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The MHC class I-like CD1d glycoprotein is a member of the CD1 family of Ag-presenting molecules and is responsible for the selection of NKT cells. A number of ligands that can be presented by CD1d to NKT or other CD1d-restricted T cells have been identified. These include glycolipids from a marine sponge, bacterial glycolipids, normal endogenous glycolipids, tumor-derived phospholipids and glycolipids, and nonlipidic molecules. The presentation of many of these molecules can have immunopotentiating effects, such as serving as an adjuvant against malaria or resulting in a more rapid clearance of certain virus infections. They can also be protective in autoimmune diseases or cancer or can be deleterious. This review will highlight these ligands in a discussion of their potential use against (and role in the pathogenesis of) these diseases. The Journal of Immunology, 2006, 177: 769–775.

It is said that looks are not everything. Nonetheless, how things look (i.e., structure) can have profound effects on biological functions. The folding and overall structure of MHC class I and class I-like molecules possessing activities ranging from Ag presentation (1) to the transport of maternal Abs via a neonatal FcR (2–4) to iron transport (5), demonstrate that this particular “look” goes a long way, and is thus critical. The majority of these molecules are involved in Ag presentation. In addition to the MHC class I and class II Ag presentation pathways, the presentation of nonpeptidic ligands (almost all are lipids) occurs by CD1 molecules. The CD1 family consists of two groups based on amino acid homology (6): group 1 includes CD1a, b, and c, whereas CD1d is the sole member of group 2. In the mouse, there are two CD1d molecules, CD1d1 and CD1d2. Although the group 1 CD1 molecules can present Ags to a wide variety of T cells, CD1d molecules present their Ags mostly to a unique T cell population called NKT cells. The majority of these T cells are called canonical or invariant NKT (iNKT) cells with a specific TCR. The majority of these cells are called canonical or invariant NKT (iNKT) cells with a specific TCR

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In 1997, the first report suggesting that CD1d could present Ag demonstrated that a hydrophobic peptide called p99 could bind to murine CD1d1 and stimulate CD8+ T cells generated against the CD1d1/p99 complex (9). Interestingly, 2 years later, the crystal structure of CD1d1 was solved, and it indeed possessed a highly hydrophobic Ag binding groove (10). However, in 1998, we (Sebastian Joyce, Robert Cotter, Amina Woods, Jonathan Yewdell, Jack Bennink, and I) extracted a natural ligand from CD1d1 (11). Rather than a peptide (which is frankly what we had anticipated we would find), we detected GPI as a major natural ligand (Fig. 1). We went on to show that phosphatidylinositol (PI) is also bound (12). Similarly, it was found that PI is a ligand naturally bound to human CD1d as well (13). More recently, CD1d1 was found to have phosphatidylcholine (PC) bound in a crystal structure derived from CD1d1 secreted from Drosophila cells (14). Thus, glyco- and phospholipids are natural ligands bound to CD1d molecules. What is their purpose in the grand scheme of CD1d-mediated Ag presentation? Although these molecules can bind to CD1d, they can only in rare circumstances stimulate canonical NKT cells (15). We suspected that the role they play is analogous to the function played by the invariant chain-derived CLIP peptide for MHC class II-mediated Ag presentation. Thus, the natural, endogenous glyco- and phospholipids reside in the CD1d

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3 Abbreviations used in this paper: iNKT, invariant NKT; PI, phosphatidylinositol; PC, phosphatidylcholine; iGb3, isoglobotrihexosylceramide; EAE, experimental allergic encephalomyelitis; α-GalCer, α-galactosylceramide; β-GaLGa, β-galactosylceramide; OCH, α-GalCer with a truncated sphingosine chain; GSL-1, α-glucuronoxylceramide; PIM, phosphatidylinositol mannoside; PBPB, phenylpentamethylydihydrobenzo furansulfonate.
Ag binding groove until in the correct (presumably late endocytic) compartment, where these ligands are replaced by a (glyco)lipid that, when presented on the cell surface by CD1d, stimulate NKT cells (16–18). For MHC class II molecules, the exchange of CLIP for an antigenic peptide in the MIIC compartment requires an acidic pH environment (19). Similarly, we found that inhibiting such acidification in these compartments substantially impairs CD1d-mediated Ag presentation (16). In line with our observations, Luc Teyton and Albert Bendelac, and Peter Cresswell independently showed direct evidence for such an exchange of an endogenous CD1d ligand mediated by a family of lipid transfer proteins termed saposins (20, 21). Soon after these reports, the Bendelac laboratory found that isoglobotrihexosylceramide (iGb3), a lysosomal glycosphingolipid, is a natural CD1d-presented ligand that can stimulate NKT cells (22). In mice deficient in the enzyme /H9252-hexosaminidase b, important for iGb3 generation, NKT cell development is impaired substantially (22). In contrast to that which occurs with iNKT cells, some noncanonical NKT cells appear to be able to recognize lipids that are potentially loaded onto CD1d in the endoplasmic reticulum (20, 21, 23–26).

Some natural cellular lipids can bind to CD1d and stimulate NKT cells or other CD1d-restricted T cells; several of these have effects in various disease states. For example, the natural lipid sulfatide found in myelin is presented by the group 1 CD1 molecules CD1a, b, and c (27, 28). This has important relevance in multiple sclerosis, as autoimmune responses to myelin occur in these patients, and such responses are believed to contribute to disease pathogenesis. In a mouse model, it has been shown that sulfatide can be presented by CD1d to T cells other than NKT cells, as determined by the use of sulfatide-loaded CD1d1 tetramers (29). Furthermore, and most importantly, treatment of wild-type (but not CD1d-deficient) mice with sulfatide prevented the development of experimental allergic encephalomyelitis (EAE) in that study. The recent solving of the crystal structure of CD1d1 complexed with sulfatide (30) will be helpful in understanding how this lipid is presented to CD1d-restricted, sulfatide-specific T cells.

Two natural lipid components of the cell membrane, the disialoganglioside GD3 and PI, have been shown to be CD1d-presented Ags capable of stimulating (at least some) NKT cells.

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**FIGURE 1.** “Natural” ligands of CD1d. An array of lipids derived from mammalian cells that can bind to CD1d and stimulate or inhibit (or not activate) CD1d-restricted T cells. Gg3Cer, gangliotriaosylceramide; PE, phosphatidylethanolamine.
In the case of GD3 derived from the melanoma line SK-MEL-28, it appears as though it can be cross-presented by murine APCs to NKT cells (31). Furthermore, injection of GD3 into wild-type (but not CD1d-deficient) mice resulted in an increased number of NKT cells in the spleen, and these were detectable using GD3-loaded CD1d1 dimers. In the case of PI, one NKT cell hybridoma out of a panel of nine expressing an invariant TCR (Vα14)α18 was strongly stimulated by soybean-derived PI when added to plate-bound purified CD1d1 (15). This NKT cell hybridoma called 24.8.A (and none of the others) was also stimulated to various degrees by phosphatidylethanolamine, phosphatidic acid, phosphatidylserine, phosphatidylinositol, PC, and monogalactosyl diglyceride. Furthermore, 24.8.A was able to recognize cd1d1 cDNA-transfected murine P815 (mastocytoma), EL-4, RMA/S (both T cells), and A20 (B cell) tumor cell lines (15). Of note, this group went on to show that changes in the degree of unsaturation of the phosphatidylethanolamine (the CD1d-presented Ag best able to stimulate 24.8.A) acyl chain affected its recognition by the NKT cell hybridoma. This is consistent with acyl chain structure playing an important role in phospholipid Ag presentation by CD1d (32). Interestingly, the 24.8.A NKT cell hybridoma could also “see” purified CD1d1 without added ligand. However, with a recent report showing that the crystal structure of Drosophila cell-expressed CD1d1 contains PC as a natural ligand (14), perhaps CD1d is never really “empty,” and this result may have been predictable in hindsight. Regardless of the very wide range of Ags recognized by this particular NKT cell hybridoma, it is nonetheless an interesting example of CD1d-presented ligands and their ability to be recognized by components of the innate immune response.

Lipids binding to CD1d can also have inhibitory functions. As a case in point, the murine T cell lymphoma line L5178Y-R sheds glycolipids, with gangliotriaosylceramide being predominant (33, 34). We found that gangliotriaosylceramide inhibited CD1d-mediated Ag presentation to NKT cells (35), suggesting one means by which a tumor can evade an important component of the innate antitumor immune response.

α-Galactosylceramide (α-GalCer) and α-GalCer analogs

In 1997, a group led by Masaru Taniguchi found that α-GalCer, a glycolipid extracted from the marine sponge Agelas maurusianus (36), could stimulate NKT cells to rapidly produce both Th1 and Th2 cytokines in a CD1d-dependent manner (7, 37). α-GalCer has an α-anomeric sugar attached to acyl and sphingosine chains (Fig. 2). α-GalCer-mediated stimulation of NKT cells interestingly results in an internalization of the TCR (38–40), originally thought to be a “loss” (41–46). The Kawano et al. (37) study opened a floodgate of reports showing that α-GalCer could be effective as an antitumor immunopotentiator in a wide range of tumor model systems (reviewed in Ref. 47), as well as in other disease states. For example, in the murine B16 melanoma, or those tumors induced by methylcholanthrene, the administration of α-GalCer to tumor-bearing mice had profound in vivo effects by inhibiting metastatic disease, as well as the elimination of the primary tumor. Analysis of the antitumor efficacy of α-GalCer, coupled with the site-directed mutagenesis of the murine CD1d1 molecule (48), helped increase our understanding of how α-GalCer bound to CD1d1 and also provided clues regarding the mechanisms behind NKT cell activation by this glycolipid. α-GalCer was also shown to inhibit or ameliorate autoimmune diseases including...
treatment of mice with 
and other lipids from this genus can bind to CD1d, those li-
other pathogens (68, 69). In 
duced sphingosine chain results in IL-4 production with a reduction in IFN-
mined sphingosine chain (OCH). It was found that OCH was 
sentially in half, generating a molecule called OCH with a trun-
in assessing the effect of NKT cell activation on EAE (51). In 
results in a large number of NKT cells infiltrating the site of 
secretion (52). The requirements for the binding of this 
(53–57) used a differ-
was found that the C-glycoside induced more of a Th1 (i.e., IFN-γ response, was longer lasting, and was actually a better 
avor in vitro, as well as antimicrobial and antitumor activity in vivo. For example, modifying the acyl chain of the 
molecule KRN 7000 to create a dimermaturated 20 
jug from the bacteria (and 
unique aspect of GSL-1 as opposed to 
the lack of LPS substantially helps) extends the number of lab-
if it is the case for sulfatide, for example (29). In 
and Th2 cytokines (7). Thus, the idea behind the use of α-GalCer in 
response toward the production of Th2 cytokines (49), it appears as 
the polarization of NKT cells (i.e., Th1 vs Th2) is 

either incomplete or ineffective (50). Interestingly, work by 
the Yamamura laboratory tried the approach of an α-GalCer analog in 
EAE than the parental α-GalCer. This effect corre-
related with a higher level of IL-4 production induced by 
administration of OCH than with α-GalCer (51). Other analogs of 
have been shown to have various effects on NKT cell 
activation in vitro, as well as antimicrobial and antitumor activity in vivo. For example, modifying the acyl chain of the 
α-GalCer analog were less stringent as well, illustrating how changes in 
this chain can also have profound effects on NKT cell acti-
vation. In contrast to the effects of acyl chain modification on 
α-GalCer activity, Tsuji and colleagues (53–57) used a differ-
ent approach. In their studies, they used a C-glycosidic form of 
α-GalCer in analyses of the adjuvant properties of α-GalCer. It 
was found that the C-glycoside induced more of a Th1 (i.e., IFN-γ) response, was longer lasting, and was actually a better 
advantage than the parental compound itself in murine models of 
mesa of malaria and metastatic melanoma (53–57). Furthermore, 
α-GalCer administration has been shown to be mostly protective 
avtivity against viruses (58–61), bacteria (62–67), and other pathogens (68, 69). In Trypanosoma cruzi, although GPIs 
and other lipids from this genus can bind to CD1d, those li-
gands do not stimulate NKT cells (70). However, although 
treatment of mice with α-GalCer is protective against infection with 
T. cruzi (71), it actually impairs the immune response against 
a DNA vaccine specific for this pathogen (72).

Ortaldo and colleagues (73), as well as Parekh et al. (74), have 
found that a 12 carbon acyl chain form of galactosylceramide 
((β-GalCer (C12)) can stimulate NKT cells in a CD1d-dependent 
manner. It has been shown previously that the activation of 
NKT cells by α-GalCer results in concomitant NK cell stimu-
lution (75, 76). The administration of the β-GalCer (C12) in 
vivo caused the apparent loss of NK cells as found with α-Gal-
Cer (73), but without cytokine expression or activation of NK 
cells. Tetramers loaded with β-GalCer (C12) could stain NKT 
cells, although lower numbers were detected as compared with 
when α-GalCer-loaded tetramers are used (38–40, 73). How-
ever, some contamination with minute amounts of α-GalCer 
cannot be completely ruled out in either of the β-GalCer prepa-
rations (73, 74). In another study, the s.c. administration of 
β-GalCer (but not α-GalCer) to wild-type C57BL/6 mice re-
sulted in a large number of NKT cells infiltrating the site of 
injection, similar to the CD1d-independent granulomas in-
duced by the s.c. injection of mycobacterial PI dimannoside 
(77). Thus, β-GalCer may have some similar yet distinctly dif-
ferent actions on NKT cells.

Endogenous microbial NKT cell “activators”

Last year, three groups independently found that a predomi-
nant glycosphingolipid from the LPS-free Sphingomonas (S. 
capsulata, S. paucimobilis, and S. wittichii) and Ehrlichia muris 
bacteria could stimulate human and/or murine NKT cells in a 
CD1d-dependent manner (78–81). All of these groups identi-
ified α-glucuronosylceramide (GSL-1) as this glycosphingo-
lipid, with α-galacturonosylceramide also able to activate NKT 
cells (78, 79). Interestingly, in the case of S. paucimobilis, the 
tetracylgcosylated glycosphingolipid from S. paucimobilis (not or 
poorly recognized by NKT cells) is processed into the active 
GSL-1 in low pH compartments, probably lysosomes (80). The 
relative ease in the purification of GSL-1 from the bacteria (and 
the lack of LPS substantially helps) extends the number of lab-
oratories who can use this ligand in their studies. An additional 
unique aspect of GSL-1 as opposed to α-GalCer is the NKT cell 
populations identified by CD1d1 tetramers or dimers loaded with 
this glycolipid. Approximately one-half of those NKT cells 
that are α-GalCer/CD1d1 tetramer positive are GSL-1/CD1d1 
dimer (or tetramer) positive. Furthermore, the demonstration 
that the clearance of these bacteria from animals that are NKT 
cell deficient is impaired (78, 79) shows that GSL-1 presenta-
tion by CD1d is important in a pathogen model system. Thus, 
GSL-1 is not simply “just another CD1d ligand.”

Two other sources of microbial products have been shown to 
be able to activate NKT cells. The Bonneville and Schaible 
groups reported that PI mannoside (PIM) could stimulate both 
human and murine NKT cells (82). Additionally, PIM-loaded 
CD1d tetramers could stain both human (PBL) and mouse (liver) NKT cells, although the latter detected a substantially 
smaller population than CD1d tetramers loaded with α-GalCer 
(~0.3 vs 32%, respectively). Despite this interesting observa-
cion clearly identifying PIM as a CD1d ligand, how PIM actu-
ally participates in the immune response against mycobacteria is 
not yet clear, as it has been shown that CD1d-deficient mice do 
not significantly differ in survival from wild-type mice when 
injected with M. tuberculosis (83), whereas Jα18 (NKT cell)- 
deficient mice appear to be more susceptible (84). In the con-
text of a Leishmania infection, it was found that lipophospho-
glycan (present on the surface of this organism) and related 
glycoinositol phospholipids could bind to CD1d and stimulate 
IFN-γ production in a CD1d-dependent manner (85). In that 
study, a defective granulomatous response in CD1d-deficient 
mice infected with L. donovani was observed, with a concomi-
taneous elevation in parasite burden relative to wild-type mice.

Synthetic and nonmicrobial CD1d ligands

It was mentioned above that the α-GalCer analog OCH has 
been used in autoimmune disease models and that its efficacy in 
these models is dependent on a Th2 cytokine “bias” by NKT 
cells (51). The predominant NKT cells that are activated by 
α-GalCer, GSL-1, and other microbial glycolipids are the 
inNKT cells. Certainly, there could be other CD1d-restricted T 
cells that recognize CD1d presenting something other than 
these ligands, as is the case for sulfatide, for example (29). In 
fact, work from the Moody laboratory has shown that human 
noninvariant T cells that are CD1d-restricted can be activated 
by ligands that are neither peptides nor lipids. They found that 
phenyl pentamethyldihydrobenzofuran sulfonates (PPBFs; Fig.
3) could stimulate a clonotypic population of human T cells (Vα2/Vβ21 TCR) that were very sensitive to changes in the hydroxylation and methylation patterns at the terminal ends of these molecules (86). Interestingly, PPBFs did not stimulate NKT cells, nor did α-GalCer stimulate the PPBF-reactive T cells. The structural relationship of PPBFs with sulfa drugs capable of inducing hypersensitivity reactions in humans may suggest that some of these responses are CD1d mediated and that the range of ligands capable of being presented by CD1d is not limited.

The chicken OVA molecule has been used for analyzing Ag processing and presentation through “classical” MHC class I and II molecules. In the context of CD1d, it has been shown that CTL can be induced in response to OVA in an MHC-unrestricted manner, and their cytolytic activity can be inhibited by Abs to CD1d (87).

Interestingly, it has recently been shown that PC and phosphatidylethanolamine from plant pollen can stimulate human CD1d- (and CD1a)-specific T cells (88). The T cells that responded to PC or phosphatidylethanolamine were rather diverse and only rarely included iNKT cells. Of intriguing note, T cell responses to these pollen phospholipids were mainly observed in allergic patients during pollen season, whereas normal (nonallergic) subjects rarely displayed such reactivity. This could also be related (at least to a limited degree) to recent work by Umetsu and his colleagues (89) in asthma patients.

**Final thoughts**

It is clear the ligands capable of being presented by CD1d molecules to T cells go well beyond α-GalCer and related compounds. Besides its application as a parental compound, α-GalCer has quite useful as a “backbone” or “foundation,” as

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**FIGURE 3.** Pathogen-derived and “other” CD1d ligands. CD1d ligands from *Sphingomonas* spp., *Leishmania donovani*, and *Mycobacterium tuberculosis*, as well as synthetic PPBF, capable of stimulating various CD1d-restricted T cell subpopulations. LPG, lipophosphoglycan from *L. donovani*; PIM4, PIM from *M. tuberculosis*. The number of carbons in the chains indicated as “R” is presented in the illustration next to the respective structures.
illustrated by the enhanced efficacy of OCH in EAE, and increase in adjuvant activity when α-GalCer is introduced as its C-glycosidic form in malaria and tumor model systems. α-GalCer, either as a free compound, or pulsed on dendritic cells, has been given to patients with various forms of cancer in phase I trials (90–94). Although none have reported clinical responses, increased NKT cell activation and cytokine production was evident, suggesting that the potential for antitumor efficacy is there in more refined future studies. CD1d ligands completely unrelated to α-GalCer have been identified (and perhaps more of them are to be discovered), as well as the T cell subpopulation(s) stimulated by them. This opens up additional avenues for investigations aimed at understanding other diseases at a more basic level, in addition to the translation of those results to the clinic, where patients present with symptoms of unknown etiology. As a case in point, it has been shown recently by Umetsu and colleagues (89) that the majority of CD4+ T cells in the lungs of patients with moderate to severe asthma are, in fact, iNKT cells. Thus, it is conceivable that natural glycolipid Ags such as iGb3 (22), or bacterially-derived glycolipids such as GSL-1 (78–81), could cause the enhanced number of pulmonary NKT cells in this disease. Certainly, then, the CD1d pathway of Ag presentation has the promise of utility in a number of different disorders or conditions, and for which we might be able to exploit their CD1d dependence for novel treatments or preventative strategies.

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References

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