CD1d-Restricted Natural Killer T Cells

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Natural killer T (NKT) cells that express the semi-invariant T-cell receptor are innate-like lymphocytes whose functions are controlled by self and foreign glycolipid ligands. Such ligands are presented by the antigen-presenting, MHC class I-like molecule CD1d, which belongs to a family of lipid antigen-presenting molecules collectively called CD1. Activation of NKT cells in vivo results in rapid release of copious amounts of effector cytokines and chemokines with which they regulate innate and adaptive immune responses to pathogens, certain types of cancers and self-antigens. The nature of CD1d-restricted ligands, the manners in which they are recognised and the unique effector functions of NKT cells suggest an immunoregulatory role for this T-cell subset. Their ability to respond fast and our ability to steer NKT cell cytokine responses to altered lipid ligands make them an important target for vaccine design and immunotherapies against autoimmune diseases. This article summarises our current understanding of CD1d-restricted NKT cell biology and how these innate-like lymphocytes control inflammation.

**Introduction**

The immune system, as a constituent of the ten physiologic systems of the body, has evolved to sense perturbations in the *milieu intérieur* (homeostasis) and to actuate an appropriate response so as to restore a set homeostatic state unique to an organism. Homeostasis can be disrupted by both internal and external stressors – such as toxic substances or microbial and parasitic infections – all of which are known to incite tissue injury. Containment and removal of the stressor – which are essential for initiating tissue repair – are accomplished initially by the more archaic, multimodular innate immune system. The evolutionarily younger, adaptive immune system consists of B and T lymphocytes – which are recruited to assist in the healing process should the innate mechanisms fail to contain and clear the inciter. The quick-acting innate system – elements of which are seen in early metazoans – senses an altered homeostatic state using pattern recognition receptors (PRRs). In contrast, the slow-responding, adaptive immune system uses antigen-specific receptors that are expressed clonally by B and T lymphocytes – B-cell receptors (BCRs and antibodies; see also: B Lymphocytes: Receptors; Antibodies) and T-cell receptors (TCRs; see also: T-Cell Receptors), respectively – to sense alterations in homeostasis.

Straddling the two immune systems are the innate-like lymphocytes that are comprised of cells expressing the classical lymphocyte receptors (BCR and TCR). These innate-like lymphocytes exhibit innate-like recognition principles and ‘trigger-ready’ functional responses. They are comprised of T cells (γδ T cells, natural killer T (NKT) cells, mucosal-associated invariant T lymphocytes and CD8αα intestinal intraepithelial lymphocytes) and B cells (B-1 B cells and marginal zone B cells; see also: B Lymphocytes; B1- and CD5-Positive B Cells). Current evidence suggests that several of these innate-like lymphocytes, including NKT cells, have evolved to jump-start and
fine-tune the nature and magnitude of the innate and adaptive immune responses. While each immune module plays a specific role, multiple modules act in concert resulting in an inflammatory response that is essential in maintaining homeostasis (Kotas and Medzhitov, 2015). This article focuses on the major subset of CD1d-restricted NKT cells because much has been learnt about them.

NKT cells – which express both NK- and T-cell phenotypic and functional features – are thymus-derived, innate-like lymphocytes whose functions are regulated by self- and non-self-lipid ligands presented by CD1d molecules. The majority of NKT cells express an invariant TCR α-chain generated by TRAV11*02 (mouse Vα14i) or TRAV10 (human Vα24i) to TRAJ18 (Jα18) rearrangement. The invariant α-chain pairs predominantly with mouse TRBV13-2*01 (Vβ8.2), TRBV29*02 (Vβ7), TRBV1 (Vβ2) or human TRBV25-1 (Vβ11) β-chain to form a functional semi-invariant TCR. A small subset – referred to as type II NKT cells – expresses a more diverse TCR repertoire; these, however, are the focus of other articles (Macho-Fernandez and Brigl, 2015; Marrero et al., 2015; Rhost et al., 2012; Terabe and Berzofsky, 2014). NKT cells regulate microbial and tumour immunity as well as autoimmune and inflammatory diseases by their ability to rapidly secrete large amounts of immunoregulatory cytokines and to upregulate costimulatory molecules to alert and modulate the effector functions of myeloid and lymphoid cells (reviewed by Bendelac et al., 2007; Van Kaer, 2005).

**CD1d Structure Dictates Lipid Ligand Presentation**

**CD1d-restricted ligands and three modes of NKT-cell activation**

CD1d belongs to a protein family called CD1 (see also: *Glycolipid Presentation by CD1*), which was originally discovered as a thymocyte differentiation antigen. Molecular cloning and nucleotide sequence analyses subsequently revealed that CD1 resembles a family of MHC class I-like molecules (see also: *Major Histocompatibility Complex (MHC): Mouse*) comprised of three groups: group I (CD1a, CD1b and CD1c) CD1 expressed by humans but not by mice or rats; group II (CD1d) CD1 expressed by many mammals including primates and rodents and group III (CD1e) expressed by humans but not by mice (Brigl and Brenner, 2004).

**Exogenous lipid agonists**

The original report by Brenner and colleagues demonstrating CD1-restriction of *Mycobacterium tuberculosis* (see also: *Mycobacteria: Biology*)-reactive T cells and the recognition of *Mtb* lipids indicated that CD1 molecules present lipid ligands (Brigl and Brenner, 2004). Much of our understanding of NKT-cell biology has been gleaned from numerous *in vitro* and *in vivo* studies using the synthetic lipid αGalCer (KRN7000) and its analogues that closely resemble the marine sponge (see also: *Porifera (Sponges): Recent Knowledge and New Perspectives*) Agelas mauritianus-derived agelasphin 9b (Table 1; and references therein). αGalCer was originally isolated from *A. mauritianus*. It has now become clear that several microbes – for example, the gut bacterium *Bacteroides fragilis* (see also: *The Genus Bacteroides*) and the fungus *Aspergillus fumigatus*, the agent of airway hyperactivity (AHR; see also: *Allergy*), and perchance the commensals that populate *A. mauritianus* – and some mammals including mouse and rat biosynthesise αGalCer and/or related compounds (Table 1; and references therein). Hence, αGalCer may be more prevalent than previously thought and the biology of NKT cells gleaned by probing with this compound may be relevant to the physiology of this T-cell subset. As αGalCer is a potent agonist, NKT-cell activation occurs directly in a TCR-dependent manner without need for additional signals, suggesting a TCR-dominated mode of NKT-cell activation (Figure 1).

*Sphingomonas spp.* – a Gram-negative α-Proteobacteria that lack lipopolysaccharide (LPS)- synthetise α-galacturonosylceramide and α-galacturonosylceramide (αGalACer) that resemble αGalCer (Table 1). αGalACer by itself activates NKT cells in a CD1d-restricted manner but this activity is weak; hence, robust NKT-cell activation requires the presence of a second signal provided by inflammatory cytokines resulting from innate activation of dendritic cells (DCs) via their PRRs (Brigl et al., 2011; see also: *Pattern Recognition Receptor*). This two-signal mode of NKT-cell activation is termed TCR- and cytokine-mediated activation (Figure 1) – a feature that is important for NKT-cell activation by other microbial and self-lipid agonists as discussed below.

NKT cells also recognise diacylglycerol-based microbial lipids; for example, α-galactosyl (αGalDAG) and α-glucosyl (αGlcDAG) diacylglycerol, which are cell wall components synthesised by *Borrelia burgdorferi* – the agent of Lyme disease (see also: *Spirochaetes*) and *Streptococcus pneumoniae*, respectively. So also, NKT cells are activated by *Helicobacter pylori*-derived cholesteryl-6-O-acyl α-glucoside. Hence, NKT cells have broad ligand specificity (Table 1; and references therein).

**Endogenous lipid agonists**

NKT cells are also autoreactive – that is, they react to self-lipids presented by the host APCs in conjunction with a second signal (Benelac et al., 2007; Brennan et al., 2013). An initial search for the endogenous NKT-cell agonist revealed that neither cells deficient in βGlcCer synthase and transiently expressing CD1d nor cell-free CD1d–βGlcCer complexes activate mouse NKT-cell-derived hybridomas (Stanic et al., 2003a). This finding suggested that a cellular, βGlcCer-derived glycosphingolipid (GSL; see also: *Glycolipids: Distribution and Biological Function; Animal Glycolipids; Membrane Lipid Biosynthesis*) is an endogenous mouse NKT-cell agonist. Current evidence suggests that both α- and β-linked GSLs – for example, cellular αGalCer, βGalCer, βGlcCer, isoglobotrihexosylceramide (iGb3), ganglioside D3 (GD3) – as well as glycosphingolipids (GPLs; see also: *Glycosphingolipids*) – for example, phosphatidyl (Ptd)-inositol, Ptd-ethanolamine and lyso-Ptd-choline – are agonists for either a subset or for all mouse and/or human NKT cells (Table 1; and references therein).
### Table 1: Structure and properties of selected synthetic, microbial and self-NKT-cell agonists

<table>
<thead>
<tr>
<th>Lipid (class$^a$)</th>
<th>Chain Length$^b$</th>
<th>Structure</th>
<th>Agonist$^c$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$GalCer (GSL)</td>
<td>C18 C24:1</td>
<td><img src="image" alt="Structure" /></td>
<td>IFN-$\gamma$, IL-4 self</td>
<td>Kain <em>et al.</em> (2014)</td>
</tr>
<tr>
<td>Agel 9b (GSL)</td>
<td>C17 (C16-Me) phyto C24</td>
<td><img src="image" alt="Structure" /></td>
<td>Antitumour; <em>Agelas mauritianus</em></td>
<td>Morita <em>et al.</em> (1995), Natori <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>$\alpha$GalCer (GSL)</td>
<td>C18-phyto C26</td>
<td><img src="image" alt="Structure" /></td>
<td>Very strong; robust IFN-$\gamma$ IL-4 and other cytokines; synthetic analogue of Agel 9b (KRN7000)</td>
<td>Kawano <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>$\alpha$-C-GalCer (GSL)</td>
<td>C18-phyto C26</td>
<td><img src="image" alt="Structure" /></td>
<td>Weak (mo$^a$)-to-none (hu); IFN-$\gamma$; synthetic</td>
<td>Schmieg <em>et al.</em> (2003)</td>
</tr>
<tr>
<td>OCH (GSL)</td>
<td>C9-phyto C24</td>
<td><img src="image" alt="Structure" /></td>
<td>Weak (mo)-to-none (hu); IL-4 (low-to-no IFN-$\gamma$); synthetic</td>
<td>Miyamoto <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>C20-diene (GSL)</td>
<td>C18-phyto C20:2</td>
<td><img src="image" alt="Structure" /></td>
<td>Strong; IL-4 (low-to-no IFN-$\gamma$); synthetic</td>
<td>Yu <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>$\alpha$GalA Cer (GSL)</td>
<td>C18-phyto C14</td>
<td><img src="image" alt="Structure" /></td>
<td>Weak; <em>Sphingomonas</em> spp.</td>
<td>Kinjo <em>et al.</em> (2005), Mattner <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Asp B</td>
<td>C20:2-C9 Me C16-C2 OH</td>
<td><img src="image" alt="Structure" /></td>
<td>Weak; <em>Aspergillus fumigatus</em></td>
<td>Albacker <em>et al.</em> (2013)</td>
</tr>
</tbody>
</table>
αGlc-acyl-Chol C14

Strong; binds a small NKT-cell subset (mo); *Helicobacter pylori* Chang *et al.* (2011)

βGlcCer (GSL) C18

C24:1

Strong; self Brennan *et al.* (2011)

iGb3 (GSL) C18-

C24

Weak (mo)-to-none (hu); self Zhou *et al.* (2004b)

αGalDAG (GGL) sn1-C18:1

sn2-C16

Weak (mo)-to-none (hu); *Borrelia burgdorferi* Kinjo *et al.* (2006)

αGlcDAG (GGL) sn1-C18:1

sn2-C16

Weak; *Streptococcus pneumoniae* Kinjo *et al.* (2011)

PtdIno (GPL) sn1-C18:1

sn2-C18:1

Week (mo)-to-no (hu); self Gumperz *et al.* (2000), Mallevaey *et al.* (2011)

Lyso-PtdCho (GPL) sn1-C16

sn2-lyso

Weak (hu)-to-none (mo); GM-CSF (no IL-4, IFN-γ); self Fox *et al.* (2009)

αGal, agelasphin; Asp B, asparamide B; Chol, cholesterol; DAG, diacylglycerol; GalCer, galactosylceramide; GalACer, galacturonosylceramide; GlcCer, glucosylceramide; PtdCho, phosphatidylcholine; PtdIno, phosphatidylinositol; sn, stereo nomenclature for glycerolipids; GGL, glycosylglycerolipid; GPL, glycerophospholipid; GSL, glycosphingolipid.

b Sphingosine/phytosphingosine chain length indicated first and N-acyl chain length second.

c Agonist strength based on the reference by Joyce *et al.* (2011).

d Relative potency in comparison to αGalCer; mo, mouse; hu, human.
Figure 1 Three distinct strategies activate NKT cells. Potent NKT-cell agonists – such as αGalCer – directly activate NKT cells without the need for a second signal in a TCR-signalling dominated manner (a). Alternatively, microbes containing TLR ligands such as LPS activate NKT cells by inducing IL-12 production by DC, which amplifies weak responses elicited upon the recognition of CD1d bound with self-glycolipids by the NKT CR. Several endogenous lipid agonists have been identified and characterised (Table 1). Some microbes such as Sphingomonas capsulata, which are α-Proteobacteria, synthesise α-anomeric glycolipids for their cell walls. These glycolipids, when presented by CD1d, weakly activate NKT cells directly. In the presence of a second signal – generally a proinflammatory cytokine such as IL-12 – it strongly activate NKT cells (b). Intriguingly, NKT cells can be activated solely by cytokines – mainly IL-12 plus IL-18 – in a TCR-independent manner (c).

The importance of self-lipid recognition was realised in studies demonstrating human and mouse NKT-cell activation by DCs cocultivated with the Gram-positive Staphylococcus aureus, the Gram-negative Salmonella typhimurium or A. fumigatus. While S. aureus and S. typhimurium are not known to contain NKT-cell lipid agonists, A. fumigatus contains an αGalCer-related GSL, asparamide B (Albacker et al., 2013). Asparamide B activity being weak on NKT cells is bolstered by IL-12 secreted by DC upon ligation of dectin-1 by the fungal β-1,3 glucans (Albacker et al., 2013; Cohen et al., 2011). Because β-1,3 glucans from Candida, Histoplasma and Alternaria spp. (see also: Fungal Infections in Humans) also activate NKT cells in a dectin-1-dependent manner, this mode of NKT-cell activation may be a common sensing mechanism of fungal infections (Cohen et al., 2011). Similar to A. fumigatus, NKT-cell activation by S. typhimurium resulted from self-ligand recognition through bacterial LPS-mediated stimulation of DCs through toll-like receptor (TLR)4 (see also: Toll-like Receptors; Biochemistry of Toll-Like Receptors) and the secretion of IL-12 (Brigl et al., 2003). The self-ligands include mammalian αGalCer and perhaps iGb3 (Kain et al., 2014; Mattner et al., 2005; Zhou et al., 2004b).

NKT cells also respond to a sialylated endogenous lipid when DCs are activated by CpG, a TLR9 ligand, and produce interferon (IFN)-α (Paget et al., 2007) (see also: An Overview of Cytokine Regulation of Inflammation and Immunity) indicating that the second signal for NKT-cell activation can be relayed by multiple inflammatory cytokines. In this regard, it is noteworthy that viral infections also activate NKT cells (Table 2 and references therein). As viruses do not carry genes for lipid biosynthesis, NKT-cell activation perhaps occurs via self-lipid(s) recognition in the presence of proinflammatory cytokine(s) such as type I IFN that is elicited by almost all viral infections.

NKT cells also respond to a combination of inflammatory cytokines such as IL-12 and IL-18 in the absence of a CD1d-restricted agonist (Nagarajan and Kronenberg, 2007; Tynnik et al., 2008; Wesley et al., 2008). This latter mechanism – termed cytokine-driven NKT-cell activation (Figure 1) – is important for immunity to cytomegalovirus (Wesley et al., 2008) (see also: Cytomegaloviruses). Summarily then, NKT cells have evolved multiple ways to sense microbial stressors including direct recognition of CD1d-restricted exogenous glycolipids. Alternatively, they sense stressors indirectly, either through the recognition of a CD1d-self-lipid complex or in a CD1d-independent manner, in the presence of inflammatory cytokines (Figure 1). The nature of the inflammatory cytokine serving the second signal depends on the APC and the PRR activated by the microbial pathogen-associated molecular patterns (PAMPs; Innate Immune Mechanisms: Nonself Recognition).
### Table 2  Role of NKT cells in microbial infection and immunity

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Antigen</th>
<th>NKT-cell role</th>
<th>Model</th>
<th>Route of infection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive</strong></td>
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<td></td>
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<tr>
<td>S. pneumoniae</td>
<td>αGalDAG</td>
<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n., i.t.</td>
<td>Brigl et al. (2011), Kawakami et al. (2003), Kinjo et al. (2011)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>ND</td>
<td>Not protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.v.</td>
<td>Kwiecinski et al. (2013)</td>
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<tr>
<td><strong>Gram-negative</strong></td>
<td></td>
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<td></td>
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<tr>
<td>P. aeruginosa</td>
<td>ND</td>
<td>Protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n.</td>
<td>Nieuwenhuis et al. (2002)</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>ND</td>
<td>Not protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.t.</td>
<td>Berntman et al. (2005), Brigl et al. (2003), Mattner et al. (2005)</td>
</tr>
<tr>
<td>H. pylori</td>
<td>αGlc-acyl-cholesterol</td>
<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>p.o.</td>
<td>Ito et al. (2013)</td>
</tr>
<tr>
<td>C. trachomatis (muridarum)</td>
<td>GLXA</td>
<td>Detrimental</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n. intravaginal</td>
<td>Bilenki et al. (2005), Habbeddine et al. (2013), Peng et al. (2012)</td>
</tr>
<tr>
<td>C. pneumonia</td>
<td>ND</td>
<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n.</td>
<td>Joyee et al. (2007)</td>
</tr>
<tr>
<td>L. pneumophila</td>
<td>ND</td>
<td>Detrimental</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.t.</td>
<td>Bilenki et al. (2005), Habbeddine et al. (2013), Peng et al. (2012)</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>ND</td>
<td>Detrimental</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n.</td>
<td>Hill et al. (2015)</td>
</tr>
<tr>
<td>subsp. tularensis SchuS4</td>
<td>ND</td>
<td>Detrimental</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n.</td>
<td></td>
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<tr>
<td>subsp. holarctica LVS</td>
<td>ND</td>
<td>Not protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>s.c., i.d.</td>
<td></td>
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<tr>
<td>F. novicida</td>
<td>ND</td>
<td>Not protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td><strong>α-Proteobacteria</strong></td>
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<tr>
<td>S. capsulata</td>
<td>αGlcACer</td>
<td>Protective (low dose)</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.v.</td>
<td>Kinjo et al. (2005), Mattner et al. (2005), Sriram et al. (2005)</td>
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<td></td>
<td></td>
<td>Detrimental (high dose)</td>
<td></td>
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<tr>
<td>N. aromaticivorans</td>
<td>αGalACer</td>
<td>Primary biliary cirrhosis</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.v.</td>
<td>Mattner et al. (2008)</td>
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<tr>
<td><strong>Spirochetes</strong></td>
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<tr>
<td>B. burgdorferi</td>
<td>αGalDAG</td>
<td>Protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.d.</td>
<td>Kumar et al. (2000), Tupin et al. (2008)</td>
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<tr>
<td><strong>Mycobacteria</strong></td>
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<tr>
<td>M. tuberculosis</td>
<td>ND</td>
<td>Not protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.v. aerosol</td>
<td>Behar et al. (1999), Sada-Ovalle et al. (2008)</td>
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<tr>
<td></td>
<td></td>
<td>Protective&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Cell transfer</td>
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<td><strong>Viruses</strong></td>
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<tr>
<td>HSV-1</td>
<td>ND</td>
<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Scarification</td>
<td>Cornish et al. (2006), Grubor-Bauk et al. (2003)</td>
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<td></td>
<td></td>
<td>Not protective&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<tr>
<td>HSV-2</td>
<td>ND</td>
<td>Protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Intravaginal</td>
<td>Ashkar and Rosenthal (2003)</td>
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<td>Sendai virus</td>
<td>ND</td>
<td>Detrimental</td>
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<td>i.n.</td>
<td>Kim et al. (2008)</td>
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<td>RSV</td>
<td>ND</td>
<td>Protective</td>
<td>CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>Johnson et al. (2002)</td>
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<td>Influenza virus H1N1 and H3N2</td>
<td>ND</td>
<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.n.</td>
<td>De Santo et al. (2008), Ho et al. (2008), Kok et al. (2012), Paget et al. (2011, 2012)</td>
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<td>HBV</td>
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<td>Protective</td>
<td>Ja18&lt;sup&gt;−/−&lt;/sup&gt;, CD1d&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>i.v.</td>
<td>Zeissig et al. (2012)</td>
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</table>
CD1d assembly with NKT-cell agonists

CD1d molecules are expressed by several haematopoietic cells especially APCs (see also: Antibigen Processing) – such as DCs (see also: Dendritic Cells (T-lymphocyte Stimulating)), macrophages (Mφ; see also: Macrophages) and B cells (see also: B Lymphocytes), with the highest levels being found on marginal zone B cells (Brossay et al., 1997; Dougan et al., 2007a; Roark et al., 1998) – and CD4+8+ thymocytes. Hepatocytes, intestinal epithelial cells (see also: Lymphocytes: Intraperithelial), granulocytes, keratinocytes, adipocytes and haematopoietic stem cells also express CD1d molecules (De Santo et al., 2010; Dougan et al., 2007a; Huh et al., 2013; Kotsianidis et al., 2006; Mandal et al., 1998). Antigen recognition and activation of NKT cells are influenced by the levels of CD1d expressed on the surface of APCs (Skold et al., 2005). CD1d on the surface of APCs is enhanced by IFN-γ (see also: An Overview of Cytokine Regulation of Inflammation and Immunity) and either TNF-α (see also: Tumour Necrosis Factors) or TLR2 or TLR4 ligands (Brigl et al., 2011; Skold et al., 2005). Not surprisingly, a few pathogens have evolved mechanisms to lower CD1d expression levels for NKT-cell-targeted immune evasion (Hage et al., 2005; Sanchez et al., 2005; Yuan et al., 2006). The ensuing briefly describes how CD1d molecules assemble with NKT-cell agonists and how microbes have evolved ways to evade their presentation.

Assembly and exchange

CD1d is a heterodimer consisting of a heavy chain that is noncovalently associated with the light-chain β2-microglobulin (β2m) (see also: Glycolipid Presentation by CD1). The folding and assembly of CD1d occurs within the endoplasmic reticulum (ER) and is facilitated by the chaperones calnexin and calreticulin and the thiol reductase ERP57 (Figure 2a) (Kang and Cresswell, 2002). Because the antigen-binding groove (ABG) of CD1d is largely nonpolar and hydrophobic (Figure 3) (Giabbané et al., 2005; Koch et al., 2005; Zajonc et al., 2005a, 2005b; Zeng et al., 1997), it is thought to assemble with phospholipids indigenous to the ER (Cox et al., 2009; De Silva et al., 2002; Haig et al., 2011; Joyce et al., 1998; Park et al., 2004; Yuan et al., 2009) perhaps by a process facilitated by the ER-resident lipid-transfer protein (LTP) microsomal triglyceride transfer protein (MTP; Figure 2a; Brozovic et al., 2004; Dougan et al., 2005, 2007b). Thence, by virtue of a short motif (Tyr-Gln-Gly-Val-Leu and Tyr-Gln-Asp-Ile-Arg in human and mouse CD1d, respectively) contained within the cytoplasmic tail, CD1d negotiates the plasma membrane, via the Golgi complex, en route to the MHC class II compartment (MIIC) where it assembles with NKT-cell antigens (Joyce and Van Kaeer, 2003). Alternatively, CD1d can arrive directly at the MIIC by assembly with MHC class II-associated invariant chain (ii), which contains a MHC-targeting motif (Jayawardena-Wolf et al., 2001).

Assembly with antigens involves the exchange of ER-derived lipid or ii-bound CD1d with GSLs in the MIIC. These exchange and reassembly processes are facilitated by MIIC-resident LTPs such as saposins A, B, C and D, G_{ex} activator (Kang and Cresswell, 2004; Yuan et al., 2007; Zhou et al., 2004a) and Niemann–Pick type C2 protein (Schrantz et al., 2007), as well as cathepsins L and S (Honey et al., 2002; Riese et al., 2001). In addition, MTP appears to play an as yet undefined role in returning CD1d back to the cell surface upon negotiating the MIIC (Sagiv et al., 2007).

The presentation of bacterial cell wall lipids by CD1d molecules depends on bringing them inside the APC (Figure 2b). This can occur by one of two ways: by phagocytosis of the bacterium itself or by receptor-mediated or fluid-phase endocytosis (micro- or macroinocytosis) and eventual maturation of the phagosome or endosome to form the MIIC. The internalised bacterium is then broken down by MIIC-resident hydrolases, thus making lipids available for CD1d-restricted presentation. In the case of shed or secreted bacterial lipids, soluble LTPs that regulate cellular lipid homeostasis, such as apolipoproteins, bind such microbial lipids, assemble into very-low-density lipoprotein (VLDL) complexes and by LDLR (low-density lipoprotein-receptor)-mediated endocytosis arrive in the MIIC.
Figure 2  Topologic biochemistry and the assembly of CD1d with NKT-cell antigen. (a) CD1d assembly with lipids begins within the rough endoplasmic reticulum (ER) with the assistance of several chaperones. Partially folded \( \alpha \)-chain–\( \beta \)2m complex is then thought to bind ER-resident lipids with the assistance of lipid-transfer proteins (LTP) such as microsomal triglyceride transfer protein (MTP), a protein that facilitates the assembly of apolipoprotein B. Upon complete assembly, the CD1d–lipid complexes egress from the ER and negotiate the secretory pathway to the plasma membrane. By virtue of late endosome/lysosome-targeting motif (tyrosine-glutamine-glycine-valine-leucine and tyrosine-glutamine-aspartate-isoleucine-arginine in human and mouse CD1d, respectively) within the cytoplasmic tail of CD1d, it recycles through the MHC class II-enriched compartment (MIIC). During its time in the MIIC (late endosomes/lysosomes), CD1d exchanges its ER-loaded lipids for antigenic glycolipids that activate NKT cells \( \textit{in vivo} \). The extraction of bound lipids from CD1d and the loading of antigenic glycolipids are facilitated by lysosomal LTP such as Saposins (Sap), GM2 activator (GM2A) and Niemann–Pick C-2 (not shown), which are essential for the enzymatic catabolism of glycolipids. (b) Infection of Mφ and DC delivers microbes to the CD1d-containing lysosomes. Differential interference contrast (DIC) picture of Mφ observed under a light microscope showing the gross cellular outline; the prominent structure within this cell is its nucleus. Cellular organelles and their contents are observed by confocal fluorescence microscopy. In the micrographs shown, the lysosome is stained red because it is marked with a fluorescent dye, Lyso-tracker; the microbe, in this case \textit{Borrelia burgdorferi}, stained with the fluorescent dye PKH-II, is seen green; CD1d, which is detected with a specific monoclonal antibody 1B1 tagged with the fluorescent dye allophycocyanin, is stained blue. Where \textit{B. burgdorferi} colocalises with lysosomes and CD1d colocalised appear white in the merged picture. (Figure 2a; Freigang \textit{et al.}, 2012; \textit{and} \textit{en Elzen \textit{et al.}}, 2005). Thus, the topological distribution of cellular and microbial lipids and LTPs dictates the types of antigens that assemble with CD1d.

### Microbial subversion

Because NKT cells play a critical role in protective immune responses against a variety of microbes, it is not surprising that pathogens, especially viruses that establish latency, have devised ways to interfere with the CD1d-restricted antigen-presentation pathway. Most accomplish this mainly by interfering with intracellular CD1d trafficking. For example, the modulator of immune recognition (MIR)-1 and MIR-2 proteins of Kaposi sarcoma-associated herpesvirus are ubiquitin ligases that ubiquitinylate (see also: Ubiquitin Pathway) the cytoplasmic tail of human CD1d, which triggers endocytosis of surface CD1d thereby reducing cell-surface CD1d expression. The Nef protein of human immunodeficiency virus also reduces CD1d expression perhaps by increased endocytosis of cell-surface CD1d molecules coupled with inhibition of CD1d transport to the cell surface. Similarly, in cells infected with herpes simplex virus 1 (see also: Herpes Simplex: Viruses and Infections), CD1d molecules accumulate in the MIIC owing to a defect in...
recycling CD1d molecules back from endosomal compartments to the cell surface. Vesicular stomatitis virus and vaccinia virus also impair antigen presentation by CD1d, perhaps by interfering with intracellular trafficking of CD1d molecules induced by mitogen-activated protein kinase signalling. Some bacteria have also devised strategies to evade CD1d-restricted antigen presentation. For example, infection of monocytes with *M. tuberculosis* also results in CD1d downmodulation by reducing CD1d mRNA expression, implying regulation at the transcriptional level. These findings indicate that NKT-cell-based immune recognition may play a significant role in viral and bacterial immunity (reviewed by Van Kaer and Joyce, 2006).

**Structures of CD1d–lipid complexes**

The CD1d heavy chain folds into five domains: the extracellular α1, α2 and α3 domains, which are membrane-anchored by the transmembrane region that culminates in a short cytoplasmic tail (Figure 3a). Solution of the three-dimensional structures of mouse and human CD1d molecules, which differ subtly from one another, in complex with several lipid ligands revealed that the α1 and α2 domains of the heavy chain fold into a superdomain to form the ABG (Figure 3). The ABG is laterally confined by two antiparallel α-helices; these helices are supported at the bottom by an 8-stranded antiparallel β-sheet platform. The membrane-proximal immunoglobulin-like α3 domain and the noncovalently associated light chain support the superdomain (Figure 3a). In these regards, the topology of CD1d resembles peptide-antigen-presenting MHC class I molecules (reviewed by Rossjohn et al., 2015).

**Display of αGalCer and its analogues**

The arrangement of the amino acids that make up the ABG is such that the narrow apical entrance leads into two deep-seated pockets (A′ and F′; Figure 3). The two pockets are lined predominantly by hydrophobic amino acid residues and, hence, permit the binding of lipid hydrocarbon tails of varying lengths. The N-acyl chain of αGalCer and related compounds – OCH and αGalACer – tucks into the large A′ pocket while the long-chain

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**Figure 3**  The structures of CD1d-restricted glycolipid antigens. (a) Lateral view of the three-dimensional structure of mouse CD1d bound to the potent agonist αGalCer. The CD1d molecule is made up of a heavy chain that is noncovalently associated with a light-chain β2-microglobulin (β2m). The membrane distal α1 and α2 domains of the heavy chain fold in such a manner that they form an apical antigen-binding groove into which bind lipid ligands. The α3 domain, which adopts an immunoglobulin constant domain-like fold, is intermediary between the antigen-binding domain and the transmembrane domain that anchors the heterodimer into the plasma membrane. (b) Apical en face view of the antigen-binding domain shows the solvent-exposed sugar of αGalCer. The antigen-binding groove contains two deep pockets – A′ and F′ – which bind the hydrocarbon tails of the N-acyl chain and the phytosphingosine base, respectively. (c, d) Close-up three-dimensional view of mouse CD1d-αGalCer structure reveals critical amino acid side chains within hydrogen-bonding distance from atoms of the glycolipid antigen. Mouse (Mo) and human (Hu) sequences are shown for comparison, with arrows indicating hydrogen bonds. The three-dimensional structures were generated with Chimaera (a and c) or PyMol (b) software using published X-ray crystallographic coordinates (1Z5L, and 2AKR) deposited with the Protein Data Bank.
base of GSLs fits into the F’ pocket (Figure 3). This binding mode exposes the polar head group above the ABG (Figure 3). Moreover, the charged amino acids at the entrance of the ABG form a conserved hydrogen-bond network with polar atoms of the head groups of these α-anomeric GSLs. The same CD1d residues also form hydrogen bonds with β-anomeric GSLs, such as iGb3 (Zajonc et al., 2005b, 2008). This hydrogen-bond network provides stability to the CD1d–lipid interaction. Thus, the physicochemical architecture of the ABG dictates how the polar epitope is disposed for recognition by the Var14i/Va24i TCR (reviewed by Rossjohn et al., 2015).

**Display of αGalDAG**

Microbial αGalDAG antigens are structurally similar to αGalCer in that they also have an α-anomeric galactose attached to a lipid backbone (Table 1). However, in contrast to αGalCer, the DAG backbone is characterised by two fatty acids esterified to both the sn-1 and sn-2 position of a glycerol moiety, while the α-anomeric galactose is attached to the sn-3 position. Borrellial αGalDAG lipids bind to CD1d in two different orientations, depending on the nature of the acyl chains linked to sn-1 and sn-2 positions of glycerol. As a result, the lipid backbone is important in the formation of a TCR epitope as certain αGalDAGs are NKT-cell agonists and others not, because they bind in the opposite orientation (Wang et al., 2010). B. burgdorferi glycolipid 2c (BbGl-2c), which is bound with the sn-1 oleic acid (C_{18:1}) in the A’ pocket and the sn-2 palmitic acid (C_{16:0}) in the F’ pocket, is a mouse NKT-cell agonist, while BbGl-2f that binds in a reversed orientation with the sn-2-linked oleic acid (C_{18:1}) in the A’ pocket and the sn-1-linked linoleic acid (C_{18:2}) in the F’ pocket does not activate mouse NKT cells (Kinjo et al., 2006). In contrast, BbGl-2f is a human NKT-cell agonist (Kinjo et al., 2006). Even though chemical modifications such as unsaturations do not directly make contact with the TCR, by virtue of affecting the orientation of the hexose sugar, they contribute to the formation of an NKT-cell epitope. Similar changes in the ceramide backbone of αGalCer analogues do not lead to alternative GSL binding orientation, as the ceramide backbone is bound in a fixed orientation through a conserved hydrogen-bond network. Hence, αGalDAG presentation reveals the first time, striking differences between mouse and human glycolipid antigen recognition that could not have been appreciated using strong agonists, such as αGalCer.

**iGb3 display**

iGb3 binds CD1d in a distinct manner such that the first hexose – that is, glucose – is solvent-exposed and projects up and away from the ABG as a result of its β linkage (Zajonc et al., 2005b). This contrasts the more intimate binding of the galactosyl headgroup of αGalCer to CD1d (Koch et al., 2005; Zajonc et al., 2005a). Moreover, the upward projection of the first glucose of iGb3 results in an almost perpendicular exposition of the two terminal galactoses [Glc–β1→4Gal–α1→3Gal] of iGb3 out of the ABG as revealed by the structure of the mouse CD1d–iGb3 complex (Zajonc et al., 2008). Nevertheless, the β-linked glucose, and whose 4’-hydroxyl is equatorially disposed, perhaps results in poor binding to CD1d owing to the loss in hydrogen bonding with CD1d. Taken together, the presentation principles for α- and β-linked glycolipids are distinct (reviewed by Rossjohn et al., 2015).

**Display of phosphoglycerolipids**

Lyso-Ptd-choline, but not Ptd-choline, is a human NKT-cell agonist (Fox et al., 2009). As Lyso-Ptd-choline consists of only one acyl chain, that is, at sn1-glycerol, it tucks into human CD1d’s F’ pocket leaving the A’ pocket bare. In this case, the A’ pocket was shown to bind two short, C11 and C6 spacer lipids. Strikingly, this binding mode presented a previously unseen CD1d conformation that especially impacted the α1-helix, which had moved away from the a2-helix as compared to α-linked GSL-bound CD1d (Lopez-Sagaseta et al., 2012). Such a dramatic conformational change has yet to be seen in any mouse lipid-bound CD1d structures including even that of complexes containing Ptdlno and PtdCho (Giabbi et al., 2005; Lopez-Sagaseta et al., 2012; Malleaeya et al., 2011). Hence, these numerous structures of CD1d-lipid complexes unveiled how both the nature of the hydrocarbon moiety and the head group impact the presentation of diverse self- and nonself-lipids to NKTCR to activate T cells that express them.

**NKTCR/CD1d-lipid recognition logic**

In contrast with TCR/pMHC complexes – wherein the receptor docks diagonal on the antigen (reviewed by Rossjohn et al., 2015) (see also: T-Cell Receptors; Antigen Recognition by T Lymphocytes) – the NKTCR docks parallel onto the extreme C-terminal end of the CD1d ABG, above the F’ pocket, using three of the six complementarity determining regions (CDRs) – CDR1α, CDR3α and CDR2β – while almost excluding CDR2α, CDR1β and CDR3β from the interface. This docking mode enables a lock-and-key interaction with the α-linked galactose epitope. Furthermore, alanine-scanning mutagenesis of the mouse Var14i TCR, the crystal structures of Var14i-Vβ8.2 and Var14i-Vβ7 and Var14i-Vβ2 cocomplexed with mouse CD1d–αGalCer revealed that the mouse NKTCR interfaces its ligand in a manner similar to the Var24i TCR–ligand interaction (reviewed by Rossjohn et al., 2015).

The above germline-encoded recognition logic raises the question of how the mouse Var14i and human Var24i TCRs recognise structurally distinct ligands such as iGb3, GD3, PtdlnoMan₁₄, PtdIno, PtdEtN and lyso-PtdCho. Alanine-scan mutants of Var14i TCR revealed that the NKTCR recognises many α-linked GSLs (αGalCer, OCH, αGalAcCer, αGalDAG and iGb3, which contains an α-linked terminal galactose) by means of a ‘hot spot’ of germline-encoded amino acids within CDR1α, CDR3α and CDR2β loops (Scott-Browne et al., 2007). The recent structure of mCD1d-PtdIno bound to an autoreactive Var14i TCR, in which the β-chain was mutated to increase affinity toward the self-antigen, surprisingly revealed that CDR3α residues do not directly contact the solvent-exposed phospho-inositol. Yet the NKTCR maintains a conserved footprint on CD1d by allowing additional residues in CDR2α to contact the inositol head group (Malleaeya et al., 2011).

The recent solution of the Var14i-Vβ8.2/mouse CD1d–αGalDAG and αGalAcCer crystal structures revealed...
that the TCR has the capacity to induce structural changes in both CD1d and the ligand orientation to maintain the conserved TCR footprint (Li et al., 2010). Similar to αGalCer and αGalACer, the TCR contacts αGalDAG exclusively through CDR1α and CDR3α. In each of these ternary structures, CDR1α and CDR3α residue Gly96 contacts the 2′-OH through a main chain carbonyl, while Arg95 contacts the 3″-OH of the ceramide backbone of both αGalCer and αGalACer. However, this hydrogen-bond interaction is lost in the αGalDAG/CD1d structure due to the differences in the lipid backbone of αGalDAG (Li et al., 2010). These findings suggest that the interaction of NKTCR with structurally distinct α-linked ligands is accomplished by similar recognition logic, which involves the germine-encoded ‘hot spot’ composed of amino acids within CDR1α, CDR3α and CDR2β loops (reviewed by Rossjohn et al., 2015).

A key advance in understanding how the NKTCR recognises diverse NKT-cell agonists was the solution of the structures of mouse NKTCR cocomplexed with CD1d-iGb3, CD1d-βGlcCer and CD1d-PtdIno as well as the Va24i/Vp11-TCR with human CD1d-lyso-PtdCho. A theme that emerged is that the NKTCR induced a significant conformational change within the ligand – CD1d and/or the bound lipid – to maintain the conserved footprint over the complex. In the case of CD1d-βGlc and CD1d-βGlcCer structures, the NKTCR flattened the sugars that protrude up from the ABG owing to the β-linkage of the lipid-proximal sugar moiety (reviewed by Rossjohn et al., 2015). In a similar vein, akin to the reorientation of the head group of αGalDAG for NKT-cell recognition, the NKTCR reorients the phospholipid head group to interact with the CD1d-PtdIno and CD1d-PtdCho ligands (Lopez-Sagaseta et al., 2012; Mallevaey et al., 2011). The energy dependence of conformational changes to maintain germine-encoded NKTCR/CD1d-ligand recognition logic provides insight into why the interactions between the Va14i or Va24i TCR and CD1d-self-lipid ligands are of low affinity and, hence, make poor NKT-cell agonists.

It is surprising that despite conserved NKTCR–ligand binding, the equilibrium binding affinity towards microbial glycolipids can vary up to 600-fold compared to αGalCer (Li et al., 2010). Essentially two factors have been identified that affect both the association rate of the TCR and the dissociation rate. Firstly, the need to reorient the galactose of borrelial αGalDAG results in a reduced TCR association rate (Li et al., 2010). Secondly, upon TCR binding onto mouse CD1d-αGalDAG or αGalACer, the NKTCR induces a structural change in mouse CD1d above the F′′′ roof, namely the formation of the F′′′ roof (Li et al., 2010). The F′′′ roof is already preformed upon αGalCer binding to mouse and human CD1d, but not when other known NKT-cell agonists bind (reviewed by Rossjohn et al., 2015) and as such, the TCR does not invest energy into keeping the roof closed upon binding. This results in a more stable complex, indicated by a reduced TCR dissociation rate. The F′′′ roof is also closed in the previously mentioned ternary complexes of the various αGalCer analogues, as well as in the PtdIno structure (Aspeslagh et al., 2011; Mallevaey et al., 2011; Wun et al., 2011) but because structures without bound TCR were not solved, it is not clear whether the F′′′ roof is already preformed in those CD1d molecules before TCR engagement. In summary, the agonistic potency of αGalCer and related compounds appears consistent with the extent to which the F′′′ roof is preformed as well as the ability of the glycolipids to induce further structural changes within CD1d that could enhance CD1d-ligand stability or CD1d-TCR binding stability. Those factors could in turn dictate the biologic outcome upon engaging different ligands, in addition to the pharmacological differences of the glycolipids.

NKT-cell DC synapse: synaptic transmission of information and transactivation of innate and adaptive immune system cells

NKT-cell DC synapse

Conventional T cells and APCs as well as NK cells and target cells form immune synapses in preparation for eliciting an appropriate effector response (Fooksman et al., 2010; Roda-Navarro, 2009). And so do NKT cells and APCs/CD1d-containing plasmatic membranes (Bezbradica et al., 2006; McCarthy et al., 2007), the specificity of which lies within NKTCR/ligand interactions. In addition, the quality of signalling across the NKT-cell-APC synapse depends on the kinetic parameters of NKTCR/ligand interactions (McCarthy et al., 2007).

Conventional T cells polarise certain cytokines, cytokine receptors and lytic granules to the immune synapse (Clark et al., 2003; Griffiths et al., 2010; Huse et al., 2006; Maldonado et al., 2004). So also, activated NKT cells rapidly polarise IFN-γ and lytic granules to the immune synapse (Bezbradica et al., 2006; McCarthy et al., 2007). In this way, they engage in synaptic transmission of effector molecules to modulate inflammatory responses to changes in cellular lipid content.

Transactivation

NKT cells respond very rapidly, within the first several hours of antigen recognition, as do other cells of the innate immune system (e.g. neutrophils, MΦ, DCs and NK cells). Upon activation, NKT cells elaborate copious amounts of effector cytokines and chemokines (Figure 4). The quality of the cytokine response depends on the type of NKT-cell antigen that elicits the response. For example, the synthetic agonist αGalCer, within 30–90 min, elicits a wide variety of cytokines (Figure 4). However, derivatives of αGalCer that are modified for lipid chain length or unsaturation predominantly elicit an IL-4 cytokine response from NKT cells (Miyamoto et al., 2001; Yu et al., 2005). In contrast, other αGalCer variants that have an altered glycosidic linkage, a chemically modified acyl chain or a modified sphingoid base potently elicit an IFN-γ-biased response from NKT cells (Table 1; and references therein). The distinct NKT-cell cytokine response elicited by distinct antigens is currently being harnessed to tailor downstream immune responses that optimally benefit the host.
DC, MΦ and PMN. A key feature of NKT-cell activation by αGalCer is cross-talk with other cells of the immune system. DCs, which are critical for the activation of naïve conventional T cells, are essential for glycolipid antigen presentation and NKT-cell activation (Arora et al., 2014; Bai et al., 2012; Bezbradica et al., 2005; Bialecki et al., 2011; Fujii et al., 2002; Macho-Fernandez et al., 2014). DCs are the immediate target of this activation, that is, NKT-DC cross-talk results in the stimulation and maturation of DC through activated NKT-cell-derived IFN-γ and CD154 (CD40 ligand on NKT cells)–CD40 (on DC) interaction (Kitamura et al., 1999; Vincent et al., 2002). This NKT-cell-DC cross-talk leads to NK-cell transactivation (Carnaud et al., 1999), enhanced responses to protein antigens by B cells (Singh et al., 1999), CD4 and CD8 T cells (Fujii et al., 2003; Hermans et al., 2003), the licencing of DCs for cross-presentation (Semmling et al., 2010) and the controlling of the number and phenotype of DCs after bacterial infection or tumour induction (Shekhar et al., 2015; Shimizu et al., 2013). It is through these bidirectional interactions that NKT cells and DCs cooperate to amplify and direct both innate and adaptive immune responses and are therefore the basis for efforts aimed at developing NKT-cell-targeted adjuvants and immunotherapies (Carreno et al., 2014; Singh et al., 2014; Van Kaer et al., 2011; Wu et al., 2009; Yu and Porcelli, 2005).

Tissue-resident MΦ – such as Kupffer cells of the liver – play important roles in resident NKT-cell activation to control downstream antigen-specific responses (Barral et al., 2010, 2012; Lee et al., 2010; Schmieg et al., 2005). IFN-γ and TNF-α secreted by activated NKT cells in turn stimulate MΦ, which attain microbicidal activity and promote delayed-type hypersensitivity (Nieuwenhuis et al., 2002; Sada-Ovalle et al., 2008). So also, this source of TNF-α is known to recruit DCs that have captured antigens from the skin to the local draining lymph node where they subserve conventional T-cell activation.

Osteopontin secreted by activated NKT cells can activate PMN leukocytes (see also: Neutrophils). PMNs are among the first cells recruited to sites of infection or injury. In models of lung infection, early recruitment of PMNs to the lungs depended on IFN-γ secreted by activated NKT cells (Kawakami et al., 2003; Nakamatsu et al., 2007; Nieuwenhuis et al., 2002). So also, PMN recruitment during influenza virus infection required an NKT-cell-derived IL-17 response (Michel et al., 2007). Aside from recruitment, activated NKT cells can control PMN function under certain conditions. CD1d-dependent PMN and NKT-cell interactions can reverse acute phase protein serum amyloid A-induced development of IL-10-producing immunosuppressive PMNs thereby promoting instead the production of IL-12 by PMNs (De Santo et al., 2010). Such effects are reciprocal, in that PMNs can also suppress NKT-cell responses in both mice and humans (Wingender et al., 2012a).

NK cells. After in vivo administration of αGalCer, NK cells (see also: Natural Killer (NK) Cells) rapidly produce IFN-γ. This NK-cell activation depends on CD1d not for
NKT-Cell Development

The unique phenotypic and functional features of NKT cells – especially the secretion of immunoregulatory cytokines amongst recent thymic emigrants – suggested that these cells might follow a distinct ontogenetic path. Hence, where NKT cells develop and what lineage-specific factor(s) specify, commit and maintain NKT cells are central to understanding NKT-cell biology.

Their absence in thymectomised and athymic mu/mu mice suggested that NKT cells develop in the thymus (see also: The Thymic Niche and Thymopoiesis). Similarly, genetically altered mice deficient in γc – the common chain utilised by IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (see also: Interleukins) receptors (R) – or IL-7 receptor-a chain that are arrested at the double-negative (DN2/DN3) stage of thymocyte ontogeny (see also: Lymphoid Development) do not develop NK1.1+ T cells (Boebeane et al., 1997). The rescue of NKT-cell ontogeny by enforced Bcl-2 expression within IL-7Ra chain-deficient DN2 thymocytes further suggested that commitment and development of this lineage perhaps occurred in the thymus (Boebeane et al., 1997; Gordy et al., 2011). These findings, in conjunction with the development of NK1.1+ T cells in mice that do not otherwise develop these T cells upon accepting a wild-type thymus graft, provided compelling evidence that NKT-cell development occurs in the thymus from the same precursor pool as conventional CD4 and CD8 T cells. These cells subsequently populated the liver – wherein they are disproportionately represented in high numbers (reviewed by Godfrey et al., 2010).

Having established that NK1.1+ T cells develop in the thymus, questions about when they were committed to this lineage and how they are selected remained. Early studies revealed that the selection of NK1.1+ T cells depends on double-positive (DP) thymocytes – that is, cells that coexpress CD4 and CD8. This interaction of developing NKT cells with DP cells was essential for positive selection, which involved both self-lipid-bound CD1d/TCR and homotypic/heterotypic SLAM-SLAM interactions that led to downstream PKCθ-NF-κB and SAP-Fyn activation (see also: Lymphocyte Activation: Signal Transduction), respectively, that are essential to NKT maturation (reviewed by Godfrey et al., 2010).

The finding that purified immature DP thymocytes contained Va14 and Ja18 rearrangements suggested that commitment to the NKT-cell lineage occurred at the DP thymocyte stage. The transfer of highly purified DP-high, TCR β-chain and tetramer-negative thymocytes into Ja18-deficient mice that do not contain NKT cells hosted the development of these cells. These findings coupled with genetic evidence that the Va14 and Ja18 rearrangement occurred at a late DP stage firmly the idea that NKT-cell commitment, as were conventional CD4 and CD8 T cells, occurred at the DP stage (reviewed by Godfrey and Berzins, 2007).

Commitment to the NKT-cell lineage leads to a unique transcriptional programming initiated by stimulation of the semi-invariant TCR (Figure 5). TCR signalling turns on the master transcription factor (see also: Transcriptional Gene Regulation in Eukaryotes), PLZF (promyelocytic leukaemia
CD1d-Restricted Natural Killer T Cells

NKT-Cell Functions

NKT-cell subsets and the division of labour

The wide range of functions that NKT cells elaborate upon activation lie in their ability to produce proinflammatory and regulatory cytokines and their capacity to interact with a variety of innate and adaptive immune cells – see section titled ‘Transactivation’. Current evidence indicates that NKT cells are a heterogeneous population that consists of at least four distinct subsets – NKT1, NKT2, NKT10 and NKT17 on the basis of the prototypic cytokine response of each subset (Figure 6) – that are represented in different proportions in various tissues (Lee et al., 2015) and mouse strains (Hammond et al., 2001; Lee et al., 2013; Lynch et al., 2015; Sag et al., 2014; Watarai et al., 2012).

NKT1 cells

NKT1 cells produce predominantly Th1 cytokines and comprise the majority of NKT cells in mouse spleen and liver. They are either DN or CD4\(^+\); the majority express NK1.1 and the shared IL-2/IL-15R \(\beta\)-chain (CD122) and, hence, depend on T-bet (Tbx21) and IL-15 for their development (Castillo et al., 2010; Gordy et al., 2011; Matsuda et al., 2002; Watarai et al., 2012). They are identified as high T-bet and low GATA-3 expressers (Lee et al., 2015; Watarai et al., 2012). NKT1 cells predominate in mostly lymphoid tissues of the C57BL/6 mouse. In most mouse strains, this subset predominates the liver and spleen; within the spleen they localise to the red pulp (Lee et al., 2015). NKT1 cells are the predominant producers of IFN-\(\gamma\) in response to \(\alpha\)GalCer (Lee et al., 2015) and may be responsible for the antitumour effect of \(\alpha\)GalCer (Crowe et al., 2005) and in controlling intracellular infections.

Figure 5 A putative NKT-cell ontogenetic pathway. Early steps (dashed arrow), CD4 and CD8 double-negative through immature CD8 single-positive stages (not shown) that precede the CD4 and CD8 double-positive (DP) stage of thymocyte development are common to both NKT lymphocyte and conventional T-cell lineages. The ontogenetic programming of the unique features of NKT-cell function occurs at the DP stage; it begins with the rearrangement of the \(\alpha\) to \(\alpha\)-chain gene segments and after its interaction with the positively selecting ligand, CD1d/self-lipid complex. Stage-specific NKT-cell markers – for example, CD24 (heat-stable antigen), CD44 and NK1.1 (CD161) – and lineage-specific differentiation signals are indicated. IL-7 and IL-15 are cytokines that utilise specific (IL-7R \(\gamma\)-chain) and shared common \(\gamma\)-chain (IL-15R \(\gamma\)-chain) receptor protein subunits that mediate intercellular communication. IL-15 also uses IL-2R \(\gamma\)-chain that it shares with IL-2 for intercellular communication. CD1d and pre-T-cell receptor (TCR)-\(\alpha\) (pre-\(\alpha\)-chain) are structural proteins, while \(\alpha\)-chain and \(\beta\)-chain FG-loop are structural parts of the TCR essential for positive selection of NKT cells. TCR signalling turns on the master transcription factor PLZF, which controls multiple molecular events that distinguish NKT cells from all of the other thymus-derived lymphocytes. Fyn and Lck are Src (cellular protein homologous to the Rous sarcoma virus oncogene) kinases (protein phosphorylation enzymes) essential for transmitting TCR signals from the plasma membrane to inside of the cell. Fyn also transmits signals relayed from SLAM (signalling lymphocyte activation molecule) through the adapter protein SAP (SLAM-associated protein). Protein kinase C (PKC)-\(\theta\) processes TCR signalling and activates nuclear factor-\(\kappa\)B (NF-\(\kappa\)B), which is a transcription factor. Other transcription factors such as Egr-2, Ets-1, GATA3, Id2, Id3, MEK, Nur77, ROR\(\gamma\)-t and T-bet, some of which are also essential for functional differentiation of NKT-cell subsets (see Figure 6), are also depicted.
Figure 6  Four NKT-cell subsets and the division of labour. The four currently defined NKT-cell subsets are operationally defined and shadow the conventional Th1, Th2, Th17 and Treg subsets. The expression of subset-specific transcription factors and the secretion of subset-specific prototypic effector molecules define the four NKT-cell subsets. The reported location of the subsets and their functions are also shown. The text contains detailed description of each subset.

**NKT2 cells**

NKT2 cells are marked by the expression of CD4 and IL-17RB and predominantly produce Th2 cytokines upon activation. They do not express CD122 and, hence, develop independent of IL-15. NKT2 cells are highly represented in the BALB/c mouse. They localise to the thymic medulla and constitutively secrete IL-4. This NKT-cell subset localises to the T-cell area within the lymph nodes (LN)s of the BALB/c mouse but equally distributed within the T- and B-cell areas of the C57BL/6 LNs (Lee et al., 2015). Mesenteric LN NKT cells are major responders to perorally administered αGalCer (Lee et al., 2015). This subset can be activated by IL-25 and may be responsible for NKT-cell-mediated airway hyperresponsiveness (AHR) (Kim et al., 2009; Matangkasombut et al., 2009; Terashima et al., 2008; Watarai et al., 2012). AHR ensues when NKT2 cells are stimulated to secrete IL-13 and IL-4 along with the Th2 chemokines CCL17, CCL22, C10/CCL6 and eosinophil chemotactic factor-L (ECF-L) that result in the recruitment of Mφs, eosinophils, neutrophils and lymphocytes into the lungs (Terashima et al., 2008), inciting tissue damage.

**NKT10 cells**

NKT10 cells are a recently identified subset whose development and effector functions are independent of PLZF (Lynch et al., 2015). They are found in low frequency in unchallenged mice and in human PBMCs (peripheral blood mononuclear cells). NKT10 cells secrete IL-10 upon stimulation of cells that were previously exposed to αGalCer in vivo (Sag et al., 2014). As IL-10 has immunoregulatory functions, NKT10 cells may be involved in the maintenance of tolerance in immune-privileged sites or control of TREG-cell functions in adipose tissues by inducing the polarisation of antiinflammatory M2 Mφs (Lynch et al., 2015).

**NKT17 cells**

NKT17 cells are CD4- and IL-17RB-negative cells that are enriched in barrier tissues such as the lungs, skin and peripheral lymph nodes, with fewer cells in the spleen and liver (Doisne et al., 2009; Michel et al., 2007). NKT17 cells do not express CD122, develop independent of IL-15 and require IL-7 for their survival (Watarai et al., 2012; Webster et al., 2014). NKT17 cells constitutively express RORγt and, hence, are distinguishable from NKT2 cells, which do not (Michel et al., 2007). NKT17 cells are mostly found in the lungs of C57BL/6 and nonobese diabetic (NOD) mice but are a rarity in the BALB/c mouse. They predominate the LNs of the NOD mouse (Lee et al., 2015). NKT17 cells rapidly produce IL-17A in response to S. pneumoniae and K. pneumoniae infections and induce airway neutrophilia in response to synthetic glycolipid or LPS challenge (Kinjo et al., 2011; Michel et al., 2007; Price et al., 2012). Their production of IL-22 in response to influenza virus infection seems to be important in the maintenance and regeneration of damaged epithelial tissue (Paget et al., 2012). In contrast, they may
CD1d-Restricted Natural Killer T Cells

contribute to ozone-induced AHR (Pichavant et al., 2008) and the development of experimental autoimmune encephalomyelitis (EAE) in mice.

Whether humans also have the NKT-cell subsets described for mice at present remains unknown. Nonetheless, human CD4+ and DN NKT cells show functional dichotomy: The CD4+ NKT cells secrete IL-4 upon antigen stimulation. Moreover, CD4+ NKT cells producing IL-4 and IL-13 accumulate in the lungs of chronic asthmatic patients, suggesting a pathological role for this NKT-cell subset in clinical asthma. By contrast, the DN NKT cells secrete IFN-γ and TNF-α. Further, both CD4 and DN subsets upregulate perforin in the presence of inflammatory signals. The DN NKT cells also upregulate NKG2D expression, which together with perforin have the potential to bestow upon NKT cells’ cytolytic activity against infected cells and cancer cells (Gumperz et al., 2002; Lee et al., 2002).

Peripheral NKT cells localise to sites of microbial entry

In mice, NKT cells develop only after birth while in humans this occurs in utero but the timing of human NKT-cell commitment and development are as yet unknown. The latter are present in substantial frequencies in all umbilical cord blood so far studied but are found in varying frequencies in peripheral blood of adults – from barely detectable to 0.001 to up to ~5%; (Gumperz et al., 2002; Lee et al., 2002; Montoya et al., 2007). So, what befalls human peripheral blood NKT cells between birth and adulthood remains unknown. Upon development within the postnatal thymus, mouse NKT cells home to secondary lymphoid organs.

NKT cells are perhaps best studied in the mouse spleen (see also: Spleen) and liver where they are most abundant, comprising ~1–2% and 20–30% of total lymphocytes, respectively. They are also found in secondary lymphoid organs (blood, bone marrow, lymph node, spleen and liver and mucosal tissues). They account for about a million cells in each mouse lymphoid organ except for their rarity in peripheral blood and lymph nodes as well as in the lungs and intestine. Thus, NKT cells home to critical anatomical sites where they can promptly execute their immunoregulatory functions (reviewed by Bendelac et al., 2007; Godfrey et al., 2010).

In the lymph nodes (see also: Lymph Nodes), NKT cells and other innate-like lymphocytes are rare, but they strategically localise to the port of antigen entry, that is, to the lymphatic sinus, in the proximity of CD169+ subcapsular Mφ and together with other innate-like lymphocytes contribute to the evolving immune response in the lymph node. This prepositioning permits immediate sensing of lymph–borne pathogens by a mechanism involving an inflammatory response mediated by Mφ (Barral et al., 2010; Kastenmuller et al., 2012). Within the mouse spleen, NKT cells patrol the red pulp and marginal zone where B cells, DCs and Mφ are located (Barral et al., 2012). This splenic NKT-cell distribution lends to surveillance of blood-borne pathogens. Consequently, intravenous administration of the model NKT-cell agonist αGalCer results in quick recognition and the arrest of red pulp and marginal zone NKT cells and rapid IFN-γ secretion. In contrast, relatively few NKT cells are found in the white pulp; and still fewer of these become activated in the presence of the agonist in vivo (Barral et al., 2012).

As with spleen, liver NKT cells continuously survey for blood-borne pathogens as they patrol the liver sinusoids crawling over hepatic Kupffer cells and perhaps the resident stellate (Ito) cells (Geissmann et al., 2005; Winau et al., 2007). Both Kupffer and Ito cells have been shown to present αGalCer in vivo (Schmiegl et al., 2005; Winau et al., 2007). As with splenic NKT cells, hepatic NKT cells also come to a quick halt upon intravenous injection of αGalCer into mice, indicating ligand presentation by resident myeloid cells and recognition by NKT cells (Geissmann et al., 2005). Moreover, NKT cells are also recruited to the peritoneum and become activated after intraperitoneal delivery of pathogens such as Salmonella choleraesuis and Listeria monocytogenes (Matsuzaki et al., 1995; Naiki et al., 1999).

NKT cells are found in both the epithelial layer and lamina propria of the small and large intestine (Olszak et al., 2012; Wingender et al., 2012b). Their numbers in the intestinal mucosa are controlled by neonatal colonisation of commensal bacteria. Germ-free (GF) mice have increased numbers of NKT cells in the intestinal mucosa with an immature phenotype marked by hyporesponsiveness to glycolipid antigens (Wingender et al., 2012b), which is reversed by colonisation with NKT-cell antigen-bearing bacteria during early life but not in adulthood (Olszak et al., 2012). Similar increases in NKT-cell frequency are observed in the liver and lungs but not spleen or thymus of GF mice (Olszak et al., 2012). The increased NKT-cell number in GF mouse intestinal mucosa perhaps owes to increased levels of the CXCR6 ligand CXCL16 whose levels are controlled by the gut microbiota by an unknown mechanism (Olszak et al., 2012; Zeissig and Blumberg, 2013). These attributes of developing NKT cells suggest the intriguing possibility that the gut microbiota, which vary between individuals of different genetic, ethnic and geographic backgrounds (Human Microbiome Project Consortium, 2012), imparts an epistatic control over NKT-cell frequency, at least in humans.

NKT cells are enriched among T cells in the lung where they can be found positioned within the lung vasculature that are likely kept in place by LFA1/iCAM1 interactions (Thomas et al., 2011). Upon exposure to airborne lipid antigens or microbial infections – for example, αGalCer or Francisella species, respectively – NKT cells extravasated into the lung tissue, promoted inflammation that led to eosinophil-containing lymphohistiocytic granulomas, determined the nature of the adaptive immune response (Hill et al., 2015; Scanlon et al., 2011) or caused AHR that resembled human chronic obstructive pulmonary disease (COPD; Kim et al., 2008). Similar to NKT cells in the intestinal mucosa, lung NKT cells are also increased in GF mice, suggesting a role for microbial exposure during early life on the establishment of NKT-cell niches within barrier tissues (Olszak et al., 2012). In support of this notion, infection of neonatal mice with influenza virus protected them from later development of allergen-induced AHR, an effect that was associated with the expansion of DN NKT cells in the lungs (Chang et al., 2011).

NKT cells are also thought to play an important role in immune surveillance of the reproductive tract. Intraperitoneal injection of a large dose of αGalCer induces abortion in mice in a manner that is dependent on IFN-γ, TNF-α and perforin (Ito et al., 2000),
whereas in nonpregnant mice, αGalCer treatment protects against genital tract Chlamydia muridarum infection (Wang et al., 2012). Interestingly, CD1d-deficient mice are no more susceptible to intravaginal C. muridarum infection than wild-type mice, but the analysis of leukocyte numbers in infected and uninfected pregnant mice suggested a role for NKT cells in the control, recruitment or homeostasis of these populations at the maternal–foetal interface (Habbeddine et al., 2013). Collectively, the foregoing discussion suggests that NKT cells are optimally stationed to patrol ports of microbe entry and antigen exposure.

Role of NKT cells in infection and protective immunity

The ability of NKT cells to respond rapidly and activate other cells of the innate and adaptive immune systems (see section titled ‘Transactivation’) suggest that they play a significant role in infection and protective immunity. This role has been extensively investigated in mouse models of infectious diseases (Table 2).

For example, B. burgdorferi infection if left unchecked can lead to disabling symptoms as the bacteria invade the joints, heart and central nervous system (Biesiada et al., 2012). NKT cells recognise αGalDAG produced by B. burgdorferi and rapidly proliferate and produce IFN-γ and IL-4 (Kinjo et al., 2006). NKT cells that surround the outside of blood vessels interacted directly with bacteria at the blood vessel walls, limiting their dissemination into the surrounding tissue via granzyme-mediated killing (Lee et al., 2014). Consequently, the absence of NKT cells resulted in increased B. burgdorferi burden in the joints (Lee et al., 2010; Tupin et al., 2008).

S. pneumoniae asymptptomatically resides within the upper airway of humans but causes an acute inflammatory response resulting in severe disease when it gains access to the lower respiratory tract. αGlCDAG produced by S. pneumoniae activated mouse NKT cells (Kinjo et al., 2011) to secrete IFN-γ and IL-17 that promoted bacterial clearance through PMN recruitment to the infected lung (Kawakami et al., 2003).

In contrast, mice deficient in NKT cells were less susceptible to infections with Legionella pneumophila, the causative agent of Legionnaires’ disease, C. muridarum (see also: Chlamydiae), a causal agent of sexually transmitted diseases and blindness as well as childhood pneumonia and F. tularensis spp., the etiologic agent of tularemia. Poor susceptibility to these infections were not due to reduced bacterial burden, but rather to less severe tissue damage and/or due to a tempered sepsis-like response elicited by the mutant mice when compared to the wild-type counterparts (Bilenki et al., 2005; Hayakawa et al., 2008; Hill et al., 2015; Joyce et al., 2007). Hence, in these disease models, NKT cells appear to promote runaway inflammation in an effort to generate protective immunity. Collectively, the current evidence suggests that NKT cells play a beneficial role, exacerbate disease or are of little consequence (Table 2).

αGalCer-induced protection

Owing to its ability to robustly activate NKT cells, αGalCer has been investigated as a means to modulate the immune response to a variety of infectious diseases. Composite results demonstrate varied efficacy irrespective of whether or not the inciting pathogen produces an NKT-cell ligand. The beneficial role demonstrated for NKT cells recognising S. pneumoniae-derived DAG is enhanced upon αGalCer treatment. Mice so treated exhibited enhanced bacterial clearance due to increased recruitment of PMNs into the infected lung (Kawakami et al., 2003; Nakamatsu et al., 2007). This effect was dependent on IFN-γ produced by activated NKT cells and transactivated NK cells (Christaki et al., 2015) but resulted in increased production of TNF-α and MIP-2 (Nakamatsu et al., 2007). Similar results were obtained when αGalCer-treated mice were infected with C. pneumoniae (Joyee et al., 2007). Multiple treatments with αGalCer before P. aeruginosa infection resulted in increased bacterial clearance by activated alveolar macrophages (Nieuwenhuis et al., 2002). Treated mice exhibited decreased neutrophil influx, increased TNF-α production and a less severe pneumonia due to early IFN-γ production by activated NKT cells. Nonetheless, mice treated with a single dose of αGalCer failed to protect mice from pneumonia caused by P. aeruginosa infection (Kinjo et al., 2006).

αGalCer-mediated NKT-cell activation has also been shown to have a beneficial effect on the outcome of systemic experimental infections in mice. αGalCer treatment afforded protection in infection models of murine malaria (Gonzalez-Aseguinolaza et al., 2002), Cryptococcus neoformans (Kawakami et al., 2001), hepatitis B virus (Kakimi et al., 2000) and encephalomyocarditis virus (Exley et al., 2001). In each case, the protective effect of αGalCer was dependent on the rapid production of IFN-γ by NKT cells. However, αGalCer may also elicit the production of Th2 cytokines, as do mice infected with the intracellular parasite Toxoplasma gondii (Ronet et al., 2005). In these mice, αGalCer treatment resulted in reduced IFN-γ but increased IL-4, IL-13 and IL-10 that protected from lethal intestinal inflammation. Interestingly, intranasal infection of mice with C. muridarum following αGalCer treatment also resulted in a Th2-biased response, which in this instance, was detrimental (Bilenki et al., 2005; Joyee et al., 2007). Hence, the ability of αGalCer treatment to protect against lethal infectious disease is likely determined by the nature of the pathogen, the character of the protective response at the local site of infection and the subset(s) of NKT cells involved in conferring protection.

Role of NKT cells in natural immunity against tumours

αGalCer was originally described as a GSL isolated from a marine sponge with potent antimitastatic activities in mice (Natori et al., 1993). The subsequent identification of αGalCer as a ligand for NKT cells not only raised enthusiasm for therapeutic applications of NKT cells against cancer but also prompted investigators to explore the potential role of NKT cells in natural immunity against tumours. This has been investigated in the context of chemically induced tumours, transplanted tumours and tumours arising in genetically engineered animals (reviewed by Fujii et al., 2013). The vast majority of these studies have provided strong support for the notion that NKT cells exhibit natural immunity against tumours. However, one study was unable to find evidence for a role of NKT cells to carcinomas induced by
the topical carcinogen methylcholanthrene (Kammertoens et al., 2012). The antitumour activities of NKT-cell agonists have been investigated extensively in mice (reviewed by Fujii et al., 2013) and have already been explored in a variety of clinical trials, with some encouraging results (Kuni et al., 2009; Motohashi et al., 2009). Th1-biasing αGalCer analogues (Table 1) appeared to be most effective in protection against cancer metastases in mice. Mechanisms of this protection remain incompletely understood and may involve activation of a variety of downstream effectors such as NK cells, cytotoxic T cells, Th1 and Th17 cells, γδ T cells, IFN-γ and direct lysis of myeloid-lineage cells (reviewed by Fujii et al., 2013; Van Kaer et al., 2011).

Role of NKT cells in autoimmune and inflammatory diseases

NKT cells have been implicated in a variety of autoimmune and inflammatory diseases. Studies with human subjects have shown that numbers and functions of NKT cells are often profoundly impacted by autoimmunity or inflammatory disease, but a causal relationship of this phenomenon remains to be established. In the preclinical mouse studies that have investigated the role of NKT cells in autoimmune and inflammatory diseases, a recurring theme that has emerged is that the effects of NKT cells on disease are strongly influenced by the particular experimental model of disease and the genetic background of the mouse strains employed and with substantial variation among different laboratories where the studies were performed. The latter confounding factor might be potentially explained by variations in NKT-cell numbers and functions in different mouse colonies, owing to differences in endogenous microbiota (reviewed by Zeissig and Berg, 2005; Taniguchi et al., 2007; Brigl and Brenner, 2004; Kronenberg, 2005; Taniguchi et al., 2003; Van Kaer, 2007; Van Kaer et al., 2013). See also: Autoimmune Disease: Mechanisms; Autoimmune Disease: Pathogenesis; Autoimmune Disease: Animal Models

Autoimmunity

The majority of studies have provided evidence for a suppressive role of NKT cells in autoimmunity, including experimental models of type 1 diabetes, multiple sclerosis, systemic lupus erythematosus and arthritis. Consistent with these findings, αGalCer treatment usually (but not always) protects against autoimmunity, with Th2-biasing agonists typically (but not always) having superior activities (reviewed by Van Kaer, 2005; Van Kaer et al., 2011). Mechanisms involved remain to be fully explored but may include alterations in the Th1/Th17/Th2 balance of autoantigen-specific immune responses, induction of energy in pathogenic T cells, direct effects on B cells, induction of Foxp3-expressing regulatory T cells (reviewed by La Cava et al., 2006) and suppressive myeloid-lineage cells (Denney et al., 2012; Naumov et al., 2001; Parekh et al., 2013).

Colitis

NKT cells are required for the development of Th2-mediated colitis induced by the hapten oxazolone in mice (Heller et al., 2002; see also: Crohn Disease and Ulcerative Colitis). In this model, IL-13, produced by NKT cells, and innate lymphocytes such as nuocytes are responsible for the induced pathology (Camelo et al., 2012). Additional studies have shown that NKT-cell activation protects against Th1/Th17-mediated colitis induced by sodium dextran sulfate (Kim et al., 2012). A role for NKT cells in human ulcerative colitis has also been suggested (reviewed by Fuss and Strober, 2008).

Atherosclerosis

Atherosclerotic lesions in mice and humans express CD1d molecules and contain infiltrating NKT cells (Kyriakakis et al., 2010); (reviewed by Braun et al., 2010; see also: Atherosclerosis: Pathogenesis, Genetics and Experimental Models). Studies with apolipoprotein E (apoE)- and LDLR-deficient mice, and with mice fed an atherosclerotic diet have shown a pathogenic role for NKT cells in atherogenesis (reviewed by Braun et al., 2010). Consistent with these findings, most studies have shown that αGalCer exacerbates atherogenesis, although one study with LDLR-deficient mice reported disease exacerbation (van Puijvelde et al., 2009).

Metabolic disease

NKT cells are particularly abundant in tissues with high metabolic activity such as liver and adipose, suggesting that they may influence normal metabolism and metabolic disease. As already discussed, NKT10 cells (see section titled ‘NKT-Cell Subsets and the Division of Labour’) appear to be particularly enriched in adipose tissue of lean animals, especially in the omentum (Lynch et al., 2015). Studies with experimental models of nonalcoholic fatty liver disease (NAFLD; see also: Genetics of Non-alcoholic Fatty Liver Disease) have provided conflicting results, with some studies suggesting a protective role and others a pathogenic role for NKT cells (reviewed by Tajiri and Shimizu, 2012). A possible explanation for these confounding studies is that NKT cells may play a protective role early during the disease process and a pathogenic role at later stages of disease when fibrosis develops. Studies investigating the role of NKT cells in obesity-induced inflammation and insulin resistance have been equally discordant (reviewed by Wu and Van Kaer, 2013). While most studies agree that NKT cells exhibit an anitinflammatory phenotype in the adipose tissue of lean animals, some studies reported that this phenotype was retained in obese animals whereas others found that NKT cells acquired a proinflammatory phenotype during the development of obesity. Studies with diet-induced obesity or genetic models of obesity have reported no effect, amelioration or exacerbation of obesity-associated inflammation and disease by NKT cells. Similar divergent findings were obtained when investigating the effects of αGalCer on obesity-associated inflammation. While a variety of factors may contribute to these divergent findings, a likely culprit is differences in the functional status of NKT cells imparted by the endogenous microbiota present in the different animal facilities where the respective studies were performed.
Acute tissue injury and sickle-cell disease

In experimental models of acute tissue injury induced by ischemia-reperfusion in kidney and liver, NKT cells expand and become activated in the target organ (reviewed by Kinsey and Okusa, 2014). Consequently, NKT cells were shown to be required for the induction of ischemia-reperfusion injury in kidney and liver. Sickle-cell disease (see also: Sicklet Cell Disease as a Multifactorial Condition) causes widely disseminated vasocclusive episodes that bear similarity to ischemia-reperfusion. NKT cells were shown to exacerbate pulmonary inflammation and injury in an experimental model of sickle-cell disease in mice (reviewed by Field et al., 2011). These findings have prompted studies to explore the effects of NKT-cell depletion or inactivation as a means to protect patients against acute tissue injury and sickle-cell disease. Stroke is another type of ischemia that involves reperfusion injury followed by induction of systemic immune suppression. Strikingly, in a mouse model of stroke, liver NKT cells were shown to be required for the induction of immune suppression, in a mechanism involving activation of these cells by a noradrenergic neurotransmitter (Wong et al., 2011).

Allograft rejection and graft-versus-host disease

NKT cells can contribute both positively and negatively to allograft rejection (reviewed by Fratschke et al., 2009; see also: Graft Rejection: Mechanisms). These cells play a critical role in inducing tolerance to allografts following blockade of costimulatory receptors (Seino et al., 2001). They are also required for the long-term survival of corneal allografts (Sonoda et al., 2002). However, in a model of pancreatic islet transplantation in the liver, NKT cells played a pathogenic role (Toyofuku et al., 2006). Because CD1d exhibits limited polymorphism, these effects of NKT cells on allograft rejection or tolerance are likely mediated by NKT-cell activation in response to tissue injury or mediators produced by alloreactive, conventional T cells. Nevertheless, one study showed that the limited polymorphisms observed in CD1d in mice can cause allogeneic NKT-cell responses (Zimmer et al., 2009). A number of studies have provided evidence that NKT cells protect against graft-versus-host disease (GVHD) in a manner that is dependent on IL-4 production by these cells (Leveson-Gower et al., 2011).

Preterm birth

NKT cells are abundant at the foetal–maternal interface (Boyson et al., 2002). While the role of these cells in normal pregnancy remains unclear, their activation with αGalCer in pregnant mice can induce abortion (Ito et al., 2000). NKT cells have also been implicated in the capacity of LPS to induce preterm birth (Li et al., 2015, 2012; see also: Genetics of Preterm Birth). These findings therefore suggest that NKT cells may play a critical role in the induction of preterm labour in response to infection or inflammation (reviewed by Rinaldi et al., 2015).

Hypersensitivity reactions

NKT cells play a pathogenic role in AHR induced in mice using a variety of experimental models and allergens such as ovalbumin, ragweed, respiratory viruses, house dust extracts, the environmental pollutant ozone, the cytokine IL-25, various NKT-cell antigens and apoptotic respiratory epithelial cells (reviewed by Iwamura and Nakayama, 2010). Treatment with αGalCer during allergen-induced AHR either ameliorated or exacerbated disease, depending on the dose and timing of αGalCer administration (reviewed by Van Kaer et al., 2013). Whether NKT cells play a role in allergic asthma in humans has been a topic of significant controversy (reviewed by Iwamura and Nakayama, 2010). In addition to atopic disease, NKT cells have been implicated in the development of contact hypersensitivity in mice (reviewed by Askenase et al., 2004). In these models, it has been suggested that IL-4 produced by liver NKT cells contributes to B1 B-cell activation in the peritoneum, leading to production of natural IgM antibodies, which in turn activate complement and other inflammatory mediators to recruit effector T cells.

Conclusions

NKT cells localise to portals of microbial entry around cells that express CD1d, which has evolved to bind lipids. Cellular lipid gradients are tightly regulated. Internal and external stressors are known to alter this gradient. Any alterations in the gradient are displayed at the cell surface for an appraisal by NKT cells. The stability of CD1d–lipid complexes depends on whether the hydrocarbon chain occupying the F pocket permits the formation of a roof. The NKTCR interfaces its cognate ligand – CD1d-lipid complexes – in a unique mode, which involves germline-encoded ‘hot spots’. By virtue of sensitive ligand recognition – perhaps based on cooperativity (Florence et al., 2008; Stanic et al., 2003b) – NKT cells can respond quickly to changes in lipid concentration and/or structure. The NKT-cell-APC synaptic duet is driven by the binding kinetics of NKTCR/ligand interactions. Synapse formation prepares for effector functions and permits synaptic transmission of chemokines as well as effector cytokines and lytic granules. As such, they are known to regulate autoimmune diseases and microbial immunity, and hence inflammation arising from stressors from within as occurs in cancers, autoimmunity or inflammation, or from the outside as in an infection or allergic reaction. In this manner, NKT cells can regulate homeostasis.

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Further Reading


