, this therapeutic approach was also therapeutic in a model of emphysema [14] and limited the development of experimental autoimmune encephalomyelitis, a model of human multiple sclerosis [20–22]. Further utility for this Sph1P mimetic may also be in diabetes where FTY720 has been demonstrated to be effective in preventing both diabetes and islet allograft rejection in rodents and non-human primate models [23–25]. KRN7000 (α-galactosylceramide) has also been shown to be a potent *in vivo* modulator of host-graft interactions [26] and in a model of pulmonary fibrosis [27]. This expands the potential utility of KRN7000 from its originally described antitu-

mor activities [28]. In fact, a recent phase I trial of KRN7000 demonstrated no overt adverse effects in patients with advanced and recurrent non-small cell lung cancer [29]. A sphingosine truncated analog of α-galactosylceramide, OCH, has been demonstrated to be therapeutic in autoimmune encephalomyelitis [30], and in models of experimental rheumatoid arthritis [31] and colitis [32]. Additionally, OCH has inhibited the development of diabetes and insulitis in non-obese diabetic mice [33]. The mechanisms by which these ceramide metabolite analogs can regulate immunobiology are only recently being described, but in regards to FTY720 may include sequestering of lymphocytes from the circulation to lymph nodes and Peyer's patches, which involves the SphK2 isoform [34]. This redistribution effectively reduces T cell numbers at the sites of inflamed tissue or graft sites. Recent evidence suggests that FTY720 may also inhibit Sph1P lyase, which will decrease Sph1P degradation, thus providing another mechanism of action in addition to the activation of Sph1P (EDG) receptors [35]. The α-galactosylceramide analogs mediate many of their effects through the stimulation of natural killer T cells inducing T helper 2 cytokine production [17, 18].

Another emerging clinical application for modulation of ceramide and its metabolites may be diabetes. Several studies have demonstrated increases in ceramide content in insulin-resistant skeletal muscle of rats [36, 37] and humans with diabetes [38, 39]. A recent review by Summers and Nelson puts forth the hypothesis that the similarities of type 2 diabetes, metabolic syndrome X, and Cushing's syndrome may be mediated through altered sphingolipid metabolism [40]. Other groups have demonstrated that glycosphingolipids may be involved in the pathogenesis of diabetes by contributing to insulin resistance in adipocytes [41, 42] and/or being involved in renal complications [43, 44]. In addition, hyperglycemia induces the activation of SphK1 in the aorta and heart of streptozotocin-induced diabetes in rats [45]. Moreover, increases in Sph1P have been correlated with glomerular mesangial cell proliferation of streptozotocin-induced diabetic rats, which again suggests that inhibition of sphingolipid flux might be efficacious in proliferative/hypertrophic complications of diabetes [46].

While in the past ceramide had taken center stage as a modulator of inflammatory responses, the roles of ceramide kinase (CerK) and SphK are now gaining greater appreciation [47, 48]. These recent reviews summarize the regulatory roles that CerK and SphK serve in the activation, chemotaxis, degranulation, cytokine production and cytokine signaling within monocytes, macrophages, mast cells and lymphocytes [47, 48]. Recent evidence supports the role of CerK in mediating phagocytosis [49] and for CerK's product, ceramide-1-phosphate (Cer1P), as a direct substrate in activating phospholipase A_2 and thus produce arachidonic acid [50]. In fact, CerK and SphK work together to produce the prostenoid PGE_2 via arachidonic acid and COX-2 induction, respectively [51]. These data further support the notion that strategies to inhibit phosphorylated sphingolipid metabolites may have utility in inflammatory diseases.

Recently a mutated CerK-like protein has been described to be responsible for a form of retinitis pigmentosa (RP) [52]. Though the activity and regulation of this CerK-like protein remains elusive [53], modulation of ceramide levels has been demonstrated to be important in other forms of RP not caused by a genetically altered sphingolipid enzyme. By overexpressing neutral ceramidase, which forms sphingosine at the expense of ceramide, photoreceptor degeneration was suppressed in *Drosophila* models of RP [54]. Again, the dynamic balance between phosphorylated and unphosphorylated forms of ceramide or sphingosine control cellular fate. It will be interesting to investigate alterations of sphingolipid metabolites in other retinal diseases such as macular degeneration and diabetic retinopathy.

Antiviral/antimicrobial actions of ceramide

Ceramide has been shown to facilitate the entry of a variety of pathogens. In general, this is due to activation of an acid sphingomyelinase, which hydrolyses sphingomyelin into ceramide and phosphorylcholine. As a result of its biophysical properties, ceramide spontaneously self-associates and fuses into ceramide-enriched platforms (also referred to as microdomains or rafts). These platforms serve to cluster the receptors for the pathogen, and the negative membrane curvature induced by ceramide facilitates cellular invasion. Often the end result of ceramide-mediated cellular entry of pathogens is containment and/or inactivation of the pathogen.

Initial observations on the role of ceramide in pathogen entry involved infection of mucosal cells by *Neisseria gonorrhoeae* [55]. It was observed that the invasion of cells by *N. gonorrhoeae* was accompanied by the generation of ceramide, following activation of acid sphingomyelinase. Further investigations demonstrated that targeting acid sphingomyelinase by either employing chemical or antisense inactivation prevented infection [55, 56]. It was also shown that fibroblasts taken from patients with Niemann-Pick disease, which are defective in acid sphingomyelinase, are resistant to *N. gonorrhoeae* infection. Complementation studies that restore acid sphingomyelinase activity or the addition of exogenous ceramide reestablishes the ability of *N. gonorrhoeae* to infect these fibroblasts [56].

This concept of ceramide-mediating pathogen entry was further explored and confirmed by studies on *Pseudomonas aeruginosa* [57]. These studies demonstrate the *P. aeruginosa* also activates acid sphingomyelinase and

triggers the release of ceramide within minutes of infection. Ceramide platforms were shown to colocalize with both the bacteria and acid sphingomyelinase. Cells from acid sphingomyelinase-deficient mice did not form ceramide-enriched platforms and failed to internalize *P. aeruginosa*. In reconstitution studies, the addition of long-chain ceramides to acid sphingomyelinase-deficient cells restored the ability to *P. aeruginosa* to internalize. Furthermore, the addition of anti-ceramide antibodies to wild-type cells neutralized cell surface ceramide and inhibited pathogen invasion, indicating that ceramide-enriched platforms play a central role in the internalization of *P. aeruginosa*. Internalization of *P. aeruginosa* led to apoptosis of the infected cell, limiting systemic inflammatory responses and interleukin (IL)-1- induced septic death of infected mice. It was concluded that ceramide-enriched platforms may be the underlying mechanism to limit *P. aeruginosa* infection in cystic fibrosis patients and may in fact contribute to host-defense mechanisms [57].

The role of ceramide in the pathogenesis of other microbes is emerging. Infection of cells by *Staphylococcus aureus* induces a rapid activation of acid sphingomyelinase [58], which may in turn facilitate bacteria entry in a manner similar to that observed for *N. gonorrhoeae* and *P. aeruginosa*. Ceramide is also emerging as a common theme facilitating the entry process of viruses and parasites. *Plasmodium falciparum*, the causative agent of malaria, expresses its own sphingomyelinase [59], which is required for entry into erythrocytes and may function in a manner similar to what has previously been described. Viral infections, including Sindbis virus and Rhinoviruses, have also been suggested to infect via activation of acid sphingomyelinase and ceramide generation [60, 61].

The prototypical alphavirus, Sindbis virus, requires sphingolipids containing ceramide in the plasma membrane in order to fuse [60]. This requirement is confined to the actual fusion event. Preincubating cells with NH_4Cl, which prevents viral fusion by blocking the required decrease in endosomal pH, has no effect on viral binding and inhibits ceramide generation [60]. Given these results, it is tempting to speculate that Sindbis virus entry generates ceramide, which facilitates entry in a manner similar to that seen for bacterial infections. In recent studies it has been shown that infection of epithelial cells by pathogenic rhinoviruses triggers a rapid activation of acid sphingomyelinase [61]. The enzyme is transported from an intracellular compartment in a microtubule- and microfilament-dependent manner to the extracellular leaflet of the plasma membrane. Rhinovirus entry correlates with the generation of ceramide at the plasma membrane, and the virus colocalizes with these ceramide-enriched platforms. Pharmacological inhibition of acid sphingomyelinase or using target cells genetically deficient in this enzyme abrogates infection. Collectively, these studies suggest that pathogen entry is facilitated by ceramide. The rapid generation of ceramide at the extracellular leaflet of the plasma membrane restructures the membrane forming ceramide-enriched platforms that facilitate invasion.

Ceramide has also been identified as a modulator of human immunodeficiency virus-1 (HIV-1) infection [62]. Again, a link has been established between ceramide-induced increased infectivity and inactivation of the pathogen. Ceramide inhibits HIV infection at the level of membrane fusion, and the specific mechanisms of inhibition are dependent on the manner in which ceramide is generated (de novo synthetic pathway versus catabolic acid sphingomyelinase pathway). Agents that increase ceramide by stimulating de novo biosynthesis such as fenretinide were used. Fenretinide is a synthetic retinoid that specifically activates two key enzymes in the ceramide biosynthetic pathway, serine palmitoyltransferase and ceramide synthase. Treatment with fenretinide increases ceramide and inhibits HIV infection in epithelial cell lines, primary monocyte derived macrophages and purified $CD4^+$ T cells, without adverse toxic effects. Complementary studies employing exogenous sphingomyelinase or the addition of long-chain ceramides are in agreement, indicating that increasing ceramide inhibits HIV infection. Fenretinide treatment effectively inhibits HIV infection by redirecting the virus to the endocytic pathway, resulting in HIV inactivation [63]. In contrast, the massive rapid generation of ceramide that follows sphingomyelinase treatment results in a significant reduction of the effective lateral diffusion rate of CD4, the primary receptor for HIV. Since HIV fusion requires receptor mobility and the sequential engagement of CD4 and coreceptor [64], immobilizing CD4 most likely inhibits viral fusion at the crucial step of coreceptor engagement. As sphingomyelinase directly targets sphingomyelin on the outer leaflet of the plasma membrane, in contrast to fenretinide which increases de novo ceramide biosynthesis, the magnitude and subcellular membrane localization of ceramide may differ with both approaches. This, in turn, could result in the different inhibitory mechanisms observed. Regardless, both strategies increase ceramide and inhibit HIV fusion, demonstrating a novel approach to inhibiting HIV infection.

It should be no surprise that ceramide metabolites also serve as modulators of HIV-1 infection and pathogenesis. In fact, glycosphingolipids have been proposed to support HIV-1 infection by functioning as an alternate receptor and/or by regulating viral transmission and membrane fusion as recently reviewed elsewhere [65].

Optimizing sphingolipids as therapeutics

While it may be therapeutically desirable to utilize ceramide and other sphingolipid metabolites for the treatment of various diseases, their clinical utility is limited by

the physical properties of these lipids, which diminish solubility and bioavailability. Recently, the Kester laboratory [66] and the Mayer laboratory [67] have demonstrated increased efficacy of exogenous ceramides on various cancer cell lines *in vitro* through liposomal delivery. More significantly, ceramide-enriched liposomes increased survival in an ascites tumor mouse model [68] as well as significantly inhibited the growth of solid tumors in syngeneic mouse tumor models and human xenograft models of breast adenocarcinoma [69]. The formulation of nanoliposomes, with diameters less than 80 nm, has allowed very high dosages of C_6-ceramide (72 mg/kg) to be delivered to solid tumors with appropriate pharmacokinetics and with no observable side effects [69]. As another example of the use of nanotechnology to enhance delivery and targeting for ERT, Muzykantov's laboratory demonstrated that intercellular adhesion molecule-1 (ICAM-1) targeting of a latex bead nanocarrier more effectively delivers acid sphingomyelinase to Niemann-Pick cells [70]. An alternative strategy to optimize the utility of sphingolipid metabolites as therapeutics has been to directly modify the sphingolipid itself. One example is the creation of cationic pyridinium ceramides, which are relatively water soluble. These pyridinium-conjugated ceramide analogs have been demonstrated to be fourfold more effective in inhibiting cell growth of human head and neck squamous cell carcinoma cells *in vitro* [71]. This finding held true in hepatocarcinoma HepG2 cells and MCF7 breast cancer cells, where these positively charged C_6-ceramide analogs were much more effective at reducing cellular viability compared with the unmodified electroneutral C_6-ceramide [72]. Additional sphingolipid analogs and inhibitors have also been generated that demonstrate apoptotic ability. The Gatt laboratory recently generated non-natural analogs of ceramide, sphingosine and trimethylsphingosine, which elevate ceramide levels and induce apoptosis [73, 74]. Another strategy is to design water soluble non-lipid small molecule inhibitors of critical enzymes/targets regulating sphingolipid metabolism. Potentially orally available inhibitors of human sphingosine kinase have proven efficacious in a syngenic adenocarcinoma mouse model [75]. The potential utility of small molecule inhibitors as well as sphingoid mimetics may be enhanced with the use of nanoscale drug delivery modalities that can improve pharmacokinetic profiles and tissue-specific targeting. The advent of novel drug-delivery methodologies coupled with the synthesis and validation of sphingolipid analogs as therapeutics offers the promise of a new class of drugs for cancer chemotherapy, immunosuppression and inflammation.

1 Futerman A. H. and Hannun Y. A. (2004) The complex life of simple sphingolipids. EMBO Rep. **5:** 777–782
2 Kester M. and Kolesnick R. (2003) Sphingolipids as therapeutics. Pharmacol. Res. **47:** 365–371
3 Ruvolo P. P. (2003) Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol. Res. **47:** 383–392
4 Hannun Y. A. and Obeid L. M. (2002) The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J. Biol. Chem. **277:** 25847–25850
5 Goni F. M., Contreras F. X., Montes L. R., Sot J. and Alonso A. (2005) Biophysics (and sociology) of ceramides. Biochem. Soc. Symp. 177–188
6 Cremesti A. E., Goni F. M. and Kolesnick R. (2002) Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? FEBS Lett. **531:** 47–53
7 Kronke M. (1999) Biophysics of ceramide signaling: interaction with proteins and phase transition of membranes. Chem. Phys. Lipids. **101:** 109–121
8 Futerman A. H. and Riezman H. (2005) The ins and outs of sphingolipid synthesis. Trends Cell Biol. **15:** 312–318
9 Cuvillier O., Pirianov G., Kleuser B., Vanek P. G., Coso O. A., Gutkind S. et al. (1996) Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature **381:** 800–803
10 Jmoudiak M. and Futerman A. H. (2005) Gaucher disease: pathological mechanisms and modern management. Br. J. Haematol. **129:** 178–188
11 Futerman A. H. and van Meer G. (2004) The cell biology of lysosomal storage disorders. Nat. Rev. Mol. Cell Biol. **5:** 554–565
12 Miranda S. R., He X., Simonaro C. M., Gatt S., Dagan A., Desnick R. J. et al. (2000) Infusion of recombinant human acid sphingomyelinase into Niemann-Pick disease mice leads to visceral, but not neurological, correction of the pathophysiology. FASEB J. **14:** 1988–1995
13 Hojjati M. R., Li Z., Zhou H., Tang S., Huan C., Ooi E. et al. (2005) Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. J. Biol. Chem. **280:** 10284–10289
14 Petrache I., Natarajan V., Zhen L., Medler T. R., Richter A. T., Cho C. et al. (2005) Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. Nat. Med. **11:** 491–498
15 Hwang D., Popat R., Bragdon C., O'Donnell K. E. and Sonis S. T. (2005) Effects of ceramide inhibition on experimental radiation-induced oral mucositis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **100:** 321–329
16 Reynolds C. P., Maurer B. J. and Kolesnick R. N. (2004) Ceramide synthesis and metabolism as a target for cancer therapy. Cancer Lett. **206:** 169–180
17 Miyake S. and Yamamura T. (2005) Therapeutic potential of glycolipid ligands for natural killer (NK) T cells in the suppression of autoimmune diseases. Curr. Drug Targets Immune Endocr. Metabol. Disord. **5:** 315–322
18 Berkers C. R. and Ovaa H. (2005) Immunotherapeutic potential for ceramide-based activators of iNKT cells. Trends Pharmacol. Sci. **26:** 252–257
19 Tedesco-Silva H., Mourad G., Kahan B. D., Boira J. G., Weimar W., Mulgaonkar S. et al. (2004) FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in de novo renal transplantation. Transplantation **77:** 1826–1833
20 Webb M., Tham C. S., Lin F. F., Lariosa-Willingham K., Yu N., Hale J. et al. (2004) Sphingosine 1-phosphate receptor agonists attenuate relapsing-remitting experimental autoimmune encephalitis in SJL mice. J. Neuroimmunol. **153:** 108–121
21 Rausch M., Hiestand P., Foster C. A., Baumann D. R., Cannet C. and Rudin M. (2004) Predictability of FTY720 efficacy in experimental autoimmune encephalomyelitis by *in vivo* macrophage tracking: clinical implications for ultrasmall superparamagnetic iron oxide-enhanced magnetic resonance imaging. J. Magn. Reson. Imaging **20:** 16–24

22 Fujino M., Funeshima N., Kitazawa Y., Kimura H., Amemiya H., Suzuki S. et al. (2003) Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. J. Pharmacol. Exp. Ther. **305:** 70–77

23 Maki T., Gottschalk R., Ogawa N. and Monaco A. P. (2005) Prevention and cure of autoimmune diabetes in nonobese diabetic mice by continuous administration of FTY720. Transplantation **79:** 1051–1055

24 Fu F., Hu S., Deleo J., Li S., Hopf C., Hoover J. et al. (2002) Long-term islet graft survival in streptozotocin- and autoimmune-induced diabetes models by immunosuppressive and potential insulinotropic agent FTY720. Transplantation **73:** 1425–1430

25 Wijkstrom M., Kenyon N. S., Kirchhof N., Kenyon N. M., Mullon C., Lake P. et al. (2004) Islet allograft survival in nonhuman primates immunosuppressed with basiliximab, RAD and FTY720. Transplantation **77:** 827–835

26 Morecki S., Panigrahi S., Pizov G., Yacovlev E., Gelfand Y., Eizik O. et al. (2004) Effect of KRN7000 on induced graft-vs-host disease. Exp. Hematol. **32:** 630–637

27 Kimura T., Ishii Y., Morishima Y., Shibuya A., Shibuya K., Taniguchi M. et al. (2004) Treatment with alpha-galactosylceramide attenuates the development of bleomycin-induced pulmonary fibrosis. J. Immunol. **172:** 5782–5789

28 Kobayashi E., Motoki K., Uchida T., Fukushima H. and Koezuka Y. (1995) KRN7000, a novel immunomodulator and its antitumor activities. Oncol. Res. **7:** 529–534

29 Ishikawa A., Motohashi S., Ishikawa E., Fuchida H., Higashino K., Otsuji M. et al. (2005) A phase I study of alpha-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. Clin. Cancer Res. **11:** 1910–1917

30 Miyamoto K., Miyake S. and Yamamura T. (2001) A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. Nature **413:** 531–534

31 Chiba A., Oki S., Miyamoto K., Hashimoto H., Yamamura T. and Miyake S. (2004) Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosine-truncated analog of alpha-galactosylceramide. Arthritis Rheum. **50:** 305–313

32 Ueno Y., Tanaka S., Sumii M., Miyake S., Tazuma S., Taniguchi M. et al. (2005) Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of valpha14 natural killer T cells in mice. Inflamm. Bowel Dis. **11:** 35–41

33 Mizuno M., Masumura M., Tomi C., Chiba A., Oki S., Yamamura T. et al. (2004) Synthetic glycolipid OCH prevents insulitis and diabetes in NOD mice. J. Autoimmun. **23:** 293–300

34 Zemann B., Kinzel B., Muller M., Reuschel R., Mechtcheriakova D., Urtz N. et al. (2005) Sphingosine kinase type 2 is essential for lymphodepletion induced by the immunomodulatory drug FTY720. Blood [Epub, ahead of print]

35 Bandhuvula P., Tam Y. Y., Oskouian B. and Saba J. D. (2005) The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. J. Biol. Chem. **280:** 33697–33700

36 Turinsky J., O'Sullivan D. M. and Bayly B. P. (1990) 1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat *in vivo*. J. Biol. Chem. **265:** 16880–16885

37 Gorska M., Dobrzyn A., Zendzian-Piotrowska M. and Gorski J. (2004) Effect of streptozotocin-diabetes on the functioning of the sphingomyelin-signalling pathway in skeletal muscles of the rat. Horm. Metab. Res. **36:** 14–21

38 Straczkowski M., Kowalska I., Nikolajuk A., Dzienis-Straczkowska S., Kinalska I., Baranowski M. et al. (2004) Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes **53:** 1215–1221

39 Adams J. M. 2nd, Pratipanawatr T., Berria R., Wang E., DeFronzo R. A., Sullards M. C. et al. (2004) Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes **53:** 25–31

40 Summers S. A. and Nelson D. H. (2005) A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X and Cushing's syndrome. Diabetes **54:** 591–602

41 Tagami S., Inokuchi Ji J., Kabayama K., Yoshimura H., Kitamura F., Uemura S. et al. (2002) Ganglioside GM3 participates in the pathological conditions of insulin resistance. J. Biol. Chem. **277:** 3085–3092

42 Kabayama K., Sato T., Kitamura F., Uemura S., Kang B. W., Igarashi Y. et al. (2005) TNFalpha-induced insulin resistance in adipocytes as a membrane microdomain disorder: involvement of ganglioside GM3. Glycobiology **15:** 21–29

43 Zador I. Z., Deshmukh G. D., Kunkel R., Johnson K., Radin N. S. and Shayman J. A. (1993) A role for glycosphingolipid accumulation in the renal hypertrophy of streptozotocin-induced diabetes mellitus. J. Clin. Invest. **91:** 797–803

44 Masson E., Troncy L., Ruggiero D., Wiernsperger N., Lagarde M. and Bawab S. E. (2005) a-Series gangliosides mediate the effects of advanced glycation end products on pericyte and mesangial cell proliferation: a common mediator for retinal and renal microangiopathy? Diabetes **54:** 220–227

45 Wang L., Xing X. P., Holmes A., Wadham C., Gamble J. R., Vadas M. A. et al. (2005) Activation of the sphingosine kinase-signaling pathway by high glucose mediates the proinflammatory phenotype of endothelial cells. Circ. Res. **97:** 891–899

46 Geoffroy K., Troncy L., Wiernsperger N., Lagarde M. and El Bawab S. (2005) Glomerular proliferation during early stages of diabetic nephropathy is associated with local increase of sphingosine-1-phosphate levels. FEBS Lett. **579:** 1249–1254

47 Baumruker T., Bornancin F. and Billich A. (2005) The role of sphingosine and ceramide kinases in inflammatory responses. Immunol. Lett. **96:** 175–185

48 Kee T. H., Vit P. and Melendez A. J. (2005) Sphingosine kinase signalling in immune cells. Clin. Exp. Pharmacol Physiol. **32:** 153–161

49 Hinkovska-Galcheva V., Boxer L. A., Kindzelskii A., Hiraoka M., Abe A., Goparju S. et al. (2005) Ceramide 1-phosphate, a mediator of phagocytosis. J. Biol. Chem. **280:** 26612–26621

50 Subramanian P., Stahelin R. V., Szulc Z., Bielawska A., Cho W. and Chalfant C. E. (2005) Ceramide 1-phosphate acts as a positive allosteric activator of group IVA cytosolic phospholipase A2 alpha and enhances the interaction of the enzyme with phosphatidylcholine. J. Biol. Chem. **280:** 17601–17607

51 Pettus B. J., Kitatani K., Chalfant C. E., Taha T. A., Kawamori T., Bielawski J. et al. (2005) The coordination of prostaglandin E2 production by sphingosine-1-phosphate and ceramide-1-phosphate. Mol. Pharmacol. **68:** 330–335

52 Tuson M., Marfany G. and Gonzalez-Duarte R. (2004) Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). Am. J. Hum. Genet. **74:** 128–138

53 Bornancin F., Mechtcheriakova D., Stora S., Graf C., Wlachos A., Devay P. et al. (2005) Characterization of a ceramide kinase-like protein. Biochim. Biophys. Acta **1687:** 31–43

54 Acharya U., Patel S., Koundakjian E., Nagashima K., Han X. and Acharya J. K. (2003) Modulating sphingolipid biosynthetic pathway rescues photoreceptor degeneration. Science **299:** 1740–1743

55 Grassme H., Gulbins E., Brenner B., Ferlinz K., Sandhoff K., Harzer K. et al. (1997) Acidic sphingomyelinase mediates entry of N. gonorrhoeae into nonphagocytic cells. Cell **91:** 605–615

56 Hauck C. R., Grassme H., Bock J., Jendrossek V., Ferlinz K., Meyer T. F. et al. (2000) Acid sphingomyelinase is involved in CEACAM receptor-mediated phagocytosis of Neisseria gonorrhoeae. FEBS Lett. **478:** 260–266

57 Grassme H., Jendrossek V., Riehle A., von Kurthy G., Berger J., Schwarz H. et al. (2003) Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. Nat. Med. **9:** 322–330

58 Esen M., Schreiner B., Jendrossek V., Lang F., Fassbender K., Grassme H. et al. (2001) Mechanisms of Staphylococcus aureus induced apoptosis of human endothelial cells. Apoptosis **6:** 431–439
59 Hanada K., Mitamura T., Fukasawa M., Magistrado P. A., Horii T. and Nishijima M. (2000) Neutral sphingomyelinase activity dependent on Mg2+ and anionic phospholipids in the intraerythrocytic malaria parasite Plasmodium falciparum. Biochem. J. **346:** 671–677
60 Jan J. T., Chatterjee S. and Griffin D. E. (2000) Sindbis virus entry into cells triggers apoptosis by activating sphingomyelinase, leading to the release of ceramide. J. Virol. **74:** 6425–6432
61 Grassme H., Riehle A., Wilker B. and Gulbins E. (2005) Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms. J. Biol. Chem. **280:** 26256–26262
62 Finnegan C. M., Rawat S. S., Puri A., Wang J. M., Ruscetti F. W. and Blumenthal R. (2004) Ceramide, a target for antiretroviral therapy. Proc. Natl. Acad. Sci. USA **101:** 15452–15457
63 Finnegan C. M. and Blumenthal R. (2005) Fenretinide inhibits HIV infection by promoting viral endocytosis. Antiviral. Res. [Epub, ahead of print]
64 Gallo S. A., Finnegan C. M., Viard M., Raviv Y., Dimitrov A., Rawat S. S. et al. (2003) The HIV Env-mediated fusion reaction. Biochim. Biophys. Acta **1614:** 36–50
65 Rawat S. S., Johnson B. T. and Puri A. (2005) Sphingolipids: modulators of HIV-1 infection and pathogenesis. Biosci. Rep. **25:** 329–343
66 Stover T. and Kester M. (2003) Liposomal delivery enhances short-chain ceramide-induced apoptosis of breast cancer cells. J. Pharmacol. Exp. Ther. **307:** 468–475
67 Shabbits J. A. and Mayer L. D. (2003) Intracellular delivery of ceramide lipids via liposomes enhances apoptosis *in vitro*. Biochim Biophys Acta **1612:** 98–106
68 Shabbits J. A. and Mayer L. D. (2003) High ceramide content liposomes with *in vivo* antitumor activity. Anticancer. Res. **23:** 3663–3669
69 Stover T. C., Sharma A., Robertson G. P. and Kester M. (2005) Systemic delivery of liposomal short-chain ceramide limits solid tumor growth in murine models of breast adenocarcinoma. Clin. Cancer Res. **11:** 3465–3474
70 Muro S., Schuchman E. H. and Muzykantov V. R. (2006) Lysosomal enzyme delivery by ICAM-1-targeted nanocarriers bypassing glycosylation- and clathrin-dependent endocytosis. Mol. Ther. **13:** 135–141
71 Rossi M. J., Sundararaj K., Koybasi S., Phillips M. S., Szulc Z. M., Bielawska A. et al. (2005) Inhibition of growth and telomerase activity by novel cationic ceramide analogs with high solubility in human head and neck squamous cell carcinoma cells. Otolaryngol. Head Neck Surg. **132:** 55–62
72 Novgorodov S. A., Szulc Z. M., Luberto C., Jones J. A., Bielawski J., Bielawska A. et al. (2005) Positively charged ceramide is a potent inducer of mitochondrial permeabilization. J. Biol. Chem. **280:** 16096–16105
73 Dagan A., Wang C., Fibach E. and Gatt S. (2003) Synthetic, non-natural sphingolipid analogs inhibit the biosynthesis of cellular sphingolipids, elevate ceramide and induce apoptotic cell death. Biochim. Biophys. Acta **1633:** 161–169
74 Darroch P. I., Dagan A., Granot T., He X., Gatt S. and Schuchman E. H. (2005) A lipid analogue that inhibits sphingomyelin hydrolysis and synthesis, increases ceramide and leads to cell death. J. Lipid. Res. **46:** 2315–2324
75 French K. J., Schrecengost R. S., Lee B. D., Zhuang Y., Smith S. N., Eberly J. L. et al. (2003) Discovery and evaluation of inhibitors of human sphingosine kinase. Cancer Res. **63:** 5962–5969