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Introduction to Thematic Minireview Series: Novel Bioactive Sphingolipids*

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Sphingosine was named by J. L. W. Thudichum for its enigmatic properties. This descriptor has applied to sphingolipids for over a century because new enigmas continue to surface. This JBC minireview series presents articles about three novel subspecies of sphingolipids, α-galactosylceramides, 4,5-dihydroceramides, and 1-deoxysphingolipids, that have important activities but, until recently, remained undetected (or at least understudied) in the shadow of very closely related compounds. They also serve as a reminder that important metabolites still lie "off the radar screen" in reports of global and comprehensive metabolomic profiling.

Sphingolipids are one of the eight major categories of lipids (1) and are defined by the presence of a sphingoid base backbone (2, 3), with sphingosine (Fig. 1) displayed most frequently. The backbone is usually derivatized with an amide-linked fatty acid and/or a headgroup attached to the hydroxyl on carbon 1, as also depicted in Fig. 1 (4).

These compounds have long been considered to be enigmas (mysterious and puzzling riddles) beginning with the initial naming of "sphingosin" in the 1880s by J. L. W. Thudichum (5). However, a substantial number of mysteries have been clarified over the past several decades as much has been learned about their structures and biophysical properties (6–8), biosynthesis and turnover (4, 9–11), interactions with proteins (12–14), and roles in cell-cell communication and signaling (13, 15–17). So have all the enigmas been solved?

This minireview series illustrates how subtle structural features of sphingolipids still have the capacity to surprise. The articles describe findings with naturally occurring deviations from three "hallmarks" of mammalian sphingolipid structure: the β-glycosidic linkage of the first carbohydrate attached to ceramide, the 4,5-double bond of the sphingoid base, and the hydroxyl on carbon 1 of the sphingoid base (Fig. 1).

The first minireview in the series, "The alpha and omega of galactosylceramides in T cell immune function" by Birkholz *et al.* (18), describes a monohexosylceramide that differs from the predominant glycosphingolipids made by mammals in having an α-linked rather than the β-linked sugar shown in the figure. A subset of T lymphocytes called natural killer T cells (NKT cells)[2] recognizes glycolipids, predominantly α-linked glycosphingolipids, when they are bound to the cell surface protein CD1d. By activating NKT cells, synthetic α-linked glycosphingolipids can have profound effects on T cell immune responses. Structure-function studies have found fascinating contributions from the lipid backbone (19), and clinical trials of some of these derivative compounds as vaccine adjuvants are planned. Similar compounds are found in soil bacteria, and an interesting recent discovery has been that α-galactosylceramides are produced by *Bacteroides fragilis*, a prominent member of the human gut microflora (20). This expands the possible impact of these compounds beyond just their pharmaceutical (and basic research) applications into their possible roles in affecting gastrointestinal immune function and the complex relationship of the host with the microbiome. Self-glycosphingolipids with β-linked sugars are much weaker antigens, but they can also stimulate NKT cells. Recent work suggests, however, that mammalian cells may also produce small amounts of α-linked glycosphingolipids, and these may contribute to the tightly regulated but essential self-reactivity of NKT cells.

The second minireview, "Dihydroceramides: from bit players to lead actors" by Siddique *et al.* (21), discusses intermediates of *de novo* sphingolipid biosynthesis that do not have the 4,5-double bond of sphingosine (Fig. 1). Until recently, they were thought to be essentially inert (indeed, they were added to cells as controls for studies of the bioactivity of ceramides), but advanced detection techniques that facilitated the resolution of ceramides and dihydroceramides led to the surprising finding that drug effects previously attributed to ceramides were in fact driven by the dihydroceramides (22). Indeed, experimental inhibition (23) or depletion (24) of the desaturase that converts dihydroceramides to ceramides in mammalian cells revealed distinct and non-overlapping functions of these endogenous sphingolipids. Independent roles for dihydroceramides are emerging as players in autophagy, hypoxia, and metabolic control, and they have been implicated in the etiology or treatment of diabetes, cancer, and neurodegenerative diseases (24).

The third minireview, "1-Deoxysphingolipids encountered exogenously and made *de novo*: dangerous mysteries inside an enigma" by Duan and Merrill (25), describes a subcategory of sphingoid bases that lack the hydroxyl group on the first carbon (Fig. 1). These types of compounds were known to be produced by fungi and other organisms (3), and to be of health interest as mycotoxins (26) and as potential anticancer compounds that

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[2] The abbreviation used is: NKT cells, natural killer T cells.

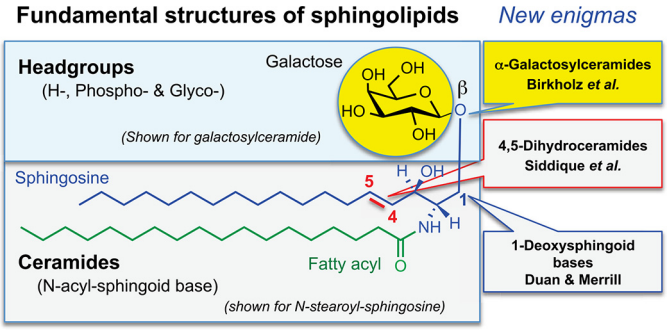

FIGURE 1. **Fundamental structures of sphingolipids and the topics discussed in this minireview series.** Sphingolipids are defined by having a sphingoid base (shown for sphingosine) that is often derivatized with an amide-linked fatty acid and/or headgroup of the general types shown.

surfaced in screens of aquatic organisms (27). Thus, it was surprising to learn that mammals can also make 1-deoxy-sphingolipids (28) because wild-type serine palmitoyltransferase can accommodate L-alanine in addition to L-serine, and furthermore, that mutations that increase L-alanine utilization and 1-deoxysphingolipid production cause sensory neuropathies (29, 30). Elevations in these bioactive compounds have also been found in diabetes (31), non-alcoholic steatohepatitis (32), and when serine biosynthesis is defective (33), and can be envisioned for other conditions where metabolic changes or diet alter the amounts.

The findings with these novel compounds illustrate that our understanding of structure and function for sphingolipids is still in its infancy. This should be kept in mind when interpreting claims of "global" and "comprehensive" profiling of the metabolome because most of the bioactive sphingolipids, including ones yet to be discovered, still lie "off the radar screen" of such analyses.

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