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Raising the Roof: The Preferential Pharmacological Stimulation of Th1 and Th2 Responses Mediated by NKT Cells

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Abstract

Natural killer T (NKT) cells serve as a bridge between the innate and adaptive immune systems, and manipulating their effector functions can have therapeutic significances in the treatment of autoimmunity, transplant biology, infectious disease, and cancer. NKT cells are a subset of T cells that express cell-surface markers characteristic of both natural killer cells and T cells. These unique immunologic cells have been demonstrated to serve as a link between the innate and adaptive immune systems through their potent cytokine production following the recognition of a range of lipid antigens, mediated through presentation of the major histocompatibility complex (MHC) class I like CD1d molecule, in addition to the NKT cell's cytotoxic capabilities upon activation. Although a number of glycolipid antigens have been shown to complex with CD1d molecules, most notably the marine sponge derived glycolipid alpha-galactosylceramide (α -GalCer), there has been debate as to the identity of the endogenous activating lipid presented to the T-cell receptor (TCR) via the CD1d molecule on antigen-presenting cells (APCs). This review aims to survey the use of pharmacological agents and subsequent structure–activity relationships (SAR) that have given insight into the binding interaction of glycolipids with both the CD1d molecules as well as the TCR and the subsequent immunologic response of NKT cells. These studies not only elucidate basic binding interactions but also pave the way for future pharmacological modulation of NKT cell responses.

Keywords

NKT cells; CD1d molecules; lipid antigens

1. INTRODUCTION

Natural killer T (NKT) cells are a unique subset of lymphocytes that serve as a link between the innate and adaptive immune system through their ability to not only recognize lipid

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antigens but also through their expeditious release of a number of different cytokines. NKT cells possess a classical $\alpha\beta$ T-cell receptor (TCR) that is able to recognize self and foreign glycolipid antigens presented via the nonclassical class I major histocompatibility complex (MHC) molecule, CD1d. Although the initial classification of NKT cells was due to their expression of cell-surface markers of both NK cells and T cells, they are officially defined as TCR $\alpha\beta$ ⁺ cells that are CD1d-restricted.¹

NKT cells have been demonstrated to play a role in autoimmune disease,² tumor surveillance,^{3,4} hematological cancers,⁵ infectious disease, and inflammatory conditions such as ischemia reperfusion injury.⁶ These effects are mediated through two defined subsets of NKT cells. Type I NKT cells (also known as invariant NKT cells, or iNKT cells) express an invariant V α 14J α 18 TCR in mice and V α 24J α 18 TCR in humans. Type II NKT cells are CD1d-restricted T cells that express a more diverse set of α chains in their TCR. The two types of NKT cells often exert opposing effects especially in tumor immunity, where Type II cells generally suppress tumor immunity while Type I NKT cells enhance antitumor immune responses.⁷ In mice, there are further subsets of NKT cell populations including CD4⁺ and CD4⁻ CD8⁻ double negative (DN); all of which have specific functions in the mediation of disease.³ In addition, there is heterogeneity within CD4⁺ iNKT cells with regard to the expression of the IL-17 receptor B (IL-17RB), a receptor for IL-25.^{8,9} The CD4⁺ IL-17RB⁺ iNKT cells have been reported to produce high levels of IL-13 and IL-4 but little IFN- γ in response to IL-25 stimulation. A recently characterized subset of iNKT cells within the NK1.1⁻ CD4⁻ subset is the retinoic acid receptor-related orphan receptor (ROR) γ ⁺ iNKT cells, which produce IL-17A and IL-22. Historically, human NKT cells have been characterized according to their expression of CD4 or lack thereof,^{11–13} however, recent studies have further defined human NKT cell subsets into CD4⁺, CD8⁺, and CD4⁻ CD8⁻ DN.¹⁴ In addition, these studies have highlighted the distinct Th1 and Th2 cytokine profiles of these subsets. CD4⁺ NKT cells produce Th1 and Th2 cytokines, whereas the CD4⁻ subset, which includes both CD8⁺ and DN, primarily produces Th1 cytokines. It has been reported that NKT cells from human peripheral blood mononuclear cells make up 0.089% of the lymphocyte population in the blood.¹³ Studies from our lab have found that NKT cells in healthy donors typically range from 0.04—to 1.3% of the circulating lymphocytes. Notably, studies from our lab and others have found that the percentage of human NKT cells in the peripheral blood is highly variable. Most of these circulating NKT cells express CD161, and a small percentage of NKT cells express CD56. In addition, 49% of the NKT cells are DN, 27% CD4⁺, and 24% CD8⁺. Interestingly, NKT cells are phenotypically similar to effector T cells because they express nonlymphoid tissue homing chemokine receptors such as CCR2, CCR5, and CXCR3.

Perhaps the most intriguing aspect of NKT cells is their ability to generate both Th1 and Th2 responses. Following activation, NKT cells produce Th1-type cytokines, including IFN- γ and TNF- α as well as Th2-type cytokines, including IL-4 and IL-13, and at least in mice, they appear to constitutively express mRNA for these cytokines, even before activation with exogenous antigens. Moreover, individual NKT cells are able to make both Th1- and Th2-type cytokines simultaneously following stimulation *in vivo*. Due to their ability to rapidly produce a diverse array of cytokines NKT cells can, under certain conditions, polarize the

immune response in a Th1 direction, while in other cases NKT cells drive Th2 responses. In addition, these lymphocytes have been reported to be immunosuppressive in some systems, usually via their production of the Th2-type cytokines, while in other systems, they appear to promote enhanced cell-mediated immunity via production of Th1-type cytokines. The mechanisms that determine the cytokine polarity of the NKT cell response and the influence of the NKT cell response in the context of initiating or regulating the host's innate and adaptive immune system are still not well understood.¹⁵ IL-12 has been shown to stimulate IFN- γ and CD40L expression on NKT cells, which is then responsible for downstream production of additional IL-12 and IFN- γ by antigen presenting and cytotoxic cells, generating a pro-inflammatory environment.¹⁶ Conversely, exogenous IL-7 can elicit IL-4 and IL-13 production to induce a Th2 response.¹⁷

NKT cells have been described as Th0 cells in some situations because of their ability to secrete both Th1 and Th2 cytokines.¹⁸ In experimental settings, the prototypical glycolipid antigen used is the marine sponge-derived α -galactosylceramide (α -GalCer; Fig. 1). When administered in vivo or in vitro, α -GalCer stimulates a mixture of Th1- and Th2-type cytokine responses from NKT cells.¹⁹ Because of the wide range of disease states that can benefit from this type of immunologic response, α -GalCer has been investigated in regards to its therapeutic abilities, most notably in Phase I trials for solid tumors in humans.²⁰ However, due to the varied Th1/Th2 response induced by α -GalCer and its tendency to induce anergy in NKT cells,²¹ there has been a push in the medicinal chemistry literature to find pharmacological agents that can modulate the strength of response mediated by NKT cells and specifically induce either Th1 or Th2 responses. This review aims to look at the structure–activity relationships (SAR) of glycolipid antigens in addition to the efforts made to modulate cytokines responses upon stimulation of NKT cells, specifically in relation to preferential Th1/Th2 cytokine profiles (Table 1). These studies are invaluable toward utilizing NKT cells as a therapeutic modality.

2. BINDING OF GLYCOLIPIDS TO CD1d AND PRESENTATION TO EFFECTOR CELLS

CD1 proteins are a distinct lineage of antigen-presenting molecules that function in an analogous fashion to the classical antigen-presenting MHC molecules; however in contrast to MHC molecules that present peptide antigens, CD1 molecules have evolved to present lipid and glycolipid antigens to T cells.²² The human CD1 locus contains five distinct genes, CD1A–E, which have an overall structure similar to class I MHC including their association with β 2-microglobulin. CD1 proteins are divided into two groups: group 1 CD1 (CD1a, CD1-b, and CD1-c) and group 2 CD1 (CD1d). The human *cd1e* gene shares characteristics of both groups, thus it is considered to be intermediate. While Group 1 and 2 CD1 molecules are found on the cell surface, CD1e is found intracellularly and has been shown to aid in the loading of antigen in endocytic compartments.²³ There have been no studies reported on any mammalian species that entirely lacks CD1 genes, although the number and complexity of CD1 molecules vary between species. For example, there are only two murine CD1 genes, *CD1d1* and *CD1d2*. The two murine genes are arranged in opposite orientations and they share 90–95% sequence identity, which suggests an evolutionarily recent gene duplication

event.²⁴ However, only *CD1d1* is functional in mice and is referred to as CD1d. Group 1 CD1-restricted human T cells can recognize mycobacterial cell wall lipids and are elicited as part of the immune response to *Mycobacterium tuberculosis*. Notably, while Group 1 CD1 proteins are expressed, it was believed that CD1d molecules and NKT cells were lacking in ruminants.^{25,26} However, a recent study by Nguyen et al. has shown that although the two bovine *CD1D* genes have structural features that suggest that they cannot be translated into functional proteins, bovine CD1d molecules are expressed on the cell surface and are indeed functional.²⁷ The authors provide additional evidence that demonstrate that bovine CD1d molecules present lipid antigens that are smaller than α -GalCer. This review will focus on CD1d-restricted NKT cells.

CD1d molecules are composed of heterodimers comprised a heavy chain containing three α domains, and a β 2-microglobulin subunit. CD1d molecules are synthesized in the endoplasmic reticulum (ER) and form a complex with β 2-microglobulin. In the ER, CD1d associates with endogenous glycolipids, such as glycosylphosphatidylinositol (GPI)²⁸ and phosphatidylcholine (PC).²⁹ The CD1d-antigen complex traffics through the Golgi complex and onto the cell surface. These GPI-loaded molecules can be recognized by noncanonical, type II NKT cells, but not by canonical or type I *i*NKT cells. A Tyr-based endosomal targeting sequence (YXX Φ) causes the CD1d-antigen complex to reenter the cell and traffic to endocytic compartments, where an endogenous “placeholder” antigen can be exchanged for an activating antigen.^{30–33}

Although MHC class I and MHC class II molecules present highly specific antigens (nonameric or 10–24 amino acid peptides, respectively) to T cells, CD1d molecules present a promiscuous lipid antigen repertoire. This arises due to a difference in antigen-binding grooves in terms of both size and amino acid hydrophobicity. MHC antigen presenting molecules have a shallow binding groove populated with a rich diversity of hydrophobic and hydrophilic residues that facilitates the wide variety of possible peptide antigens that the host may encounter during its lifetime. In contrast CD1d, and to some degree all CD1 molecules, possess a deep binding groove that can accommodate the lipid tails of glycolipids. For α -GalCer, and indeed all other glycosphingolipids analyzed, the phytosphingosine tail is contained in the F' groove of CD1d, while the amide-linked fatty acid is placed into the A' pocket (Fig. 2).³⁴ The sugar moiety is exposed to solvent until it binds to the invariant TCR of NKT cells. Because of the hydrophobic and relatively accommodating size of the F' and A' pockets, CD1d is able to recognize a variety of glycolipid substrates.

TCR interaction with the CD1d-antigen complex is the final interaction that must take place in order to elicit the diverse functions of NKT cells through the TCR pathway. SAR and computational modeling of α -GalCer interactions with the NKT TCR revealed that when the 2-, 3-, or 4-hydroxyl groups of the galactose were modified by either truncation, substitution, or installment of additional sugars, activation of NKT cells was eliminated or diminished,^{35–37} while modifications of the 6 OH were tolerated (vide infra).³⁸ This leads to the unsurprising observation that even minor modifications to the sugar group can cause profound affinity differences in antigen/CD1d interaction with the TCR.³⁹

NKT cell TCR interactions with the CD1d molecule and antigen have been elucidated based on crystal structures of the ternary complex of human CD1d- α -GalCer-NKT TCR^{40,41} and crystal structures of other α -GalCer derivatives in simultaneous complex with mouse CD1d and NKT cell TCR.⁴² In all cases, there is a conserved binding mode where the CDR1 α loop of the invariant TCR α chain interacts with the lipid antigen and CD3 α binds both lipid antigen and CD1d. The β portion (V β 8, V β 7, V β 2 in mice, and V β 11 in humans) of the TCR interacts with CD1d via the CDR2 β loop. In general, these interactions appear to hold true for both human and mouse ternary interactions, although the β subunit can impart slightly different conformational changes that may alter antigen specificity.⁴³ Amazingly, when comparing the different conformations of CD1d containing the “placeholder” antigen versus CD1d bound with α -GalCer, there is essentially no change in spatial arrangement of the residues in CD1d, implying a lock-and-key mode for antigen binding. Conversely, there have been recently published crystal structures of CD1d with nonnatural antigens that demonstrated an induced-fit model for CD1d and antigen, and this conformational change skewed NKT cells toward a Th1 response. The authors suggest that this change from a lock-and-key mode of binding to an induced-fit model can be utilized to alter Th0, Th1, and Th2 responses in NKT cells.⁴⁴ After CD1d-antigen engagement, the NKT TCR initiates classical intracellular TCR signaling cascades beginning with ZAP-70 and Lck.

3. ANTIGEN STIMULATION OF NKT CELLS

Despite the conserved nature of the NKT TCR, NKT cells are capable of recognizing a range of antigens, including phospholipids,⁴⁵ vinyl ether-linked phospholipids,⁴⁶ α -linked, and β -linked glycolipids (Fig. 1).⁴⁷ α -Linked glycolipids are seen as being indicative of a microbial presence as α -linked sugars are not seen in normal mammalian metabolic functions. The β -linked antigens are capable of being recognized because the NKT cell TCR and CD1d force the β -linked sugars to adopt a flattened “ α -like” conformation.⁴⁸ This induced torsional strain in polyglycosylated lipids such as isoglobotrihexosylceramide (iGb3), which is compensated by the interaction of the terminal sugar with the CD1d molecule. The impressive scope of antigens has been reviewed elsewhere.^{45,47}

4. STRUCTURE–ACTIVITY RELATIONSHIPS OF α -GalCer

Since the discovery of the prototypical NKT agonist α -GalCer (KRN 7000) resulting from a medicinal chemistry endeavor⁴⁹ performed around glycolipid natural products⁵⁰ from the marine sponge *Agelas mauritanus* nearly two decades ago, there has been a flurry of interest into its diverse SAR. And yet, only the crudest of structural predictions can currently be made regarding the biological effects of α -GalCer analogs. Several review articles have dissected the SAR of α -GalCer by structural region, namely the polar galactose head group, sugar anomeric configuration, polar ceramide core, and two highly lipophilic tails.^{51–53} Here, analogs of α -GalCer shall be classified by the cytokine profile they elicit.

A. The Th1/Th2 Axis

The driving forces behind NKT cell-induced Th1 and Th2 responses are many and intricate. The first consideration is the avidity of binding between lipid and CD1d, the molecule. A more intimate binding of lipid antigen and CD1d allows for a longer interaction with the

NKT TCR, allowing for a skewing toward a Th1 cytokine profile. These longer association times between lipid and CD1d do not seem to affect the binding affinity for the TCR, but rather the duration of the response. Th2 responses seem to arise from not only a shortened antigen interaction time with CD1d, but also from a reduced requirement of intracellular loading of lipid in the endosome.⁵⁴ Conversely, the more hydrophobic lipid antigens, such as α -GalCer-C26:0, can be loaded onto CD1d and then directed to lipid-rafts on the cell's outer membrane. Truncated α -GalCer analogs such as OCH are less discriminately loaded on the cell surface onto CD1d and less likely to reside in a lipid raft, thus altering interaction and activation of antigen-presenting cell (APC) and NKT cell. This type of lipid raft-dependent Th1/Th2 bias has been demonstrated in the analogous MHC class I and MHC class II molecules.⁵⁵ Additionally, cytokine profiles after NKT stimulation may not be driven merely by the TCR stimulation and local release of cytokines, but also by the downstream interaction with other immune cells.⁵⁶ As a result, many efforts have been made to utilize these differences in antigen-binding characteristics to develop small molecules that elicit a biased Th1 or Th2 response (Fig. 3). In the case of α -GalCer, cytokine responses can also be modified depending on the APCs involved.⁵⁷

B. Structural Features Necessary for α -GalCer-Mediated NKT Activation

The α -anomeric configuration of galactose to ceramide is the most intriguing structural quality of α -GalCer primarily because such linkages are rare if not nonexistent in mammalian cells, and yet essential for galactose ceramide activity (Fig. 4). Early SAR studies focused on the identity of the sugar moiety, and concluded that the α -linkage was necessary for the immunostimulative effects.^{49,50,58–60} These studies also examined the stereochemistry and substitution of the sugar hydroxyl groups, and found that galactose is preferred. The hydroxyl group at position 2 is the most resistant to change—with its methylation, stereo inversion, or fluoride substitution greatly diminishing NKT activation.^{58,61,62} Crystal structure data have illuminated the importance of the 2-OH in mediating important hydrogen bonds to Asp151 of the α 2 helix of CD1d and Gly96 of the CDR3 α loop of the TCR.^{36,40,63–66} Moreover, the 2-OH is the least exposed hydroxyl of the sugar to bulk water, reinforcing the strength and importance of these hydrogen bonds for TCR recognition. The equatorial hydroxyl group at the 3 position also responds poorly to substitution but the condensation of a sulfate ester is well tolerated and produces NKT cell stimulation similar to α -GalCer.⁶⁷ The axial stereochemistry of the hydroxyl at the 4 position of galactose is preferred, but not necessary for detectable activity, and α -glucosylceramide has been shown to possess some activity.⁵⁸ The 6 position is the most exposed to bulk water, and several studies have shown that derivation at this position is well tolerated.^{38,44,68–73}

The glycolipid antigen must bind to CD1d for TCR activation and a majority of the CD1d– α -GalCer-binding energy comes from the desolvation of the highly lipophilic phytosphingosine and ceramide chains binding to the F' and A' pockets, respectively (Fig. 2A).⁴⁰ Therefore, it is not surprising that there are minimum length requirements for each of the two alkyl chains to elicit NKT activation. Additionally, the phytosphingosine tail, which binds between the α 1 and α 2 helices, acts to raise the roof of the of the F' pocket (zone), which plays a major role in TCR recognition.⁷⁴ The spatial relationship between the lipid

tails and the sugar head group affects NKT activation and cytokine profile, and the 2',3',4'-acyl amino diol of the ceramide backbone responsible for their presentation has been an intense region of derivation. Amide⁷⁵⁻⁷⁷ and hydroxy^{78,79} isosteres, stereo,^{80,81} deoxy compounds,^{60,82} and rigid analogs⁸³ have shown that there is no substitute for the 3-hydroxyl group. The elimination or substitution of the 3-OH breaks a hydrogen bond to the nearby Asp80 of CD1d, which may be involved in glycolipid loading and can affect antigen presentation. The substitution of the 3-OH with an amine group to enhance electrostatic contacts yielded an increased affinity, but affected antigen presentation leading to a reduction in cytokine production.⁷⁹ The 4-OH is far less critical and the 4-deoxy analog of α -GalCer is equipotent to the parent. The amide proton participates in the stabilization of the sugar head group by hydrogen bond donating to the alcohol of Thr154 of the α 2 helix of CD1d, which in turn stabilizes the position of Asp151 creating a hydrogen bond network affecting galactose presentation (Fig. 2B).

C. α -GalCer Analogs That Elicit a Th1 Cytokine Profile

The general rule of thumb is that the ratio of Th1 to Th2 cytokines is dependent on stability of the CD1d–glycolipid–TCR ternary complex.⁵² Glycolipids with slower dissociation rates from CD1d have an increased IFN- γ /IL-4 ratio, skewing the cytokine profile toward a more pronounced Th1-biased response. Within 2 hr of TCR activation by α -GalCer, NKT cells begin secreting large concentrations of IL-4, which is triggered through Notch-regulated conserved noncoding-2 (CNS-2) enhancer activation, but this spike in Th2 cytokine release quickly subsides.⁸⁴ Only after prolonged TCR activation does the cytokine profile shift toward a Th1 response, with a peak in IFN- γ production following 16 hr of stimulation. The stability of the α -GalCer-CD1d binary complex, with a half-life in excess of one day, may then explain the Th1 profile bias observed for α -GalCer. Groups intent on maximizing a Th1 cytokine response as immunostimulants or anticancer therapies, therefore attempt to generate analogs of α -GalCer that bind more tightly to CD1d in order to maximize IFN- γ production.

The ostensible relationship between lipid-CD1d stability and Th1 signal enhancement is a workable hypothesis for medicinal chemists to strengthen. There are several regions of α -GalCer that can be modified to enhance lipid-CD1d avidity (Fig. 4). Wong and co-workers have developed aryl-containing analogs that favor a Th1 cytokine profile (Fig. 5).^{68,85-87} The terminal phenyl groups interact favorably with aromatic residues in the lipophilic A'- and F'-binding regions of CD1d meant to accept the two lipid tails of the phytosphingosine and ceramide alkyl chains and presumably reduce the entropic cost of binding.^{85,86,88} Polarizing the terminal arenes with electron withdrawing groups for donating substituents at the para positions only enhanced affinity for CD1d and for a Th1-biased cytokine release.⁸⁷ Members of this aromatic library outperformed α -GalCer in murine breast and lung cancer models, and stand as primary evidence for the potential of Th1-biased α -GalCer analogs being therapeutically feasible as immunostimulating agents.⁶⁸ Interestingly, there were very minor conformational changes in regards to the CD1d and galactose orientation thus suggesting that the Th1 bias was derived solely because of the longer association time of the antigen.^{86,89} More recently, a large library of compounds with a various positioning of phenyl groups in the fatty acid region showed a sizeable IFN- γ :IL-4 and IFN- γ :IL-10 ratio in

mice and humans, in addition to higher avidity and stability with the NKT TCR.⁹⁰ These skewed cytokine responses resulted in a greater reduction of solid tumor in a murine melanoma model. The stability gained by the addition of a terminal aryl ring also allows for the deletion of the 3'- and 4'-hydroxyl groups without loss of activity.⁹¹

The 6 position of the galactose can easily be modified without loss of activity, because the hydroxymethyl present in α -GalCer does not form any major contacts with either CD1d or the TCR. Replacement of the hydroxymethyl with a carboxylate, an ethyl group, or by capping to formamethyl ether yields an increased Th1 response (Fig. 5).^{68,72} These substitutions have been hypothesized to function by removing an intramolecular hydrogen bond being donated from the 6 position to the axial 4-OH of galactose, and modeling suggests increased electron density on the 4-OH oxygen may strengthen TCR interactions. The discovery that aryl amide and aryl urea substitution at the 6 position of galactose yields Th1 selectivity opens a new avenue for structural diversity (Fig. 5). A crystal structure of NU- α -GalCer bound to mCD1d shows that urea acts as a linker to present the naphthalene into a previously unutilized pocket between the α 1 and α 2 helices of CD1d (Fig. 6A and B).⁴⁴ This region of mCD1d does not make contact with the TCR, but these interactions stabilize the glycolipid-mCD1d complex leading to longer TCR stimulation, an increased IFN- γ :IL-4 ratio, and the outperformance of α -GalCer in a B16 melanoma lung metastasis model. However, because the naphthyl urea binds a pocket of mCD1d that does not interact with the TCR, this anchor pocket is less conserved than other areas of the antigen-presenting domain. hCD1d has bulkier amino acid residues that form a smaller anchor pocket (mMet69 $\rightarrow$ hIle69, mGly155 $\rightarrow$ hTrp153, and mMet162 $\rightarrow$ hTrp160), which may explain why NU- α -GalCer performs somewhat less profoundly in human NKT cells. One can easily imagine using the induced-fit structure of mCD1d bound with NU- α -GalCer to model hCD1d and design ligands with anchor substituents that are better suited for human biological activity.

Much attention has been given to the nature of the O-glycosidic bond in α -GalCer, and C (sp, sp², and sp³),⁹²⁻⁹⁴ S,^{95,96} N,⁹⁷ and oxime⁹⁸ glycosides have been synthesized and evaluated. Of these, only the C-linked glycosides have yet shown much promise. α -C-GalCer, a direct replacement of a methylene for the glycosidic oxygen, produces a strongly Th1-biased cytokine response in vivo, and like NU- α -GalCer is more effective than α -GalCer in a murine B16 melanoma lung metastasis model.⁹² Kinetic studies show that α -C-GalCer has a longer half-life with efficacies up to 4 days post injection compared to the single day efficacies of α -GalCer, which is most likely due to the resistance of C-glycosides to α -galactosidases.⁹⁴ However, α -C-GalCer underperforms in vitro with marginal cytokine production in human NKT cells.⁹² This discrepancy between murine and human activity is believed to arise from differences in the TCRs rather than the affinities of α -C-GalCer for mCD1d and hCD1d, but is yet poorly understood. Chemically, differences in galactose presentation between the crystal structures of α -GalCer and α -C-GalCer bound to CD1d are twofold: The C-O bond lengths of the glycosidic bond and the first bond of the sphingosine backbone are 8-9% longer compared to same bonds in α -C-GalCer due to the increased strength of the C-O bonds versus C-C bonds and the dihedral angle of the O-glycosidic bond adopts a more sterically favored configuration compared to the more eclipsing arrangement for α -C-GalCer (Fig. 7).^{40,44} The dihedral angle of the O-glycosidic bond is

stabilized to a preferred arrangement due to the stereoelectronic anomeric effect; thus, rotation into a pseudoeclipsing conformation, as seen in the crystal structure for α -C-GalCer, is more costly for α -GalCer and does not occur. The distance and angle distortion between the galactose and ceramide backbone caused by the C-glycosidic linkage must be drastic enough to lead to a loss in human NKT cell activation. Vinyl analogs by the research groups of Franck and Tsuji mitigate these conformational issues.⁹² The shortened carbon E double bond (the Z configuration was inactive) in GSK127 and GSK152 restores some human NKT cell activity. These rigid analogs provide insight into the sensitivity of the glycosidic region in the selectivity of murine or human NKT activation. GSK152 was the most active vinyl-linked glycolipid tested in human NKT cells, but had no measurable activity in murine in vitro or in vivo assays. Conversely, GSK127 was less effective in inducing human NKT activation, but elicited greater in vivo IFN- γ cytokine production than α -GalCer in mice. α -C-alkyne-GalCer has also been synthesized and has been demonstrated to possess near equal affinity to CD1d as α -C-GalCer, however, in vivo activity was reduced comparatively.^{93,99}

Carbon replacement of the pyran oxygen in the galactose ring produces potent cyclitol derivatives with a strong Th1 profile as well.¹⁰⁰ RCAI-56, a direct deoxy derivative of α -GalCer, shows comparable IL-4 release to α -GalCer, but produces high levels of IFN- γ that peak between 30 and 40 hr after administration in mice in vivo. In addition, 6-OH modifications to the galactose that result in an increased Th1 bias in α -GalCer-mediated responses have also been shown to function similarly for this cyclitol framework. Aminocyclitols have been demonstrated to be effective carbohydrate replacements,^{101–103} and have thus been used as galactose mimetics in α -GalCer derivatives.^{104,105} Modeling of the aminocyclitol/CD1d complex suggests that the amine attached to the six-membered ring, which is protonated at physiological pH, mimics the anomeric oxygen of the galactose ring in α -GalCer and forms a coulombic interaction with Asp151. The binding mode of aminocyclitol HS138 is interesting in that it better adopts a gluco-rather than a galacto-configuration when bound to CD1d. This observation of conformation can account for the reduced affinity for CD1d for this lipid when compared to the NKT activity of the related α -glucosylphytoceramide.⁵⁸ In attempts to find compounds that adopted a more galacto-configuration, another aminocyclitol HS161 with a hydroxyl in the 6' position (the position usually occupied by the anomeric oxygen of α -GalCer) was synthesized and found to have a pronounced effect on NKT cell IFN- γ production (Fig. 5). Conversely, the incorporation of a hydroxyl group in the 6' position in compound HS138 showed a reduced interaction with the NKT cell TCR, which is presumed to be due to an unfavorable interaction with Pro28 of the V α portion of the NKT TCR. Other Th1 stimulating sugar derivatives forgoing an anomeric oxygen include carbacyclic compounds RCAI-RCAI-101¹⁰⁶ and cyclitol compounds RCAI-59, RCAI-60, and RCAI-92.¹⁰⁷

α -C-GalCer and its derivatives stand as an exception to the relationship between glycolipid-CD1d stability and Th1 bias, since it elicits an enhanced Th1 cytokine profile despite a faster dissociation rate.⁵⁶ One way this is currently rationalized is that the enhanced bioavailability caused by the biochemically stable C-glycosidic bond allows for prolonged TCR activation by an increased pool of C-glycoside in vivo. This reasoning has also been

employed to explain a similar discrepancy between in vitro and in vivo activity of aminocyclitol analogs, such as HS161.⁹⁷ The absence of the pyran oxygen makes these N-glycosides resistant to α -galactosidases. Whatever the reason, drug development programs interested in furthering Th1 cytokine bias and longevity should show interest in such scaffolds. Studies are indubitably underway examining the additive effects of C-linked analogs with aromatic substituents in the ceramide tail and at the 6 position of galactose to produce more potent analogs as potential anticancer and immunostimulating therapies.

D. α -GalCer Analogs That Elicit a Th2 Cytokine Profile

Although stabilizing the CD1d–glycolipid–TCR complex favors a Th1 response, destabilizing the ternary complex tips the balance back toward a Th2 cytokine profile and potential immunomodulatory therapeutic effects. This takes advantage of the rapid release of IL-4 from activated NKT cells, while subsequent breakdown of the ternary complex reduces IFN- γ production. Analogs that fall into the Th2 category often do so by weakening and subsequently hastening the glycolipid–CD1d dissociation, while maintaining proper antigen presentation (Fig. 8).

Much work has been done concerning modifications of the lipid chain of α -GalCer derivatives. Lengthening the acyl and phytosphingosine chains of α -GalCer induces higher IFN- γ production, relative to IL-4 levels, in both murine and human NKT cells.¹⁰⁸ Further studies concerning truncated and low-potency α -GalCer analogs suggested that these weak NKT agonists would be useful for stimulating a Th2 response due to their high levels of IL-13 secretion in human NKT cells but not IL-4.¹⁰⁹ These low-potency compounds are also attractive as it has been shown that high avidity lipids can induce anergy in NKT cells.^{21,110} In both cases, the Th2 responses are thought to be the result of shorter stimulation through the NKT TCR due to a reduced interaction between the antigen–CD1d complex and the NKT TCR, and have been seen with structural modifications of groups other than those in the lipid region.^{111,112}

Lipid tail truncation of either the sphingosine or ceramide chains has been correlated with decreased CD1d affinity and Th2 bias and is well documented.^{52,108,113} OCH contains a C-9 phytosphingosine core and is the best representative from the truncated libraries, and prevents the development of the autoimmune disease experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (Fig. 8).^{114–116} The shorter phytosphingosine chain reduces the affinity of the OCH for CD1d, while maintaining TCR stimulation. This might seem surprising since the phytosphingosine tail appears to play a role in lifting the F' pocket roof prior to α -GalCer-mediated TCR activation. Crystal structures of OCH bound to CD1d indicate the presence of a recruited short chain fatty acid bound with OCH in the F' pocket, functionally raising the roof, and allowing for NKT activation until OCH dissociates from the CD1d–low MW fatty acid complex (Fig. 6C).^{42,56} C20:2 is truncated only by six carbons on the acyl chain, yet produces a pronounced Th2 bias, releasing more IL-4 than α -GalCer.¹¹⁴ The activity of C20:2 lies in the Z double bond configurations, which provide C20:2 with an increased ability to be rapidly loaded onto CD1d.⁵⁴ Once loaded, the bent configuration of the ceramide tail matches well to the curvature of the A' pocket (Fig. 2A).^{42,88}

Long-chained α -GalCer analogs have also been discovered that draw out a Th2-biased cytokine profile. Lee et al. found that 1,2,3-triazoles are suitable isosteres for the amide present in the ceramide backbone (Fig. 9).⁷⁷ Modeling studies showed that the triazole is capable of hydrogen bond accepting from the nearby Thr154. This is notable because the amide in α -GalCer hydrogen bond donates to Thr154. By accepting a hydrogen bond from Thr154 the alcohol is no longer capable of stabilizing Asp151, and the interaction between Asp151 and the 2-OH of galactose is predicted to break. It seems likely that by breaking this hydrogen bond to CD1d, the triazole glycolipids weaken their association with CD1d causing a Th2 profile.

This hypothesis is reinforced by the replacement of the amide with an ester function group, which can only hydrogen bond accept and also produced a Th2-biased profile.⁷⁶ Conversely, α,α -difluoro substitution of the amide should enhance the amide hydrogen bond to Thr154 to stabilize the glycolipid-CD1d complex, yet this derivative produced a Th2 bias.⁷⁵ It is unclear why the difluoro derivative elicits such a cytokine profile, but it is also unclear how the amide is orientated during binding and whether the amide is still in position to hydrogen bond donate effectively. It will be of interest in the future to examine how other nontriazole heterocycles perform as amide isosteres.

There continues to be a healthy interest in developing either a primary or adjunct immunomodulatory glycolipid therapy utilizing the early Th2 cytokine preference of activated NKT cells. Several α -GalCer analogs such as OCH and C20:2 show promise, and might be aided by structural modifications at the amide position of the ceramide backbone. Adding water solubility in general appears to favor a Th2 profile, and substituting the 6 position of the galactose with amide-linked PEG increases bioavailability while most likely raising the dissociation constant by stabilizing the glycolipid in water.¹¹⁷ However, translating activity to human NKT cells remains exceedingly hard to predict, where the performance of C20:2 was copacetic and OCH failed to stimulate a Th2 profile cytokine profile.¹¹⁸

5. EXOGENOUS ANTIGENS

The existence of a highly conserved TCR that detects antigens from a sea sponge led researchers to the hypothesis of, and subsequent search for, more relevant naturally occurring ligands. The role of NKT cells in bacterial infections has largely been elucidated through homozygous knockouts of the J α 18 (formerly known as J α 281) segment of the NKT cell invariant TCR α chain. Treatment of mice with anti-CD1d antibody before challenge and CD1d^{-/-} animals are also used, although results in the loss of both Type I and Type II NKT cells, compared to J α 18^{-/-}, which are only deficient in Type I NKT cells.¹¹⁹ Infection with *Listeria monocytogenes* of mice pretreated with anti-CD1d monoclonal antibodies showed a reduction in listeriosis.¹²⁰ This was attributed to the reduction of Th1 cytokines produced by NKT cells. J α 18^{-/-} mice have also shown an increase in bacterial load and a reduction in both survival rate and level of TNF- α induced upon *Streptococcus pneumoniae* challenge versus wild-type mice.¹²¹ *Pseudomonas aeruginosa* infection was increased in CD1d^{-/-} mice, compared to wild-type controls, and was marked by increased bacterial load in the lungs as well as a reduced neutrophil count in the bronchoalveolar

lavage fluid in the knockout mice.¹²² Infection with *Borrelia burgdorferi*, the causative agent of Lyme disease, showed indications of arthritis in CD1d^{-/-} mice as evidenced by a thickening of the knee and tibiotarsal joints.¹²³ Notably, an increase in arthritis has been reported in J α 18^{-/-} mice on a BALB/c background¹²⁴ and increased carditis has been found in J α 18^{-/-} on the C57BL/6 background.¹²⁵ Gram-negative, lipopolysaccharide-negative bacteria including *Sphingomonas capsulata* and *Ehrlichia muris* have been shown to stimulate NKT cell expansion in a CD1d-dependent, Toll-like receptor (TLR)-independent fashion.¹²⁶ NKT cells do not always play a protective role in bacterial infections as they have been shown to favor a Th2 cytokine profile upon interaction with *Chlamydia trachomatis*, which resulted in increased pathogenesis.¹²⁷

Although mammalian glycolipids are exclusively β -linked to ceramide, there are examples of bacterial α -linked antigens that activate NKT cells. *Sphingomonas*, prevalent Gram-negative bacteria in both terrestrial and aquatic environments, have a diverse set of α -linked glucuronosyl and galacturonosylceramides that cause NKT activation at higher doses, such as GSL-1 (Fig. 9).^{126,128, 129} These galacturonosylceramides are currently the most similar bacterial antigens to the α -GalCer family, and septic shock was induced in a murine model due to cytokine release via NKT cell activation. A crystal structure of GSL bound to mCD1d not surprisingly reveals a binding position very similar to that of α -GalCer.¹³⁰

α -Galactosyldiacylglycerols isolated from *B. burgdorferi* have also been shown to bind CD1d and activate human NKT cells.¹³¹ Notably, these nonglycosphingolipid derivatives, such as BbGL-IIc, cause IL-2 secretion and NKT cell proliferation, but do not induce strong IFN- γ or IL-4 cytokine release (Fig. 9). BbGL-IIc lacks an amide to hydrogen bond donate to Thr156 and the phytosphingosine 3'- and 4'-hydroxyl groups to hydrogen bond donate to Asp80 of CD1d. A crystal structure of BbGL-IIc bound to mCD1d shows that these interactions are in fact lost (Fig. 10).¹³² More interesting, however, is the relative switch between which acyl chain that occupy the A' and F' pockets of CD1d for BbGL-IIc compared to α -GalCer. The ceramide chain of α -GalCer branches off the 2 position of the phytosphingosine backbone and fills the A' pocket, while the phytosphingosine turns to fill and raise the roof of the F' pocket (Fig. 2). The opposite occurs for BbGL-IIc, with the sn-2 chain that branches off the 2 position filling the F' pocket, and the backbone sn-1 acyl chain curling into the larger A' zone. While this explains the preference for the unsaturation in the sn-2 chain, the crystal structure suggests this may rotate the galactose to a position unfavorable for antigen presentation.¹³² Modeling indicates that the galactose can rotate and form the necessary interactions with CD1d prior to TCR recognition; however, this would suggest an induced-fit model, and that the energetic cost of binding would need to be paid prior to ternary complex formation.¹³¹ Diacylglycerols can bind with the sn-2 position in either the F' pocket or the A' pocket. In regards to the *S. pneumoniae* antigen, it has been shown that sn-2 is in the A' pocket.^{133,134} This is because diacylglycerols lack the extensive set of hydrogen bonds that help to lock in glycosphingolipids, and therefore show more flexible binding.

Phospholipids have also been shown to activate NKT cells in a CD1d-dependent manner, the most studied being a tetramannosylated form of phosphatidylinositol isolated from *Mycobacterium bovis* known as PIM4 (Fig. 9).¹³⁵ PIM4 was significantly less active than α -

GalCer, however, there was a Th1-biased cytokine profile, activating the NKT cells against an infection. The crystal structure of PIM2, a dimannoside derivative, illustrates that the glycerol backbone binds to CD1d similarly to BbGL-II, with the sn-1 and sn-2 chains filling the A' and F' pockets, respectively (Fig. 10). The inositol head group does not match the position of the galactose ring in α -GalCer. First, the phosphate linker is chelated by Arg79 and Ser76, which lifts the inositol up and out of position. This phosphate chelation appears to be common for phospholipids in general, as a crystal structure of PC bound to CD1d shows the phosphate acting in the same way.⁶³ Second, the inositol is linked through an equatorial hydroxyl group distorting its presentation to the TCR relative to α -GalCer. A ternary crystal structure of CD1d–PIM2–TCR would be invaluable in determining the induced fit of the inositol-mannoside for TCR recognition. Other phospholipids isolated from *Leishmania donovani* and *Entamoeba histolytica* known to activate NKT cells could presumably act under a similar mechanism.^{136,137}

A 6-acyl-cholesteryl- α -glucoside (PI57) isolated from *Helicobacter pylori* elicits NKT activation in a CD1d-dependent manner.¹³⁸ PI57 has been shown to protect mice from airway hyperactivity caused by influenza A infection, illuminating the potential of a glycolipid therapeutic strategy for asthma (Fig. 9).¹³⁹ It is not currently well understood how PI57 functions, however, it has been demonstrated to break up lipid rafts. Porcelli and co-workers have pointed out that antigens requiring intracellular loading to CD1d accumulate on lipid rafts, whereas antigens that load more easily, such as α -GalCer-C20:2, are located outside of lipid rafts.^{140,141} There may be a connection between CD1d–antigen presentation on or off lipid rafts to Th1 versus Th2 bias, and this connection may go a long way in adding insight into glycolipids with biases currently unexplained by the CD1d affinity arguments.¹⁴¹

6. ENDOGENOUS ANTIGENS

Isoglobotrihexosylceramide (isogloboside, or iGb3) has been purported to be the endogenous glycolipid essential for NKT cell development in the thymus. The first evidence to support this conclusion was the observation that a deficiency in lysosomal enzymes β -hexaminidase A and B impedes NKT cell development. Subsequent experiments demonstrated that not only was iGb3 able to activate human NKT cells and mouse NKT cell hybridomas but inhibitory binding of iGb3 through isolectin B4 binding reversed its activation of NKT cells.¹⁴² These findings pointed to iGb3 as a candidate self-antigen glycolipid in mammals. While it is accepted that iGb3 is an agonist of NKT cells, its role as the endogenous lipid for NKT cells has been called into question due to the lack of a functional iGb3 synthase in humans.¹⁴³ Further doubts about the essential role of iGb3 in NKT cell activation stem from studies showing that mice deficient in iGb3 synthase have normal NKT cell development.¹⁴⁴ Regardless of the exact role of iGb3 in NKT cell development, chemical manipulation of the core molecule results in the modulation of NKT cell activity. Because of the complex chemical nature of polysaccharide glycolipids, the modifications to iGb3 have been restricted to tail region changes and minor atom replacements contained in the sugar portion of the molecule. Recently, β -GluCer has been demonstrated to be an endogenous human ligand capable of stimulating NKT cell activity, with its antigenic potency highly dependent upon the presence of a C24:1 fatty acid.^{145,146}

The crystal structures of β -GalCer and iGb3 bound to CD1d have been useful in understanding how mammalian β -linked glycolipids may function versus their more potent exogenous α -linked counterparts.^{147,148} The ternary crystal structure of mCD1d–iGb3–mTCR shows in detail that the β -linked glucoside adopts an “ α -like” conformation (Fig. 11). Meanwhile, the galactosides of iGb3 make contacts with an area of CD1d referred to as the anchor pocket, which does not have direct contact with the TCR, in a manner similar to NU- α -GalCer (Fig. 6). This relatively strained conformation would imply an induced fit for TCR recognition, but the elucidation of this ternary structure will be exceedingly useful to medicinal chemists synthesizing α -C-GalCer derivatives that utilize contacts in the anchor pocket to stabilize the CD1d–glycolipid complex.

A. Synthetic iGb3 Analogs

Savage and co-workers have synthesized α anomers of the first glucose of iGb3.¹⁴⁹ These analogs elicited higher IL-2 release from mouse NKT hybridomas and human NKT cell lines, presumably due to decreased torsional strain induced by pressing the β -linked sugar in an α -like conformation while in complex with CD1d.⁴⁸ Deoxy analogs of the terminal galactose have been informative as to the binding interaction of sugar and TCR (Fig. 12).¹⁵⁰ Systematic deletion of the 2'' or 3'' hydroxyl groups of the galactose by Chen et al. showed dramatic reduction in IL-2 release from DN32.D2 mouse NKT cell hybridomas, while tolerance was shown for 4'' or 6'' deletions. These findings open up the possibility of 4'' and 6'' derivatives of iGb3 as therapeutic agents, akin to SAR studies of α -GalCer. Xia and co-workers have also synthesized a dihydroceramide (H2-iGb3) and phytosphingosine (OH-iGb3) version of iGb3 (Fig. 12).¹⁵¹ The rationale for these changes was that by altering the lipid portion of the molecule, and therefore the interaction of iGb3 with the CD1d molecule, it would be possible to increase binding affinity. This was indeed the case as the OH-iGb3 showed a slight increase in cytokine release at low concentrations versus iGb3.

7. CONCLUSION

The potential to direct NKT cell mediated cytokine responses to Th1-, Th2-, or Th17-biased immune responses has significant therapeutic implications. This has been the impetus for a multitude of studies, whereby α -GalCer remains the prototypical scaffold for maximizing TCR stimulation, from which chemical variants have been synthesized and subsequently characterized. The wealth of X-ray crystallography data, for both murine and human binary and ternary complexes, will be invaluable to the computationally aided design of CD1d ligands to elicit either Th1 or Th2 cytokine profiles. As investigation continues into the sources of cytokine profile bias, hypotheses of the cause of cytokine bias continue to grow on the molecular level. CD1d–glycolipid complex stability and structure, mechanism of glycolipid loading, and lipid raft association are beginning to shed light onto a complicated picture of antigenic structure and NKT cell mediated responses. Work from our group and others, demonstrate that alterations in glycolipid antigen structure alters the immune response initiated by *i*NKT cell activation and that understanding the structural requirements which enhance ligand binding stability to CD1d will aid in the development of novel NKT cell agonists. Given that affinity of the glycolipid is believed to play an important role in the cytokine profile bias, it is important to note that the methods used to determine this affinity

and the problems associated with it, such as antibodies used for SPR measurements are raised against the CD1d–GalCer complex, may not be optimal to study galactose-modified analogs. Moreover, recent studies suggest that other antigens in addition to glycosphingolipid antigens should be studied, and that human *i*NKT cells should be included during the screening because human and mouse NKT cells can be differentially activated by glycolipid antigens. Collectively, these studies demonstrate the feasibility of utilizing specific glycolipid antigen to preferentially induce Th1 or Th2 cytokines from *i*NKT cells. Extensive progress has been made within the past few years; thus, the challenge will soon be to translate this information into clinically relevant therapeutic strategies.

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ABBREVIATIONS

APCs	antigen-presenting cells
DN	double negative
GPI	glycosylphosphatidylinositol
iGb3	isoglobotrihexosylceramide
NKT	natural killer
TPC	phosphatidylcholine
ROR	retinoic acid receptor-related orphan receptor
SAR	structure–activity relationships
TCR	T-cell receptor
α-GalCer	α -galactosylceramide

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Biographies

James E. East is currently a postdoctoral fellow at the University of Maryland School of Medicine. He began his research career as an undergraduate at the University of Virginia under H. Mario Geysen. His early work concerned the optimization of solid phase synthesis of small molecules aimed at intracellular cancer targets. James also obtained his PhD at the University of Virginia where he continued his biological and pharmaceutical research with work targeting the lysophosphatidic acid pathway in the laboratory of Timothy Macdonald. During this time in Dr. Macdonald's lab James designed and synthesized small molecules that targeted not only the cell-surface G-protein-coupled receptors associated with

lysophosphatidic acid signaling but also the soluble protein responsible for its generation in vivo. James is currently studying the role of natural killer T cells in regulating antitumor immune responses under the direction of Tonya Webb at the University of Maryland School of Medicine.

Andrew John Kennedy was born in Danville, Pennsylvania, and earned his degree in chemistry in 2005 from Providence College. He received his PhD in organic chemistry and lipid signaling in 2011 at the University of Virginia in the laboratory of Timothy Macdonald, where he designed molecular probes to study sphingosine 1-phosphate signaling in proliferative and inflammatory disease states. Andrew is currently a postdoctoral fellow at the University of Virginia School of Medicine.

Tonya J. Webb graduated from Prairie View A&M University. She performed her doctoral studies at Indiana University School of Medicine, under the direction of Randy Brutkiewicz, PhD, a leader in the field of NKT cell biology. She was postdoctoral fellow in the laboratory of David Wilkes, MD, and worked on mechanisms regulating immune tolerance. Her second postdoctoral fellowship focused on cancer immunology at Johns Hopkins with Jonathan Schneck, MD, PhD and Mathias Oelke, PhD. She joined the faculty as a tenure track Assistant Professor at the University of Maryland School of Medicine in 2009. Her laboratory investigates the efficacy of ex vivo expanded NKT cells for cancer immunotherapy.

iGb3 - Proposed mammalian self-antigen for NKT TCR

alpha-galactosyluronosylceramide - bacterial antigen

aGalCer - prototypical NKT agonist

alpha-galactosyldiacylglycerol - bacterial antigen

Figure 1.
Stimulatory antigens of NKT cells.

Figure 2. Crystal structure of the CD1d- α -GalCer-TCR ternary complex.⁴⁰ (A) α -GalCer (shown as a space-filling model) bound to CD1d (shown in green) and the TCR (shown in yellow). (B) The hydrogen bond network of α -GalCer to both CD1d and the TCR.

Figure 3.
The NKT cell Th1/Th2 axis

Figure 4. The structure–activity relationships of NKT activation, Th1-biased analogs, and Th2-biased analogs.

Figure 5.
Notable Th1-biased α -GalCer analogs.

Figure 6.

(A) Crystal structure of NU- α -GalCer (shown as a space-filling model) bound to CD1d (shown in green) and the TCR (shown in yellow). (B) A view of the naphthyl urea of NU- α -GalCer bound to the anchor pocket between the α 1 and α 2 helices of CD1d. (C) Crystal structure of OCH and captylate (C_8) bound to CD1d. Portions of the α 1 helix have been cut away to reveal ligand binding.

Figure 7.

The differences in length and dihedral angle between the crystal structures of α -GalCer⁴⁰ and α C-GalCer,⁴⁴ and the estimated bond lengths for vinyl-linked C-glycosides.

Figure 8.
Notable Th2-biased α -GalCer analogs.

GSL-1 **BbGL-IIc** **PIM₄** **P157**

Figure 9.
Known bacterial antigens.

Figure 10.

(A) Crystal structure of BbGL-IIc (shown as a stick model) bound to mCD1d (shown in green). Portions of the $\alpha1$ helix have been cut away to reveal ligand binding. (B) Crystal structure of PIM2 bound to mCD1d.

Figure 11.

Crystal structure of the mCD1d-iGb3-mTCR ternary complex. (A) iGb3 (shown as a stick model) bound to mCD1d (shown in green) and the mTCR (shown in yellow). (B) The hydrogen bond network of iGb3 to both mCD1d and the mTCR.

Figure 12.
Isoglobotrihexosylceramide and its derivatives.

Table I

CD 1 d-Binding Ligands

Antigen		Source	NKT cell subset
C-glycosidic form of α -galactosylceramide	α -C-GalCer	Synthetic	Type I, induces Th1 response
α -Galactosylceramide	α -GalCer	<i>Agleas mauritanus</i>	Type I, invariant
12 carbon acyl form of galactosylceramide	β -GalCer(C12)	Synthetic	Type I, invariant
β -D-glucopyranosylceramide	β -GlcCer	Self	Type I, invariant
1,2-Diacyl-3-O-galactosyl-sn-glycerol	BbGL-II	<i>Borrelia burgdorferi</i>	Type I, invariant
Diacylglycerol containing glycolipids	Glc-DAG-s2	<i>Streptococcus pneumonia</i> , group <i>B Streptococcus</i>	Type I, invariant
Ganglioside	GD3	Self; melanoma, other tumors	Type I, type II
Gangliotriaosylceramide	Gg3Cer	Murine lymphoma	Type I, invariant
Glycosylphosphatidylinositol	GPI	Self, <i>T. cruzi</i>	Type I, type II
α -Glucuronosylceramide	GSL-1	<i>Sphingomonas</i> , <i>E. muris</i>	Type I, invariant
α -Glucuronosylceramide	GSL-4	<i>Sphingomonas</i>	Type I, invariant
House dust extract + ovalbumin	HDE+OVA		Type I, induces Th2 response & CD4
Isoglobotrihexosylceramide	iGb3	Self	Type I, invariant
Lipophosphoglycan	LPG	<i>Leishmania</i>	Subset of type I
Lyosphosphatidylcholine	LPC	Self	Human type I (not mouse)
α -Galactosylceramide analog	OCH	Synthetic	Type I, induces Th2 response
Phosphatidylcholine	PC	Self, <i>Drosophila</i> , Cyprus pollen	24.8.A hydridoma; human type II
Phosphatidylethanolamine	PE	Self, Cyprus pollen	24.8.A hydridoma; human type II
Phosphatidylinositol	PI	Self, soybean	24.8. A hydridoma
PI dimannoside	PIM4	<i>M. tuberculosis</i>	24.8. A hydridoma
Phenyl pentamethyldihydrobenzofuran sulfonates	PPBF	Synthetic	Type II, variant
Sulfatide		Self	Type II, variant
Phosphatidylserine	PS	Self	24.8.A hydridoma
Threitolceramide		Synthetic	Type I, invariant