

Review

C. Marie Dowds, Sabin-Christin Kornell, Richard S. Blumberg* and Sebastian Zeissig*

Lipid antigens in immunity

Abstract: Lipids are not only a central part of human metabolism but also play diverse and critical roles in the immune system. As such, they can act as ligands of lipid-activated nuclear receptors, control inflammatory signaling through bioactive lipids such as prostaglandins, leukotrienes, lipoxins, resolvins, and protectins, and modulate immunity as intracellular phospholipid- or sphingolipid-derived signaling mediators. In addition, lipids can serve as antigens and regulate immunity through the activation of lipid-reactive T cells, which is the topic of this review. We will provide an overview of the mechanisms of lipid antigen presentation, the biology of lipid-reactive T cells, and their contribution to immunity.

Keywords: CD1; CD1a; CD1b; CD1c; CD1d; (NK)T.

***Corresponding authors:** Richard S. Blumberg, Division of Gastroenterology, Hepatology, and Endoscopy, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA; and Sebastian Zeissig, Department of Internal Medicine I, University Medical Center Schleswig-Holstein, Schittenhelmstraße 12, D-24105 Kiel, Germany, e-mail: rblumberg@partners.org, szeissig@1med.uni-kiel.de
C. Marie Dowds and Sabin-Christin Kornell: Department of Internal Medicine I, University Medical Center Schleswig-Holstein, Schittenhelmstraße 12, D-24105 Kiel, Germany

CD1 proteins survey subcellular compartments for lipids

Lipid antigens are presented by atypical MHC class I proteins of the CD1 family. In contrast to the polymorphic nature of classical MHC class I, CD1 genes exhibit little sequence diversity with few nonsynonymous single-nucleotide polymorphisms (Zimmer et al., 2009; Seshadri et al., 2013). All mammalian species investigated so far express at least one CD1 protein, suggesting conservation of the CD1 system in mammals (Kasmar et al., 2009). In humans, lipid antigens are presented by group 1 (CD1a, CD1b, and CD1c) and group 2 (CD1d) CD1 isoforms (Porcelli et al., 1989, 1992; Balk et al., 1991; Blumberg et al., 1991; Beckman et al., 1994; Kawano et al., 1997). Mice lack

expression of group 1 CD1 and contain two highly homologous CD1d-encoding genes (*Cd1d1* and *Cd1d2*), whereby the *Cd1d2* gene contains a frameshift in the C57BL/6 strain that prevents cell surface expression of the encoded protein (Park et al., 1998).

T cells responding to lipid antigens are CD1a-, CD1b-, CD1c-, or CD1d-restricted and, in the case of CD1d, have been termed natural killer T (NKT) cells. Based on their T cell receptor (TCR) repertoire, (NK)T cells are further distinguished into invariant NKT (iNKT) cells expressing a semi-invariant TCR and non-invariant NKT cells with a more diverse TCR repertoire (see further below).

CD1 family members are considered atypical MHC class I proteins and share selected structural and functional characteristics of classical MHC class I and class II proteins. Thus, similar to MHC class I, CD1 proteins contain three extracellular domains (α_1 , α_2 , and α_3), a transmembrane domain, and a C-terminal intracellular domain and are synthesized in the endoplasmic reticulum (ER) in a manner dependent on the ER chaperones calreticulin and calnexin as well as the thiol oxidoreductase ERp57 (Kang and Cresswell, 2002; Cohen et al., 2009). However, differences in the biosynthesis of CD1 and MHC class I exist with regard to the sequence of these interactions and their physiological implications (Kang and Cresswell, 2002). Thus, in contrast to MHC class I, calreticulin, calnexin, and ERp57 interact with the CD1d heavy chain before associating with β_2 -microglobulin (β_2m) (Kang and Cresswell, 2002). As a consequence, the binding of β_2m is less critical for folding of CD1d compared with MHC class I, which may explain the occurrence of functionally competent β_2m -independent CD1d (Balk et al., 1994; Koh et al., 2008). CD1 isoform-specific differences also exist in that ER exit of CD1b but not CD1d is β_2m -dependent (Balk et al., 1994; Sugita et al., 1997).

In the ER, CD1 loads cellular lipids common in this compartment such as glycerophospholipids (GPLs), including phosphatidylcholine (PC) and phosphatidylinositol (PI) (Park et al., 2004; Yuan et al., 2009). Although these findings were obtained with ER-retained forms of CD1d, the association of PC with secreted forms of CD1b and CD1c suggests that similar mechanisms likely apply to group 1 CD1 (Garcia-Alles et al., 2006; Haig et al., 2011). Loading of lipids

onto CD1d in the ER is facilitated by lipid transfer molecules expressed in this compartment such as microsomal triglyceride transfer protein (MTP) and may contribute to ligand-induced stabilization of CD1 during biosynthesis (Brozovic et al., 2004; Dougan et al., 2005, 2007; Kaser et al., 2008; Odyniec et al., 2010; Zeissig et al., 2010). This concept is supported by the observation that mutations in the gene encoding for MTP (*MTTP*), as found in human abetalipoproteinemia (ABL), are associated with proteasomal degradation of CD1a, CD1b, and CD1c (Zeissig et al., 2010).

Following biosynthesis in the ER, CD1 traffics through the secretory pathway to the plasma membrane, where acquired lipids are presented to lipid-reactive T cells. Interestingly, the repertoire of lipids associated with engineered, ER-retained CD1d (ER-CD1d) differs from that observed with soluble CD1d (sCD1d), which lacks the transmembrane domain and is released at the cell surface following completion of trafficking through the secretory pathway. Thus, GPLs were found to be the dominant group of lipids associated with ER-CD1d, whereas both sphingolipids and GPLs were bound to sCD1d (Cox et al., 2009; Yuan et al., 2009; Muindi et al., 2010). This indicates that either unloaded CD1 proteins proceed to the Golgi complex, where they bind sphingolipids generated in this compartment or, more likely, that lipids loaded in the ER are replaced in the Golgi either spontaneously or facilitated by lipid transfer and editing proteins yet to be identified.

In addition to pathways shared with MHC class I, CD1 trafficking patterns partially overlap with those of MHC class II. Thus, CD1 is internalized after reaching the plasma membrane and traffics through the endolysosomal pathway in a manner dependent on the CD1 isoform. CD1a, CD1c, and human CD1d are recruited to early endosomes and the endocytic recycling compartment, whereas CD1b and mCD1d show additional trafficking into lysosomes (Cohen et al., 2009). CD1 isoform-specific trafficking is thereby determined by interactions of the cytoplasmic tail of CD1 with adaptor protein (AP) 2 and 3 leading to internalization through interaction with AP-2 (all CD1 isoforms except for CD1a) and subsequent lysosomal recruitment through interaction with AP-3 (CD1b and mCD1d) (Sugita et al., 1996, 1999, 2000; Chiu et al., 1999; Briken et al., 2000; Jayawardena-Wolf et al., 2001; Chiu et al., 2002). As a consequence of differences in trafficking patterns, CD1 isoforms survey different endolysosomal compartments, where cellular GPLs and sphingolipids loaded in the secretory pathway are exchanged against other endogenous (self) or exogenous (microbial, nutritional) lipids presented to CD1-restricted T cells after recycling of CD1 to the cell surface.

Endolysosomal lipid exchange is facilitated by low pH, lipid transfer and editing proteins, and enzymes involved in lipid processing and catabolism. Acidification within the endolysosomal system ensures optimal activity of hydrolytic enzymes, stabilizes CD1 in the absence of bound lipid (Odyniec et al., 2010), and facilitates lipid loading through direct effects on the conformation of CD1 (Ernst et al., 1998; Relloso et al., 2008). In addition, lipid transfer and editing proteins such as saposin family members and GM2 activator contribute to CD1 lipid loading in the endolysosomal system through extraction of lipids from membranes and transfer onto CD1 as well as potentially through prevention of an irreversible collapse of the CD1 binding cavity in the lipid-free intermediate state (Kang and Cresswell, 2004; Winau et al., 2004; Zhou et al., 2004a,b; Yuan et al., 2007; Garzon et al., 2013). In addition, cathepsin L expression in thymocytes is required for the activation and positive selection of iNKT cells and may act through the assistance in the conversion of prosaposin into saposins (Honey et al., 2002). Further, endolysosomal enzymes involved in the processing of glycolipid headgroups as well as the lipid tails play major roles in the generation of antigenic CD1 lipids. Thus, glycosidases including α - and β -galactosidase, α -mannosidase, and β -hexosaminidase are involved in the trimming of the headgroups of complex glycosphingolipids (GSLs) and bacterial glycosylphosphoinositides, which facilitates lipid headgroup recognition by the NKT-TCR (Sieling et al., 1995; Prigozy et al., 2001; Zhou et al., 2004a,b; de la Salle et al., 2005; Gadola et al., 2006). Deficiency in lysosomal glycolipid-processing enzymes is associated with storage disorders in humans and mice (Kolter and Sandhoff, 2010) and affects lipid antigen presentation through direct effects on the processing of CD1-restricted lipid antigens and indirect effects through interference with lysosomal function (Sieling et al., 1995; Prigozy et al., 2001; Zhou et al., 2004a,b; de la Salle et al., 2005; Gadola et al., 2006). In addition to glycosidases, CD1e, the sole known group 3 CD1 member, facilitates α -mannosidase-dependent processing of glycolipids and directly transfers diacylated phosphatidylinositol mannoside onto CD1b (de la Salle et al., 2005; Facciotti et al., 2011; Garcia-Alles et al., 2011; Cala-De Paepe et al., 2012). Finally, the processing of lipid tails, for example, by phospholipase A₂ (PLA₂) enzymes, occurs in various subcellular compartments including the endolysosomal system and modulates the antigenicity of CD1 lipids (Fox et al., 2009; Zeissig et al., 2012; Paduraru et al., 2013).

Together, broad and isoform-dependent trafficking of CD1 as well as the concerted action of lipid transfer and processing enzymes facilitate a survey of subcellular

compartments for CD1 binding self-lipids and foreign lipids.

CD1 binds a broad spectrum of self-lipids

CD1 isoforms have two or four hydrophobic pockets (A', C', F', and T'), which accommodate the hydrophobic tail of lipid ligands, whereas the lipid headgroup is presented to the NKT-TCR (Moody et al., 2005; De Libero et al., 2009; Girardi and Zajonc, 2012; Rossjohn et al., 2012). Volumes of the binding grooves vary significantly between CD1 isoforms with CD1a at the lower end (1200 Å³) and CD1b at the upper end of the spectrum (2400 Å³) (Moody et al., 2005; Cohen et al., 2009; De Libero et al., 2009). Consequently, CD1b has been shown to capture exogenous lipids with combined alkyl chain lengths of 70–86 carbons, whereas the total lipid tail bound by other CD1 isoforms is in the range of 36–46 carbons (Moody et al., 2005; Cohen et al., 2009). Lipids with up to four alkyl chains were found to be associated with CD1.

In accordance with few restrictions for CD1 binding, a large spectrum of self- and foreign lipids associates with CD1. Lipidomics studies of endogenous lipids associated with individual CD1 members revealed several hundred lipids with distinct mass-to-charge ratios bound to each CD1 isoform in a single cell line *in vitro* (Cox et al., 2009; Huang et al., 2011). Of those, 5–25% of lipids were CD1 isoform-specific (Huang et al., 2011).

With regard to CD1d, the most extensively studied CD1 member, the spectrum of associated lipids reflected that of the total cellular or compartmental abundance of lipids. Thus, GPLs and sphingolipids were found to be the major groups of lipids associated with CD1d (Cox et al., 2009; Yuan et al., 2009; Muindi et al., 2010; Haig et al., 2011). Within GPLs, abundant cellular lipids such as PC and phosphatidylethanolamine (PE) as well as less abundant lipids such as phosphatidylserine, PI, phosphatidylglycerol, and phosphatidic acid were bound to CD1d (Park et al., 2004; Cox et al., 2009; Shiratsuchi et al., 2009; Yuan et al., 2009; Haig et al., 2011). Among sphingolipids, both sphingomyelin and GSLs were found to be associated with CD1d (Cox et al., 2009; Yuan et al., 2009; Muindi et al., 2010; Haig et al., 2011). A detailed characterization of cellular and CD1d-bound GSLs revealed that the relative abundance of GSLs associated with CD1d did not solely reflect their cellular abundance (Muindi et al., 2010). This suggests that the subcellular localization of CD1d and potential

lipid ligands as well as the abundance and activity of lipid processing and transfer proteins may contribute to the repertoire of CD1d-associated lipids. In accordance with the concept of compartmental effects on the CD1d lipidome, the spectrum of GPLs and sphingolipids associated with CD1d differed between CD1d molecules engineered to selectively traffic through the secretory pathway compared with those that exhibited additional endolysosomal trafficking (Yuan et al., 2009; Muindi et al., 2010). Thus, lysophospholipids were predominantly associated with CD1d proteins, which maintain the ability to undergo endolysosomal trafficking, whereas the ganglioside GM2 was only detected in CD1d engineered to selectively survey secretory compartments (Yuan et al., 2009; Muindi et al., 2010).

Similar studies on endogenous ligands have recently been performed for secreted forms of group 1 CD1, which selectively traffic through the secretory but not the endolysosomal pathway due to lack of the CD1 transmembrane domain and the intracellular C-terminus (Haig et al., 2011; Huang et al., 2011). These studies revealed a large overlap in the spectrum of lipids associated with group 1 and group 2 CD1 with some noteworthy isoform-specific distinctions. Thus, CD1b, despite having a significantly larger lipid binding groove than other CD1 isoforms, captures low-mass lipids such as diacylglycerols and deoxyceramides (Facciotti et al., 2011; Huang et al., 2011). These lipids act as scaffolds and facilitate subsequent parallel binding of lipids that would otherwise not fully occupy the CD1b-binding groove, thus contributing to optimal positioning of these lipids for T-cell recognition (Garcia-Alles et al., 2006; Facciotti et al., 2011; Huang et al., 2011). In addition, these and other lipids may act as spaceholders for subsequent exchange against lipids with long acyl chains, which fully occupy the CD1b groove (Huang et al., 2011).

Antigenicity is limited to a small subset of self-lipids

Group 1 CD1 expression is limited to professional antigen-presenting cells (APCs) including, in case of CD1c, a subset of B cells (Cohen et al., 2009). In contrast, CD1d is broadly expressed by professional and non-professional APCs including epithelial and endothelial cells (Blumberg et al., 1991; Geissmann et al., 2005; Cohen et al., 2009). As CD1d binds to a wide variety of self-lipids, it may provide a constant source of antigens for CD1d-restricted (NK)T cells, both in the thymus and the periphery. In accordance

with this concept, autoreactivity, i.e., CD1-dependent activation by APCs in the absence of exogenous antigen, is a hallmark of (NK)T cells. However, the search for self-lipid antigens involved in (NK)T cell activation revealed that the majority of CD1d-binding lipids exhibit little or no antigenicity (Fox et al., 2009; Brennan et al., 2011; Pei et al., 2011; Facciotti et al., 2012; Zeissig et al., 2012). Noteworthy examples of antigenic CD1d self-lipids that activate iNKT cells include GSLs such as isoglobotrihexosylceramide (iGb3), β -glucosylceramide (β -GlcCer), β -galactosylceramide (β -GalCer), and lysosphingomyelin as well as phospholipids such as lysophosphatidylcholine (LPC), ether-bonded lysophosphatidylethanolamine (pLPE) and lysophosphatidic acid (eLPA) (Figure 1) (Zhou et al., 2004a,b; Fox et al., 2009; Brennan et al., 2011; Facciotti et al., 2012). In addition, CD1d self-lipids that exhibit antigenicity for non-invariant NKT cells include ester-bonded lysophosphatidylethanolamine (LPE) and the GSLs sulfatide, β -GlcCer and β -GalCer, whereby the lyso-derivatives of the GSLs showed increased antigenicity (Figure 1) (Jahng et al., 2004; Roy et al., 2008; Brennan et al., 2011; Rhost et al., 2012; Zeissig et al., 2012). The relative contribution of these antigenic lipids to (NK)T cell-positive selection in the thymus and activation in the

periphery as well as the mechanisms underlying species-specific differences in self-antigen-mediated (NK)T cell activation are currently not clear and require further analysis. Similarly, the mechanisms that prevent autoimmunity in the presence of antigenic self-lipids require further study but may include the balance of antigenic and non-antigenic CD1d lipids.

Mechanistically, the discrepancy between the number of CD1d-binding lipids and those associated with (NK)T cell antigenicity is the consequence of a rather non-selective binding mode based on hydrophobic interactions between CD1d and the aliphatic lipid tail, whereas antigenicity follows more strict, but only partially understood structural requirements that allow for recognition of the CD1d-lipid complex by the NKT-TCR (De Libero et al., 2009; Girardi and Zajonc, 2012; Rossjohn et al., 2012). These structure-function relationships at the interface of the CD1d-lipid complex and the NKT-TCR have recently been the focus of excellent reviews and will only be briefly outlined here (De Libero et al., 2009; Girardi and Zajonc, 2012; Rossjohn et al., 2012). Thus, recognition of the CD1d-lipid complex by the NKT-TCR is based on interactions between the TCR and CD1d as well as the headgroup of lipids. Thereby, the molecular nature of the headgroup, its

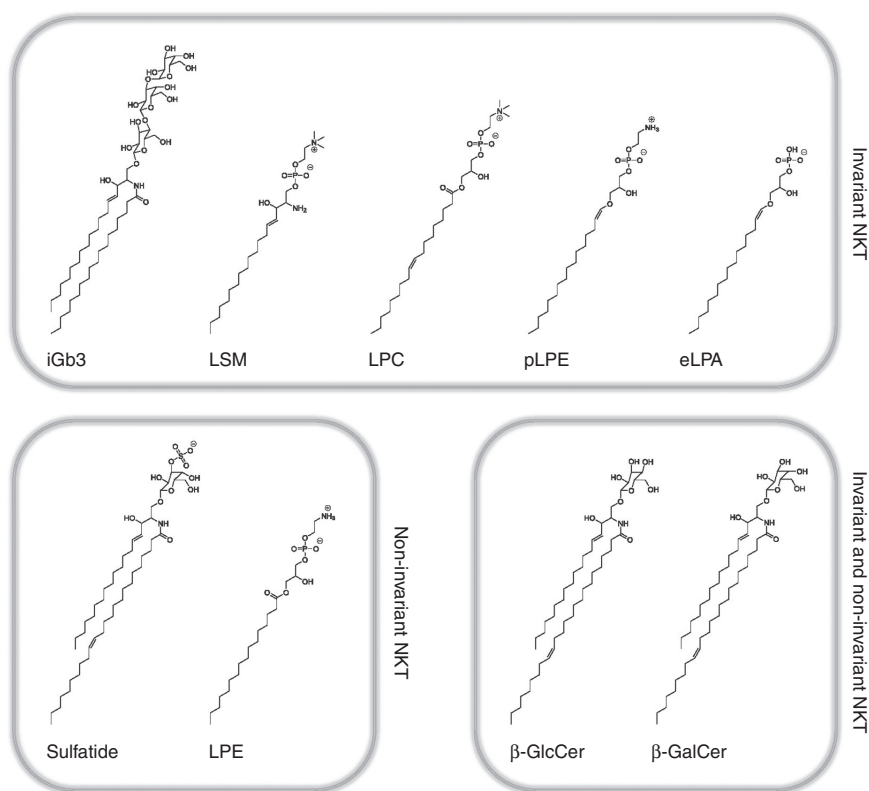


Figure 1 Self-antigens involved in the activation of invariant and non-invariant NKT cells.

size, and its linkage to the lipid backbone affect TCR recognition (De Libero et al., 2009; Girardi and Zajonc, 2012; Rossjohn et al., 2012). Moreover, the length, composition, and saturation of lipid tails modulate positioning of lipids within CD1, thus regulating TCR recognition (De Libero et al., 2009; Girardi and Zajonc, 2012; Rossjohn et al., 2012). Remarkably, the spectrum of antigenic headgroups extends from more (iGb3) or less complex (β -GlcCer) carbohydrates and phospholipid headgroups (LPC and LPE) to the minimal sugar-free phosphate anion headgroup of eLPA (Zhou et al., 2004a,b; Brennan et al., 2011; Facciotti et al., 2012; Ly et al., 2013). In case of GSLs, mammalian β -glycosidic linkage confers lower TCR affinity compared with microbial α -glycosidic linkage because it requires an induced fit of the NKT-TCR (Pellicci et al., 2011; Yu et al., 2011). Similarly, complex GSL headgroups may require enzymatic processing and trimming for TCR recognition, whereas some adaptation to bulkier hexose headgroups such as that of iGb3 can also be achieved by an induced TCR fit (Florence et al., 2009). In contrast, the mechanisms that confer specificity to NKT-TCR recognition of some but not other phospholipids and the mechanistic basis of the observed increased potency of lyso-variants of phospholipids and sphingolipids are just beginning to emerge (Lopez-Sagaseta et al., 2012).

Finally, recent technical advances including group 1 CD1 tetramers and lipidome analysis of engineered group 1 CD1 proteins provide the basis for ongoing studies of the CD1a-, CD1b-, and CD1c-associated lipidome and the mechanisms that govern recognition by group 1 CD1 TCRs (Huang et al., 2011; Kasmar et al., 2011; Ly et al., 2013; Van Rhijn et al., 2013). These studies have revealed a significant overlap in the spectrum of lipids associated with group 1 and group 2 CD1 (Huang et al., 2011). Moreover, commonalities in self-lipid recognition by group 1 and 2 CD1-restricted (NK)T cells are likely to exist, given the recognition of particular self-antigens such as sulfatide across group 1 and group 2 CD1-restricted (NK)T cells (Shamshiev et al., 2002; Jahng et al., 2004). However, it is conceivable that the spectrum of antigenic lipids associated with group 1 CD1 exceeds that of CD1d given the diversity in the TCR repertoire of group 1 CD1-restricted T cells (Grant et al., 1999).

Environmental influences shape the CD1 self-lipid repertoire

Recent studies have revealed that the repertoire of CD1 self-lipids is not static but rather subject to continuous

regulation in response to microbial, inflammatory, and likely metabolic stimuli. Such alterations may act in concert with microbiota- and inflammation-induced maturation of APCs, upregulation of CD1 expression, and CD1-dependent and CD1-independent (NK)T cell activation to contribute to CD1- and (NK)T cell-dependent immunity. Moreover, metabolic factors such as serum lipids and lipoproteins are involved in the regulation of CD1 expression and further contribute to the control of CD1-dependent immunity (Gogolak et al., 2007; Leslie et al., 2008).

Microbial infection and exposure to toll-like receptor (TLR) agonists affects cellular lipid metabolism and leads to alterations in the spectrum and antigenicity of CD1 lipids, particularly through the regulation of sphingolipid metabolism (De Libero et al., 2005; Paget et al., 2007; Salio et al., 2007; Muindi et al., 2010; Brennan et al., 2011). Recent studies have provided a comprehensive characterization of microbial-induced alterations in the sphingolipid self-antigen repertoire associated with CD1d and revealed β -GlcCer as a potent self-antigen for (NK)T cells, which is induced upon bacterial infection or TLR stimulation (Muindi et al., 2010; Brennan et al., 2011). In addition, it has recently been demonstrated that microbial-induced alterations in the endogenous CD1d lipid repertoire are not limited to bacterial infection. Thus, hepatitis B virus (HBV) infection is, among other broad alterations in the hepatocyte lipid profile, associated with increased abundance of PE. PE binds to CD1d but is non-antigenic and requires HBV-dependent induction of selected secretory PLA₂ enzymes for hydrolysis into LPE (Figure 1), which is strongly antigenic for a subgroup of non-invariant NKT cells (Zeissig et al., 2012). Noteworthy, alterations in the abundance of several antigenic CD1d lipids, including β -GlcCer and lysophospholipids, are not restricted to specific microbial infections but represent common signatures of inflammatory and malignant diseases (Fuchs et al., 2012; Santos and Schulze, 2012). It is therefore likely that the CD1 self-lipid repertoire is under continuous metabolic and immune-mediated control, with broad implications for (NK)T cell-dependent immunity at steady state and during inflammatory, malignant, and metabolic disorders.

Microbial organisms contain potent CD1 lipid antigens

In addition to shaping the repertoire of CD1 self-lipids, environmental and particularly microbial exposure provides a wealth of exogenous lipid antigens, which

contributes to antimicrobial responses of lipid-reactive T- and (NK)T cells in the context of local or systemic infections (Kinjo and Kronenberg, 2009; Brennan et al., 2013).

The structural complexity of microbial-derived CD1 lipid antigens considerably exceeds that of self-lipid antigens. Thus, seminal studies by Brenner, Porcelli, Moody, and others revealed that group 1 CD1 associates with a wide variety of mycobacterial phospholipids, glycolipids, mycolic acids, lipopeptides, mycoketides, and isoprenoids, which act as antigens for group 1 CD1-restricted T cells (Moody et al., 2005; Cohen et al., 2009). Similarly, various group 2 CD1 (CD1d)-binding, (NK)T cell-activating lipids have been described and include α -linked GSLs derived from *Sphingomonas* species (e.g., GSL-1; Figure 2) (Kinjo et al., 2005; Mattner et al., 2005; Sriram et al., 2005), diacylglycerol-containing glycolipids derived from *Borrelia burgdorferi* (e.g., Bb-GLIc; Figure 2) (Kinjo et al., 2006), *Streptococcus pneumoniae* and group B *Streptococcus* (Kinjo et al., 2011), phospholipids derived from mycobacteria and *Corynebacterium* spp. [e.g., *Corynebacterium glutamicum* diphosphatidylglycerol (Cg-DPG); Figure 2] (Fischer et al., 2004; Tatituri et al., 2013), a cholesterol ester produced by *Helicobacter pylori* (PI-57; Figure 2) (Chang et al., 2011), and lipophosphoglycans derived from *Leishmania donovani* (Amprey et al., 2004), and *Entamoeba histolytica* (Lotter et al., 2009).

Exogenous antigens typically associate with a particular CD1 isoform and load onto CD1 either at the plasma membrane or in the endolysosomal pathway. The specificity of lipid binding to a particular CD1 isoform relates both to the structural requirements of these interactions and differences in the compartmental distribution of CD1 isoforms and lipids. Thus, CD1a contains a shallow and laterally oriented F' pocket, which, for example,

facilitates binding and presentation of the peptide moiety of the lipopeptide didehydroxymycobactin (Moody et al., 2004; Zajonc et al., 2005). In contrast, binding of mycobacteria-associated long-chain mycolic acids to CD1b is facilitated by the large antigen binding groove of CD1b and specific trafficking of these lipids to late endosomes, where CD1b is located and where loading is facilitated by acidic pH (Moody et al., 2002; Rellosio et al., 2008). In case of mycobacterial mannosyl phosphomycoketide, specific binding to CD1c is conferred by methyl branches found in foreign mycoketides but not mammalian self-lipids (Scharf et al., 2010; Ly et al., 2013). These examples of specific interactions between microbial lipids and CD1 illustrate the unique ability of CD1 to survey different subcellular compartments for exogenous, microbial lipid antigens in a CD1 isoform-dependent manner.

In the context of an infection, antigen-dependent and antigen-independent pathways contribute to the activation of lipid-reactive T cells and particularly CD1d-restricted (NK)T cells (see below). For these reasons, the contribution of an individual microbial-derived CD1 lipid antigen to immune-mediated control of a particular infection is often difficult to assess. However, studies using group 1 and group 2 CD1 tetramers loaded with microbial lipids could demonstrate the presence of (NK)T cells reactive or cross-reactive to these lipids in humans and mice (Fischer et al., 2004; Kinjo et al., 2005; Mattner et al., 2005; Sriram et al., 2005; Kinjo et al., 2006; Kasmar et al., 2011; Ly et al., 2013; Van Rhijn et al., 2013). Moreover, expansion of mycobacterial lipid-reactive group 1 CD1-restricted T cells in humans and mice following *M. tuberculosis* infection suggests that these T cells and the lipid antigens recognized participate in the antimicrobial immune response *in vivo* (Folio et al., 2009; Van Rhijn et al., 2013). Future studies in animal models will be critical to delineate the contribution of individual lipid antigens to antimicrobial immune responses through genetic and chemical manipulation of metabolic pathways associated with the generation of these lipids.

Lipid-reactive T cells

Endogenous and exogenous lipids presented by CD1 activate subsets of lipid-reactive T cells in a TCR-restricted manner. Thus, group 1 CD1 presents lipid antigens to CD1a-, CD1b-, and CD1c-restricted T cells, whereas group 2 CD1 presents lipids to CD1d-restricted (NK)T cells (Porcelli et al., 1989, 1992; Balk et al., 1991; Blumberg et al., 1991; Beckman et al., 1994; Kawano et al., 1997).

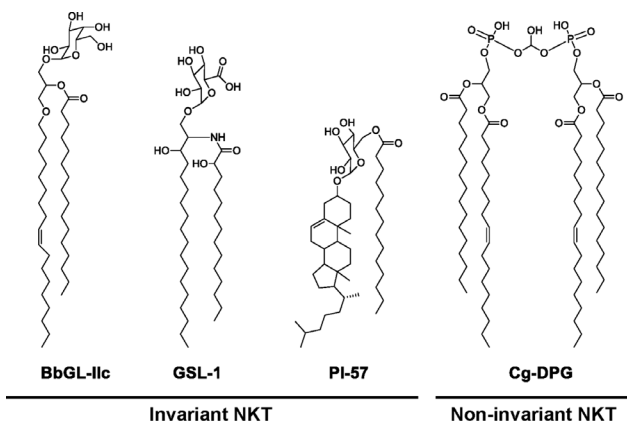


Figure 2 Selected microbial lipid antigens that activate invariant and non-invariant NKT cells.

CD1d-restricted (NK)T cells

CD1d-restricted (NK)T cells are distinguished, based on their TCR expression, into invariant and non-invariant NKT cells, which differ with regard to the antigens recognized and the functions exerted. Invariant, or type I, (NK)T cells are characterized by the expression of a semi-invariant TCR composed of V α 14-J α 18 in mice and V α 24-J α 18 in humans, paired with a limited set of V β chains (Bendelac et al., 2007). iNKT cells recognize the marine sponge glycolipid α -GalCer and can be directly detected using CD1d-tetramers loaded with this lipid (Matsuda et al., 2000), which has provided unique insight into the phenotype and function of this (NK)T cell subset. iNKT cells exhibit co-expression of T-cell and NK-cell markers and are characterized by an effector memory phenotype (Bendelac et al., 2007). In accordance with these phenotypic characteristics, iNKT cells exhibit rapid, innate-like activation and secrete abundant amounts of T_H1 [interferon (IFN) γ], T_H2 [interleukin (IL) 4, 13, and 10], and T_H17 cytokines (IL-17) with broad effects on other innate and adaptive immune cells and central roles in antimicrobial and cancer immunity as well as the regulation of autoimmunity and metabolism (Bendelac et al., 2007; Brennan et al., 2013).

Non-invariant, or type II, NKT cells are also CD1d-restricted but do not express the semi-invariant TCR. Compared with iNKT cells, less is known about non-invariant NKT cells due to the lack of specific markers for these cells. Initial studies on non-invariant NKT cells in MHC class II-deficient mice as well as subsequent studies on a subset of non-invariant NKT cells that recognize sulfatide revealed an oligoclonal TCR repertoire with predominant usage of V β 8 paired with a limited number of different V α chains (V α 8, V α 3, and V α 1) (Cardell et al., 1995; Park et al., 2001; Arrenberg et al., 2010). In addition to differences in the TCR repertoire between invariant and non-invariant NKT cells, the antigens recognized by these cells (see above), the mode of their recognition, and the phenotype and function of (NK)T cell subsets are distinct. Thus, the ternary complex of CD1d, sulfatide, and the non-invariant NKT-TCR revealed fundamental differences in interaction compared with the iNKT-CD1d complex (Girardi et al., 2012; Patel et al., 2012). Specifically, the type I NKT-TCR adopts a tilted and parallel docking mode over the F'-pocket of CD1d, whereas the type II NKT-TCR docked diagonally above the A'-pocket of CD1d (Girardi et al., 2012; Patel et al., 2012). Functionally, non-invariant NKT cells lack constitutive expression of the early activation marker CD69 and exhibit regulatory actions in part through suppression of iNKT cell activation (Terabe et al., 2000; Jahng et al., 2004; Halder et al., 2007; Berzofsky and Terabe, 2009; Arrenberg et al., 2010). However,

LPE-reactive non-invariant NKT cells can support anti-microbial immunity both directly *via* IFN- γ secretion and indirectly *via* activation of iNKT cells and conventional T cells, suggesting the presence of functional heterogeneity even within the non-invariant NKT cell subset (Zeissig et al., 2012). Finally, additional complexity is provided by the recent discovery of atypical (NK)T cell subsets, which are α -GalCer/CD1d-tetramer-reactive, but do not express murine V α 14 or human V α 24 (Constantinides et al., 2011; Uldrich et al., 2011).

Group 1 CD1-restricted T cells

In contrast to (NK)T cells, group 1 CD1-restricted T cells do not exist in mice due to the lack of CD1a, CD1b, and CD1c. For this reason, functional studies of group 1 CD1-restricted T cells were initially limited to the analysis of T cell clones expanded from human peripheral blood. These studies revealed that group 1 CD1-restricted T cells express either $\alpha\beta$ - or $\gamma\delta$ -TCRs, can be grouped based on co-receptor expression into CD4⁺, CD8⁺, or double-negative T cells, and consistently secrete IFN- γ upon activation, whereas secretion of T_H2 and T_H17 cytokines exhibits significant CD1 isoform-dependent and inter-individual variation (Porcelli et al., 1989; Vincent et al., 2005; de Jong et al., 2010; de Lalla et al., 2011). Further, these cells resemble conventional MHC class I- and II-restricted T cells rather than iNKT cells in that they express a diverse TCR V α and V β repertoire and, at least in peripheral blood, express markers characteristic of naive T cells (Grant et al., 1999; Vincent et al., 2005; de Jong et al., 2010; de Lalla et al., 2011). Similar to conventional MHC class I- and II-restricted T cells, the ratio of memory to naive group 1 CD1-restricted T cells increases from birth to adulthood with an increased percentage of cells expressing memory markers in peripheral tissues, suggesting antigen-dependent, activation-induced transition from naive to memory phenotype (de Jong et al., 2010; de Lalla et al., 2011).

Although studies of group 1 CD1-restricted T cell clones provided critical insight into the mechanisms of TCR-dependent recognition of foreign lipid antigens, it remained unclear whether the phenotype, function, and frequency of group 1 CD1-restricted T cells within these clonal populations reflected the situation found *in vivo*. The development of group 1 CD1 tetramers therefore represented a major breakthrough in the field because it provided the opportunity to directly detect group 1 CD1-restricted T cells among peripheral blood mononuclear cells (Kasmar et al., 2011; Ly et al., 2013; Van Rhijn

et al., 2013). These studies revealed that T cells recognizing mycobacterial lipids in the context of CD1b and CD1c can be detected among polyclonal T cells in peripheral blood, whereas CD1b-restricted autoreactivity is not observed in contrast to CD1a (de Jong et al., 2010; de Lalla et al., 2011; Van Rhijn et al., 2013). Moreover, this work led to the recognition of an invariant CD1b-restricted germline-encoded, mycolyl lipid-reactive (GEM) T cell subset expressing a semi-invariant TCR composed of TRAV1-2 and TRAJ9 paired with a limited set of V β chains (Van Rhijn et al., 2013). Despite the expression of a semi-invariant TCR, GEM T cells are phenotypically and functionally distinct from iNKT cells in that they do not consistently express the NK-cell marker CD161, lack baseline expression of the activation marker CD69, and are increased in numbers in patients with tuberculosis in accordance with infection-induced expansion of GEM T cells (Van Rhijn et al., 2013). In addition, recently developed group 1 CD1 transgenic mice confirmed that group 1-restricted T cells resemble conventional T cells rather than iNKT cells and exhibit delayed immune responses and infection-induced expansion (Folio et al., 2009).

In conclusion, pioneering studies of lipid-reactive T cell clones and the recent development of group 1 CD1 tetramers and transgenic mice (see below) provided critical insight into the biology of CD1a-, CD1b-, and CD1c-restricted T cells. This work revealed that group 1 CD1-restricted T cells phenotypically and functionally resemble conventional T cells rather than iNKT cells and recognize a wide variety of mycobacterial lipid antigens. Future studies using tetramers and animal models will be important to obtain further insight into the frequency, TCR repertoire, and function of CD1a-, CD1b-, and CD1c-restricted T cells *in vivo*.

Antigen-dependent and antigen-independent pathways to activation of CD1-restricted (NK)T cells

Antigen-dependent and antigen-independent pathways to CD1-restricted, (NK)T cell-dependent immunity have been described (Figure 3). As such, a large number of microbial lipids can bind to CD1 and activate CD1-restricted T cells *in vitro* and *in vivo*, thus providing the basis for antigen-driven, CD1-restricted antimicrobial immune responses (Figure 3, left). In addition, CD1 expression can be modulated upon infection, which can either facilitate antigen presentation through upregulation of CD1 or contribute

to immune evasion through interference with this process (Amprey et al., 2004; Shinya et al., 2004; Berntman et al., 2005; Cho et al., 2005; Hage et al., 2005; Lin et al., 2005; Renukaradhya et al., 2005; Sanchez et al., 2005; Skold et al., 2005; Chen et al., 2006; Raghuraman et al., 2006; Yuan et al., 2006; Donovan et al., 2007; Kawana et al., 2007; Raftery et al., 2008).

Moreover, microbial exposure can affect the CD1 self-lipid repertoire and its antigenicity, thereby contributing to protective antimicrobial immunity through (NK)T cell activation (De Libero et al., 2005; Paget et al., 2007; Salio et al., 2007; Muindi et al., 2010; Brennan et al., 2011; Zeissig et al., 2012).

In addition to direct, antigen-dependent activation of lipid-reactive T cells, indirect cytokine-driven mechanisms exist, which promote the activation of CD1d-restricted iNKT cells independent of microbial lipid antigens. Thus, recognition of microbe-associated microbial patterns (MAMPs) through pattern recognition receptors such as TLRs and dectin 1 on dendritic cells (DCs) is associated with production of TLR4-dependent IL-12 and IL-18, dectin 1-dependent IL-12, and TLR9-dependent IL-12 and type I IFN. These cytokines potentially activate iNKT cells, which is further enhanced by CD1d-restricted presentation of self-lipid antigens (Figure 3, second from left) (Brigl et al., 2003, 2011; Mattner et al., 2005; Montoya et al., 2006; Brennan et al., 2011; Cohen et al., 2011). Depending on the context, cytokines can be sufficient for indirect stimulation of iNKT cells, even in the absence of CD1d signals (Figure 3, second from right) (Nagarajan and Kronenberg, 2007; Tyznik et al., 2008). In addition to these mechanisms, TLR engagement and APC- and iNKT cell-dependent cytokine secretion promote CD1d-restricted antigen presentation through differentiation and maturation of DCs (Fujii et al., 2003; Hermans et al., 2003, 2007; Hegde et al., 2007). Cytokine-mediated iNKT cell activation thus enables iNKT cell-dependent recognition of bacteria and viruses devoid of CD1d-restricted lipid antigens and provides a mechanistic explanation for broad, iNKT cell-dependent immune responses against a wide variety of bacterial, viral, fungal, and protozoan pathogens. Moreover, lipid antigen- and cytokine-dependent pathways of iNKT cell activation are not exclusive but act together in iNKT cell-mediated antimicrobial immune responses *in vivo*. Thus, even in the case of infection with microbes containing antigenic CD1d lipids, cytokine-mediated stimulation of iNKT cells critically contributes to the overall immune response (Brigl et al., 2003, 2011). Finally, neurotransmitters may provide a third pathway to iNKT cell activation in addition to antigen- and cytokine-dependent mechanisms (Figure 3, right) (Wong et al., 2011).

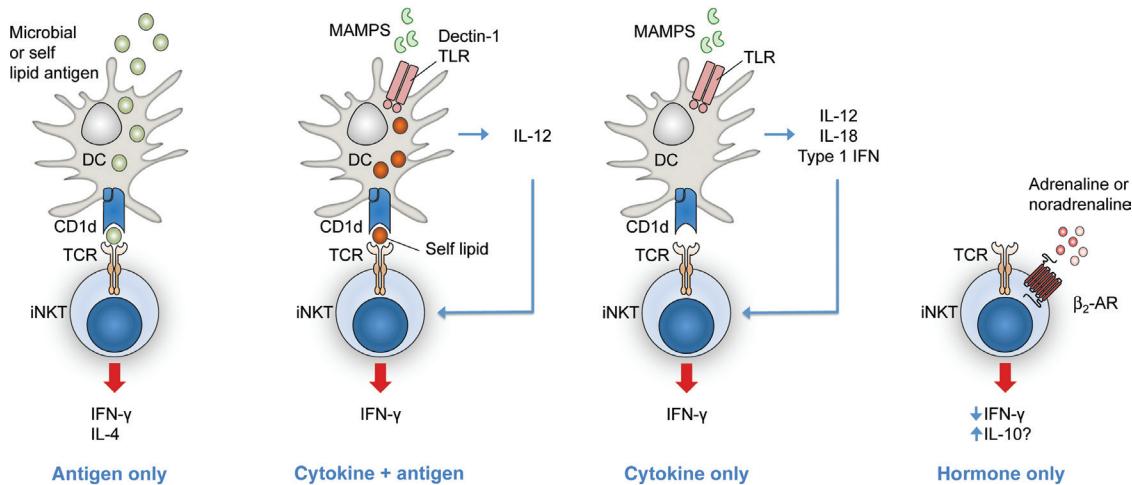


Figure 3 Direct and indirect mechanisms of iNKT cell activation or modulation. β_2 -AR, β_2 -adrenergic receptor; DC, dendritic cell.

In conclusion, the activation of lipid-reactive (NK)T cells in infection is facilitated by regulation of CD1 expression, alterations in the CD1 self-lipid profile, modulation of APC function, and antigen- and cytokine-dependent iNKT cell activation.

Murine studies of lipid-reactive T cells in immunity, cancer, and metabolism

Our knowledge of the physiological and pathophysiological roles of lipid-reactive T cells is largely based on the study of CD1d-restricted (NK)T cells in mice. These studies revealed that (NK)T cells play critical roles in antimicrobial immunity, cancer immunosurveillance, the modulation of immune-mediated or autoimmune diseases, and the regulation of lipid and carbohydrate metabolism. Selective aspects of the functions of (NK)T cells and group 1 CD1-restricted T cells in immunity are discussed in the following sections. A more comprehensive description of this topic has been provided by several excellent, recent reviews (Berzofsky and Terabe, 2009; Cohen et al., 2009; Kinjo and Kronenberg, 2009; Vivier et al., 2012; Brennan et al., 2013).

(NK)T cells in antimicrobial immunity

Protective as well as pathogenic roles of (NK)T cells have been described in the immune response to a large number of bacterial, viral, fungal, and protozoan infections and

in a subset of cases, direct, antigen-driven and indirect, cytokine-mediated (NK)T cell activation could be delineated (Tupin et al., 2007; Cohen et al., 2009). Thus, microbial-derived, CD1d-restricted lipid antigens implicated in antimicrobial immunity were found in *Sphingomonas* spp. (Kinjo et al., 2005; Mattner et al., 2005; Sriram et al., 2005), *B. burgdorferi* (Kinjo et al., 2006), *S. pneumoniae*, group B *Streptococcus* (Kinjo et al., 2011), *Mycobacterium tuberculosis* (Fischer et al., 2004; Tatituri et al., 2013), *H. pylori* (Chang et al., 2011), *L. donovani* (Amprey et al., 2004), and *E. histolytica* (Lotter et al., 2009). In contrast, TLR-induced cytokine-driven responses mediate iNKT cell activation in response to bacteria and viruses devoid of lipid antigens and also contribute to immunity against bacteria containing CD1d-restricted lipid antigens (Brigl et al., 2003, 2011; Mattner et al., 2005; Montoya et al., 2006; Nagarajan and Kronenberg, 2007; Tyznik et al., 2008). Another example of combined direct and indirect effects on (NK)T cell activation is HBV infection, where early HBV-induced alterations in the self-lipid repertoire are recognized by a subset of non-invariant NKT cells, which consequently leads to broad, IL-12-dependent activation of other immune cells including iNKT cells (Zeissig et al., 2012).

Although a vast number of studies aimed at the delineation of the roles of invariant and non-invariant NKT cells in antimicrobial immunity, these studies were based on comparative analysis of CD1d- and $\alpha 18$ -deficient mice and may need reassessment given the recent discovery of unanticipated, broad defects in the TCR repertoire of $\alpha 18$ knockout mice (Bedel et al., 2012). CD1d knockout mice (*Cd1d*^{−/−} *Cd1d2*^{−/−}) lack both invariant and non-invariant NKT cells due to loss of CD1d-restricted positive selection in the thymus (Chen et al., 1997; Gapin et al., 2001; Benlagha

et al., 2005). In contrast, $J\alpha 18$ knockout mice exhibit a deletion of the $J\alpha$ segment of the semi-invariant iNKT cell receptor (Traj18^{-/-}) and were developed to selectively delete iNKT cells (Cui et al., 1997). However, a recent study revealed that $J\alpha 18$ knockout mice exhibit an unanticipated loss of a significant fraction of $J\alpha$ regions associated with lack of about 60% of TCR α diversity (Bedel et al., 2012). Impaired immunity in Traj18^{-/-} mice may therefore relate to lack of iNKT cells as well as defects in the conventional T cell repertoire, which limits conclusions derived from the study of these mice. For these reasons, the relative contributions of invariant and non-invariant NKT cells to antimicrobial immunity are largely unclear at present.

Finally, in accordance with the critical role of (NK)T cells in antimicrobial immunity, immune evasion strategies exist, particularly for viral agents. Thus, HIV infection is associated with internalization and trans-Golgi retention of CD1d in a manner dependent on the HIV Nef and gp120 proteins (Cho et al., 2005; Hage et al., 2005; Chen et al., 2006). Moreover, HIV infection is associated with (NK)T cell exhaustion through the modulation of PD-1 expression and PD-1-independent pathways (Moll et al., 2009). Similar observations of evasion of CD1d-restricted immunity have been made for other viruses such as lymphocytic choriomeningitis virus, vesicular stomatitis virus, herpes simplex virus, Kaposi sarcoma-associated herpesvirus and bacteria including *Leishmania* and *Chlamydia* species (Lin et al., 2005; Sanchez et al., 2005; Raftery et al., 2006; Yuan et al., 2006; Kawana et al., 2007; Renukaradhya et al., 2008).

(NK)T cells in cancer immunosurveillance

α -GalCer, a marine sponge-derived GSL, was originally identified in a screen for tumor-modulating compounds, where it was associated with protection in the B16 melanoma model (Kobayashi et al., 1995; Motoki et al., 1996). α -GalCer was later demonstrated to act through the activation of iNKT cells and to suppress tumor development in an IFN- γ - and IL-12-dependent manner (Kawano et al., 1997; Kitamura et al., 1999). In addition, related ceramide-based lipids such as β -mannosylceramide elicit similar anti-oncogenic actions but act through independent iNKT cell-mediated pathways dependent on TNF- α and nitric oxide (O'Konek et al., 2011). Even in the absence of exogenous, stimulatory lipid antigens, iNKT cells contribute to natural immunosurveillance in solid and hematopoietic cancers including fibrosarcoma induced by methylcholanthrene

(Smyth et al., 2000; Crowe et al., 2002, 2005), adenocarcinomas, sarcomas, and hematopoietic tumors induced by loss of p53 (Swann et al., 2009), colorectal cancer (Yoshioka et al., 2012), hepatocellular carcinoma (Anson et al., 2012), and neuroblastoma (Song et al., 2009). Studies in neuroblastoma also revealed that iNKT cells may not only interfere with tumor growth through direct tumor cell cytotoxicity but can act indirectly through killing of tumor-associated, oncogenic macrophages (Song et al., 2009). Although initial cancer studies were performed in $J\alpha 18$ knockout mice, with limitations as described above, CD1d-deficient mice exhibited a similar phenotype, and the transfer of total (NK)T cells or purified iNKT cells was associated with protection thus supporting a role of iNKT cells in anticancer immunity (Crowe et al., 2002, 2005; Song et al., 2009; Izhak et al., 2013). One remarkable exception to the rule of anti-oncogenic effects of iNKT cells is the model of azoxymethane/oxazolone-induced colitis-associated colorectal cancer, which is mediated by iNKT cells in a process dependent on IL-13 secretion, induction of CD11b^{hi} Gr1^{low} macrophages, and transforming growth factor- β (TGF- β)-dependent suppression of tumor immunosurveillance (Schiechl et al., 2011).

In contrast to protective roles of iNKT cells, non-invariant NKT cells were suggested to contribute to tumor growth in an oncogenic manner in models of fibrosarcoma as well as mammary, colorectal, and renal cell carcinoma (Terabe et al., 2000, 2003; Izhak et al., 2013). These actions were mediated by IL-13-induced expression of TGF- β by CD11b⁺ Gr1⁺ cells and suppression of tumor-specific CD8⁺ T cells (Terabe et al., 2000, 2003; Izhak et al., 2013). In the majority of these studies, the protective effects of CD1d deletion were ascribed to the loss of non-invariant NKT cells, as tumor development in $J\alpha 18$ -deficient mice resembled that observed in wildtype mice. Although the defects in the conventional TCR repertoire may have affected tumor cell growth, recent studies based on selective activation of invariant and non-invariant NKT cell subsets and reconstitution of iNKT cells in $J\alpha 18$ -deficient mice revealed similar results and support the concept of opposing roles of NKT cell subsets in cancer (Izhak et al., 2013). Noteworthy, invariant and non-invariant NKT cell subsets cross-regulate each other not only in terms of function but also frequency, thus adding another layer of complexity to the role of (NK)T cells in malignancy (Izhak et al., 2013).

Although the aforementioned studies were performed in mice, indirect evidence suggests similar roles of (NK)T cells in human malignancy. Thus, iNKT cell infiltration in colorectal cancer and neuroblastoma correlates with increased survival (Metelitsa et al., 2004; Tachibana et al., 2005), whereas low levels of circulating iNKT cells predict

poor clinical outcomes in head and neck squamous cell carcinoma (Molling et al., 2007). Moreover, iNKT cells obtained from human cancer patients exhibit impaired proliferative responses, decreased secretion of IFN- γ , and an increased ratio of regulatory IL-4 to anti-oncogenic IFN- γ (Tahir et al., 2001; Yanagisawa et al., 2002; Dhodapkar et al., 2003; Bricard et al., 2009).

Given the protective roles of iNKT cells in oncogenesis, therapeutic modulation of (NK)T cells has been investigated in human malignancy. Direct injection of α -GalCer into patients with solid tumors proved largely unsuccessful due to limited iNKT cell responses (Giaccone et al., 2002). Subsequent approaches included the transfer of α -GalCer-pulsed autologous DCs, which was also associated with considerable, inter-individual variation in the response observed (Ishikawa et al., 2005; Motohashi et al., 2006, 2009; Uchida et al., 2008; Nagato et al., 2012). Therefore, combination therapy may be required for efficacious (NK)T cell-based immunotherapy in cancer. In accordance with this concept, a subset of patients with multiple myeloma exhibited tumor regression upon treatment with α -GalCer-loaded dendritic cells in combination with lenalidomide (Richter et al., 2013).

Together, these studies demonstrate a critical role of (NK)T cells in tumor development and warrant further optimization of (NK)T cell-based immunotherapy in human malignancy.

(NK)T cells in the regulation of immune-mediated diseases

(NK)T cells were suggested to contribute to a variety of human immune-mediated diseases and their murine models including type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, autoimmune hepatitis, primary biliary cirrhosis, sarcoidosis, asthma, and inflammatory bowel disease (IBD). Owing to the complexity of this field and the controversies that have arisen (Subleski et al., 2011), we will focus on the role of (NK)T cells in IBD, where recent studies have provided critical insight into the regulation of mucosal immune responses by (NK)T cells at the interface between the host and the environment.

We and others could demonstrate that (NK)T cells play critical roles in human IBD and mouse models of intestinal inflammation (Saubermann et al., 2000; Heller et al., 2002; Fuss et al., 2004; Zeissig et al., 2007; Schiechl et al., 2011; Camelo et al., 2012; Olszak et al., 2012). Thus, oxazolone colitis, a model of human ulcerative colitis

(UC), is dependent on CD1d and CD1d-restricted (NK)T cells (Heller et al., 2002; Schiechl et al., 2011; Olszak et al., 2012). Similar findings were made for human UC, where key pathogenic cytokines such as IL-13 originate from (NK)T cells and contribute to impaired epithelial barrier function and sustained intestinal inflammation (Fuss et al., 2004; Heller et al., 2005). Although studies in oxazolone colitis reported a central, pathogenic role of iNKT cells (Heller et al., 2002), this work was based on α 18-deficient mice and is associated with the respective limitations outlined above (Bedel et al., 2012). In contrast, pathogenic IL-13 in human UC originates from α -GalCer/CD1d-tetramer-negative T cells and thus presumably non-invariant NKT cells (Fuss et al., 2004). In accordance with a predominant role of non-invariant NKT cells in intestinal inflammation, transgenic expression of a non-invariant TCR led to spontaneous intestinal inflammation in mice, particularly in the presence of overexpression of CD1d (Liao et al., 2012). The biological relevance of these findings is highlighted by the fact that genetic polymorphisms associated with susceptibility to IBD relate to genes enriched in expression by NKT cells (Jostins et al., 2012). Further, a variety of genes with critical roles in lipid metabolism have been highlighted by expression analysis and genome-wide association studies in IBD and may at least partially contribute to disease susceptibility through modulation of the activation of lipid-reactive T cells (Jostins et al., 2012; Planell et al., 2013). Finally, a randomized, placebo-controlled, multicenter study recently revealed the efficacy of oral PC in UC (Karner et al., 2012). Given that PC binds to CD1d but does not activate human (NK)T cells (Fox et al., 2009), interference with CD1d-restricted mucosal (NK)T cell activation may contribute to the therapeutic efficacy of PC, a concept that awaits verification.

It has recently been demonstrated that intestinal inflammation in oxazolone colitis is not dependent on microbial antigens as germfree mice exhibited increased rather than decreased intestinal inflammation (Olszak et al., 2012). These findings suggest that a yet to be identified self-antigen may initiate and mediate chronic, (NK)T cell-dependent intestinal inflammation. Moreover, these studies revealed that exposure to the intestinal microbiota during early but not adult life leads to a persistent decrease in intestinal iNKT cell numbers and protection from (NK)T cell-mediated intestinal inflammation during adult life (Olszak et al., 2012). The microbiota therefore plays critical roles in the modulation of (NK)T cells at mucosal surfaces, a phenomenon similarly observed in the lung (Olszak et al., 2012). Future studies will be required to delineate the cellular origin and the nature of presumed

self-antigens, which initiate (NK)T cell-dependent inflammation at mucosal surfaces. Similarly, antigen-dependent and antigen-independent environmental modulators of postnatal, mucosal (NK)T cell development await further characterization.

(NK)T cells in the regulation of lipid and carbohydrate metabolism

(NK)T cells are affected by dysregulation of lipid metabolism and, conversely, also actively control lipid and carbohydrate metabolism, thus reflecting a bidirectional process of regulation. Moreover, (NK)T cells modulate disorders at the interface of metabolism and inflammation such as obesity, metabolic syndrome, and non-alcoholic fatty liver disease (NAFLD). Thus, obesity is associated with reduced numbers of iNKT cells in peripheral blood, liver, and adipose tissue in humans and mice, whereas weight loss leads to increasing numbers of peripheral iNKT cells (Guebre-Xabier et al., 2000; Lynch et al., 2009, 2012; Kotas et al., 2011; Ji et al., 2012). Moreover, NAFLD is associated with reduced CD1d expression by hepatocytes and impaired hepatocyte-dependent iNKT cell activation (Yang et al., 2007).

In accordance with bidirectional regulation of (NK)T cells and lipid metabolism, CD1d and $\alpha 18$ deficiency in mice, independent of low- or high-fat diet, is associated with adipocyte hypertrophy, increased leptin as well as decreased adiponectin levels, and insulin resistance (Lynch et al., 2012; Schipper et al., 2012). On a high fat diet, CD1d- and $\alpha 18$ -deficient mice also exhibit increased weight gain and hepatic steatosis (Lynch et al., 2012). In further support of a mechanistic link between iNKT cells and the regulation of glucose and lipid metabolism, transfer of iNKT cells or α -GalCer treatment was associated with reduced adipocyte size, decreased leptin and increased adiponectin levels, increased M2 macrophage polarization, anti-inflammatory IL-10 and IL-4 signaling, and improved insulin sensitivity (Ji et al., 2012; Lynch et al., 2012). Importantly, both hepatocytes and adipocytes express CD1d and can activate iNKT cells in a CD1d-restricted manner (Yang et al., 2007; Schipper et al., 2012). This raises the possibility that obesity-induced alterations in the adipocyte and hepatocyte CD1d self-lipid repertoire may regulate metabolism and obesity-associated inflammation *via* modulation of iNKT cell activation. Although differences in the extent and outcome of iNKT cell-mediated metabolic modulation were observed between studies (Mantell et al., 2011; Wu

et al., 2012), these discrepancies may relate to differences in the intestinal microbiota, which is modulated by host metabolism and regulates iNKT cell function (Olszak et al., 2012; Tremaroli and Backhed, 2012; Wingender et al., 2012).

Together, these studies demonstrate a tight connection between metabolism and immunity and reveal a central role of lipid-reactive T cells in the bidirectional regulation of this process.

Murine studies of group 1 CD1-restricted T cells in immunity

Although a large number of studies in rodent models characterized the role of CD1d-restricted (NK)T cells *in vivo*, similar studies of CD1a-, CD1b-, and CD1c-restricted T cells were prevented by the lack of group 1 CD1 expression in mice. Our understanding of the function of group 1 CD1-restricted T cells is therefore largely based on the analysis of human T cell clones. These studies revealed the recognition of various mycobacterial lipid antigens by group 1 CD1-restricted T cells, suggesting a potential role of these cells in immunity to mycobacteria and particularly to *M. tuberculosis* (Moody et al., 2005; Cohen et al., 2009).

The recent development of mice with transgenic expression of human group 1 CD1 provided a crucial step toward the characterization of CD1a-, CD1b-, and CD1c-restricted T cells *in vivo* (Felio et al., 2009). Initial studies in these mice demonstrated that group 1 CD1-restricted T cells both phenotypically and functionally resemble conventional T cells rather than iNKT cells and exhibit delayed immune responses (Felio et al., 2009). In accordance with previous *ex vivo* and *in vitro* studies, which suggested a central role of CD1a, CD1b, and CD1c in the recognition of *M. tuberculosis*, infection of transgenic mice induced group 1 CD1-restricted T cell responses directed against *M. tuberculosis* with T cell-dependent recognition of previously characterized *M. tuberculosis*-derived lipids (Felio et al., 2009). Interestingly, preliminary studies revealed that clearance of *M. tuberculosis* did not differ between mice with and without group 1 CD1 expression. Further studies are therefore required to delineate the contribution of group 1 CD1 to the immune response against *M. tuberculosis* and other microorganisms and to define potential roles of CD1a-, CD1b-, and CD1c-restricted T cells in the modulation of autoimmunity, cancer immunosurveillance, and the regulation of metabolic function.

The biological role of lipid-reactive T cells in human immunity

Murine studies revealed a critical role of CD1d-restricted (NK)T cells in antimicrobial immunity and demonstrated susceptibility of CD1d-deficient mice to a vast number of microorganisms (Tupin et al., 2007; Brennan et al., 2013). However, similar observations of broad immunodeficiency were made in mice deficient in TLRs, TLR adaptors, and NK cells, whereas patients carrying mutations in these pathways exhibited few, selected immune defects (Orange, 2002; Zhang et al., 2007; von Bernuth et al., 2008). These studies therefore suggested that the immune response in inbred mice may not fully recapitulate human immunity – a finding recently confirmed in a systematic approach (Casanova and Abel, 2007; Seok et al., 2013).

To define the role of CD1 in human immunity, patients with the rare Mendelian disorder abetalipoproteinemia (ABL) were studied. ABL is characterized by mutations in MTP, an ER-resident lipid transfer protein involved in the generation of apolipoprotein B (ApoB)-containing lipoprotein particles (Wetterau et al., 1992; Berriot-Varoqueaux et al., 2000; Abumrad and Davidson, 2012). Due to deficiency in MTP, ABL patients exhibit defects in the secretion of chylomicrons and very low-density lipoprotein, which is associated with hypolipidemia and severe neurological defects (Berriot-Varoqueaux et al., 2000; Abumrad and Davidson, 2012). In addition to its role in lipoprotein metabolism, MTP can directly transfer phospholipids onto CD1d and is critical for the function of group 1 and group 2 CD1 (Brozovic et al., 2004; Dougan et al., 2005, 2007; Kaser et al., 2008). Accordingly, these studies revealed broad defects in group 1 and group 2 CD1 function in ABL (Zeissig et al., 2010). Group 1 CD1, particularly CD1a and CD1c, underwent proteasomal degradation in patients with ABL, possibly as a consequence of impaired ligand-dependent CD1 stabilization, a fate similarly observed for apolipoprotein B (ApoB) (Berriot-Varoqueaux et al., 2000; Zeissig et al., 2010). CD1d escaped proteasomal degradation and showed normal cell surface expression but was unable to load and present exogenous lipid antigens and did not elicit (NK)T cell auto-reactivity. Consequently, iNKT cells were either not detectable or showed severe phenotypic alterations in ABL. In contrast to CD1, MHC class I- and class II-restricted antigen presentation and conventional T- and B-cell function were unaltered. ABL is thus characterized by selective and severe defects in CD1 and provides a unique opportunity to study the biological relevance of CD1-restricted (NK)T cells.

Although fewer than 100 patients with ABL have been clinically characterized, several cases of heart failure due to lesions resembling chronic interstitial myocarditis, fatal

pulmonary infections, and rare malignancies have been described in ABL (Berriot-Varoqueaux et al., 2000). Given the central role of (NK)T cells in immunity to pulmonary and cardiotropic microbial pathogens as well as cancer immunity in mice, these findings support the concept of immune-related defects due to deficiency in MTP and CD1 (Zeissig et al., 2010; Zeissig and Blumberg, 2012). However, the spectrum of immune defects in ABL is remarkably restricted, which contrasts with observations in CD1d-deficient mice. Although such conclusions are limited to frequently encountered microorganisms given the low incidence of ABL, they reflect findings made for human and murine TLR and NK cell deficiency as outlined above (Orange, 2002; Zhang et al., 2007; von Bernuth et al., 2008). Together, these observations suggest that CD1-restricted (NK)T cells have critical and indispensable contributions to human and murine immunity but that mechanisms that compensate for the loss of functional CD1 prevent broad immunodeficiency, at least in humans.

Concluding remarks

Significant insight into the role of lipid-reactive T cells has been gained over the past 25 years. As such, antigen-dependent and antigen-independent pathways to the activation of lipid-reactive T cells were described, a large number of self and microbial lipid antigens were identified, and the roles of lipid-reactive T cells in immunity were characterized. Despite these significant advances, however, further studies are required to characterize the dynamics of the CD1 lipodome in health and disease, to gain further insight into the physiological role of group 1 CD1-restricted T cells *in vivo*, to delineate the functions of invariant and non-invariant NKT cells, and to define the mechanisms of (NK)T cell activation in autoimmune diseases and cancer and their modulation by environmental influences such as the microbiota.

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C. Marie Dowds studied Biosciences at the University of Kaiserslautern, Germany. After completion of her master's degree in the group of Dr. Stefan Kins, she joined the laboratory of Dr. Zeissig at the Department of Internal Medicine, Kiel University (Kiel, Germany) as PhD student. In her PhD studies, Dowds investigates the role of NKT cells and lipid antigens in intestinal inflammation.

Sabin-Christin Kornell studied Biology at the Christian-Albrechts-University of Kiel and received her master's degree in cell biology and physiology in 2013. In May 2013, Kornell started her PhD studies in Dr. Zeissig's group at the Department of Internal Medicine, Kiel University, Germany (Kiel, Germany).

Richard S. Blumberg, MD, is Professor of Medicine at Harvard Medical School, Chief of the Division of Gastroenterology, Hepatology and Endoscopy at Brigham and Women's Hospital, co-Director of the Biomedical Research Institute at Brigham and Women's Hospital and co-Director of the Harvard Digestive Diseases Center. He received his medical degree from Jefferson Medical College (Philadelphia, PA), internship and residency at The New York Hospital, Cornell Medical Center followed by fellowship training in infectious diseases at Massachusetts General Hospital, gastroenterology at Brigham and Women's Hospital and molecular immunology at Dana Farber Cancer Institute, all in Boston. His work focuses on the mechanisms that control homeostasis and inflammation at mucosal surfaces.

Sebastian Zeissig, MD, received his medical degree from the Charité Berlin, Germany. After a postdoctoral fellowship at the same institution, Dr. Zeissig joined the laboratory of Richard S. Blumberg as postdoctoral fellow to investigate the role of CD1d-restricted NKT cells in infectious hepatitis and immunodeficiency. Since 2010, Dr. Zeissig is leading the Laboratory of Mucosal Immunology at the Department of Internal Medicine, Kiel University (Kiel, Germany), where he continues to investigate the role of lipid antigens in intestinal and hepatic immunity.