

# 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.**

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## Advanced article

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## 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 ( $\gamma\delta$  T cells, natural killer T (NKT) cells, mucosal-associated invariant T lymphocytes and CD8 $\alpha\alpha$  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 nonself-lipid ligands presented by CD1d molecules. The majority of NKT cells express an invariant TCR  $\alpha$ -chain generated by TRAV11\*02 (mouse V $\alpha$ 14i) or TRAV10 (human V $\alpha$ 24i) to TRAJ18 (Ja18) rearrangement. The invariant  $\alpha$ -chain pairs predominantly with mouse TRBV13-2\*01 (V $\beta$ 8.2), TRBV29\*02 (V $\beta$ 7), TRBV1 (V $\beta$ 2) or human TRBV25-1 (V $\beta$ 11)  $\beta$ -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 CD1d-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  $\alpha$ GalCer (KRN7000) and its analogues that closely resemble the marine sponge (see also: **Porifera (Sponges): Recent Knowledge and New Perspectives**) *Agelas mauritanus*-derived agelasphin 9b (Table 1;

and references therein).  $\alpha$ GalCer was originally isolated from *A. mauritanus*. 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. mauritanus* – and some mammals including mouse and rat biosynthesise  $\alpha$ GalCer and/or related compounds (Table 1; and references therein). Hence,  $\alpha$ GalCer 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  $\alpha$ GalCer 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).

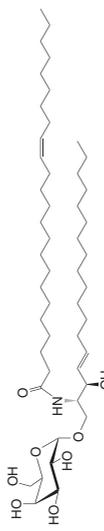
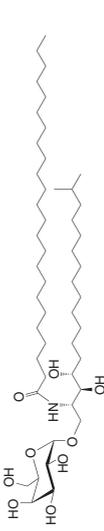
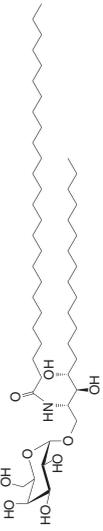
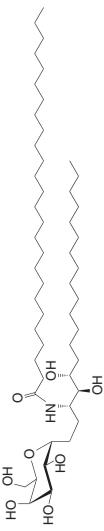
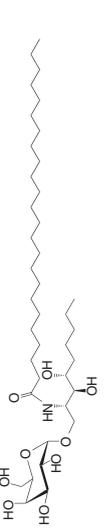
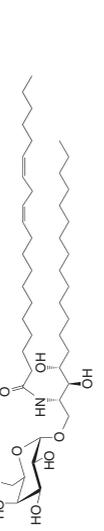
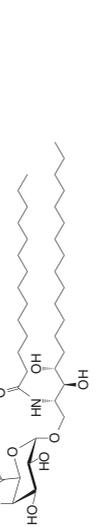
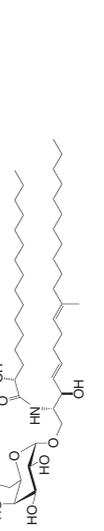
*Sphingomonas* spp. – a Gram-negative  $\alpha$ -Proteobacteria that lack lipopolysaccharide (LPS)- synthesise  $\alpha$ -glucuronosylceramide and  $\alpha$ -galacturonosylceramide ( $\alpha$ GalACer) that resemble  $\alpha$ GalCer (Table 1).  $\alpha$ GalACer by itself activates NKT cells in a CD1d-restricted 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.

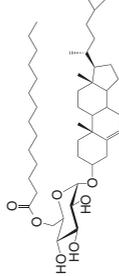
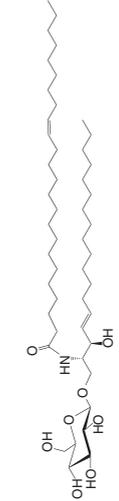
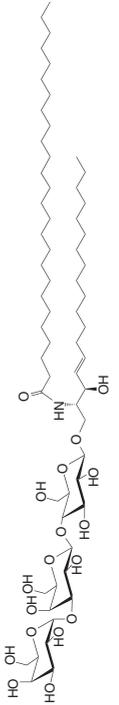
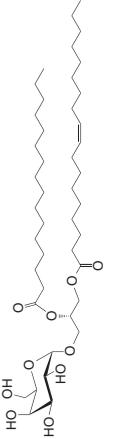
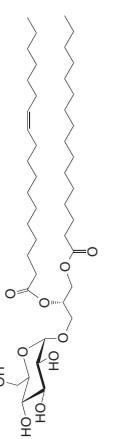
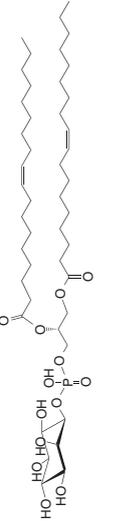
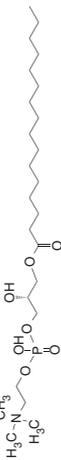
NKT cells also recognise diacylglycerol-based microbial lipids; for example,  $\alpha$ -galactosyl ( $\alpha$ GalDAG) and  $\alpha$ -glucosyl ( $\alpha$ GlcDAG) diacylglycerol, which are cell wall components synthesised by *Borrelia burgdorferi* – the agent of Lyme disease (see also: **Spirochaetes**) – and *Streptococcus pneumoniae*, respectively. So also, NKT cells are activated by *Helicobacter pylori*-derived cholesteryl-6-O-acyl  $\alpha$ -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 (Bendelac *et al.*, 2007; Brennan *et al.*, 2013). An initial search for the endogenous NKT-cell agonist revealed that neither cells deficient in  $\beta$ GlcCer synthase and transiently expressing CD1d nor cell-free CD1d- $\beta$ GlcCer complexes activate mouse NKT-cell-derived hybridomas (Stanic *et al.*, 2003a). This finding suggested that a cellular,  $\beta$ GlcCer-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  $\alpha$ - and  $\beta$ -linked GSLs – for example, cellular  $\alpha$ GalCer,  $\beta$ GalCer,  $\beta$ GlcCer, isoglobotrihexosylceramide (iGb3), ganglioside D3 (GD3) – as well as glycerophospholipids (GPLs; see also: **Glycerophospholipids**) – 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

Lipid (class <sup>a</sup> )	Chain Length <sup>b</sup>	Structure	Agonist <sup>c</sup>	References
$\alpha$ GalCer (GSL)	C18 C24:1		IFN- $\gamma$ , IL-4 self	Kain <i>et al.</i> (2014)
Agel 9b (GSL)	C17 (C <sub>16</sub> -Me) phyto C24		Antitumour; <i>Agelas mauritianus</i>	Morita <i>et al.</i> (1995), Natori <i>et al.</i> (1993)
$\alpha$ GalCer (GSL)	C18-phyto C26		Very strong; robust IFN- $\gamma$ IL-4 and other cytokines; synthetic analogue of Agel 9b (KRN7000)	Kawano <i>et al.</i> (1997)
$\alpha$ -C-GalCer (GSL)	C18-phyto C26		Weak (mo <sup>6</sup> -to-none (hu); IFN- $\gamma$ ; synthetic	Schmieg <i>et al.</i> (2003)
OCH (GSL)	C9-phyto C24		Weak (mo)-to-none (hu); IL-4 (low-to-no IFN- $\gamma$ ); synthetic	Miyamoto <i>et al.</i> (2001)
C20-diene (GSL)	C18-phyto C20:2		Strong; IL-4 (low-to-no IFN- $\gamma$ ); synthetic	Yu <i>et al.</i> (2005)
$\alpha$ GalA Cer (GSL)	C18-phyto C14		Weak; <i>Splingomonas</i> spp.	Kinjo <i>et al.</i> (2005), Mattner <i>et al.</i> (2005)
Asp B	C20:2-C <sub>9</sub> Me C16-C <sub>2</sub> OH		Weak; <i>Aspergillus fumigatus</i>	Albacker <i>et al.</i> (2013)

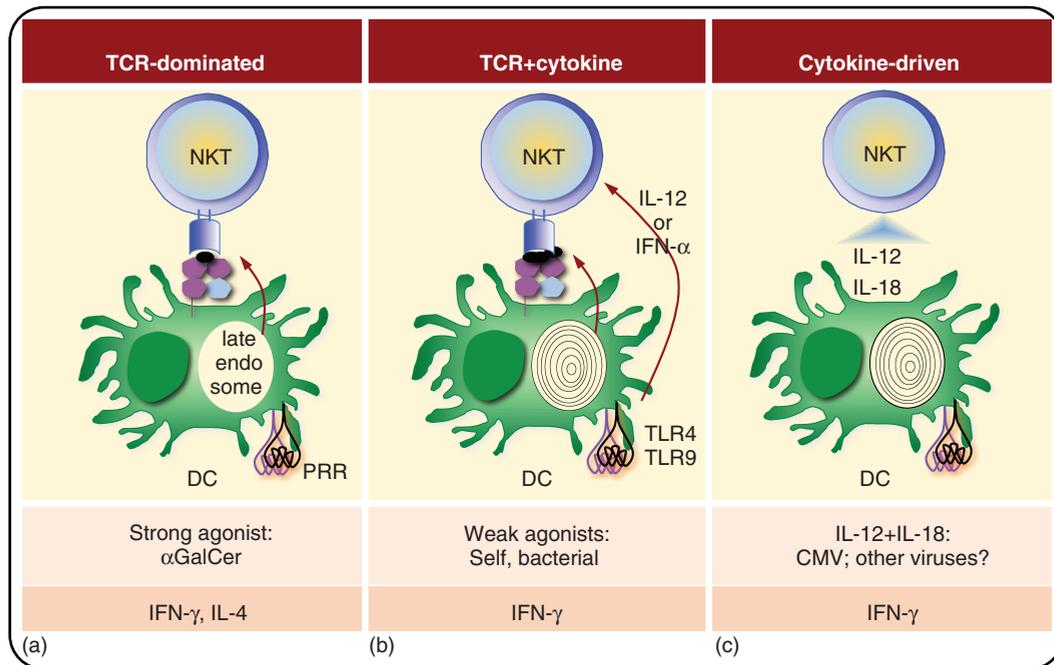
$\alpha$ Glc-acyl-Chol	C14		Strong; binds a small NKT-cell subset (mo); <i>Helicobacter pylori</i>	Chang <i>et al.</i> (2011)
$\beta$ GlcCer (GSL)	C18 C24:1		Strong; self	Brennan <i>et al.</i> (2011)
iGb3 (GSL)	C18- C24		Weak (mo)-to-none (hu); self	Zhou <i>et al.</i> (2004b)
$\alpha$ GalDAG (GGL)	<i>sn</i> 1-C18:1 <i>sn</i> 2-C16		Weak (mo)-to-none (hu); <i>Borrelia burgdorferi</i>	Kinjo <i>et al.</i> (2006)
$\alpha$ GlcDAG (GGL)	<i>sn</i> 1-C18:1 <i>sn</i> 2-C16		Weak; <i>Streptococcus pneumoniae</i>	Kinjo <i>et al.</i> (2011)
PtdIno (GPL)	<i>sn</i> 1-C18:1 <i>sn</i> 2-C18:1		Weak (mo)-to-no (hu); self	Gumperz <i>et al.</i> (2000), Mallevaey <i>et al.</i> (2011)
Lyso-PtdCho (GPL)	<i>sn</i> 1-C16 <i>sn</i> 2-lyso		Weak (hu)-to-none (mo); GM-CSF (no IL-4, IFN- $\gamma$ ); self	Fox <i>et al.</i> (2009)

<sup>a</sup>Agel, 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, glycolglycerolipid; GPL, glycerophospholipid; GSL, glycosphingolipid.

<sup>b</sup>Sphingosine/phytosphingosine chain length indicated first and *N*-acyl chain length second.

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

<sup>d</sup>Relative potency in comparison to  $\alpha$ GalCer; mo, mouse; hu, human.



**Figure 1 Three distinct strategies activate NKT cells.** Potent NKT-cell agonists – such as  $\alpha$ GalCer – 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 NKTcr. Several endogenous lipid agonists have been identified and characterised (**Table 1**). Some microbes such as *Sphingomonas capsulata*, which are  $\alpha$ -Proteobacteria, synthesise  $\alpha$ -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  $\alpha$ GalCer-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  $\beta$ -1,3 glucans (Albacker *et al.*, 2013; Cohen *et al.*, 2011). Because  $\beta$ -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  $\alpha$ GalCer 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)- $\alpha$  (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; Tyznik *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

Microbe	Antigen <sup>a</sup>	NKT-cell role <sup>b</sup>	Model	Route of infection	References
<b>Gram-positive</b>					
<i>S. pneumoniae</i>	$\alpha$ GalDAG	Protective	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.n., i.t.	Brigl <i>et al.</i> (2011), Kawakami <i>et al.</i> (2003), Kinjo <i>et al.</i> (2011)
<i>S. aureus</i>	ND	Not protective	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.v.	Kwiecinski <i>et al.</i> (2013)
<b>Gram-negative</b>					
<i>P. aeruginosa</i>	ND	Protective Not protective	$CD1d^{-/-}$ $J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.n. i.t.	Nieuwenhuis <i>et al.</i> (2002)
<i>S. typhimurium</i>	ND	Not protective	$CD1d^{-/-}$	p.o.	Berntman <i>et al.</i> (2005), Brigl <i>et al.</i> (2003), Mattner <i>et al.</i> (2005)
<i>H. pylori</i>	$\alpha$ Glc-acyl-cholesterol	Protective	$J\alpha 18^{-/-}$	p.o.	Ito <i>et al.</i> (2013)
<i>C. trachomatis</i> ( <i>muridarum</i> )	GLXA	Detrimental Not protective	$CD1d^{-/-}$	i.n. intravaginal	Bilenki <i>et al.</i> (2005), Habbeddine <i>et al.</i> (2013), Peng <i>et al.</i> (2012)
<i>C. pneumoniae</i>	ND	Protective	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.n.	Joyee <i>et al.</i> (2007)
<i>L. pneumophila</i>	ND	Detrimental	$J\alpha 18^{-/-}$	i.t.	Bilenki <i>et al.</i> (2005), Habbeddine <i>et al.</i> (2013), Peng <i>et al.</i> (2012)
<i>F. tularensis</i> subspp. <i>tularensis</i> <i>SchuS4</i>	ND	Detrimental	$CD1d^{-/-}$ ; <sup>c</sup>	i.n.	Hill <i>et al.</i> (2015)
<i>F. novicida</i>	ND	Detrimental Not protective	$CD1d^{-/-}$ $CD1d^{-/-}$	i.n. s.c., i.d.	
<b><math>\alpha</math>-Proteobacteria</b>					
<i>S. capsulata</i>	$\alpha$ GlcACer	Protective (low dose) Detrimental (high dose)	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.v.	Kinjo <i>et al.</i> (2005), Mattner <i>et al.</i> (2005), Sriram <i>et al.</i> (2005)
<i>N. aromaticivorans</i>	$\alpha$ GalACer	Primary biliary cirrhosis	$CD1d^{-/-}$	i.v.	Mattner <i>et al.</i> (2008)
<b>Spirochetes</b>					
<i>B. burgdorferi</i>	$\alpha$ GalDAG	Protective	$CD1d^{-/-}$	i.d.	Kumar <i>et al.</i> (2000), Tupin <i>et al.</i> (2008)
<b>Mycobacteria</b>					
<i>M. tuberculosis</i>	ND	Not protective Protective <sup>c</sup>	$CD1d^{-/-}$ Cell transfer	i.v. aerosol	Behar <i>et al.</i> (1999), Sada-Ovalle <i>et al.</i> (2008)
<b>Viruses</b>					
HSV-1	ND	Protective Not protective <sup>c</sup>	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$ $CD1d^{-/-}$	Scarification	Cornish <i>et al.</i> (2006), Grubor-Bauk <i>et al.</i> (2003)
HSV-2	ND	Protective	$CD1d^{-/-}$	Intravaginal	Ashkar and Rosenthal (2003)
Sendai virus	ND	Detrimental	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.n.	Kim <i>et al.</i> (2008)
RSV	ND	Protective	$CD1d^{-/-}$	i.n.	Johnson <i>et al.</i> (2002)
Influenza virus H1N1 and H3N2	ND	Protective	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.n.	De Santo <i>et al.</i> (2008), Ho <i>et al.</i> (2008), Kok <i>et al.</i> (2012), Paget <i>et al.</i> (2011, 2012)
HBV	ND	Protective	$J\alpha 18^{-/-}$ , $CD1d^{-/-}$	i.v.	Zeissig <i>et al.</i> (2012)

Table 2 (continued)

Microbe	Antigen <sup>a</sup>	NKT-cell role <sup>b</sup>	Model	Route of infection	References
<b>Fungi</b>					
<i>A. fumigatus</i>	Asper-amide-B	Detrimental (AHR) <sup>c</sup> Protective (early)	CD1d <sup>-/-</sup>	i.n. i.t.	Albacker <i>et al.</i> (2013)
<b>Parasites</b>					
<i>P. berghei</i>	ND	Detrimental	CD1d <sup>-/-</sup>	i.d.	Hansen <i>et al.</i> (2003)
<i>T. gondii</i>	ND	Protective Detrimental <sup>c</sup>	J $\alpha$ 18 <sup>-/-</sup> , CD1d <sup>-/-</sup> J $\alpha$ 18 <sup>-/-</sup> , CD1d <sup>-/-</sup> ; V $\alpha$ 14 <sup>tg</sup>	p.o.	Roberts <i>et al.</i> (2004), Spence <i>et al.</i> (2001)

<sup>a</sup>See Table 1 for antigen structure.

<sup>b</sup>Differential outcomes in the different studies may have arisen from the use of different microbial/parasite strains.

<sup>c</sup>BALB/c background of mouse strains used in these studies; others were in C57BL/6 background.

AHR, airway hyperreactivity; ND, not determined; i.d., intradermal; i.n., intranasal; i.p., intraperitoneal; i.v., intravenous; i.t., intratracheal; p.o., per-oral; s.c. subcutaneous.

## CD1d assembly with NKT-cell agonists

CD1d molecules are expressed by several haematopoietic cells especially APCs (see also: **Antigen Processing**) – such as DCs (see also: **Dendritic Cells (T-lymphocyte Stimulating)**), macrophages (M $\phi$ ; 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<sup>+</sup>8<sup>+</sup> thymocytes. Hepatocytes, intestinal epithelial cells (see also: **Lymphocytes: Intraepithelial**), 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- $\gamma$  (see also: **An Overview of Cytokine Regulation of Inflammation and Immunity**) and either TNF- $\alpha$  (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.

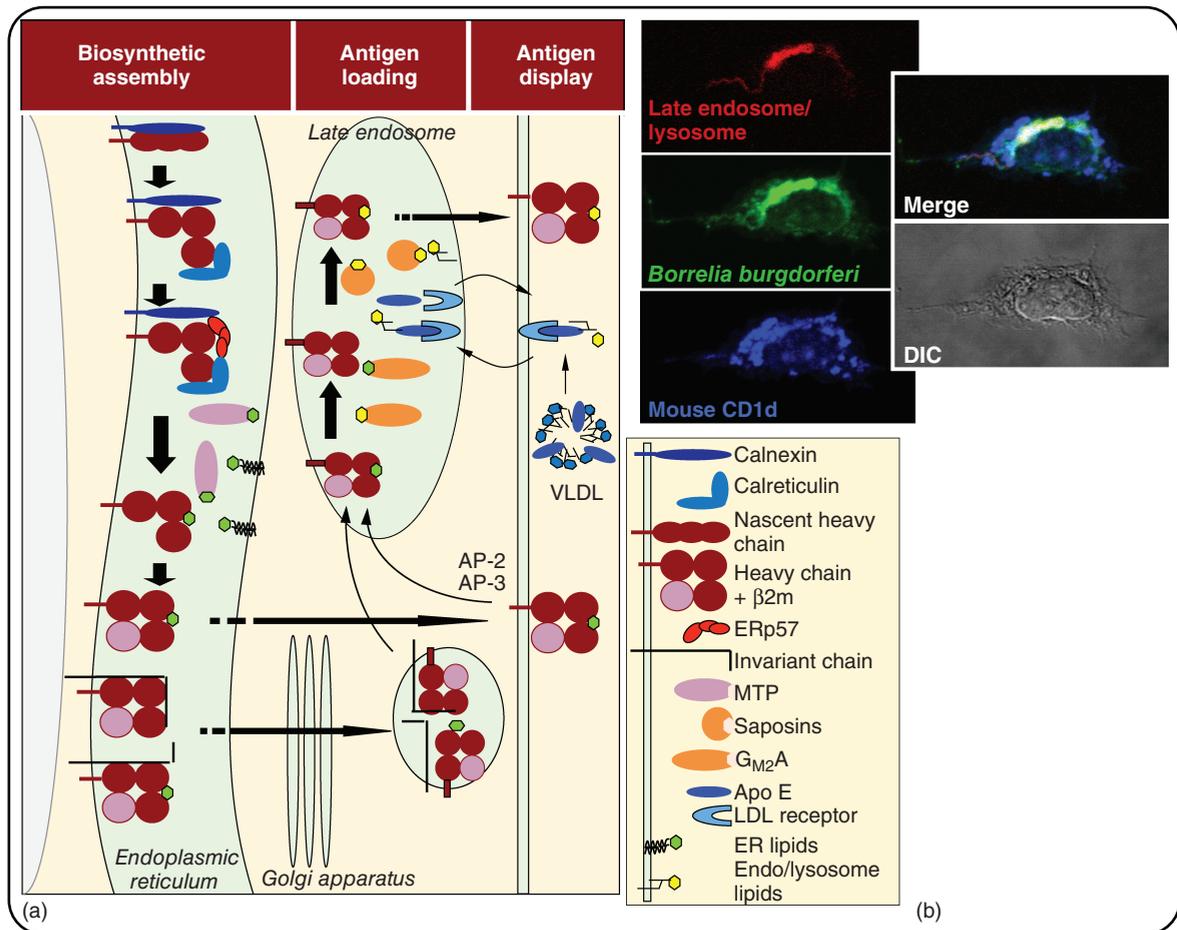
### Assembly and exchange

CD1d is a heterodimer consisting of a heavy chain that is noncovalently associated with the light-chain  $\beta$ 2-microglobulin ( $\beta$ 2m) (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) (Giabai *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 Kaer, 2003). Alternatively, CD1d can arrive directly at the MIIC by assembly with MHC class II-associated invariant chain (Ii), which contains a MIIC-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<sub>M2</sub> 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 macropinocytosis) 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 *in vivo*. The extraction of bound lipids from CD1d and the loading of antigenic glycolipids are facilitated by lysosomal LTP such as Saposins (Sap),  $G_{M2}$  activator ( $G_{M2}A$ ) and Niemann-Pick C-2 (not shown), which are essential for the enzymatic catabolism of glycolipids. (b) Infection of M $\phi$  and DC delivers microbes to the CD1d-containing lysosomes. Differential interference contrast (DIC) picture of M $\phi$  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 *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 *B. burgdorferi* colocalises with lysosomes and CD1d colocalised appear white in the merged picture.

(Figure 2a; Freigang *et al.*, 2012; van den Elzen *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 down modulation 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).

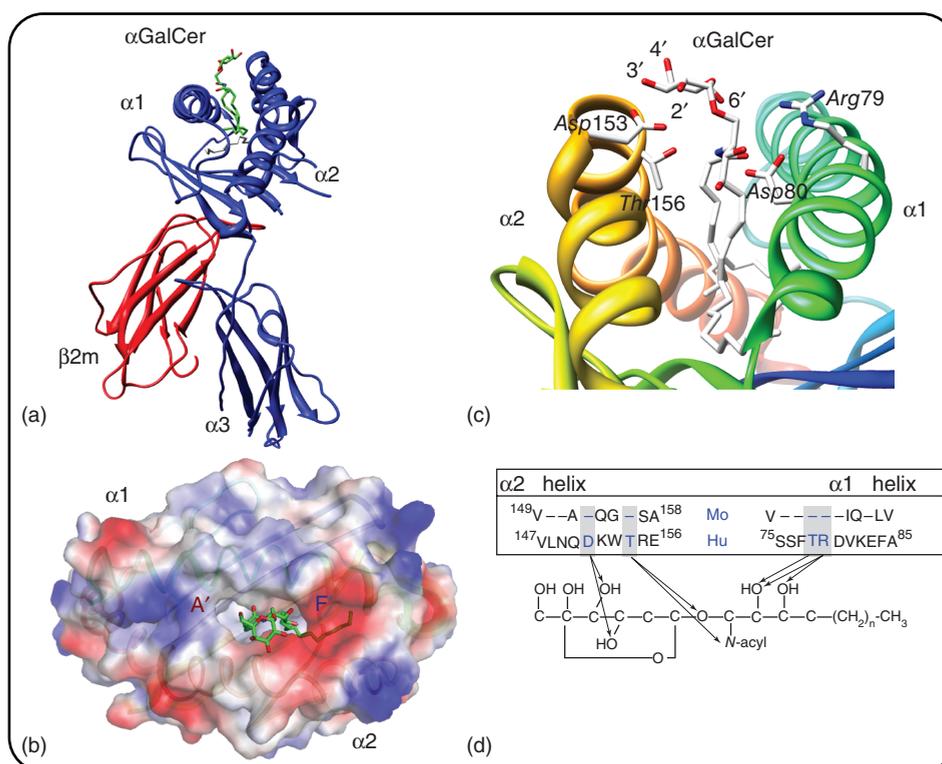
## Structures of CD1d–lipid complexes

The CD1d heavy chain folds into five domains: the extracellular  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  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  $\alpha 1$  and  $\alpha 2$  domains of the heavy chain fold into a superdomain to form the ABG (**Figure 3**). The ABG is laterally confined by two antiparallel  $\alpha$ -helices; these helices are supported at the bottom by an 8-stranded antiparallel  $\beta$ -sheet platform. The membrane-proximal immunoglobulin-like  $\alpha 3$  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 $\alpha$ GalCer 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  $\alpha$ GalCer and related compounds – OCH and  $\alpha$ GalACer – tucks into the large  $A'$  pocket while the long-chain



**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  $\alpha$ GalCer. The CD1d molecule is made up of a heavy chain that is noncovalently associated with a light-chain  $\beta 2$ -microglobulin ( $\beta 2m$ ). The membrane distal  $\alpha 1$  and  $\alpha 2$  domains of the heavy chain fold in such a manner that they form an apical antigen-binding groove into which bind lipid ligands. The  $\alpha 3$  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  $\alpha$ GalCer. 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– $\alpha$ GalCer 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 Chimera (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  $\alpha$ -anomeric GSLs. The same CD1d residues also form hydrogen bonds with  $\beta$ -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 V $\alpha$ 14i/V $\alpha$ 24i TCR (reviewed by Rossjohn *et al.*, 2015).

### Display of $\alpha$ GalDAG

Microbial  $\alpha$ GalDAG antigens are structurally similar to  $\alpha$ GalCer in that they also have an  $\alpha$ -anomeric galactose attached to a lipid backbone (**Table 1**). However, in contrast to  $\alpha$ GalCer, 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  $\alpha$ -anomeric galactose is attached to the *sn*-3 position. Borrelial  $\alpha$ GalDAG 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  $\alpha$ GalDAGs 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<sub>18:1</sub>) in the A' pocket and the *sn*-2 palmitic acid (C<sub>16:0</sub>) 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<sub>18:1</sub>) in the A' pocket and the *sn*-1-linked linoleic acid (C<sub>18:2</sub>) 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  $\alpha$ GalCer 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,  $\alpha$ GalDAG presentation reveals for the first time, striking differences between mouse and human glycolipid antigen recognition that could not have been appreciated using strong agonists, such as  $\alpha$ GalCer.

### 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  $\beta$  linkage (Zajonc *et al.*, 2005b). This contrasts the more intimate binding of the galactosyl headgroup of  $\alpha$ GalCer 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- $\beta$ (1–4)Gal- $\alpha$ (1–3)Gal] of iGb3 out of the ABG as revealed by the structure of the mouse CD1d–iGb3 complex (Zajonc *et al.*, 2008). Nevertheless, the  $\beta$ -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  $\alpha$ - and  $\beta$ -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 *sn*1-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  $\alpha$ 1-helix, which had moved away from the  $\alpha$ 2-helix as compared to  $\alpha$ -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 PtdIno and Ptd-Cho (Giabbai *et al.*, 2005; Lopez-Sagaseta *et al.*, 2012; Mallevaey *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) – CDR1 $\alpha$ , CDR3 $\alpha$  and CDR2 $\beta$  – while almost excluding CDR2 $\alpha$ , CDR1 $\beta$  and CDR3 $\beta$  from the interface. This docking mode enables a lock-and-key interaction with the  $\alpha$ -linked galactose epitope. Furthermore, alanine-scanning mutagenesis of the mouse V $\alpha$ 14i TCR, the crystal structures of V $\alpha$ 14i-V $\beta$ 8.2 and V $\alpha$ 14i-V $\beta$ 7 and V $\alpha$ 14i-V $\beta$ 2 cocomplexed with mouse CD1d– $\alpha$ GalCer revealed that the mouse NKTCR interfaces its ligand in a manner similar to the V $\alpha$ 24i TCR–ligand interaction (reviewed by Rossjohn *et al.*, 2015).

The above germline-encoded recognition logic raises the question of how the mouse V $\alpha$ 14i and human V $\alpha$ 24i TCRs recognise structurally distinct ligands such as iGb3, GD3, PtdInoMan<sub>4</sub>, PtdIno, PtdEtN and lyso-PtdCho. Alanine-scan mutants of V $\alpha$ 14i TCR revealed that the NKTCR recognises many  $\alpha$ -linked GSLs ( $\alpha$ GalCer, OCH,  $\alpha$ GalACer,  $\alpha$ GalDAG and iGb3, which contains an  $\alpha$ -linked terminal galactose) by means of a 'hot spot' of germline-encoded amino acids within CDR1 $\alpha$ , CDR3 $\alpha$  and CDR2 $\beta$  loops (Scott-Browne *et al.*, 2007). The recent structure of mCD1d–PtdIno bound to an autoreactive V $\alpha$ 14i TCR, in which the  $\beta$ -chain was mutated to increase affinity toward the self-antigen, surprisingly revealed that CDR3 $\alpha$  residues do not directly contact the solvent-exposed phospho-inositol. Yet the NKTCR maintains a conserved footprint on CD1d by allowing additional residues in CDR2 $\alpha$  to contact the inositol head group (Mallevaey *et al.*, 2011).

The recent solution of the V $\alpha$ 14i-V $\beta$ 8.2/mouse CD1d– $\alpha$ GalDAG and  $\alpha$ GalACer 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  $\alpha$ GalCer and  $\alpha$ GalACer, the TCR contacts  $\alpha$ GalDAG exclusively through CDR1 $\alpha$  and CDR3 $\alpha$ . In each of these ternary structures, CDR1 $\alpha$  Asn30 hydrogen bonds with the 2' and 3' hydroxyls of the galactose or galacturonic acid of the GSL. However, for  $\alpha$ GalDAG, this conserved interaction with the TCR required a reorientation of the galactose moiety. CDR3 $\alpha$  residue Gly96 contacts the 2'-OH through a main chain carbonyl, while Arg95 contacts the 3''-OH of the ceramide backbone of both  $\alpha$ GalCer and  $\alpha$ GalACer. However, this hydrogen-bond interaction is lost in the  $\alpha$ GalDAG/CD1d structure due to the differences in the lipid backbone of  $\alpha$ GalDAG (Li *et al.*, 2010). These findings suggest that the interaction of NKTCR with structurally distinct  $\alpha$ -linked ligands is accomplished by similar recognition logic, which involves the germline-encoded 'hot spot' composed of amino acids within CDR1 $\alpha$ , CDR3 $\alpha$  and CDR2 $\beta$  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- $\beta$ GlcCer and CD1d-PtdIno as well as the V $\alpha$ 24i/V $\beta$ 11-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-iGb3 and CD1d- $\beta$ GlcCer structures, the NKTCR flattened the sugars that protrude up from the ABG owing to the  $\beta$ -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  $\alpha$ GalDAG 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 germline-encoded NKTCR/CD1d-ligand recognition logic provides insight into why the interactions between the V $\alpha$ 14i or V $\alpha$ 24i 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  $\alpha$ GalCer (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  $\alpha$ GalDAG results in a reduced TCR association (Li *et al.*, 2010). Secondly, upon TCR binding onto mouse CD1d- $\alpha$ GalDAG or - $\alpha$ GalACer, the NKTCR induces a structural change in mouse CD1d above the F' pocket, namely the formation of the F' roof (Li *et al.*, 2010). The F' roof is already preformed upon  $\alpha$ GalCer 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  $\alpha$ GalCer 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  $\alpha$ GalCer 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

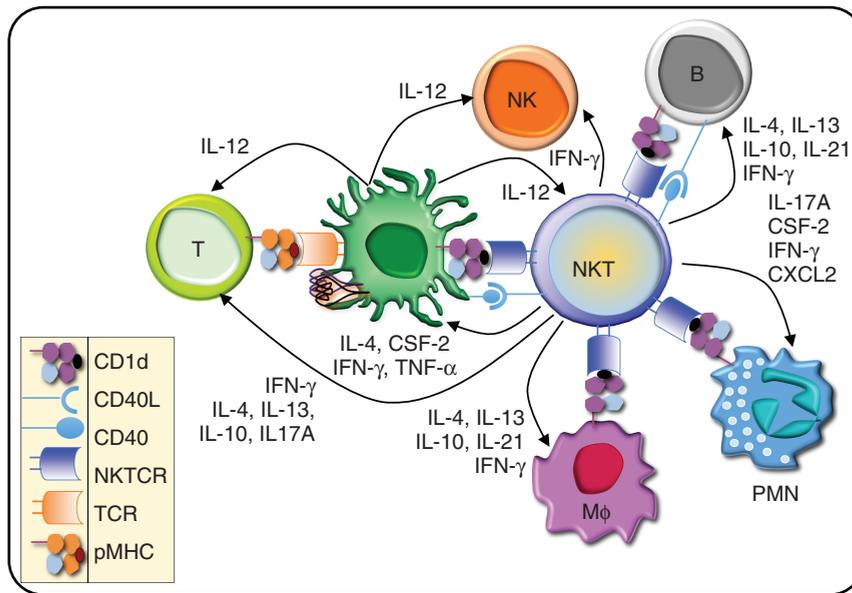
### NKT-cell DC synapse

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 planar membranes (Bezradica *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- $\gamma$  and lytic granules to the immune synapse (Bezradica *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, M $\phi$ , 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  $\alpha$ GalCer, within 30–90 min, elicits a wide variety of cytokines (Figure 4). However, derivatives of  $\alpha$ GalCer 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  $\alpha$ GalCer variants that have an altered glycosidic linkage, a chemically modified acyl chain or a modified sphingoid base potentially elicit an IFN- $\gamma$ -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.



**Figure 4 The immunological effector functions of NKT cells.** The interactions between the invariant natural killer (NKT) cell receptor and its cognate antigen and interactions between costimulatory molecules CD28 and CD40 and their cognate ligands CD80/86 (B7.1/7.2) and CD40L, respectively, activate NKT cells. Activated NKT cells participate in cross-talk between members of the innate and the adaptive immune system by deploying cytokine/chemokine messengers. Upon activation *in vivo*, NKT cells rapidly secrete a variety of cytokines/chemokines. These cytokines/chemokines influence the polarisation of CD4<sup>+</sup> T cells towards T helper (Th)1 or Th2 cells as well as the differentiation of precursor CD8<sup>+</sup> T cells to effector lymphocytes and B cells to antibody-secreting plasma cells. Some of these cytokines/chemokines facilitate the recruitment, activation and differentiation of macrophages and dendritic cells, which result in the production of interleukin (IL)-12 and possibly other factors. IL-12, in turn, stimulates NK cells to secrete IFN- $\gamma$ . Thus, activated NKT cells have the potential to enhance as well as temper the immune response. This schematic rendition of NKT-cell effector functions is an adaptation of past reviews (Brennan *et al.*, 2013; Florence *et al.*, 2008; Van Kaer, 2005).

**DC, M $\phi$  and PMN.** A key feature of NKT-cell activation by  $\alpha$ GalCer 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- $\gamma$  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 licensing 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 M $\phi$  – 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; Schmiege *et al.*, 2005). IFN- $\gamma$  and TNF- $\alpha$  secreted by activated NKT cells in turn stimulate M $\phi$ , which attain microbicidal activity and promote delayed-type hypersensitivity (Nieuwenhuis *et al.*, 2002; Sada-Ovalle *et al.*, 2008). So also, this source of TNF- $\alpha$  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 leucocytes (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- $\gamma$  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  $\alpha$ GalCer, NK cells (see also: **Natural Killer (NK) Cells**) rapidly produce IFN- $\gamma$ . This NK-cell activation depends on CD1d not for

direct NKT-cell–NK-cell interactions. But rather for reciprocal activation of DC–NKT cells and IL-12 production by NKT-cell-matured DCs (Carnaud *et al.*, 1999). It is this transactivation of NK cells that is thought to be responsible for the antitumour effect of  $\alpha$ GalCer (Fujii *et al.*, 2013). Such a NKT-cell–DC–NK-cell axis has also been proposed to be partly responsible for the Th1 versus Th2 biasing effects of certain  $\alpha$ GalCer derivatives, perhaps through the modulation of costimulatory and inhibitory molecules on the surface of APCs (Arora *et al.*, 2014).

**B lymphocytes.** Marginal zone B cells have the highest expression levels of CD1d, suggesting a role in activating NKT cells (Bosma *et al.*, 2012; Roark *et al.*, 1998; **see also: B Lymphocytes**). However, the contribution of B cells to NKT-cell activation during infection is not well understood (Bezbradica *et al.*, 2005; Bialecki *et al.*, 2009; Mattner *et al.*, 2008), but they have been suggested to play a role in NKT-cell homeostasis (Bosma *et al.*, 2012). Similar to conventional T cells, NKT cells can enhance and sustain the humoral immune response (Chaudhry and Karadimitris, 2014) through their ability to produce an array of cytokines including IL-4, IL-6, IL-13 and IL-21 (Coquet *et al.*, 2008).

Coadministration of protein antigens and  $\alpha$ GalCer bolstered antibody titres in mice (Chaudhry and Karadimitris, 2014; Singh *et al.*, 1999). This adjuvant effect impacted both T-dependent and T-independent antigens (Lang *et al.*, 2006). Such B-cell help from NKT cells occurs through cognate or noncognate interactions. Noncognate help – a common response to soluble protein antigens coadministered with soluble  $\alpha$ GalCer – involved NKT-cell licensing of APCs to promote the activation of T follicular helper cells (**see also: T-Lymphocyte Responses: Development**), which in turn promoted germinal centre formation, class-switch recombination, somatic hypermutation and the generation of long-lived plasma and memory cells (Tonti *et al.*, 2009).

Cognate interactions between NKT cells and B cells by haptenated  $\alpha$ GalCer or  $\alpha$ GalCer and protein antigen linked to bead particles (Barral *et al.*, 2008; King *et al.*, 2012; Leadbetter *et al.*, 2008) induced Bcl-6 expression and differentiation of NKT cells toward follicular helper cells (NKTfh; Chang *et al.*, 2012; King *et al.*, 2012). B-cell help from these NKTfh cells contributed to rapid antibody production and some affinity maturation but generated no long-lived memory B cells. Hence, NKT cells may play a role in early IgM and IgG responses during acute infection.

In summary, DCs, M $\phi$ s, B cells and PMNs are capable of presenting lipid agonists and, hence, activating NKT cells. Such activation is reciprocal, mediated by cell–cell interactions and/or soluble mediators, whereby NKT cells control downstream immune responses to soluble antigens and microbial pathogens. It appears that reciprocal activation of CD8<sup>+</sup> DCs, which are a major producer of IL-12, may be best suited to transactivate NK cells and the functional differentiation of antigen-specific precursors towards Th1 and CD8<sup>+</sup> T cells. Likewise, NKT-cell activation by B cells may result in a Th2-type cytokine response that can help elicit B-cell functions. How NKT-cell activation by M $\phi$  and PMN activation impacts downstream immune responses remains to be seen.

## NKT-Cell Development

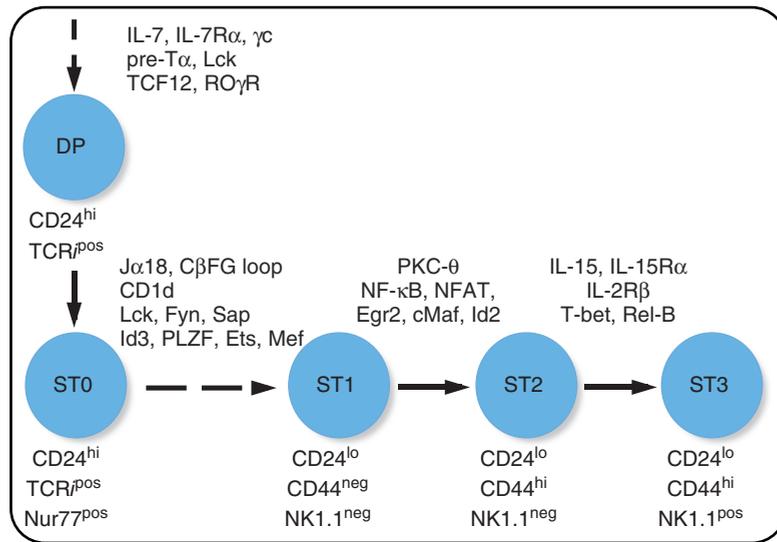
The unique phenotypic and functional features of NKT cells – especially the secretion of immunoregulatory cytokines amongst recent thymic emigrants – suggested that these cells might follow a distinct ontogenetic path. Hence, where NKT cells develop and what lineage-specific factor(s) specify, commit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

Their absence in thymectomised and athymic *nu/nu* mice suggested that NKT cells develop in the thymus (**see also: The Thymic Niche and Thymopoiesis**). Similarly, genetically altered mice deficient in  $\gamma$ c – 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- $\alpha$  chain that are arrested at the double-negative (DN)2/DN3 stage of thymocyte ontogeny (**see also: Lymphoid Development**) do not develop NK1.1<sup>+</sup> T cells (Boesteanu *et al.*, 1997). The rescue of NKT-cell ontogeny by enforced Bcl-2 expression within IL-7R $\alpha$  chain-deficient DN2 thymocytes further suggested that commitment and development of this lineage perhaps occurred in the thymus (Boesteanu *et al.*, 1997; Gordy *et al.*, 2011). These findings, in conjunction with the development of NK1.1<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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 PKC $\theta$ –NF- $\kappa$ B 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  $\beta$ -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 firmed 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



**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  $V\alpha 14$  to  $J\alpha 18$  TCR  $\alpha$ -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 $\alpha$  and IL-15R $\alpha$ , respectively) and the shared common  $\gamma$  chain ( $\gamma$ c) receptor protein subunits that mediate intercellular communication. IL-15 also uses IL-2R $\beta$  that it shares with IL-2 for intercellular communication. CD1d and pre-T-cell receptor (TCR)- $\alpha$  (pre-T $\alpha$ ) are structural proteins, while  $J\alpha 18$  segment and  $C\beta$  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, MEF, 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.

and zinc finger), encoded by *Zbtb16*, which controls multiple molecular events that distinguish NKT cells from all of the other thymus-derived lymphocytes (Kovalovsky *et al.*, 2008; Savage *et al.*, 2008). Hence, NKT cells progress from a NK1.1-negative to -positive stage. The NK1.1-negative NKT cells undergo several rounds of cell division, upon which some remain within the thymus while the majority emigrate and populate the peripheral lymphoid organs. Both the thymic and peripheral NK1.1-negative NKT cells undergo further maturation and become NK1.1-positive NKT cells. During this maturation process, they acquire distinct effector cytokine secretion behaviour (reviewed by Godfrey *et al.*, 2010).

## 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<sup>+</sup>; 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.*, 2013). NKT1 cells predominate in most 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.

NKT1	NKT2	NKT17	NKT10
			
CD4 <sup>POS</sup> /NEG, IL-12R NK1.1 CXCR3, CXCR6	CD4, CD27, IL-25R NK1.1 <sup>NEG</sup> , IL-17RB CCR4, CXCR6	CD127 <sup>H1</sup> , IL-23R, NK1.1 <sup>NEG</sup> , CCR4, CCR6, CXCR6,	CD4, CD49a NK1.1 <sup>NEG</sup> , SLAMF6, PD1
INF- $\gamma$ TNF- $\alpha$ , (IL-4) granzyme, perforin	IL-4, IL-13 IL-6, (Csf-2)	IL-17A IL-21, IL-22	IL-10 IL-2
Liver Spleen (red pulp)	Lymph nodes (T $\pm$ B cell areas)	Lungs, skin Lymph nodes	Adipose tissue Spleen
Anti-tumor immunity	IL-25 induced AHR	Virus/ozone-induced AHR; tissue repair; limit systemic pathogen spread	Immune tolerance; resolve inflammation

**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 (LNs) 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  $\alpha$ GalCer (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 *M* $\phi$ s, 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  $\alpha$ GalCer *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 T<sub>REG</sub>-cell functions in adipose tissues by inducing the polarisation of antiinflammatory M2 *M* $\phi$ s (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 ROR $\gamma$ t 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

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<sup>+</sup> and DN NKT cells show functional dichotomy: The CD4<sup>+</sup> NKT cells secrete IL-4 upon antigen stimulation. Moreover, CD4<sup>+</sup> 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- $\gamma$  and TNF- $\alpha$ . 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 post-natal 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<sup>+</sup> subcapsular M $\phi$  and together with other innate-like lymphocytes contribute to the evolving immune response in the lymph node. This positioning permits immediate sensing of lymph-borne pathogens by a mechanism involving an inflammatory response mediated by M $\phi$  (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 M $\phi$  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  $\alpha$ GalCer results in quick recognition and the arrest of red pulp and marginal zone NKT cells and rapid IFN- $\gamma$  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  $\alpha$ GalCer *in vivo* (Schmiege *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  $\alpha$ GalCer 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,  $\alpha$ GalCer 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  $\alpha$ GalCer induces abortion in mice in a manner that is dependent on IFN- $\gamma$ , TNF- $\alpha$  and perforin (Ito *et al.*, 2000),

whereas in nonpregnant mice,  $\alpha$ GalCer 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 forgoing 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  $\alpha$ GalDAG produced by *B. burgdorferi* and rapidly proliferate and produce IFN- $\gamma$  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* asymptotically 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.  $\alpha$ GlcDAG produced by *S. pneumoniae* activated mouse NKT cells (Kinjo *et al.*, 2011) to secrete IFN- $\gamma$  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 fatal 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; Joyee *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).

### $\alpha$ GalCer-induced protection

Owing to its ability to robustly activate NKT cells,  $\alpha$ GalCer 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  $\alpha$ GalCer 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- $\gamma$  produced by activated NKT cells and transactivated NK cells (Christaki *et al.*, 2015) but resulted in increased production of TNF- $\alpha$  and MIP-2 (Nakamatsu *et al.*, 2007). Similar results were obtained when  $\alpha$ GalCer-treated mice were infected with *C. pneumoniae* (Joyee *et al.*, 2007). Multiple treatments with  $\alpha$ GalCer 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- $\alpha$  production and a less severe pneumonia due to early IFN- $\gamma$  production by activated NKT cells. Nonetheless, mice treated with a single dose of  $\alpha$ GalCer failed to protect mice from pneumonia caused by *P. aeruginosa* infection (Kinjo *et al.*, 2006).

$\alpha$ GalCer-mediated NKT-cell activation has also been shown to have a beneficial effect on the outcome of systemic experimental infections in mice.  $\alpha$ GalCer 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  $\alpha$ GalCer was dependent on the rapid production of IFN- $\gamma$  by NKT cells. However,  $\alpha$ GalCer 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,  $\alpha$ GalCer treatment resulted in reduced IFN- $\gamma$  but increased IL-4, IL-13 and IL-10 that protected from lethal intestinal inflammation. Interestingly, intranasal infection of mice with *C. muridarum* following  $\alpha$ GalCer 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  $\alpha$ GalCer 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

$\alpha$ GalCer was originally described as a GSL isolated from a marine sponge with potent antimetastatic activities in mice (Natori *et al.*, 1993). The subsequent identification of  $\alpha$ GalCer 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 (Kunii *et al.*, 2009; Motohashi *et al.*, 2009). Th1-biasing  $\alpha$ GalCer 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,  $\gamma\delta$  T cells, IFN- $\gamma$  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 Blumberg, 2014). Here, we will briefly review the most recent pertinent studies and we refer to published reviews for earlier work (Bendelac *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,  $\alpha$ GalCer 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 anergy 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  $\alpha$ GalCer 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 antiinflammatory 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  $\alpha$ GalCer 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: **Sickle Cell Disease as a Multifactorial Condition**) causes widely disseminated vasoocclusive 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 Pratschke *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  $\alpha$ GalCer 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  $\alpha$ GalCer during allergen-induced AHR either ameliorated or exacerbated disease, depending on the dose and timing of  $\alpha$ GalCer 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 ligand 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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## References

Albacker LA, Chaudhary V, Chang YJ, *et al.* (2013) Invariant natural killer T cells recognize a fungal glycosphingolipid that can induce airway hyperreactivity. *Nature Medicine* **19**: 1297–1304.

- Arora P, Baena A, Yu KO, *et al.* (2014) A single subset of dendritic cells controls the cytokine bias of natural killer T cell responses to diverse glycolipid antigens. *Immunity* **40**: 105–116.
- Ashkar AA and Rosenthal KL (2003) Interleukin-15 and natural killer and NKT cells play a critical role in innate protection against genital herpes simplex virus type 2 infection. *Journal of Virology* **77**: 10168–10171.
- Askenase PW, Szczepanik M, Itakura A, Kiener C and Campos RA (2004) Extravascular T-cell recruitment requires initiation begun by Valpha14+ NKT cells and B-1 B cells. *Trends in Immunology* **25**: 441–449.
- Aspeshlagh S, Li Y, Yu ED, *et al.* (2011) Galactose modified iNKT cell agonists stabilized by an induced fit of CD1d prevent tumor metastasis. *EMBO Journal* **30** (11): 2294–2305.
- Bai L, Constantinides MG, Thomas SY, *et al.* (2012) Distinct APCs explain the cytokine bias of alpha-galactosylceramide variants in vivo. *Journal of Immunology* **188**: 3053–3061.
- Barral P, Eckl-Dorna J, Harwood NE, *et al.* (2008) B cell receptor-mediated uptake of CD1d-restricted antigen augments antibody responses by recruiting invariant NKT cell help in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 8345–8350.
- Barral P, Polzella P, Bruckbauer A, *et al.* (2010) CD169(+) macrophages present lipid antigens to mediate early activation of iNKT cells in lymph nodes. *Nature Immunology* **11**: 303–312.
- Barral P, Sanchez-Nino MD, van Rooijen N, Cerundolo V and Batista FD (2012) The location of splenic NKT cells favours their rapid activation by blood-borne antigen. *EMBO Journal* **31**: 2378–2390.
- Behar SM, Dascher CC, Grusby MJ, Wang CR and Brenner MB (1999) Susceptibility of mice deficient in CD1D or TAP1 to infection with *Mycobacterium tuberculosis*. *Journal of Experimental Medicine* **189**: 1973–1980.
- Bendelac A, Savage PB and Teyton L (2007) The biology of NKT cells. *Annual Review of Immunology* **25**: 297–336.
- Berntman E, Rolf J, Johansson C, Anderson P and Cardell SL (2005) The role of CD1d-restricted NK T lymphocytes in the immune response to oral infection with *Salmonella typhimurium*. *European Journal of Immunology* **35**: 2100–2109.
- Bezbradica JS, Stanic AK, Matsuki N, *et al.* (2005) Distinct roles of dendritic cells and B cells in Valpha18 natural T cell activation in vivo. *Journal of Immunology* **174**: 4696–4705.
- Bezbradica JS, Gordy LE, Stanic AK, *et al.* (2006) Granulocyte-macrophage colony-stimulating factor regulates effector differentiation of invariant natural killer T cells during thymic ontogeny. *Immunity* **25**: 487–497.
- Bialecki E, Paget C, Fontaine J, *et al.* (2009) Role of marginal zone B lymphocytes in invariant NKT cell activation. *Journal of Immunology* **182**: 6105–6113.
- Bialecki E, Macho Fernandez E, Ivanov S, *et al.* (2011) Spleen-resident CD4+ and CD4- CD8alpha- dendritic cell subsets differ in their ability to prime invariant natural killer T lymphocytes. *PLoS One* **6**: e26919.
- Biesiada G, Czepiel J, Leśniak MR, Garlicki A and Mach T (2012) Lyme disease: review. *Archives of Medical Science* **8**: 978–982.
- Bilenki L, Wang S, Yang J, *et al.* (2005) NK T cell activation promotes Chlamydia trachomatis infection in vivo. *Journal of Immunology* **175**: 3197–3206.
- Boesteanu A, Silva AD, Nakajima H, *et al.* (1997) Distinct roles for signals relayed through the common cytokine receptor gamma chain and interleukin 7 receptor alpha chain in natural T cell development. *Journal of Experimental Medicine* **186**: 331–336.
- Bosma A, Abdel-Gadir A, Isenberg DA, Jury EC and Mauri C (2012) Lipid-antigen presentation by CD1d(+) B cells is essential for the maintenance of invariant natural killer T cells. *Immunity* **36**: 477–490.
- Boysson JE, Rybalov B, Koopman LA, *et al.* (2002) CD1d and invariant NKT cells at the human maternal-fetal interface. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 13741–13746.
- Braun NA, Covarrubias R and Major AS (2010) Natural killer T cells and atherosclerosis: form and function meet pathogenesis. *Journal of Innate Immunity* **2**: 316–324.
- Brennan PJ, Tatituri RV, Brigl M, *et al.* (2011) Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. *Nature Immunology* **12**: 1202–1211.
- Brennan PJ, Brigl M and Brenner MB (2013) Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nature Reviews Immunology* **13**: 101–117.
- Brigl M, Bry L, Kent SC, Gumperz JE and Brenner MB (2003) Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nature Immunology* **4**: 1230–1237.
- Brigl M and Brenner MB (2004) CD1: antigen presentation and T cell function. *Annual Review of Immunology* **22**: 817–890.
- Brigl M, Tatituri RV, Watts GF, *et al.* (2011) Innate and cytokine-driven signals, rather than microbial antigens, dominate in natural killer T cell activation during microbial infection. *Journal of Experimental Medicine* **208**: 1163–1177.
- Brossay L, Jullien D, Cardell S, *et al.* (1997) Mouse CD1 is mainly expressed on hemopoietic-derived cells. *Journal of Immunology* **159**: 1216–1224.
- Brozovic S, Nagaishi T, Yoshida M, *et al.* (2004) CD1d function is regulated by microsomal triglyceride transfer protein. *Nature Medicine* **10**: 535–539.
- Camelo A, Barlow JL, Drynan LF, *et al.* (2012) Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13. *Journal of Gastroenterology* **47**: 1198–1211.
- Carnaud C, Lee D, Donnars O, *et al.* (1999) Cutting edge: cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. *Journal of Immunology* **163**: 4647–4650.
- Carreno LJ, Kharkwal SS and Porcelli SA (2014) Optimizing NKT cell ligands as vaccine adjuvants. *Immunotherapy* **6**: 309–320.
- Castillo EF, Acero LF, Stonier SW, Zhou D and Schluns KS (2010) Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation. *Blood* **116**: 2494–2503.
- Chang YJ, Kim HY, Albacker LA, *et al.* (2011) Influenza infection in suckling mice expands an NKT cell subset that protects against airway hyperreactivity. *Journal of Clinical Investigation* **121**: 57–69.
- Chang PP, Barral P, Fitch J, *et al.* (2012) Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses. *Nature Immunology* **13**: 35–43.
- Chaudhry MS and Karadimitris A (2014) Role and regulation of CD1d in normal and pathological B cells. *Journal of Immunology* **193**: 4761–4768.
- Christaki E, Diza E, Giamarellos-Bourboulis EJ, *et al.* (2015) NK and NKT Cell Depletion Alters the Outcome of Experimental Pneumococcal Pneumonia: Relationship with Regulation of Interferon- $\gamma$  Production. *Journal of Immunological Research* **2015**: 532717. DOI: 10.1155/2015/532717.

- Clark RH, Stinchcombe JC, Day A, *et al.* (2003) Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. *Nature Immunology* **4**: 1111–1120.
- Cohen NR, Tatituri RV, Rivera A, *et al.* (2011) Innate recognition of cell wall beta-glucans drives invariant natural killer T cell responses against fungi. *Cell Host & Microbe* **10**: 437–450.
- Coquet JM, Chakravarti S, Kyriassoudis K, *et al.* (2008) Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 11287–11292.
- Cornish AL, Keating R, Kyriassoudis K, *et al.* (2006) NKT cells are not critical for HSV-1 disease resolution. *Immunology and Cell Biology* **84**: 13–19.
- Cox D, Fox L, Tian R, *et al.* (2009) Determination of cellular lipids bound to human CD1d molecules. *PLoS One* **4**: e5325.
- Crowe NY, Coquet JM, Berzins SP, *et al.* (2005) Differential antitumor immunity mediated by NKT cell subsets in vivo. *Journal of Experimental Medicine* **202**: 1279–1288.
- De Santo C, Salio M, Masri SH, *et al.* (2008) Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans. *Journal of Clinical Investigation* **118**: 4036–4048.
- De Santo C, Arcott R, Booth S, *et al.* (2010) Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. *Nature Immunology* **11**: 1039–1046.
- De Silva AD, Park JJ, Matsuki N, *et al.* (2002) Lipid protein interactions: the assembly of CD1d1 with cellular phospholipids occurs in the endoplasmic reticulum. *Journal of Immunology* **168**: 723–733.
- Denney L, Kok WL, Cole SL, *et al.* (2012) Activation of invariant NKT cells in early phase of experimental autoimmune encephalomyelitis results in differentiation of Ly6Chi inflammatory monocyte to M2 macrophages and improved outcome. *Journal of Immunology* **189**: 551–557.
- Doisne JM, Becourt C, Amniai L, *et al.* (2009) Skin and peripheral lymph node invariant NKT cells are mainly retinoic acid receptor-related orphan receptor ( $\gamma$ )t+ and respond preferentially under inflammatory conditions. *Journal of Immunology* **183**: 2142–2149.
- Dougan SK, Salas A, Rava P, *et al.* (2005) Microsomal triglyceride transfer protein lipidation and control of CD1d on antigen-presenting cells. *Journal of Experimental Medicine* **202**: 529–539.
- Dougan SK, Kaser A and Blumberg RS (2007a) CD1 expression on antigen-presenting cells. *Current Topics in Microbiology and Immunology* **314**: 113–141.
- Dougan SK, Rava P, Hussain MM and Blumberg RS (2007b) MTP regulated by an alternate promoter is essential for NKT cell development. *Journal of Experimental Medicine* **204**: 533–545.
- van den Elzen P, Garg S, Leon L, *et al.* (2005) Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* **437**: 906–910.
- Exley MA, Bigley NJ, Cheng O, *et al.* (2001) CD1d-reactive T-cell activation leads to amelioration of disease caused by diabetogenic encephalomyocarditis virus. *Journal of Leukocyte Biology* **69**: 713–718.
- Field JJ, Nathan DG and Linden J (2011) Targeting iNKT cells for the treatment of sickle cell disease. *Clinical Immunology (Orlando, Fla.)* **140**: 177–183.
- Florence WC, Bhat RK and Joyce S (2008) CD1d-restricted glycolipid antigens: presentation principles, recognition logic and functional consequences. *Expert Reviews in Molecular Medicine* **10**: e20.
- Fooksman DR, Vardhana S, Vasiliver-Shamis G, *et al.* (2010) Functional anatomy of T cell activation and synapse formation. *Annual Review of Immunology* **28**: 79–105.
- Fox LM, Cox DG, Lockridge JL, *et al.* (2009) Recognition of lyso-phospholipids by human natural killer T lymphocytes. *PLoS Biology* **7**: e1000228.
- Freigang S, Landais E, Zadorozhny V, *et al.* (2012) Scavenger receptors target glycolipids for natural killer T cell activation. *Journal of Clinical Investigation* **122**: 3943–3954.
- Fujii S, Shimizu K, Kronenberg M and Steinman RM (2002) Prolonged IFN-gamma-producing NKT response induced with alpha-galactosylceramide-loaded DCs. *Nature Immunology* **3**: 867–874.
- Fujii S, Shimizu K, Smith C, Bonifaz L and Steinman RM (2003) Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. *Journal of Experimental Medicine* **198**: 267–279.
- Fujii S, Shimizu K, Okamoto Y, *et al.* (2013) NKT cells as an ideal anti-tumor immunotherapeutic. *Frontiers in Immunology* **4**: 409.
- Fuss IJ and Strober W (2008) The role of IL-13 and NK T cells in experimental and human ulcerative colitis. *Mucosal Immunology* **1** (Suppl 1): S31–S33.
- Geissmann F, Cameron TO, Sidobre S, *et al.* (2005) Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. *PLoS Biology* **3**: e113.
- Giabbai B, Sidobre S, Crispin MD, *et al.* (2005) Crystal structure of mouse CD1d bound to the self ligand phosphatidylcholine: a molecular basis for NKT cell activation. *Journal of Immunology* **175**: 977–984.
- Godfrey DI and Berzins SP (2007) Control points in NKT-cell development. *Nature Reviews Immunology* **7**: 505–518.
- Godfrey DI, Stankovic S and Baxter AG (2010) Raising the NKT cell family. *Nature Immunology* **11**: 197–206.
- Gonzalez-Aseguinolaza G, Van Kaer L, Bergmann CC, *et al.* (2002) Natural killer T cell ligand alpha-galactosylceramide enhances protective immunity induced by malaria vaccines. *Journal of Experimental Medicine* **195**: 617–624.
- Gordy LE, Bezbradica JS, Flyak AI, *et al.* (2011) IL-15 regulates homeostasis and terminal maturation of NKT cells. *Journal of Immunology* **187**: 6335–6345.
- Griffiths GM, Tsun A and Stinchcombe JC (2010) The immunological synapse: a focal point for endocytosis and exocytosis. *Journal of Cell Biology* **189**: 399–406.
- Grubor-Bauk B, Simmons A, Mayrhofer G and Speck PG (2003) Impaired clearance of herpes simplex virus type 1 from mice lacking CD1d or NKT cells expressing the semivariant V alpha 14-J alpha 281 TCR. *Journal of Immunology* **170**: 1430–1434.
- Gumperz JE, Roy C, Makowska A, *et al.* (2000) Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity* **12**: 211–221.
- Gumperz JE, Miyake S, Yamamura T and Brenner MB (2002) Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *Journal of Experimental Medicine* **195**: 625–636.

- Habbeddine M, Verbeke P, Delarbre C, *et al.* (2013) CD1d-restricted NKT cells modulate placental and uterine leukocyte populations during chlamydial infection in mice. *Microbes and Infection* **15**: 928–938.
- Hage CA, Kohli LL, Cho S, *et al.* (2005) Human immunodeficiency virus gp120 downregulates CD1d cell surface expression. *Immunology Letters* **98**: 131–135.
- Haig NA, Guan Z, Li D, *et al.* (2011) Identification of self-lipids presented by CD1c and CD1d proteins. *Journal of Biological Chemistry* **286**: 37692–37701.
- Hammond KJ, Pellicci DG, Poulton LD, *et al.* (2001) CD1d-restricted NKT cells: an interstrain comparison. *Journal of Immunology* **167**: 1164–1173.
- Hansen DS, Siomos MA, Buckingham L, Scalzo AA and Schofield L (2003) Regulation of murine cerebral malaria pathogenesis by CD1d-restricted NKT cells and the natural killer complex. *Immunity* **18**: 391–402.
- Hayakawa K, Tateda K, Fuse ET, *et al.* (2008) Paradoxically high resistance of natural killer T (NKT) cell-deficient mice to *Legionella pneumophila*: another aspect of NKT cells for modulation of host responses. *Journal of Medical Microbiology* **57**: 1340–1348.
- Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS and Strober W (2002) Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* **17**: 629–638.
- Hermans IF, Silk JD, Gileadi U, *et al.* (2003) NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. *Journal of Immunology* **171**: 5140–5147.
- Hill TM, Gilchuk P, Cicek BB, *et al.* (2015) Border patrol gone awry: lung NKT cell activation by Francisella tularensis exacerbates tularemia-like disease. *PLoS Pathogens* **11**: e1004975.
- Ho LP, Denney L, Luhn K, *et al.* (2008) Activation of invariant NKT cells enhances the innate immune response and improves the disease course in influenza A virus infection. *European Journal of Immunology* **38**: 1913–1922.
- Honey K, Benlagha K, Beers C, *et al.* (2002) Thymocyte expression of cathepsin L is essential for NKT cell development. *Nature Immunology* **3**: 1069–1074.
- Huh JY, Kim JI, Park YJ, *et al.* (2013) A novel function of adipocytes in lipid antigen presentation to iNKT cells. *Molecular and Cellular Biology* **33**: 328–339.
- Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207–214.
- Huse M, Lillemeier BF, Kuhns MS, Chen DS and Davis MM (2006) T cells use two directionally distinct pathways for cytokine secretion. *Nature Immunology* **7**: 247–255.
- Ito K, Karasawa M, Kawano T, *et al.* (2000) Involvement of decidual Valpha14 NKT cells in abortion. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 740–744.
- Ito Y, Vela JL, Matsumura F, *et al.* (2013) *Helicobacter pylori* cholesteryl alpha-glucosides contribute to its pathogenicity and immune response by natural killer T cells. *PLoS One* **8**: e78191.
- Iwamura C and Nakayama T (2010) Role of NKT cells in allergic asthma. *Current Opinion in Immunology* **22**: 807–813.
- Jayawardena-Wolf J, Benlagha K, Chiu YH, Mehr R and Bendelac A (2001) CD1d endosomal trafficking is independently regulated by an intrinsic CD1d-encoded tyrosine motif and by the invariant chain. *Immunity* **15**: 897–908.
- Johnson TR, Hong S, Van Kaer L, Koezuka Y and Graham BS (2002) NK T cells contribute to expansion of CD8(+) T cells and amplification of antiviral immune responses to respiratory syncytial virus. *Journal of Virology* **76**: 4294–4303.
- Joyce S, Woods AS, Yewdell JW, *et al.* (1998) Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. *Science* **279**: 1541–1544.
- Joyce S and Van Kaer L (2003) CD1-restricted antigen presentation: an oily matter. *Current Opinion in Immunology* **15**: 95–104.
- Joyce S, Girardi E and Zajonc DM (2011) NKT cell ligand recognition logic: molecular basis for a synaptic duet and transmission of inflammatory effectors. *Journal of Immunology* **187**: 1081–1089.
- Joyce AG, Qiu H, Wang S, *et al.* (2007) Distinct NKT cell subsets are induced by different Chlamydia species leading to differential adaptive immunity and host resistance to the infections. *Journal of Immunology* **178**: 1048–1058.
- Kain L, Webb B, Anderson BL, *et al.* (2014) The identification of the endogenous ligands of natural killer T cells reveals the presence of mammalian alpha-linked glycosylceramides. *Immunity* **41**: 543–554.
- Kakimi K, Guidotti LG, Koezuka Y and Chisari FV (2000) Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *Journal of Experimental Medicine* **192**: 921–930.
- Kammertoens T, Qin Z, Briesemeister D, Bendelac A and Blankenstein T (2012) B-cells and IL-4 promote methylcholanthrene-induced carcinogenesis but there is no evidence for a role of T/NKT-cells and their effector molecules (Fas-ligand, TNF-alpha, perforin). *International Journal of Cancer* **131**: 1499–1508.
- Kang SJ and Cresswell P (2002) Calnexin, calreticulin, and ERp57 cooperate in disulfide bond formation in human CD1d heavy chain. *Journal of Biological Chemistry* **277**: 44838–44844.
- Kang SJ and Cresswell P (2004) Saposins facilitate CD1d-restricted presentation of an exogenous lipid antigen to T cells. *Nature Immunology* **5**: 175–181.
- Kastenmuller W, Torabi-Parizi P, Subramanian N, Lammermann T and Germain RN (2012) A spatially-organized multicellular innate immune response in lymph nodes limits systemic pathogen spread. *Cell* **150**: 1235–1248.
- Kawakami K, Kinjo Y, Yara S, *et al.* (2001) Activation of Valpha14(+) natural killer T cells by alpha-galactosylceramide results in development of Th1 response and local host resistance in mice infected with *Cryptococcus neoformans*. *Infection and Immunity* **69**: 213–220.
- Kawakami K, Yamamoto N, Kinjo Y, *et al.* (2003) Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against *Streptococcus pneumoniae* infection. *European Journal of Immunology* **33**: 3322–3330.
- Kawano T, Cui J, Koezuka Y, *et al.* (1997) CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* **278**: 1626–1629.
- Kim EY, Battaile JT, Patel AC, *et al.* (2008) Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nature Medicine* **14**: 633–640.
- Kim HY, Pichavant M, Matangkasombut P, *et al.* (2009) The development of airway hyperreactivity in T-bet-deficient mice requires CD1d-restricted NKT cells. *Journal of Immunology* **182**: 3252–3261.
- Kim CJ, Nazli A, Rojas OL, *et al.* (2012) A role for mucosal IL-22 production and Th22 cells in HIV-associated mucosal immunopathogenesis. *Mucosal Immunology* **5**: 670–680.

- King IL, Fortier A, Tighe M, *et al.* (2012) Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. *Nature Immunology* **13**: 44–50.
- Kinjo Y, Wu D, Kim G, *et al.* (2005) Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* **434**: 520–525.
- Kinjo Y, Tupin E, Wu D, *et al.* (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nature Immunology* **7**: 978–986.
- Kinjo Y, Illarionov P, Vela JL, *et al.* (2011) Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. *Nature Immunology* **12**: 966–974.
- Kinsey GR and Okusa MD (2014) Expanding role of T cells in acute kidney injury. *Current Opinion in Nephrology and Hypertension* **23**: 9–16.
- Kitamura H, Iwakabe K, Yahata T, *et al.* (1999) The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *Journal of Experimental Medicine* **189**: 1121–1128.
- Koch M, Stronge VS, Shepherd D, *et al.* (2005) The crystal structure of human CD1d with and without  $\alpha$ -galactosylceramide. *Nature Immunology* **6**: 819–826.
- Kok WL, Denney L, Benam K, *et al.* (2012) Pivotal advance: invariant NKT cells reduce accumulation of inflammatory monocytes in the lungs and decrease immune-pathology during severe influenza A virus infection. *Journal of Leukocyte Biology* **91**: 357–368.
- Kotas ME and Medzhitov R (2015) Homeostasis, inflammation, and disease susceptibility. *Cell* **160**: 816–827.
- Kotsianidis I, Silk JD, Spanoudakis E, *et al.* (2006) Regulation of hematopoiesis in vitro and in vivo by invariant NKT cells. *Blood* **107**: 3138–3144.
- Kovalovsky D, Uche OU, Eladad S, *et al.* (2008) The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. *Nature Immunology* **9**: 1055–1064.
- Kronenberg M (2005) Toward an understanding of NKT cell biology: progress and paradoxes. *Annual Review of Immunology* **23**: 877–900.
- Kumar H, Belperron A, Barthold SW and Bockenstedt LK (2000) CD1d deficiency impairs murine host defense against the spirochete, *Borrelia burgdorferi*. *Journal of Immunology (Cutting Edge)* **165**: 4797–4801.
- Kunii N, Horiguchi S, Motohashi S, *et al.* (2009) Combination therapy of in vitro-expanded natural killer T cells and alpha-galactosylceramide-pulsed antigen-presenting cells in patients with recurrent head and neck carcinoma. *Cancer Science* **100**: 1092–1098.
- Kwieceński J, Rhost S, Löfbom L, *et al.* (2013) Sulfatide Attenuates Experimental *Staphylococcus aureus* Sepsis through a CD1d-Dependent Pathway. *Infection and Immunity* **81**: 1114–1120.
- Kyriakakis E, Cavallari M, Andert J, *et al.* (2010) Invariant natural killer T cells: linking inflammation and neovascularization in human atherosclerosis. *European Journal of Immunology* **40**: 3268–3279.
- La Cava A, Van Kaer L and Fu Dong S (2006) CD4+ CD25+ Tregs and NKT cells: regulators regulating regulators. *Trends in Immunology* **27**: 322–327.
- Lang GA, Exley MA and Lang ML (2006) The CD1d-binding glycolipid alpha-galactosylceramide enhances humoral immunity to T-dependent and T-independent antigen in a CD1d-dependent manner. *Immunology* **119**: 116–125.
- Leadbetter EA, Brigl M, Illarionov P, *et al.* (2008) NK T cells provide lipid antigen-specific cognate help for B cells. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 8339–8344.
- Lee PT, Benlagha K, Teyton L and Bendelac A (2002) Distinct functional lineages of human V(alpha)24 natural killer T cells. *Journal of Experimental Medicine* **195**: 637–641.
- Lee WY, Moriarty TJ, Wong CH, *et al.* (2010) An intravascular immune response to *Borrelia burgdorferi* involves Kupffer cells and iNKT cells. *Nature Immunology* **11**: 295–302.
- Lee YJ, Holzzapfel KL, Zhu J, Jameson SC and Hogquist KA (2013) Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nature Immunology* **14**: 1146–1154.
- Lee W-Y, Sanz M-J, Wong CHY, *et al.* (2014) Invariant natural killer T cells act as an extravascular cytotoxic barrier for joint-invading Lyme *Borrelia*. *Proceedings of the National Academy of Sciences* **111**: 13936–13941.
- Lee YJ, Wang H, Starrett GJ, *et al.* (2015) Tissue-specific distribution of iNKT cells impacts their cytokine response. *Immunity* **43**: 566–578.
- Leveson-Gower DB, Olson JA, Sega EI, *et al.* (2011) Low doses of natural killer T cells provide protection from acute graft-versus-host disease via an IL-4-dependent mechanism. *Blood* **117**: 3220–3229.
- Li Y, Girardi E, Wang J, *et al.* (2010) The Valpha14 invariant natural killer T cell TCR forces microbial glycolipids and CD1d into a conserved binding mode. *Journal of Experimental Medicine* **207**: 2383–2393.
- Li LP, Fang YC, Dong GF, Lin Y and Saito S (2012) Depletion of invariant NKT cells reduces inflammation-induced preterm delivery in mice. *Journal of Immunology* **188**: 4681–4689.
- Li L, Yang J, Jiang Y, Tu J and Schust DJ (2015) Activation of decidual invariant natural killer T cells promotes lipopolysaccharide-induced preterm birth. *Molecular Human Reproduction* **21**: 369–381.
- Lopez-Sagasetta J, Sibener LV, Kung JE, Gumperz J and Adams EJ (2012) Lysophospholipid presentation by CD1d and recognition by a human Natural Killer T-cell receptor. *EMBO Journal* **31**: 2047–2059.
- Lynch L, Michelet X, Zhang S, *et al.* (2015) Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of Treg cells and macrophages in adipose tissue. *Nature Immunology* **16**: 85–95.
- Macho-Fernandez E, Cruz LJ, Ghinnagow R, *et al.* (2014) Targeted delivery of alpha-galactosylceramide to CD8alpha+ dendritic cells optimizes type I NKT cell-based antitumor responses. *Journal of Immunology* **193**: 961–969.
- Macho-Fernandez E and Brigl M (2015) The extended family of CD1d-restricted NKT cells: sifting through a mixed bag of TCRs, antigens, and functions. *Frontiers in Immunology* **6**: 362.
- Maldonado RA, Irvine DJ, Schreiber R and Glimcher LH (2004) A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature* **431**: 527–532.
- Mallevaey T, Clarke AJ, Scott-Browne JP, *et al.* (2011) A molecular basis for NKT cell recognition of CD1d-self-antigen. *Immunity* **34**: 315–326.

- Mandal M, Chen X-R, Alegre M-L, *et al.* (1998) Tissue distribution, regulation and intracellular localization of murine CD1 molecules. *Molecular Immunology* **35**: 525–536.
- Marrero I, Ware R and Kumar V (2015) Type II NKT cells in inflammation, autoimmunity, microbial immunity, and cancer. *Frontiers in Immunology* **6**: 316.
- Matangkasombut P, Marigowda G, Ervine A, *et al.* (2009) Natural killer T cells in the lungs of patients with asthma. *Journal of Allergy and Clinical Immunology* **123**: 1181–1185.
- Matsuda JL, Gapin L, Sidobre S, *et al.* (2002) Homeostasis of V alpha 14i NKT cells. *Nature Immunology* **3**: 966–974.
- Matsuzaki G, Li XY, Kadena T, *et al.* (1995) Early appearance of T cell receptor  $\alpha\beta^+$  CD4<sup>-</sup> CD8<sup>-</sup> T cells with a skewed variable region repertoire after infection with *Listeria monocytogenes*. *European Journal of Immunology* **25**: 1985–1991.
- Mattner J, Debord KL, Ismail N, *et al.* (2005) Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **434**: 525–529.
- Mattner J, Savage PB, Leung P, *et al.* (2008) Liver autoimmunity triggered by microbial activation of natural killer T cells. *Cell Host & Microbe* **3**: 304–315.
- McCarthy C, Shepherd D, Fleire S, *et al.* (2007) The length of lipids bound to human CD1d molecules modulates the affinity of NKT cell TCR and the threshold of NKT cell activation. *Journal of Experimental Medicine* **204**: 1131–1144.
- Michel ML, Keller AC, Paget C, *et al.* (2007) Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia. *Journal of Experimental Medicine* **204**: 995–1001.
- Miyamoto K, Miyake S and Yamamura T (2001) A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* **413**: 531–534.
- Montoya CJ, Pollard D, Martinson J, *et al.* (2007) Characterization of human invariant natural killer T subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11. *Immunology* **122**: 1–14.
- Morita M, Motoki K, Akimoto K, *et al.* (1995) Structure-activity relationship of alpha-galactosylceramides against B16-bearing mice. *Journal of Medicinal Chemistry* **38**: 2176–2187.
- Motohashi S, Nagato K, Kunii N, *et al.* (2009) A phase I-II study of alpha-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer. *Journal of Immunology* **182**: 2492–2501.
- Nagarajan NA and Kronenberg M (2007) Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *Journal of Immunology* **178**: 2706–2713.
- Naiki Y, Nishimura H, Kawano T, *et al.* (1999) Regulatory role of peritoneal NK1.1<sup>+</sup>  $\alpha\beta$  T cells in IL-12 production during *Salmonella* infection. *Journal of Immunology* **163**: 2057–2063.
- Nakamatsu M, Yamamoto N, Hatta M, *et al.* (2007) Role of interferon-gamma in Valpha14<sup>+</sup> natural killer T cell-mediated host defense against *Streptococcus pneumoniae* infection in murine lungs. *Microbes and Infection* **9**: 364–374.
- Natori T, Koezuka Y and Higa T (1993) Agelasphins, Novel a-galactosylceramides from the marine sponge *Agelas mauritanicus*. *Tetrahedron Letters* **34**: 5591–5592.
- Naumov YN, Bahjat KS, Gausling R, *et al.* (2001) Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 13838–13843.
- Nieuwenhuis EE, Matsumoto T, Exley M, *et al.* (2002) CD1d-dependent macrophage-mediated clearance of *Pseudomonas aeruginosa* from lung. *Nature Medicine* **8**: 588–593.
- Olszak T, An D, Zeissig S, *et al.* (2012) Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* **336**: 489–493.
- Paget C, Mallevaey T, Speak AO, *et al.* (2007) Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. *Immunity* **27**: 597–609.
- Paget C, Ivanov S, Fontaine J, *et al.* (2011) Potential role of invariant NKT cells in the control of pulmonary inflammation and CD8<sup>+</sup> T cell response during acute influenza A virus H3N2 pneumonia. *Journal of Immunology* **186**: 5590–5602.
- Paget C, Ivanov S, Fontaine J, *et al.* (2012) Interleukin-22 is produced by invariant natural killer T lymphocytes during influenza A virus infection: potential role in protection against lung epithelial damages. *Journal of Biological Chemistry* **287**: 8816–8829.
- Parekh VV, Wu L, Olivares-Villagomez D, Wilson KT and Van Kaer L (2013) Activated invariant NKT cells control central nervous system autoimmunity in a mechanism that involves myeloid-derived suppressor cells. *Journal of Immunology* **190**: 1948–1960.
- Park JJ, Kang SJ, De Silva AD, *et al.* (2004) Lipid-protein interactions: biosynthetic assembly of CD1 with lipids in the endoplasmic reticulum is evolutionarily conserved. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 1022–1026.
- Peng Y, Zhao L, Shekhar S, *et al.* (2012) The glycolipid exoantigen derived from *Chlamydia muridarum* activates invariant natural killer T cells. *Cellular and molecular immunology* **9**: 361–366.
- Pichavant M, Goya S, Meyer EH, *et al.* (2008) Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. *Journal of Experimental Medicine* **205**: 385–393.
- Pratschke J, Stauch D and Kotsch K (2009) Role of NK and NKT cells in solid organ transplantation. *Transplant International: Official Journal of the European Society for Organ Transplantation* **22**: 859–868.
- Price AE, Reinhardt RL, Liang HE and Locksley RM (2012) Marking and quantifying IL-17A-producing cells in vivo. *PLoS One* **7**: e39750.
- van Puijvelde GH, van Wanrooij EJ, Hauer AD, *et al.* (2009) Effect of natural killer T cell activation on the initiation of atherosclerosis. *Thrombosis and Haemostasis* **102**: 223–230.
- Rhost S, Sedimbi S, Kadri N and Cardell SL (2012) Immunomodulatory type II natural killer T lymphocytes in health and disease. *Scandinavian Journal of Immunology* **76**: 246–255.
- Riese RJ, Shi GP, Villadangos J, *et al.* (2001) Regulation of CD1 function and NK1.1<sup>+</sup> T cell selection and maturation by cathepsin S. *Immunity* **15**: 909–919.
- Rinaldi SF, Rossi AG, Saunders PT and Norman JE (2015) Immune cells and preterm labour: do invariant NKT cells hold the key? *Molecular Human Reproduction* **21**: 309–312.
- Roark JH, Park SH, Jayawardena J, *et al.* (1998) CD1.1 expression by mouse antigen-presenting cells and marginal zone B cells. *Journal of Immunology* **160**: 3121–3127.
- Roberts TJ, Lin Y, Spence PM, Van Kaer L and Brutkiewicz RR (2004) CD1d1-dependent control of the magnitude of an

- acute antiviral immune response. *Journal of Immunology* **172**: 3454–3461.
- Roda-Navarro P (2009) Assembly and function of the natural killer cell immune synapse. *Frontiers in Bioscience* **14**: 621–633.
- Ronet C, Darce S, Leite de Moraes M, *et al.* (2005) NKT cells are critical for the initiation of an inflammatory bowel response against *Toxoplasma gondii*. *Journal of Immunology* **175**: 899–908.
- Rossjohn J, Gras S, Miles JJ, *et al.* (2015) T cell antigen receptor recognition of antigen-presenting molecules. *Annual Review of Immunology* **33**: 169–200.
- Sada-Ovalle I, Chiba A, Gonzales A, Brenner MB and Behar SM (2008) Innate invariant NKT cells recognize *Mycobacterium tuberculosis*-infected macrophages, produce interferon-gamma, and kill intracellular bacteria. *PLoS Pathogens* **4**: e1000239.
- Sag D, Krause P, Hedrick CC, Kronenberg M and Wingender G (2014) IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset. *Journal of Clinical Investigation* **124**: 3725–3740.
- Sagiv Y, Bai L, Wei DG, *et al.* (2007) A distal effect of microsomal triglyceride transfer protein deficiency on the lysosomal recycling of CD1d. *Journal of Experimental Medicine* **204**: 921–928.
- Sanchez DJ, Gumperz JE and Ganem D (2005) Regulation of CD1d expression and function by a herpesvirus infection. *Journal of Clinical Investigation* **115**: 1369–1378.
- Savage AK, Constantinides MG, Han J, *et al.* (2008) The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* **29**: 391–403.
- Scanlon ST, Thomas SY, Ferreira CM, *et al.* (2011) Airborne lipid antigens mobilize resident intravascular NKT cells to induce allergic airway inflammation. *Journal of Experimental Medicine* **208**: 2113–2124.
- Schmieg J, Yang G, Franck RW and Tsuji M (2003) Superior protection against malaria and melanoma metastases by a C-glycoside analogue of the natural killer T cell ligand alpha-Galactosylceramide. *Journal of Experimental Medicine* **198**: 1631–1641.
- Schmieg J, Yang G, Franck RW, Van Rooijen N and Tsuji M (2005) Glycolipid presentation to natural killer T cells differs in an organ-dependent fashion. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 1127–1132.
- Schrantz N, Sagiv Y, Liu Y, *et al.* (2007) The Niemann-Pick type C2 protein loads isoglobotrihexosylceramide onto CD1d molecules and contributes to the thymic selection of NKT cells. *Journal of Experimental Medicine* **204**: 841–852.
- Scott-Browne JP, Matsuda JL, Mallevaey T, *et al.* (2007) Germline-encoded recognition of diverse glycolipids by natural killer T cells. *Nature Immunology* **8**: 1105–1113.
- Seino KI, Fukao K, Muramoto K, *et al.* (2001) Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 2577–2581.
- Semmling V, Lukacs-Kornek V, Thaiss CA, *et al.* (2010) Alternative cross-priming through CCL17-CCR4-mediated attraction of CTLs toward NKT cell-licensed DCs. *Nature Immunology* **11**: 313–320.
- Shekhar S, Joyee AG, Gao X, *et al.* (2015) Invariant natural killer T cells promote T cell immunity by modulating the function of lung dendritic cells during *Chlamydia pneumoniae* infection. *Journal of Innate Immunity* **7**: 260–274.
- Shimizu K, Asakura M, Shinga J, *et al.* (2013) Invariant NKT cells induce plasmacytoid dendritic cell (DC) cross-talk with conventional DCs for efficient memory CD8+ T cell induction. *Journal of Immunology* **190**: 5609–5619.
- Singh N, Hong S, Scherer DC, *et al.* (1999) Cutting edge: activation of NK T cells by CD1d and  $\alpha$ -galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype. *The Journal of Immunology* **163**: 2373–2377.
- Singh AK, Gaur P and Das SN (2014) Natural killer T cell anergy, co-stimulatory molecules and immunotherapeutic interventions. *Human Immunology* **75**: 250–260.
- Skold M, Xiong X, Illarionov PA, Besra GS and Behar SM (2005) Interplay of cytokines and microbial signals in regulation of CD1d expression and NKT cell activation. *Journal of Immunology* **175**: 3584–3593.
- Sonoda KH, Taniguchi M and Stein-Streilein J (2002) Long-term survival of corneal allografts is dependent on intact CD1d-reactive NKT cells. *Journal of Immunology* **168**: 2028–2034.
- Spence PM, Sriram V, Van Kaer L, Hobbs JA and Brutkiewicz RR (2001) Generation of cellular immunity to lymphocytic choriomeningitis virus is independent of CD1d1 expression. *Immunology* **104**: 168–174.
- Sriram V, Du W, Gervay-Hague J and Brutkiewicz RR (2005) Cell wall glycosphingolipids of *Sphingomonas paucimobilis* are CD1d-specific ligands for NKT cells. *European Journal of Immunology* **35**: 1692–1701.
- Stanic AK, De Silva AD, Park JJ, *et al.* (2003a) Defective presentation of the CD1d1-restricted natural Va14Ja18 NKT lymphocyte antigen caused by  $\beta$ -D-glucosylceramide synthase deficiency. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 1849–1854.
- Stanic AK, Shashidharamurthy R, Bezbradica JS, *et al.* (2003b) Another view of T cell antigen recognition: cooperative engagement of glycolipid antigens by Va14Ja18 natural T(iNKT) cell receptor [corrected]. *Journal of Immunology* **171**: 4539–4551.
- Tajiri K and Shimizu Y (2012) Role of NKT cells in the pathogenesis of NAFLD. *International Journal of Hepatology* **2012**: 850836.
- Taniguchi M, Harada M, Kojo S, Nakayama T and Wakao H (2003) The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annual Review of Immunology* **21**: 483–513.
- Terabe M and Berzofsky JA (2014) The immunoregulatory role of type I and type II NKT cells in cancer and other diseases. *Cancer Immunology, Immunotherapy* **63**: 199–213.
- Terashima A, Watarai H, Inoue S, *et al.* (2008) A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *Journal of Experimental Medicine* **205**: 2727–2733.
- Thomas SY, Scanlon ST, Griewank KG, *et al.* (2011) PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions. *Journal of Experimental Medicine* **208**: 1179–1188.
- Tonti E, Galli G, Malzone C, *et al.* (2009) NKT-cell help to B lymphocytes can occur independently of cognate interaction. *Blood* **113**: 370–376.
- Toyofuku A, Yasunami Y, Nabeyama K, *et al.* (2006) Natural killer T-cells participate in rejection of islet allografts in the liver of mice. *Diabetes* **55**: 34–39.
- Tupin E, Benhnia MR, Kinjo Y, *et al.* (2008) NKT cells prevent chronic joint inflammation after infection with *Borrelia burgdorferi*. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 19863–19868.

- Tyznik AJ, Tupin E, Nagarajan NA, *et al.* (2008) The mechanism of invariant NKT cell responses to viral danger signals. *Journal of Immunology (Cutting Edge)* **181**: 4452–4456.
- Van Kaer L (2005)  $\alpha$ -Galactosylceramide therapy for autoimmune diseases: prospects and obstacles. *Nature Reviews Immunology* **5**: 31–42.
- Van Kaer L and Joyce S (2006) Viral evasion of antigen presentation: not just for peptides anymore. *Nature Immunology* **7**: 795–797.
- Van Kaer L (2007) NKT cells: T lymphocytes with innate effector functions. *Current Opinion in Immunology* **19**: 354–364.
- Van Kaer L, Parekh VV and Wu L (2011) Invariant NK T cells: potential for immunotherapeutic targeting with glycolipid antigens. *Immunotherapy* **3**: 59–75.
- Van Kaer L, Parekh VV and Wu L (2013) Invariant natural killer T cells as sensors and managers of inflammation. *Trends in Immunology* **34**: 50–58.
- Vincent MS, Leslie DS, Gumperz JE, *et al.* (2002) CD1-dependent dendritic cell instruction. *Nature Immunology* **3**: 1163–1168.
- Wang J, Li Y, Kinjo Y, *et al.* (2010) Lipid binding orientation within CD1d affects recognition of *Borrelia burgdorferi* antigens by NKT cells. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 1535–1540.
- Wang H, Zhao L, Peng Y, *et al.* (2012) Protective role of alpha-galactosylceramide-stimulated natural killer T cells in genital tract infection with *Chlamydia muridarum*. *FEMS Immunology and Medical Microbiology* **65**: 43–54.
- Watarai H, Sekine-Kondo E, Shigeura T, *et al.* (2012) Development and function of invariant natural killer T cells producing T(h)2- and T(h)17-cytokines. *PLoS Biology* **10**: e1001255.
- Webster KE, Kim HO, Kyparissoudis K, *et al.* (2014) IL-17-producing NKT cells depend exclusively on IL-7 for homeostasis and survival. *Mucosal Immunology* **7**: 1058–1067.
- Wesley JD, Tessmer MS, Chaukos D and Brossay L (2008) NK cell-like behavior of Valpha14i NK T cells during MCMV infection. *PLoS Pathogens* **4**: e1000106.
- Winau F, Hegasy G, Weiskirchen R, *et al.* (2007) Ito cells are liver-resident antigen-presenting cells for activating T cell responses. *Immunity* **26**: 117–129.
- Wingender G, Hiss M, Engel I, *et al.* (2012a) Neutrophilic granulocytes modulate invariant NKT cell function in mice and humans. *Journal of Immunology* **188**: 3000–3008.
- Wingender G, Stepniak D, Krebs P, *et al.* (2012b) Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. *Gastroenterology* **143**: 418–428.
- Wong CH, Jenne CN, Lee WY, Leger C and Kubers P (2011) Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science* **334**: 101–105.
- Wu L, Gabriel CL, Parekh VV and Van Kaer L (2009) Invariant natural killer T cells: innate-like T cells with potent immunomodulatory activities. *Tissue Antigens* **73**: 535–545.
- Wu L and Van Kaer L (2013) Contribution of lipid-reactive natural killer T cells to obesity-associated inflammation and insulin resistance. *Adipocyte* **2**: 12–16.
- Wun KS, Cameron G, Patel O, *et al.* (2011) A molecular basis for the exquisite CD1d-restricted antigen specificity and functional responses of natural killer T cells. *Immunity* **34**: 327–339.
- Yu KO, Im JS, Molano A, *et al.* (2005) Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of alpha-galactosylceramides. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 3383–3388.
- Yu KO and Porcelli SA (2005) The diverse functions of CD1d-restricted NKT cells and their potential for immunotherapy. *Immunology Letters* **100**: 42–55.
- Yuan W, Dasgupta A and Cresswell P (2006) Herpes simplex virus evades natural killer T cell recognition by suppressing CD1d recycling. *Nature Immunology* **7**: 835–842.
- Yuan W, Qi X, Tsang P, *et al.* (2007) Saposin B is the dominant saposin that facilitates lipid binding to human CD1d molecules. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 5551–5556.
- Yuan W, Kang SJ, Evans JE and Cresswell P (2009) Natural lipid ligands associated with human CD1d targeted to different subcellular compartments. *Journal of Immunology* **182**: 4784–4791.
- Zajonc DM, Cantu C 3rd, Mattner J, *et al.* (2005a) Structure and function of a potent agonist for the semi-invariant natural killer T cell receptor. *Nature Immunology* **6**: 810–818.
- Zajonc DM, Maricic I, Wu D, *et al.* (2005b) Structural basis for CD1d presentation of a sulfatide derived from myelin and its implications for autoimmunity. *Journal of Experimental Medicine* **202**: 1517–1526.
- Zajonc DM, Savage PB, Bendelac A, Wilson IA and Teyton L (2008) Crystal structures of mouse CD1d-iGb3 complex and its cognate Valpha14 T cell receptor suggest a model for dual recognition of foreign and self glycolipids. *Journal of Molecular Biology* **377**: 1104–1116.
- Zeissig S and Blumberg RS (2013) Commensal microbiota and NKT cells in the control of inflammatory diseases at mucosal surfaces. *Current Opinion in Immunology* **25**: 690–696.
- Zeissig S, Murata K, Sweet L, *et al.* (2012) Hepatitis B virus-induced lipid alterations contribute to natural killer T cell-dependent protective immunity. *Nature Medicine* **18**: 1060–1068.
- Zeissig S and Blumberg RS (2014) Commensal microbial regulation of natural killer T cells at the frontiers of the mucosal immune system. *FEBS Letters* **588**: 4188–4194.
- Zeng Z, Castano AR, Segelke BW, *et al.* (1997) Crystal structure of mouse CD1: an MHC-like fold with a large hydrophobic binding groove. *Science* **277**: 339–345.
- Zhou D, Cantu C 3rd, Sagiv Y, *et al.* (2004a) Editing of CD1d-bound lipid antigens by endosomal lipid transfer proteins. *Science* **303**: 523–527.
- Zhou D, Mattner J, Cantu C 3rd, *et al.* (2004b) Lysosomal glycosphingolipid recognition by NKT cells. *Science* **306**: 1786–1789.
- Zimmer MI, Nguyen HP, Wang B, *et al.* (2009) Polymorphisms in CD1d affect antigen presentation and the activation of CD1d-restricted T cells. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 1909–1914.

## Further Reading

- Bendelac A, Savage PB and Teyton L (2007) The biology of NKT cells. *Annual Review of Immunology* **25**: 297–336.
- Brennan PJ, Brigl M and Brenner MB (2013) Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nature Reviews Immunology* **13**: 101–117.
- Godfrey DI and Berzins SP (2007) Control points in NKT-cell development. *Nature Reviews Immunology* **7**: 505–518.
- Kotas ME and Medzhitov R (2015) Homeostasis, inflammation, and disease susceptibility. *Cell* **160**: 816–827.

- Macho-Fernandez E and Brigl M (2015) The extended family of CD1d-restricted NKT cells: sifting through a mixed bag of TCRs, antigens, and functions. *Frontiers in Immunology* **6**: 362.
- Rossjohn J, Gras S, Miles JJ, *et al.* (2015) T cell antigen receptor recognition of antigen-presenting molecules. *Annual Review of Immunology* **33**: 169–200.
- Terabe M and Berzofsky JA (2014) The immunoregulatory role of type I and type II NKT cells in cancer and other diseases. *Cancer Immunology, Immunotherapy* **63**: 199–213.
- Van Kaer L, Parekh VV and Wu L (2011) Invariant NK T cells: potential for immunotherapeutic targeting with glycolipid antigens. *Immunotherapy* **3**: 59–75.
- Van Kaer L, Parekh VV and Wu L (2013) Invariant natural killer T cells as sensors and managers of inflammation. *Trends in Immunology* **34**: 50–58.
- Wu L and Van Kaer L (2013) Contribution of lipid-reactive natural killer T cells to obesity-associated inflammation and insulin resistance. *Adipocyte* **2**: 12–16.