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The ins and outs of type I iNKT cell development

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Natural killer T (NKT) cells are innate-like lymphocytes that bridge the gap between the innate and adaptive immune responses. Like innate immune cells, they have a mature, effector phenotype that allows them to rapidly respond to threats, compared to adaptive cells. NKT cells express T cell receptors (TCRs) like conventional T cells, but instead of responding to peptide antigen presented by MHC class I or II, NKT cell TCRs recognize glycolipid antigen in the context of CD1d. NKT cells are subdivided into classes based on their TCR and antigen reactivity. This review will focus on type I iNKT cells that express a semi invariant Vα14Jα18 TCR and respond to the canonical glycolipid antigen, α-galactosylceramide. The innate-like effector functions of these cells combined with their T cell identity make their developmental path quite unique. In addition to the extrinsic factors that affect iNKT cell development such as lipid:CD1d complexes, co-stimulation, and cytokines, this review will provide a comprehensive delineation of the cell intrinsic factors that impact iNKT cell development, differentiation, and effector functions – including TCR rearrangement, survival and metabolism signaling, transcription factor expression, and gene regulation.

1. Introduction

1.1. Discovery

NKT cells were originally identified in mice as a mature (CD44+) population of double negative (DN: CD4-CD8-) or CD4+ thymocytes that expressed αβ T cell receptors (TCRs), and predominantly utilized the Vβ8 β chain (Fowlkes et al., 1987; Hayakawa et al., 1992). Simultaneously, a population of T cells with higher than normal frequency of Vα14 were discovered (Koseki et al., 1991, 1990). Eventually, these two high frequency TCR chains were linked together to define semi-invariant NKT (iNKT) cell TCRs. In mice, Vα14Jα18 pairs with three different β chains (Vβ8, 7, and 2), whereas in humans, Vα24Jα18 pairs with Vβ11 (Dellabona et al., 1994; Lantz and Bendelac, 1994). In conventional T cells, CD4 + TCRs interact with MHC class II while CD8 + TCRs interact with MHC class I. Prior to the discovery of the precise antigen presentation molecule, NKT cells were immediately singled out as unique because DN and CD4+ cells were restricted by an MHC class I family molecule (Bendelac et al., 1994; Bix et al., 1993; Cardell et al., 1995). It was quickly discovered that mouse and human NKT cells are selected on CD1d, an MHC class Ib antigen presentation molecule that presents glycolipid antigen, expressed on double positive (DP: CD4+CD8+) thymocytes (Bendelac, 1995; Bendelac et al., 1995; Coles and Raulet, 1994; Exley et al., 1997; Kawano et al., 1997). CD1-restriction unites a diverse population of T cells. NKT cells express γδ or αβ TCRs (Spada et al., 2000). Currently, αβ NKT cells are subdivided into two distinct subsets: type I and type II. Type I NKT cells are activated by the quintessential agonist, α-galactosylceramide (α-GalCer), and are therefore detectable by α-GalCer-loaded CD1d tetramers. Type I NKT cells include iNKT cells (detectable by their specific TCR α chain) as well as NKT cells with diverse TCRs that recognize α-GalCer:CD1d complexes. Type II NKT cells have diverse TCRs and do not respond to α-GalCer (Behar et al., 1999). This review will focus on murine type I iNKT cells unless otherwise specified.

1.2. Development

Like conventional T cells, iNKT cells develop in the thymus. They pass through the four DN stages with β chain rearrangement occurring at DN3 and α chain rearrangement occurring at the DP stage. However, at the DP stage of development, they are selected on CD1d-expressing DP cortical thymocytes, not thymic epithelial cells (Coles and Raulet, 2000). As shown in Fig. 1, iNKT cell development is divided into four stages (0 through 3, in numerical order) (Gapin, 2016). The initial description of stage 3 NKT cells characterized them as mature CD44 + NK1.1+ cells (Fowlkes et al., 1987). Positive selection occurs at stage 0 (CD4+CD8+HSA+) (Benlagha et al., 2005). This causes the upregulation of the transcription factor Egr2 and subsequently the NKT

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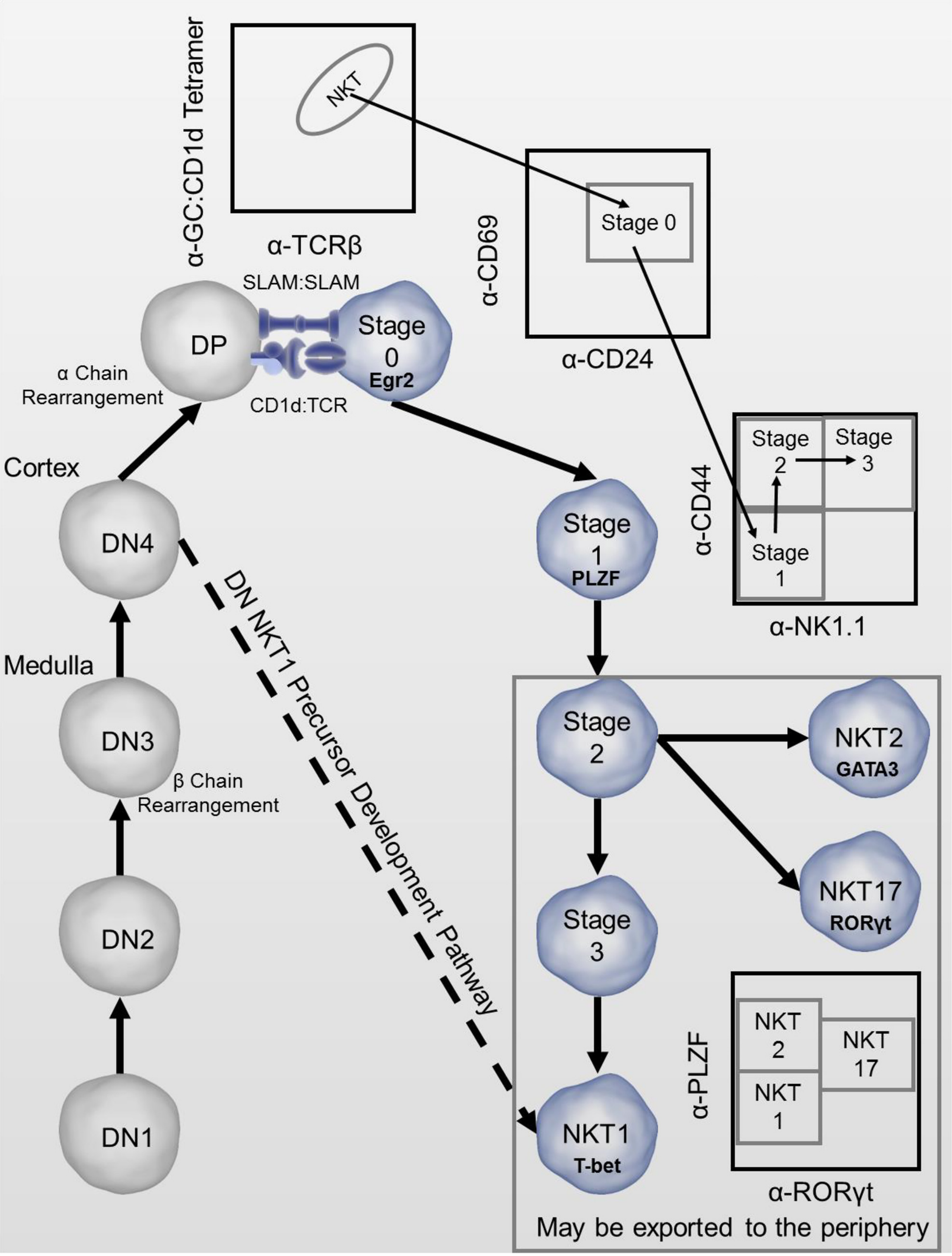

Fig. 1. NKT cell development and differentiation. NKT cells develop in the thymus and can be characterized by flow cytometry using cell surface and intracellular markers. In the most prominent developmental model, NKT cells branch off from conventional T cell development at the double positive stage where they are selected by strong SLAM:SLAM and CD1d:TCR signals that result in Egr2 upregulation and subsequently PLZF upregulation. They progress through 4 stages of development (0–3) and differentiate into NKT1, 2, and 17 cells. Both stage 2 and 3 are exported from the thymus. Another developmental pathway starts in the DN4 stage of T cell development and bypasses the double positive stage to produce mature, DN NKT1-like cells.

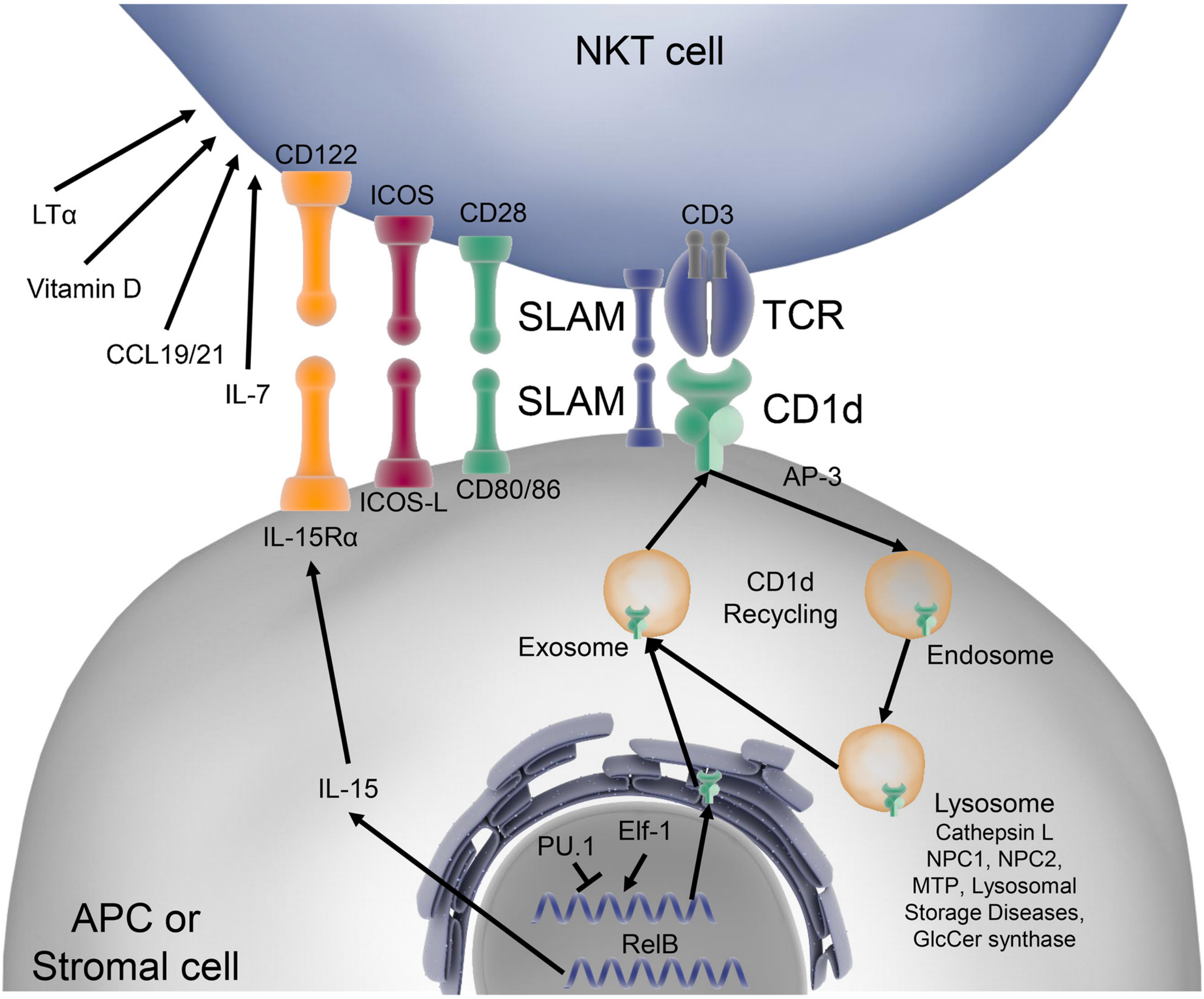

Fig. 2. Extrinsic factors that affect NKT cell development. NKT cell development requires input from other cell types. CD1d expression, recycling, and antigen exchange alters NKT cell positive and negative selection. Additionally, specific cell-cell interactions, such as CD1d:TCR, SLAM:SLAM, CD80/86:CD28, and ICOS-L:ICOS, greatly influence NKT cell population development. Moreover, factors such as lymphotoxin α, vitamin D, CCL19/21, and IL-15 have a significant impact on NKT cell development. IL-15, in particular, must be presented in trans to NKT cells by medullary thymic epithelial cells (mTECs).

cell master transcription factor, PLZF (Kovalovsky et al., 2008; Savage et al., 2008). Stage 1 (HSA-CD44-NK1.1-) and stage 2 (CD44 + NK1.1-) iNKT cells were later described (Arase et al., 1992; Benlagha et al., 2002; Pellicci et al., 2002). iNKT cell development is characterized by extensive rounds of expansion – accounting for their high frequency and mature, effector phenotype (Föhse et al., 2013). Additionally, iNKT cells migrate from the thymus at either stage 2 or stage 3 of development (Berzins et al., 2005) and become tissue resident cells (Mackay et al., 2016). An alternative pathway of iNKT cell development that does not pass through the DP stage has been demonstrated. DN4 thymocytes contain a population that expresses Vα14Jα18 mRNA and can produce mature DN iNKT cells (Dashtsoodol et al., 2008). RAG ablation under specific Cre promoters in double positive cells demonstrates that these DN4 iNKT precursors produce mature DN iNKT cells without passing through the DP stage. The DN iNKT cells produced are strongly Th1 skewed and have enhanced cytotoxicity (Dashtsoodol et al., 2017).

1.3. Differentiation

In the periphery, CD1d-mediated antigen presentation by antigen presenting cells (APCs) such as dendritic cells (DCs) results in iNKT cell activation. Upon activation, iNKT cells are known to rapidly produce a wide variety of cytokines, including IFN-γ and IL-4, and directly mediate cell lysis via perforin/granzyme and FasL. Originally, DN iNKT cells were associated with a more Th1/cytotoxic phenotype while CD4+ iNKT cells were thought to produce both Th1 and Th2-type cytokines (Bendelac et al., 1996; Gumperz et al., 2002; Lee et al., 2002). Eventually, iNKT cells were shown to differentiate into groups mirroring the T helper subsets (NKT1, NKT2 and NKT17) with each subset expressing unique genes and transcription factors (Lin et al., 2006; Niemeyer et al., 2008). Notably, there is significant plasticity amongst the subtypes (Cameron and Godfrey, 2018). Upon exiting the thymus, iNKT cells are mature, effector cells, indicating that their terminal differentiation occurs during development (Gapin, 2016). Although stage 3 was originally thought to represent mature iNKT cells, stage 2 includes mature NKT2 and NKT17 cells (Coquet et al., 2008; McNab et al., 2007; Michel et al., 2008). In fact, IL-4 secretion by stage 2 NKT2 cells promotes innate-like CD8 + T cell development and IgE production (Dickgreber et al., 2012; Lee et al., 2013).

2. Cell extrinsic effects

2.1. CD1d and lipid antigens

CD1d presents lipid antigens to NKT cells and is expressed by hematopoietic cells such as B cells, T cells, macrophages and DCs in addition to endothelial and epithelial cells (Brossay et al., 1997; Canchis et al., 1993). Interactions between CD1d and the NKT TCR are imperative for NKT cell development, and these interactions are regulated by CD1d expression, intracellular trafficking, and lipid acquisition. Increased expression of CD1d or the addition of strongly activating ligands, such as α-GalCer, resulted in alterations to Vβ usage, demonstrating changes to positive and negative selection. Importantly, this negative selection was mediated by thymic DCs rather than the DP thymocytes that mediate iNKT cell positive selection (Chun et al., 2003). To determine the APCs responsible for iNKT cell positive and negative selection, human CD1d was transgenically expressed of in either T cells (Lck promoter) or DCs and macrophages (CD11c promoter). Although CD1d on T cells only was sufficient for positive selection, the absolute number of iNKT cells was reduced and Vβ usage was altered – potentially due to slight avidity differences of mouse TCR for human CD1d. Alternatively, limiting CD1d expression to DCs and macrophages resulted in no detectable iNKT cells because these cell types cannot mediate positive selection (Schümann et al., 2005). CD1d expression is controlled by members of the Ets transcriptional regulator family. Elf-1 binds to this site as a positive regulator while Pu.1 binds to this site as a negative regulator of CD1d transcription (Fig. 2) (Choi et al., 2011). In mice, there are two CD1d genes: CD1d1 and CD1d2. When originally characterized, CD1d2 was found to be a pseudogene that does not produce protein and this was assumed to be the case for all mouse strains. Recently, it was shown that some mouse strains, namely Balb/c, express properly folded CD1d2 in the thymus and that it will select functional NKT cells. CD1d2 is slightly different structurally, resulting in different antigenic selection, and therefore a different repertoire of NKT cells (Sundararaj et al., 2018).

CD1d has a unique intracellular trafficking pattern (Fig. 2). It is synthesized in the endoplasmic reticulum, associates with the invariant chain (Ii), and traffics to the cell surface. Subsequently, CD1d is endocytosed to lysosomal compartments, exchanges lipids, and recycled to the cell surface. Mutations in the CD1d cytoplasmic tyrosine-based sorting motif decreased the ability of CD1d to recycle/internalize, exchange antigen, and positively select iNKT cells in vivo (Chiu et al., 2002). AP-3 is important for endosomal and lysosomal trafficking. AP-3 mutation or deletion resulted in increased surface expression of CD1d, altered trafficking, and decreased selection of iNKT cells in vivo (Cernadas et al., 2003; Elewaut et al., 2003a). Adenosine triphosphate binding cassette transporter 7 (ABCA7) controls lipid transport. ABCA7 deficient animals have reduced iNKT cell populations characterized by decreased Egr2, decreased proliferation, and altered maturation with fewer in stage 3 and more in stages 1 and 2. This defect is iNKT cell extrinsic and is due to decreased CD1d expression and lipid raft formation – resulting is suboptimal iNKT cell stimulation (Nowyhed et al., 2017).

Lipid availability and loading can affect NKT cell selection as demonstrated by decreased NKT cell development in Cathepsin L, NPC1 and 2, or MTP deficient mice. (Dougan et al., 2007; Honey et al., 2002; Sagiv et al., 2006; Schrantz et al., 2007). Although mice lacking Ii had normal iNKT population sizes, there was a decrease in stage 3 and an increase in stage 1 and 2 iNKT cells, altered Vβ usage, and decreased ability to suppress infection, suggesting alterations in positive selection, potentially due to differential antigen selection (Sillé et al., 2011). Mouse models of some lysosomal storage diseases – namely, mutations in β-hexosaminidase A or B, α-galactosidase A, β-galactosidase, and NPC1 – have decreased iNKT cells and altered development, specifically decreased stage 3 and increased stage 1 and 2 iNKT cells (Gadola et al., 2006; Plati et al., 2009).

The identity of the iNKT cell selecting antigen is a contested mystery with microbial antigens, α-GalCer, and isoglobotrihexosylceramide (iGb3) having been considered. (Christiansen et al., 2008; Park et al., 2000; Pellicci et al., 2003; Porubsky et al., 2007). The most recent candidates are mammalian α-linked glycosylceramides, including glucosyl (GlcCers) and galactosyl (GalCers) species, as determined using surface plasmon resonance, an antibody that recognizes α moieties bound to CD1d, and purification from mammalian cells. Although mammalian cells were originally not thought to produce these lipid species, cells deficient in the enzymes that degrade α-linked sugars were better stimulators of iNKT, suggesting that these glycolipids were synthesized and their levels were tightly controlled by degradation (Kain et al., 2014). A recent study in which GlcCer synthase was conditionally deleted in T cells, showed decreased iNKT cell populations despite normal CD1d and SLAM receptor expression. iNKT cell development proceeded normally, but the iNKT cell population had higher levels of apoptosis and lower proliferation (Popovic et al., 2017). In summary, NKT cell development can be altered by changes in CD1d expression, trafficking, antigen loading, and antigen availability (Fig. 2).

2.2. Co-Stimulation

Like conventional T cells, NKT cells require co-stimulation during development and activation. This section will cover CD3, CD28:B7, ICOS:ICOS-L, and TIM-4. CD3 is the most basic TCR associated co-stimulatory molecule and truly exerts an intrinsic effect since it does not have an extrinsic ligand. Unlike conventional T cells, iNKT cells absolutely require CD3 signaling during development. Deletion of CD3ζ or its signaling component, Lck, resulted in reduced iNKT cell populations and immature iNKT cells that failed to express a fully rearranged TCR (Baur et al., 2001). The CD3ζ chain has 10 immunoreceptor tyrosine-based activation motifs (ITAMs). While conventional T cells only require four ITAMs for development, iNKT cells require all ten. Mutation of as few as two ITAMs resulted in a dramatic population reduction, skewed development with most iNKT cells in stage 0, increased cell death, and decreased expression of Egr2 and PLZF (Becker et al., 2010). CD28 (T cell expressed) and its ligands CD80/86 (APC expressed) are the canonical costimulatory molecules. Deletion of CD28 or both CD80 and 86 resulted in decreased iNKT cell populations. Maturation was skewed with fewer iNKT cells progressing to stage 2 and 3 of development (Williams et al., 2008; Zheng et al., 2008). ICOS (expressed on T cells) and ICOS-L (expressed on APCs) are in the same co-receptor family as CD28 and CD80/86. Similarly, mice lacking ICOS or ICOS-L had decreased iNKT cell populations. Removal of ICOS was not quite as detrimental as the deletion of CD80/86, but stage 3 was slightly lower with stages 1 and 2 slightly higher. Despite being expressed on iNKT cells in the thymus and periphery, TIM-4 was not necessary for iNKT cell development and TIM4 deficient mice had normal iNKT cell populations with normal maturation (Zhang et al., 2016).

2.3. Cytokines, chemokines, and hormones

Cytokine, chemokine, and hormone receptor signaling impacts the immune system and iNKT cell development. This section will cover IL-15, IL-7, lymphotoxin, CCR7, and vitamin D. CD122 (IL-15Rβ) is a receptor for IL-2 and IL-15 expressed by NKT cells. CD122 deficiency resulted in decreased iNKT cell populations that cannot be rescued by Bcl-2 overexpression, demonstrating that IL-2/15 provide more than survival signals (Minagawa et al., 2002). A series of bone marrow chimera experiments demonstrated that iNKT cell development requires IL-15 trans-presentation by non-hematopoietic cells. iNKT cells failed to develop in IL-15Rα deficient hosts, but IL-15Rα deficient iNKT cells develop in WT hosts (Castillo et al., 2010; Chang et al., 2011). iNKT cells in IL-15 or IL-15Rα knockout mice failed to progress to stage 3 and exhibited increased cell death. iNKT development in IL-15 and IL-

15Rα deficient mice was partially rescued by Bim, Bcl-2 or Bcl-xL overexpression. Indeed, when IL-15 was added to the media of thymic iNKT cells, Bcl-2 and Bcl-xL were upregulated (Chang et al., 2011; Gordy et al., 2011). Trans-presentation of IL-15 is performed by mTECs in the thymus and DCs in the periphery (Castillo et al., 2010; White et al., 2014). IL-7 is a similarly important cytokine for T cells. Mice lacking IL-7Rα had reduced iNKT cell population sizes, normal maturation, decreased proliferation, and could be rescued by Bcl-2 overexpression (Gordy et al., 2011; Tani-ichi et al., 2013). In iNKT cells, the deubiquitinase CYLD controls IL-7Rα expression. In CYLD knockout mice, iNKT cell populations were reduced, but maturation was normal. The population reduction was due to increased apoptosis because of failure to express IL-7Rα and ICOS (Lee et al., 2010). Thus, IL-15 and IL-7 provide critical pro-survival signals to developing iNKT cells.

The lymphotoxin family has two members, LTα and LTβ, and signals through homo or hetero-trimers. Deletion of LTα, LTβ, or their receptor LTβR reduced the iNKT cell population in the periphery but only LTα deletion reduced thymic iNKT cells (Elewaut et al., 2000; Franki et al., 2006). CCR7 is the receptor for CCL19 and CCL21 and is important for the migration of developing T cells from the cortex to the medulla (Fig. 2). In mice lacking CCR7, iNKT cell populations were reduced and fewer cells progressed to stage 3 of development because the cells failed to migrate to the medulla and therefore did not receive IL-15 trans-presentation from mTECs (Cowan et al., 2014). Vitamin D receptor deficient mice had decreased iNKT cell populations characterized by decreased progression to stage 3. Interestingly, mice lacking vitamin D receptor had reduced CD1d expression in the thymus and the effect seems to be dependent on expression in the hematopoietic compartment, although it is unclear if it needs to be expressed by the developing iNKT or the selecting DP thymocyte (Yu and Cantorna, 2008). Vitamin D receptor interacts with Med1. Med1 deficiency intrinsically decreased iNKT cell development with fewer cells progressing to stage 3 of development, but these defects could be rectified by transgenic expression of Vα14Jα18. In contrast to vitamin D receptor deletion, Med1 deficient cells failed to upregulate CD122 (Yue et al., 2011).

3. Cell intrinsic effects

3.1. iNKT TCR

Due to the enhanced frequency of iNKT cell TCRs in naïve mice, it was originally thought that this particular TCR must be specifically selected by a dedicated precursor or directed rearrangement. Analysis of cloned hybrids by PCR and southern blot suggested that the iNKT TCR was not the product of directed rearrangement and was usually accompanied by an additional α chain rearrangement (Shimamura et al., 1997). iNKT cells possess a stronger requirement for pre-Tα signaling than conventional T cells – potentially because successful rearrangement occurred later during the DP stage (G. Eberl et al., 1999a and b). Analyzing gene rearrangement at different stages of development in wildtype and CD1 deficient mice confirmed that iNKT cell TCR rearrangement is similar to the rearrangement of other α chains and it conformed to the instructive model of T cell development (Gapin et al., 2001; Hager et al., 2007). Interestingly, the specific sequence of the iNKT TCR could be created through convergent recombination in which an overlap in the Vα and Jα sequences results in the same rearrangement by multiple recombination events (Greenaway et al., 2013). The enhanced frequency of these cells is now attributed to their intense expansion post-selection.

The particular pairing of α and β chains confers unique interactions to iNKT cells. In humans and mice, iNKT TCR:CD1d docking occurs in a parallel orientation with the TCR α chain primarily interacting with CD1d and the TCR β chain recognizing the glycolipid antigen head group (Borg et al., 2007; Kjer-Nielsen et al., 2006). Although all NKT cells are reactive to self-ligands, Vβ7 has higher avidity for self-ligands, such as iGb3, while Vβ8 has higher avidity of exogenous ligands, such as α-GalCer (Schümann et al., 2006; Wei et al., 2006). iNKT cell positive selection is characterized as agonistic because the TCR signaling is much stronger than that of conventional T cell selection (Kisielow et al., 2011; Moran et al., 2011). However, TCR signals that are too strong are subject to negative selection – as discovered in a mouse model with a transgenic TCRβ that conferred higher CD1d avidity (Bedel et al., 2014). The few iNKT cells that developed had decreased Egr2 and PLZF expression, in addition to reduced proliferation and effector functions, indicating the importance of TCR signal strength in iNKT cell development. Indeed in retrogenic chimera models, avidity correlated with iNKT cell selection (Cruz Tleugabulova et al., 2016). These studies indicate that iNKT cell selection occurs within a tightly controlled window.

3.2. Gene regulation

Gene regulation consists of multiple levels of control. This section will discuss the effects of NKT cell specific transcription factors (PLZF, Egr2), T helper cell transcription factors (RORγt, GATA3, T-bet), T cell development transcription factors (Notch, Wnt/β-catenin, TCF-1/LEF-1), immune cell transcription factors (Ets, AP-1, E protein), global gene regulators (HDAC3, UTX/JMJD3), and microRNAs on iNKT cell development (summarized in Table 1).

PLZF was identified during a screening for the expression of multiple BTB-ZF proteins in the immune system. PLZF was not expressed in conventional T cells, but showed temporal regulation in iNKT cells (higher in stage 1 and 2, lower in stage 3). iNKT cell populations were severely reduced in PLZF knockout mice and those that were produced failed to progress to stage 2 of development and express classic receptors such as CD122 (Kovalovsky et al., 2008; Savage et al., 2008). To investigate the effects of PLZF on NKT/T cell development, mice that express PLZF under the Lck or CD4 promoter were generated. While T cells and iNKT cells developed normally, conventional T cells displayed an effector, rather than naïve phenotype – including increased CD44 and IL-7R and decreased CD62L expression (Kovalovsky et al., 2010; Savage et al., 2011). These effects were found to be independent of SAP and Fyn expression (Kovalovsky et al., 2010). In humans, it was found that PLZF is expressed by NKT, $\gamma\delta$ T, NK and innate-like CD8 T cells and PLZF + cells were increased in individuals with autoimmunity or cancer (Eidson et al., 2011). Additionally, human iNKT cells require PLZF for development as evidenced by a patient with biallelic mutations in PLZF (Eidson et al., 2011). Using ChIP-Seq, the gene targets of PLZF were found to overlap with targets of Ets, Runx, and E protein transcription factor families. PLZF activates genes for immune effectors (Fig. 3), homing and adhesion, cytokine and chemokine receptors, and T-helper specific transcription factors and suppresses Bach2, an immune effector gene suppressor (Mao et al., 2016).

Egr2 was discovered by investigating how TCR signaling-induced calcineurin, and its downstream transcription factor NFAT, influence iNKT cell development. In a model of Lck-Cre mediated deletion of calcineurin, iNKT cells were reduced in the thymus and spleen and Egr1, 2, and 3 were not upregulated following stimulation. Egr2, but not Egr1 or 3, ablated fetal liver chimeras demonstrated reduced iNKT cell populations, characterized by decreased progression to stage 3 of development. Decreased cellularity, in spite of increased proliferation, was in part due to increased cell death of stage 0 iNKT cells (Lazarevic et al., 2009). Transgenic Egr2 expression led increased iNKT cell populations (S. Li et al., 2011a and b). In fact, strong TCR signaling, such as transgenic expression of Vα14Jα18, resulted in enhanced Egr2 expression. Using ChIP-seq and luciferase assays, Egr2 was shown to bind the promoters of PLZF as well as other iNKT cell associated genes (CD122, FasL, and Cyclin D2). Egr2 deletion resulted in decreased expression of CD122 and Id2 and increased expression of Egr1. In mice lacking both Egr1 and 2, iNKT cells were drastically reduced. These cells were almost entirely restrained to stage 1 of development and failed to express PLZF, indicating the ability of Egr1 to compensate for

Table 1
Transcription factors in NKT cell development.

	Change	Population Effect	Maturation	Cause	Differentiation
NKAP	KO	↓ Intrinsic	Stage 0 Arrest or Decreased stage 3	↓ Proliferation	Decreased NKT17
HDAC3	KO	↓ Intrinsic	Decreased Stage 3	↑ Apoptosis	Decreased NKT1
UTX JMJD3	KO	↓ Intrinsic	Stage 1 Arrest		
Ets TFs	KO	↓ Intrinsic	Decreased Stage 3	↑ Apoptosis	
AP-1	Tg Inhibitor	↓ Intrinsic	Stage 2 Arrest		
Fra-2	KO	↑ Intrinsic			Increased Vβ8.2 Usage
Id2/3	KO or Inhibition	↑ Intrinsic	Immature	↑ Proliferation	Altered Vβ Usage, Decreased NKT1, Increased NKT2 & NKT 17
E protein	KO	↓ Intrinsic	Stage 1 Arrest	↓ Proliferation	
Notch1/2	KO	↑ Intrinsic	Mostly Normal		Increased NKT17
Wnt/β-Catenin	Tg Active	Normal	Decreased Stage 3		Decreased NKT1 & NKT17
TCF-1	KO	↓ Intrinsic		Rescued by Tg Bcl-xL/TCR	
RORγt	KO	↓ Intrinsic		Rescued by Tg Bcl-xL/TCR	
Runx1	KO	↓ Intrinsic			
Gata3	KO	Normal	Increased Stage 3	↑ Apoptosis	CD4 + NKT cell decreased
ThPOK	KO	Normal	Decreased Stage 3		CD4 + NKT cell decreased, Decreased NKT1, Increased NKT17
Tbet	KO	↓ Intrinsic	Stage 2 Arrest		
Egr2	KO	↓ Intrinsic	Stage 2 Arrest	↑ Apoptosis	
Pak2	KO	↓ Intrinsic	Stage 2 Arrest	↑ Apoptosis	Decreased NKT1 & NKT2, Increased NKT17
PLZF	KO	↓ Intrinsic	Stage 1 Arrest		
YY1	KO	↓ Intrinsic	Stage 2 Arrest	↑ Apoptosis	

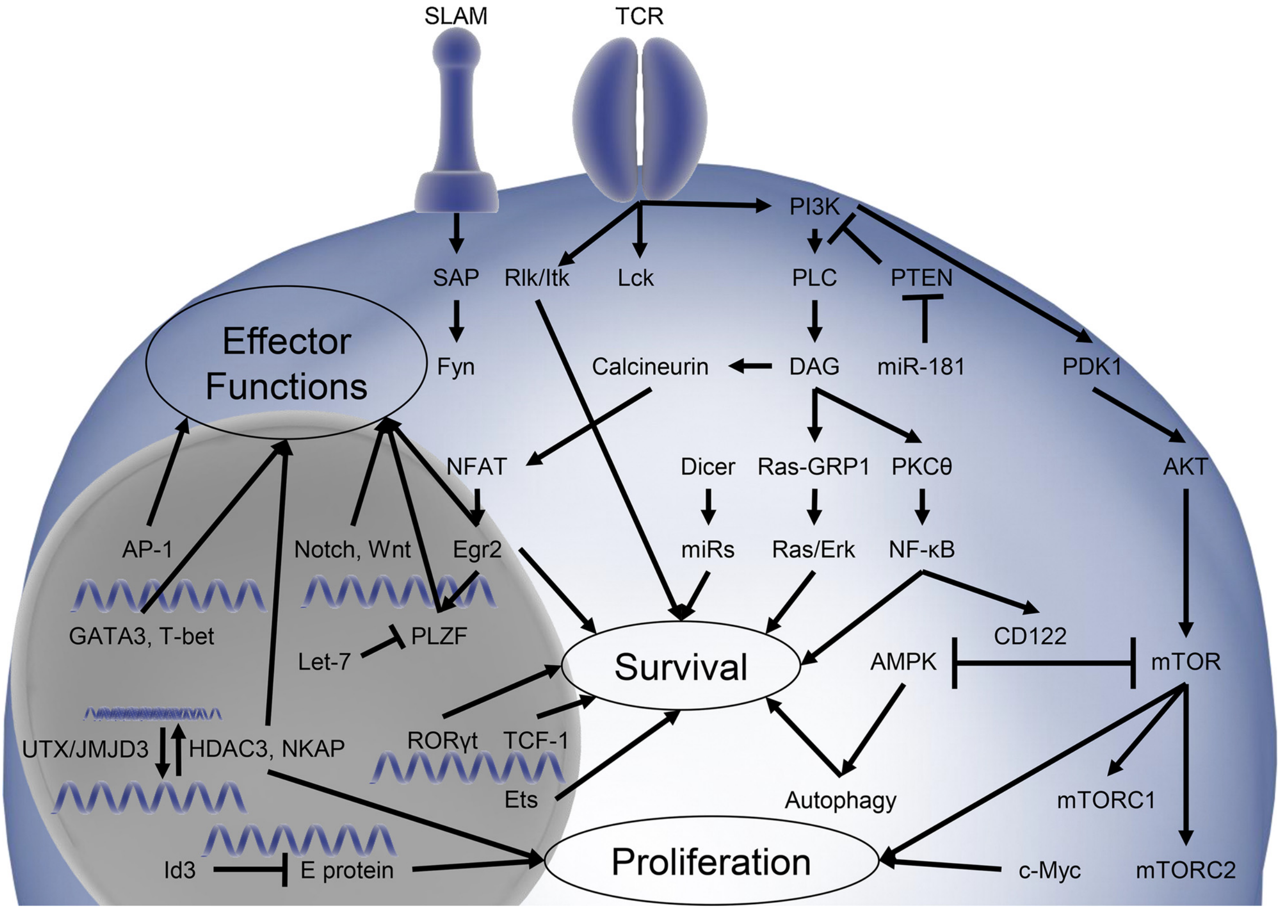

Fig. 3. NKT cells must balance many signals for development and expansion. NKT cell development involves extremely strong TCR and SLAM signals that reprogram the cell. This requires activation of survival (NF-κB) and proliferation (c-Myc) pathways as well as metabolic balancing (PI3K, mTOR, AMPK) and transcriptional activation. During this time, NKT cells also upregulate multiple lineage specific transcription factors to control differentiation and effector functions.

loss of Egr2 (Seiler et al., 2012). Genetic deletion of p21-activated kinase 2 (Pak2), a cytoskeletal remodeling gene associated with high affinity TCR signaling, resulted in decreased iNKT cells and skewed development with a higher percentage in Stage 0, 1, and 2 and a lower percentage in stage 3 in a cell intrinsic manner. Pak2 deficient iNKT cells had increased apoptosis and the defect could not be rescued by Bcl-2 expression. Pak2 deficient iNKT cells expressed less Slamf6 and had skewed differentiation with fewer NKT1 and NKT2 cells and more NKT17. These alterations were due to impaired expression of Egr2 and PLZF during iNKT development – indicating that TCR signaling proceeds through Pak2 for Egr2 upregulation (O'Hagan et al., 2015). Ying-Yang 1(YY1) is a newly discovered transcription factor that demonstrates similar temporal regulation to Egr2. Ablation of YY1 resulted in decreased iNKT cell populations and decreased progression to stage 3 of development by a cell intrinsic mechanism. Although this defect partially resulted from increased cell death, Bim expression did not rescue the phenotype. Luciferase assays combined with ChIP-seq revealed that YY1 bound and activated the PLZF promoter (Ou et al., 2018). Activation of PLZF transcription by Egr2 and YY1 is necessary early in iNKT cell development for PLZF upregulation and survival (Fig. 3).

RORγt, GATA3, and T-bet are traditionally thought of as T helper specific transcription factors. In mice lacking RORγt, iNKT cell populations were severely reduced in a cell intrinsic manner that could be rescued by Bcl-xL and Vα14Jα18 transgenic expression – demonstrating the importance of RORγt expression in the double positive stage during α chain rearrangement (Bezbradica et al., 2005; Egawa et al., 2005). Likewise, Runx1 ablation also resulted in a cell intrinsic, severe depletion of iNKT cells (Egawa et al., 2005). In mice with CD4-Cre mediated removal of GATA3, the iNKT cell population was normal in the thymus, but decreased in the periphery, potentially due to increased cell death. Despite normal cellularity, CD4+ iNKT cells were specifically depleted in the thymus and periphery and development was altered with more iNKT cells progressing to stage 3 of development. Both of these alterations were cell intrinsic (Kim et al., 2006). Conditional ablation of ThPOK phenocopied GATA3 deficiency, with a selective loss of CD4+ iNKT cells, and dual modification studies indicated that GATA3 promotes ThPOK expression (Wang et al., 2010). ThPOK regulates co-receptor expression during iNKT cell development. Thus when ThPOK was removed, CD4+ iNKT cells failed to develop and CD8+ iNKT cells developed (Engel et al., 2010). An iNKT cell developmental study with mutant ThPOK indicated that in addition to the phenotypic differences indicated above, mutant iNKT cells had a cell intrinsic selective developmental advantage in mixed bone marrow chimeras, but fewer iNKT cells progressed to stage 3 of development with decreased development of NKT1 cells and increased development of NKT17 (Enders et al., 2012). Interestingly, the additional deletion of Runx3, a controller of CD8 expression, partially rescued the expression of CD8 and moderately decreased the NKT17 skewing (Liu et al., 2014). Loss of T-bet decreased iNKT cell populations in a cell intrinsic manner as identified by both TCRβ + NK1.1+ and TCRβ+ α-GalCer-loaded tetramer staining. There was a blockade at stage 2 of development with very few iNKT cells progressing to stage 3 (Matsuda et al., 2006; Townsend et al., 2004). T-bet ablation resulted in decreased expression of IFN-γ, Granzyme B, FasL, and CD122 while forced expression of T-bet did the opposite (Matsuda et al., 2006). RORγt provides survival signals during α chain rearrangement in the DP stage. GATA3 and ThPOK induce CD4 and suppress CD8 expression (Fig. 3).

Notch, Wnt/β-catenin, and TCF-1/LEF-1 are known to be involved in early T cell development. Notch is vital for early T cell lineage commitment and development. The loss of both Notch1 and 2 resulted in increased total thymic iNKT cells, particularly in stages 2 and 3 of development, although the percentage in stage 3 was actually decreased. This defect was found to be cell intrinsic and the decreased percentage of stage 3 iNKT cells was corrected in the periphery. Additionally, mice lacking Notch 1 and 2 had increased NKT17 cells (Oh et al., 2015). Wnt/β-catenin signaling is known to be important in T lineage commitment, with activated β-catenin associating with TCF-1 for gene transcription. In mice expressing constitutively active β-catenin, iNKT cells were increased in the thymus. Similarly, CD4-Cre mediated β-catenin deletion resulted in a reduction in the percentage of iNKT cells, but not in absolute number. Activated β-catenin resulted in altered development, namely increased stage 1 and 2, but decreased stage 3 iNKT cells due to changes from the normal temporal regulation of β-catenin signaling. PLZF was upregulated while CD122 and T-bet were downregulated resulting in decreased NKT1 and NKT17 cells (Pyaram et al., 2017). Corresponding to β-catenin, TCF-1 is temporally regulated during iNKT cell development and deficiency resulted in decreased iNKT cells, but could be rescued by transgenic expression of Vα14Jα18 or Bcl-xL (Berga-Bolaños et al., 2015; Sharma et al., 2014). This defect was compounded by the additional removal of LEF-1 and both deletions resulted in cell intrinsic defects (Berga-Bolaños et al., 2015). Therefore, Notch1/2 and Wnt/β-catenin promote stage 3 iNKT cell maturation while TCF-1 provides necessary survival signals (Fig. 3).

MEF, Ets-1, and Elf-1 are Ets family members that are expressed in hematopoietic lineages. In MEF deficient mice, NKT cells, identified by NK1.1 and TCRβ expression, were reduced in the thymus and periphery and their cytolytic abilities were diminished because MEF activates the perforin promoter (Lacorazza et al., 2002). Mice lacking Ets-1 also had decreased CD4 + NK1.1+ NKT cells in the thymus and periphery (Walunas et al., 2000). Tetramer staining confirmed decreases in iNKT cell populations in Ets-1 knockout mice and showed a similar decrease in Elf-1 knockout mice that is cell intrinsic. Maturation was altered to have higher percentages in stage 0, 1, and 2 although all stages had decreased absolute cell numbers. This defect was in part due to increased cell death during stage 1 (Choi et al., 2011). BATF is a negative regulator of AP-1 and is highly expressed in lymphoid organs. In a mouse model that overexpresses BATF under the control of Lck, NK1.1+TCRβ+ NKT cells were reduced in the thymus and periphery and their effector functions (IL-4/IFN-γ production and cytolysis) were impaired (Williams et al., 2003). A follow up study confirmed these data using α-GalCer:CD1d dimers and tetramers and showed that iNKT cells were constrained in stage 2 of development (Zullo et al., 2007). CD4-Cre mediated deletion of the AP-1 family member, Fra-2, resulted in a cell intrinsic increase in iNKT cell populations in the thymus and periphery. Additionally, the use of Vβ8.2 became slightly more prominent – indicating potential changes in selection. A number of iNKT cell specific genes were dysregulated including CD122, IL-4, PLZF, TCF-1, Slamf1, and HSA (Lawson et al., 2009). The E protein transcription factors E2A and HEB and their negative regulators Id2 and Id3 are expressed in the thymus. Although Id2 removal had no effect, single germline ablation of Id3 (D'Cruz et al., 2014; Verykokakis et al., 2013) and removal of both Id2 and 3 by Lck-Cre (Li et al., 2013) or CD4-Cre (Verykokakis et al., 2013) resulted in a significant increase in thymic iNKT cells, in a cell intrinsic manner, but the majority were in stage 1 or 2 of development. Loss of Id3 resulted in increased proliferation at early stages of iNKT cell development – accounting for the increased cell number (D'Cruz et al., 2014). The percentage of cells rearranging the Vα14 TCR was increased and Vβ chain usage was skewed – indicating altered selection and potentially altered rearrangement (Li et al., 2013; Verykokakis et al., 2013). When Li et al. developed mice lacking Id2, Id3 and an E protein, iNKT cell populations were reduced to nearly normal (Li et al., 2013). Transgenic expression of an E protein fusion protein that blocks Id and sustains E protein activity altered iNKT cell development. iNKT cell populations were normal in the thymus and lymph nodes but decreased in the spleen. Additionally, thymic iNKT cells were primarily in stage 1 and 2 of development and displayed altered expression of CD122, T-bet, Runx3, GATA3 and RORγt resulting in increased differentiation of NKT17 and NKT2, but decreased NKT1 (Hu et al., 2013). The Id3 knockout model also provided evidence for decreased T-bet and therefore NKT1 cells and increased NKT2 cells (D'Cruz et al., 2014; Verykokakis et al., 2013). Id2 deficiency did not result in increased or diminished iNKT cell populations or changes in proliferation, but NKT1 cells were decreased and PLZF expression was increased (D'Cruz et al., 2014). In a model of HEB and E2A double knock out by CD4-Cre with transgenic overexpression of Vα14Jα18 TCR, iNKT cell populations were decreased in E protein deficient mice with the iNKT cells arrested in stage 0 and 1 of development. The reduction was due in part to decreased proliferation. This model also demonstrated decreased PLZF expression – because E proteins bind the PLZF promoter (D'Cruz et al., 2014). While Ets family members are important for survival, E protein members affect proliferation (Fig. 3).

Global gene changes are carried out by enzymes that add or remove post-translational modifications, such as methyl and acetyl groups, to histones. Histone deacetylase (HDAC) proteins remove acetyl groups, resulting in chromatin changes that effectively turn off transcription. NKAP is an HDAC3 associated protein that represses transcription. Conditional deletion of NKAP by CD4-Cre resulted in a severe, cell intrinsic reduction in iNKT cell populations with thymic iNKT cells primarily restrained in stage 0 of development. This defect was found to be independent of Notch signaling, but dependent upon HDAC3, as removal of HDAC3 under CD4-Cre recapitulated NKAP deficiency (Thapa et al., 2013). Gene ablation by PLZF-Cre occurs slightly later than CD4-Cre. PLZF-Cre mediated NKAP deficiency also resulted in reduced iNKT cells, with decreased progression to maturity due to decreased proliferation and skewed subset differentiation with decreased NKT17 cells. Only subset defects were rescued by transgenic expression of PLZF (Thapa et al., 2016). Mice lacking HDAC3 due to PLZF-Cre had decreased iNKT cell populations with decreased progression to maturity due to increased cell death, and reduced autophagy and nutrient receptor expression. This model also demonstrated skewed subset differentiation, but resulted in decreased NKT1 cells – likely a results of decreased stage 3 iNKT cells (Thapa et al., 2017). Conversely, HDAC4 was not required for iNKT cell development or function (Liu et al., 2017). UTX and JMJD3 are histone demethylases that remove methyl groups to turn genes on. Mice lacking JMJD3, UTX and JMJD3/UTX had decreased liver iNKT cells, but only UTX and JMJD3/UTX deficient animals had decreased thymic populations. Mice lacking both JMJD3 and UTX had the most severe development defects with a cell intrinsic block from stage 1 to stage 2 of development (Northrup et al., 2017). Collectively these studies reveal the importance of global gene regulation during stages 0 and 1 of iNKT cell development (Fig. 3).

Genes are regulated post-transcriptionally by microRNAs (miRNAs) and mRNA-binding proteins (Roquin). miRNAs bind mRNAs to block their translation and induce degradation, but to become functional, miRNAs must be processed by the enzyme Dicer. The importance of miRNA regulation was discovered in iNKT cells when Dicer was conditionally removed using Tie2-Cre by Qing-Sheng Mi's group. In this model, iNKT cells were significantly reduced in the thymus and peripheral organs. Thymic iNKT cells were primarily stage 0 with few progressing to stage 1, 2, and 3 (Zhou et al., 2009). Fedeli et al. simultaneously confirmed these results using a CD4-Cre system and mixed bone marrow chimeras to demonstrate the cell intrinsic nature of this developmental defect. iNKT cells from Dicer deficient mice were more frequently in the G2/M phase of the cell cycle and experienced much higher levels of apoptosis detected by caspase activation (Fedeli et al., 2009). Mi's group also used the CD4-Cre system in their Dicer model and confirmed an increased proportion of iNKT cells in stage 0 and increased apoptosis, as determined by Annexin V/7-AAD staining. In contrast, they found proliferation, measured by BrdU incorporation, to be normal (Seo et al., 2010). These studies show that microRNA expression is imperative for survival and population formation (Fig. 3).

Now that the effects of global repression of miRNAs were confirmed, the hunt for the precise miRNAs (miRs) that impact iNKT cell development could begin. The first miR, miR-223, to be published had no effect on iNKT cell population size or development when deleted despite being stage specifically regulated (K. Li et al., 2011a and b). miR150 also demonstrated stage specific regulation and mice lacking miR150 had a reduction in the thymic iNKT cell population, but not in the periphery. The thymic iNKT cell population was restrained in stage 2 of development with few cells progressing to stage 3 and displayed increased cell death (Zheng et al., 2012). The miR181 family includes 6 miRs. miR181a1 and miR181b1 are highly expressed in the thymus. They negatively regulate PTEN, a negative regulator of PI3K, and when removed, PI3K signaling is downregulated and metabolism is altered. Considering the sensitivity of developing iNKT cells to survival and metabolism cues, it is not surprising that iNKT cells were significantly reduced in a cell intrinsic manner due to decreased proliferation (Fig. 3). iNKT cell development was altered with few cells progressing to stage 3. Significantly, when miR181a1b1 and PTEN were simultaneously deleted, the thymic iNKT cell population was rescued (Henao-Mejia et al., 2013). Lethal 7 (Let-7) miRs are strongly expressed in thymocytes and cannot be processed by Dicer if they are bound by LIN28 proteins. Therefore, when LIN28 was expressed under the control of CD2, Let-7 miRs were not active. In this model, iNKT cells had increased Eomes, CD122, CD44, IL-4, and PLZF staining. Significantly, when PLZF was additionally deleted, IL-4 staining was reduced to wildtype control levels. iNKT cell intrinsic Let-7 transcription is induced by factors produced by the thymic stroma, including vitamin D, IL-15 and retinoic acid (Pobezinsky et al., 2015). Let-7 negatively regulates PLZF mRNA translation (Fig. 3), but does not alter the size of the iNKT cell population. For this reason, development was slightly altered in the absence of Let-7 activity with decreased progression to stage 3, resulting in increased NKT2 and NKT17 cells and decreased NKT1 cells (Pobezinsky et al., 2015). Three highly redundant miR families, miR-17 ~ 92, miR106a ~ 363, and miR106b ~ 25, were implicated in iNKT development. Germline ablation of miR106a ~ 363 and miR106b ~ 25 combined with CD4-Cre removal of miR-17 ~ 92 resulted in a triple knock out model that exhibited reduced iNKT cell populations with iNKT cells arrested in stage 2 of development. Additionally, NKT17 and NKT1 cells were reduced, and NKT2 cells increased when all three miR families were deleted (Fedeli et al., 2016). Thus, miRs contribute to iNKT cell survival signaling and subset differentiation. It will be interesting to see if long non-coding RNAs have a similar impact on iNKT cell survival and subset differentiation.

Roquin-1 and 2 are mRNA-binding proteins that have been shown to regulate gene expression post-transcriptionally in mature immune cells. Using CD4-Cre to selectively delete Roquin-1 and both Roquin-1 and 2 resulted in decreased iNKT cell populations with thymic iNKT cells restrained in stage 2 of development. Remarkably, this phenotype was not observed in mixed bone marrow chimeras indicating a cell extrinsic effect. Additionally, NKT17 cells were dramatically increased, while NKT1 subsets decreased in a cell intrinsic manner. Interestingly, all of these defects were ameliorated by forced Vα14 recombination at an early DP thymocyte stage (Drees et al., 2017).

3.3. SLAM/SAP/Fyn signaling

The surface protein signaling lymphocytic activation molecule (SLAM) signals through its adaptor protein, SLAM-associated protein (SAP), and a Src family kinase, Fyn. T cell function and development is strongly controlled by the Src family kinase, Lck. In mice lacking Lck, both T cell and iNKT cell populations were severely stunted. However, in the absence of Fyn, the T cell population appeared normal, while the iNKT cell population was significantly reduced (Gerard Eberl et al., 1999a and b). Bone marrow chimera experiments demonstrated that the reduction of iNKT cells in Fyn deficient mice was cell autonomous (Gadue et al., 1999). Transgenic Vα14Jα18 TCR expression completely restored iNKT cell populations in mice lacking Fyn, but only partial restoration was observed in Lck deficient animals, indicating that Fyn signaling provides an important additional positive signal, but can be replaced by enhanced TCR signaling (Gadue et al., 2004). Mice with a mutant SAP that cannot bind Fyn had a significant, cell intrinsic, reduction in iNKT cell numbers (Nunez-Cruz et al., 2008). Although iNKT cells were present in the absence of Fyn signaling, the population was significantly reduced, demonstrating the importance of this signaling component in iNKT cell development.

The initial characterizations of the role of SLAM and SAP in NK and T cell development were due to defects observed in individuals with X-linked lymphoproliferative disease (XLP), caused by a mutation in SAP. SAP deficient mice had a significant decrease in iNKT cells, but the population was reconstituted after bone marrow transfer from wildtype mice, indicating hematopoietic expression of SAP is crucial (Nichols et al., 2005; Pasquier et al., 2005). These results were confirmed in humans as well. SAP transduces signals for the surface receptor SLAM. The SLAM family encompasses 9 genes (Slamf1-9) and the resulting proteins typically form homotypic interactions with themselves or other SLAM molecules in some cases. An extensive series of simple, competitive, and mixed bone marrow chimera experiments showed that genetic ablation of Slamf1 or Slamf6 moderately impaired iNKT cell development in a redundant manner during the transition from stage 0 to stage 1, but deletion of Slamf2 and Slamf4 did not (Griewank et al., 2007). Interestingly, removal of SAP and Slamf6 partially rescued the iNKT cell population compared to animals lacking only SAP, except in the spleen, highlighting the potential negative signaling effects of SLAM family members (Kageyama et al., 2012). Other SLAM family members, Slamf3, Slamf7, and Slamf8 – with both Slamf7 and Slamf8 functioning independent of SAP - have inhibitory signaling effects as demonstrated by increased iNKT cell numbers in mice deficient in a single SLAM member (De Calisto et al., 2014; Sintes et al., 2013). Mice deficient in two (Slamf1,6) and three (Slamf1,5,6) SLAM family members had severely impaired iNKT cell development, although the loss of Slamf5 did not significantly reduce the population, compared to the loss of Slamf1 and 6 (De Calisto et al., 2014; Huang et al., 2016). Similarly, in Slamf5 deficient mice, there was not a statistically significant reduction in iNKT cells in the thymus or spleen (De Calisto et al., 2014). Genetic deletion of Slamf1-7 revealed greatly reduced iNKT cell populations, characterized by decreased PLZF, and alterations in the ratio of iNKT cells in stages 1, 2 and 3 of development (Chen et al., 2017). In summary, positive SAP signaling is crucial for iNKT cell development and various SLAM family members (summarized in Table 2) exert positive and negative signaling during iNKT cell development.

Although most studies focus on iNKT cell development in the presence or absence of SLAM/SAP signaling, few examine the function of the residual iNKT cell populations. In mice with mutant SAP that cannot bind Fyn, iNKT cell cytokine production appeared normal – suggesting that SAP signaling for iNKT differentiation is not through Fyn (Nunez-Cruz et al., 2008). In SAP deficient mice expressing an iNKT TCR transgene, the iNKT cell population had lower PLZF expression and was skewed with increased NKT17 and fewer NKT2 cells, and concomitant changes in RORγt and Gata3 (Michel et al., 2016). In Slamf1-7 deficient animals, the NKT1 cell population was greatly reduced while the NKT17 population was increased. These mice also had an increased percentage of NKT2 cells but a lower absolute number (Chen et al., 2017). These studies agree that in the absence of SLAM signaling through SAP, there is an overall increased in the proportion of NKT17 cells.

Table 2
SLAM family members in NKT cell development.

SLAM Family Member	Knockout NKT population effects	Natural Signaling is
Slamf1	↓	Stimulatory
Slamf2	–	No effect
Slamf3	↑	Inhibitory
Slamf4	–	No effect
Slamf5	–	No effect
Slamf6	↓	Stimulatory
Slamf7	↑	Inhibitory
Slamf8	↑	Inhibitory

3.4. Proliferation, survival, and metabolism

Two pathways implicated in survival signaling are c-myc and NF-κB. C-myc is a tumor suppressor protein known to be involved in the balance between survival, proliferation, and apoptosis. Genetic deletion of c-myc is embryonically lethal, but its effects on iNKT cell development can be studied using conditional deletion models. Studies utilizing the CD4-Cre system to delete c-myc observed a significant reduction in iNKT cells, with the remaining iNKT cells arrested in stage 1 of development. Notably, this population was also significantly reduced (Dose et al., 2009; Mycko et al., 2009). This defect was not rescued with either Bcl-2 overexpression or inactivation of p21, which eliminates the apoptotic and survival signaling implicated by c-myc loss (Dose et al., 2009; Mycko et al., 2009). Instead, c-myc ablation prevented the proliferative burst that usually occurs immediately following positive selection (Dose et al., 2009). The effects of c-myc on differentiation are controversial as one study shows that the residual iNKT cell population expressed increased PLZF and IL-4 (Mycko et al., 2009), while another group did not detect increased PLZF or IL-4 (Dose et al., 2009). Collectively, these studies demonstrate that c-Myc is an essential driver of iNKT cell expansion, which is necessary for development (Table 3, Fig. 3).

NF-κB signaling is known to be particularly important in immune cell survival and response. Deletion or mutations in nfkb1 and IκBα demonstrated cell intrinsic blocks in iNKT cell development, as shown by IκBα-mutant iNKT cells arrested in stage 2 (Sivakumar et al., 2003; A. K. Stanic et al., 2004a, Stanic et al., 2004b). Mutations in IκBα resulted in increased programmed cell death and iNKT cell populations were rescued by Bcl-xL overexpression, indicating NF-κB provides crucial survival signals (A K Stanic et al., 2004a Stanic et al., 2004b). In the absence of NF-κB signaling, death signals through tumor necrosis factor receptor 1 (TNFR1) contributed to iNKT cell death as evidenced by restoration of iNKT cell populations in mice with mutant IκBα and genetic ablation of TNFR1 (Kumar et al., 2017). Another NF-κB family member, RelA, affected iNKT cell development when it was conditionally deleted in CD4+ cells. RelA loss caused significant population reduction, arrest in stage 2, and decreased proliferation in the presence of IL-15 and IL-7, due to decreased expression of their receptors (Vallabhapurapu et al., 2008). Conversely, RelB expression was required in thymic stromal cells for iNKT cell development because it induced IL-15 production (Elewaut et al., 2003b; Sivakumar et al., 2003). NF-κB is negatively regulated by the deubiquitinating enzyme, A20. Upon CD4-Cre mediated A20 deletion, iNKT cell populations were reduced in a cell intrinsic manner. Although the percentage of iNKT cells in each stage were similar, the absolute number of iNKT cells in stage 3 were significantly reduced. In addition, NKT1 cells were reduced in A20 knock out mice (Drennan et al., 2016). Taken together these studies indicate NF-κB provides essential survival signals during iNKT cell development (Table 3, Fig. 3).

In mature conventional T cells, TCR activation of Lck/Fyn/Zap70 result in activation of several pathways, including PI3K and Tec family kinases. PI3K induces phospholipase C (PLC) production of diacylglycerol (DAG), which then activates PKCθ and the Ras/Erk pathway. PKCθ induces the activity of NF-κB. PKCθ deficient mice also had reduced iNKT cell populations with increased cells in stage 1 and 2 of development but fewer in stage 3 (A. K. Stanic et al., 2004a, Stanic et al., 2004b). Transgenic overexpression of Bcl-xL did not rescue the iNKT cell population in mice lacking PKCθ (Kumar et al., 2017). DAG kinase α and ζ are negative feedback loops to control TCR signaling. While ablation of one DAG kinase did not have a phenotype, the loss of both DAG kinases resulted in a cell intrinsic, significant decrease in iNKT cells with arrest in stage 2. This defect was in part due to increased NF-κB and Ras/Erk pathway signaling, because moderate changes in iNKT cell development were present when Ras or IKK were permanently activated (Shen et al., 2011b). Activation of the Ras/Erk pathway is initiated by DAG activation of Ras-GRP1, which activates

Table 3
Cell survival and metabolism in NKT cell development.

	Change	Population Effect	Maturation	Cause	Differentiation
c-Myc	KO	↓ Intrinsic	Stage 1 Arrest	↓ Proliferation	Potential NKT2 changes
RelA/NF-κB1/mIκBα	KO or mutation	↓ Intrinsic	Stage 2 Arrest	↑ Apoptosis, ↓ Proliferation	
RelB	KO	↓ Extrinsic		↓ IL-15 production	
A20	KO	↓ Intrinsic	Decreased Stage 3		Decreased NKT1
PKCθ	KO	↓ Intrinsic	Stage 2 Arrest		
DAG Kinase α/ζ	KO	↓ Intrinsic	Stage 2 Arrest	Increased NF-κB and Ras/Erk	
Ras-GRP1	KO	↓ Intrinsic	Decreased Stage 3	↑ Apoptosis	Decreased CD4 + NKT cells
Itk/Rlk	KO	↓ Intrinsic	Decreased Stage 3		
DOCK2/8	KO	↓ Intrinsic	Decreased Stage 3		
Fnip1	KO	↓ Intrinsic	Stage 2 Arrest	↑ Apoptosis, Metabolism changes	
Atg5/7	KO	↓ Intrinsic	Stage 1 or 2 Arrest	↑ Apoptosis	
SHIP-1	KO	↓ Extrinsic		↓ Proliferation	
mTORC1	KO	↓ Intrinsic	Stage 1 Arrest		Decreased NKT1
mTORC2	KO	↓ Intrinsic	Mostly Normal	↑ Apoptosis, ↓ Proliferation	Decreased NKT17
PDK1	KO or mutation	↓ Intrinsic	Stage 1 Arrest		

Ras. Loss of Ras-GRP1 significantly reduced iNKT cell populations, specifically there was an increase in stage 1 and 2 proportions and a decrease in stage 3. This defect was cell intrinsic and resulted from increased cell death. Interestingly, CD4+ iNKT cells were affected more prominently than DN iNKT cells (Shen et al., 2011a). In absence of RASAL3, a negative regulator of Ras, iNKT cell numbers were moderately reduced only in the liver, and development appeared normal (Saito et al., 2015).

There are three Tec family kinases: Itk, Rlk and Tec. The loss of Rlk did not significantly impact iNKT cell populations, but Itk deficiency and loss of both Rlk and Itk resulted in decreased iNKT cell populations with decreased progression to stage 3 of development (Felices and Berg, 2008). While overexpression of Rlk in Itk deficient mice restored the percentage and maturation of iNKT cells in the thymus, the absolute number of iNKT cells was not restored to wildtype control levels (Qi et al., 2012). Deletion of DOCK8, a guanine nucleotide exchange factor, resulted in decreased thymic iNKT cell populations and mature iNKT cells in a cell intrinsic manner (Crawford et al., 2013). Similarly, DOCK2 deletion resulted in a cell intrinsic decrease in iNKT cell populations with fewer progressing to stage 3 of development (Kunisaki et al., 2006). These studies reinforce the notion that the balance of survival signals plays an important role in iNKT cell development (Table 3, Fig. 3).

Proliferation requires energy and consumes many cellular resources. Cellular metabolism is controlled by two opposing pathways: mTOR and AMPK. TCR stimulation induces PI3K to activate mTOR via Akt signaling. Conversely, AMPK inhibits mTOR and activates autophagy to make energy and compile resources. Fnip1 is an adaptor molecule for AMPK. In the absence of Fnip1, iNKT cell populations were reduced in a cell autonomous manner and development was arrested at stage 2. This defect was characterized by increased sensitivity to apoptosis, altered mitochondrial homeostasis, increased mTOR activity, and decreased ATP levels (Park et al., 2014). Investigation into the role of autophagy by ablation of Atg7 via CD4-Cre, resulted in a cell intrinsic decrease of iNKT cell populations with the majority restrained in stage 2 of development. The residual iNKT cell population expressed lower levels of Bcl2, Egr2, and PLZF and were more susceptible to apoptosis (Salio et al., 2014). Another study, comparing Atg5 and Atg7 ablation utilizing Lck-Cre and CD4-Cre, observed reduced iNKT cell populations under all conditions. iNKT cell numbers were normal in stage 0 and 1 in mice lacking both, but Atg5 ablation resulted in decreased stage 2 and 3, while loss of Atg7 resulted in decreased stage 3 iNKT cells. Atg5 ablation was shown to be cell intrinsic with iNKT cells arrested in S phase and increased mitochondrial superoxide, resulting in increased susceptibility to apoptosis (Pei et al., 2015). The reallocation of resources by AMPK and autophagy are necessary for iNKT cell development (Table 3, Fig. 3).

mTOR signaling has also been shown to have an impact on iNKT cell development through both of its complexes, mTORC1 and mTORC2. To probe mTORC1 signaling, another key component of the complex, Raptor, was selectively ablated using a CD4-Cre system. The iNKT cell population was significantly reduced in a cell autonomous manner while conventional T cell development appeared normal. iNKT cells were primarily blocked in stage 1 of development with few cells progressing to stage 2 or 3 (Zhang et al., 2014). These results were confirmed by showing decreased NKT1 cell differentiation in mice lacking Raptor (Wei et al., 2014). Three studies have assessed the effects of mTORC2 signaling by removal of Rictor, an mTORC2 component, using CD4-Cre. Rictor deficient mice had moderately decreased iNKT cell populations. Although iNKT cell development was nearly normal, with a slight increase in the percentage of stage 1 cells and decrease in the percentage of stage 2 cells (Prevot et al., 2015; Sklarz et al., 2017; Wei et al., 2014). Prevot et al. demonstrated that this defect was cell intrinsic. While Wei et al. contributed the reduced cell number to increased susceptibility to cell death as evidenced by increased caspase activity and normal proliferation demonstrated by Ki67 and BrdU staining, Prevot et al. demonstrated decreased proliferation of stage 1 using Ki67 staining and normal apoptosis demonstrated by annexin V/7-AAD staining. Sklarz et al., 2018 showed decreased proliferation using Ki67 staining and BrdU incorporation and increased cell death demonstrated by increased caspase staining of stage 1 iNKT cells. Both groups observed a decrease in NKT17 cells, as demonstrated by decreased IL-17 A and RORγt in the absence of Rictor. Wei et al. rescued NKT17 cell production by breeding Rictor deficient animals with mice lacking PTEN, a negative regulator of Rictor. Additionally, Sklarz et al. showed decreased proliferation and cytotoxicity after stimulation. Conversely, Prevot et al. found a decrease in NKT2 cells, as demonstrated by decreased IL-4 production and Gata3 staining in the thymus. Sklarz et al., 2018 partially recapitulated this data by showing decreased IL-4 expression in the thymus, although IL-4 expression was increased in the lung and Gata3 staining was normal. As previously mentioned, mTOR activation can occur via PI3K induction of PDK-mediated activation of Akt. Conditional deletion of PDK1 using CD4-Cre resulted in a reduction in the iNKT cell population with the majority of cells restrained in stage 1 of development due to a failure to upregulate nutrient receptors needed to maintain growth (Finlay et al., 2010). This data was confirmed in a model that deleted only the catalytic domain of PDK1. SHIP-1 is a negative regulator of PI3K signaling. In SHIP-1 deficient mice, iNKT cell populations were decreased due to an extrinsic effect that resulted in decreased iNKT cell proliferation (Anderson et al., 2015). In summary, metabolism is finely tuned in iNKT cell development and skewing either direction can perturb both development and differentiation (Table 3, Fig. 3).

4. Discussion

The minutia of iNKT cell development weave a tangled web. Each discovery and new piece of information links formerly disparate ideas together until a picture forms. Similar to the fine line they walk between being adaptive and innate immune cells, iNKT cell development requires an exquisitely balanced sequence of events. They are essentially activated, effector cells at the end of development. Therefore they require the basic T cell development cues as well as T cell activation and effector activation components during development. Removal of any single component tips the scale and disrupts iNKT cell development, resulting in cell death, cell cycle arrest, decreased proliferation, or decreased maturation.

Within the past five years, techniques for iNKT cell characterization have improved by leaps and bounds. The use of increasing numbers of fluorophores in flow cytometry, single cell RNA-seq, and CHiP-seq have impacted the characterization of iNKT cell subset. Not only does their gene expression profile more closely mirror innate lymphocytes than conventional T helper cells, new subsets have been recognized in the thymus with identification of NKT0 or NKTp cells and further division of NKT2 cells (Engel et al., 2016; Lee et al., 2016). Applying these state of the art techniques to pathways and genes that have already been shown to influence iNKT cell development will help us determine how all of the components fit together to form a complete picture of iNKT cell development.

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References

Anderson, C.K., Salter, A.I., Toussaint, L.E., Reilly, E.C., Fugère, C., Srivastava, N., Kerr, W.G., Brossay, L., 2015. Role of SHIP1 in invariant NKT cell development and functions. J. Immunol. 195, 2149–2156. https://doi.org/10.4049/jimmunol.1500567.

Arase, H., Arase, N., Ogasawara, K., Good, R.A., Onoé, K., 1992. An NK1.1+ CD4+8- single-positive thymocyte subpopulation that expresses a highly skewed T-cell antigen receptor Vβ family. Proc. Natl. Acad. Sci. U. S. A. 89, 6506–6510. https://doi.org/10.1073/pnas.89.14.6506.

Baur, N., Nerz, G., Nil, A., Eichmann, K., 2001. Expression and selection of productively rearranged TCRβ VDJ genes are sequentially regulated by CD3 signaling in the development of NK1.1+ αβ T cells. Int. Immunol. 13, 1031–1042. https://doi.org/10.1093/intimm/13.8.1031.

Becker, A.M., Blevins, J.S., Tomson, F.L., Eitson, J.L., Medeiros, J.J., Yarovinsky, F., Norgard, M.V., van Oers, N.S.C., 2010. Invariant NKT cell development requires a full complement of functional CD3 ζ immunoreceptor tyrosine-based activation motifs. J. Immunol. 184, 6822–6832. https://doi.org/10.4049/jimmunol.0902058. (Baltimore, Md 1950).

Bedel, R., Berry, R., Mallevaey, T., Matsuda, J.L., Zhang, J., Godfrey, D.I., Rossjohn, J., Kappler, J.W., Marrack, P., Gapin, L., 2014. Effective functional maturation of invariant natural killer T cells is constrained by negative selection and T-cell antigen receptor affinity. Proc. Natl. Acad. Sci. U. S. A. 111, E119–28. https://doi.org/10.1073/pnas.1320777110.

Behar, S.M., Podrebarac, T.A., Roy, C.J., Wang, C.R., Brenner, M.B., 1999. Diverse TCRs recognize murine CD1. J. Immunol. 162, 161–167.

Bendelac, A., 1995. Positive selection of mouse NK1+ T Cells by CD1-expressing cortical thymocytes. J Exp Med 182 2091–2096. https://doi.org/10.1084/jem.182.6.2091.

Bendelac, A., Killeen, N., Littman, D.R., Schwartz, R.H., 1994. A subset of CD4+ thymocytes selected by MHC class I molecules. Science 263, 1774–1778. https://doi.org/10.1126/science.7907820.

Bendelac, A., Lantz, O., Quimby, M.E., Yewdell, J.W., Bennink, J.R., Brutkiewicz, R.R., 1995. CD1 recognition by mouse NK1+ T lymphocytes. Science 268, 863–865. https://doi.org/10.1126/science.7538697.

Bendelac, A., Hunziker, R.D., Lantz, O., 1996. Increased interleukin 4 and immunoglobulin E production in transgenic mice overexpressing NK1 T cells. J. Exp. Med. 184, 1285–1293. https://doi.org/10.1084/jem.184.4.1285.

Benlagha, K., Kyin, T., Beavis, A., Teyton, L., Bendelac, A., 2002. A thymic precursor to the NK T cell lineage. Science 296, 553–555. https://doi.org/10.1126/science.1069017.

Benlagha, K., Wei, D.G., Veiga, J., Teyton, L., Bendelac, A., 2005. Characterization of the early stages of thymic NKT cell development. J. Exp. Med. 202, 485–492. https://doi.org/10.1084/jem.20050456.

Berga-Bolaños, R., Zhu, W.S., Steinke, F.C., Xue, H.H., Sen, J.M., 2015. Cell-autonomous requirement for TCF1 and LEF1 in the development of natural killer T cells. Mol. Immunol. 68, 484–489. https://doi.org/10.1016/j.molimm.2015.09.017.

Berzins, S.P., Cochrane, A.D., Pellicci, D.G., Smyth, M.J., Godfrey, D.I., 2005. Limited correlation between human thymus and blood NKT cell content revealed by an ontogeny study of paired tissue samples. Eur. J. Immunol. 35, 1399–1407. https://doi.org/10.1002/eji.200425958.

Bezbradica, J.S., Hill, T., Stanic, A.K., Kaer, L.Van, Joyce, S., Van Kaer, L., Joyce, S., 2005. Commitment toward the natural T (iNKT) cell lineage occurs at the CD4+8+ stage of thymic ontogeny. Proc. Natl. Acad. Sci. U. S. A. 102, 5114–5119. https://doi.org/10.1073/pnas.0408449102.

Bix, M., Coles, M., Raulet, D., 1993. Positive selection of Vβ8+ CD4-8- thymocytes by class I molecules expressed by hematopoietic cells. J. Exp. Med. 178, 901–908. https://doi.org/10.1084/jem.178.3.901.

Borg, N.A., Wun, K.S., Kjer-Nielsen, L., Wilce, M.C.J., Pellicci, D.G., Koh, R., Besra, G.S., Bharadwaj, M., Godfrey, D.I., McCluskey, J., Rossjohn, J., 2007. CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. Nature 448, 44–49. https://doi.org/10.1038/nature05907.

Brossay, L., Jullien, D., Cardell, S., Sydora, B.C., Burdin, N., Modlin, R.L., Kronenberg, M., 1997. Mouse CD1 is mainly expressed on hemopoietic-derived cells. J. Immunol. 159, 1216–1224.

Cameron, G., Godfrey, D.I., 2018. Differential surface phenotype and context-dependent reactivity of functionally diverse NKT cells. Immunol. Cell Biol. https://doi.org/10.1111/imcb.12034.

Canchis, P.W., Bhan, a K., Landau, S.B., Yang, L., Balk, S.P., Blumberg, R.S., 1993. Tissue distribution of the non-polymorphic major histocompatibility complex class I-like molecule. CD1d. Immunol. 80, 561–565.

Cardell, S., Tangri, S., Chan, S., Benoist, C., Mathis, D., 1995. CD1-restricted CD4+ T Cells in major histocompatibility complex class II-deficient mice. J. Exp. Med. 182, 993–1004. https://doi.org/10.1084/jem.182.4.993.

Castillo, E.F., Acero, L.F., Stonier, S.W., Zhou, D., Schluns, K.S., 2010. Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation. Blood 116, 2494–2503. https://doi.org/10.1182/blood-2010-03-277103.

Cernadas, M., Sugita, M., van der Wel, N., Cao, X., Gumperz, J.E., Maltsev, S., Besra, G.S., Behar, S.M., Peters, P.J., Brenner, M.B., 2003. Lysosomal localization of murine CD1d mediated by AP-3 is necessary for NK T cell development. J. Immunol. 171, 4149–4155. https://doi.org/10.4049/jimmunol.171.8.4149.

Chang, C.-L., Lai, Y.-G., Hou, M.-S., Huang, P.-L., Liao, N.-S., 2011. IL-15Rα of radiation-resistant cells is necessary and sufficient for thymic invariant NKT cell survival and functional maturation. J. Immunol. 187, 1235–1242. https://doi.org/10.4049/jimmunol.1100270.

Chen, S., Cai, C., Li, Z., Liu, G., Wang, Y., Blonska, M., Li, D., Du, J., Lin, X., Yang, M., Dong, Z., 2017. Dissection of SAP-dependent and SAP-independent SLAM family signaling in NKT cell development and humoral immunity. J. Exp. Med. 214, 475–489. https://doi.org/10.1084/jem.20161312.

Chiu, Y.-H., Park, S.-H., Benlagha, K., Forestier, C., Jayawardena-Wolf, J., Savage, P.B., Teyton, L., Bendelac, A., 2002. Multiple defects in antigen presentation and T cell development by mice expressing cytoplasmic tail-truncated CD1d. Nat. Immunol. 3, 55–60. https://doi.org/10.1038/ni740.

Choi, H.-J., Geng, Y., Cho, H., Li, S., Giri, P.K., Felio, K., Wang, C.-R., 2011. Differential requirements for the Ets transcription factor Elf-1 in the development of NKT cells and NK cells. Blood 117, 1880–1887. https://doi.org/10.1182/blood-2010-09-309468.

Christiansen, D., Milland, J., Mouhtouris, E., Vaughan, H., Pellicci, D.G., McConville, M.J., Godfrey, D.I., Sandrin, M.S., 2008. Humans lack iGb3 due to the absence of functional iGb3-synthase: implications for NKT cell development and transplantation. PLoS Biol. 6, e172. https://doi.org/10.1371/journal.pbio.0060172.

Chun, T., Page, M.J., Gapin, L., Matsuda, J.L., Xu, H., Nguyen, H., Kang, H.-S., Stanic, A.K., Joyce, S., Koltun, W.A., Chorney, M.J., Kronenberg, M., Wang, C.-R., 2003. CD1d-expressing dendritic cells but not thymic epithelial cells can mediate negative selection of NKT cells. J. Exp. Med. 197, 907–918. https://doi.org/10.1084/jem.20021366.

Coles, B.M.C., Raulet, D.H., 1994. Class I dependence of the development of CD4+ CD8- NK1.1+ thymocytes. J. Exp. Med. 180, 395–399. https://doi.org/10.1084/jem.180.1.395.

Coles, M.C., Raulet, D.H., 2000. NK1.1+ T cells in the liver arise in the thymus and are selected by interactions with class I molecules on CD4+CD8+ cells. J. Immunol. 164, 2412–2418. https://doi.org/10.4049/jimmunol.164.5.2412.

Coquet, J.M., Chakravarti, S., Kyparissoudis, K., McNab, F.W., Pitt, L.A., McKenzie, B.S., Berzins, S.P., Smyth, M.J., Godfrey, D.I., 2008. Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. Proc. Natl. Acad. Sci. U. S. A. 105, 11287–11292. https://doi.org/10.1073/pnas.0801631105.

Cowan, J.E., McCarthy, N.I., Parnell, S.M., White, A.J., Bacon, A., Serge, A., Irla, M., Lane, P.J.L., Jenkinson, E.J., Jenkinson, W.E., Anderson, G., 2014. Differential requirement for CCR4 and CCR7 during the development of innate and adaptive αβT cells in the adult thymus. J. Immunol. 193, 1204–1212. https://doi.org/10.4049/jimmunol.1400993.

Crawford, G., Enders, A., Gileadi, U., Stankovic, S., Zhang, Q., Lambe, T., Crockford, T.L., Lockstone, H.E., Freeman, A., Arkwright, P.D., Smart, J.M., Ma, C.S., Tangye, S.G., Goodnow, C.C., Cerundolo, V., Godfrey, D.I., Su, H.C., Randall, K.L., Cornall, R.J., 2013. DOCK8 is critical for the survival and function of NKT cells. Blood 122, 2052–2061. https://doi.org/10.1182/blood-2013-02-482331.

Cruz Tleugabulova, M., Escalante, N.K., Deng, S., Fieve, S., Ereno-Orbea, J., Savage, P.B., Julien, J.-P., Mallevaey, T., 2016. Discrete TCR binding kinetics control invariant NKT cell selection and central priming. J. Immunol. 197, 3959–3969. https://doi.org/10.4049/jimmunol.1601382.

D'Cruz, L.M., Stradner, M.H., Yang, C.Y., Goldrath, A.W., 2014. E and Id proteins influence invariant NKT cell sublineage differentiation and proliferation. J. Immunol. 192, 2227–2236. https://doi.org/10.4049/JIMMUNOL.1302904.

Dashtsoodol, N., Watarai, H., Sakata, S., Taniguchi, M., 2008. Identification of CD4 − CD8 − double-negative natural killer T cell precursors in the thymus. PLoS One 3, e3688. https://doi.org/10.1371/journal.pone.0003688.

Dashtsoodol, N., Shigeura, T., Aihara, M., Ozawa, R., Kojo, S., Harada, M., Endo, T.A., Watanabe, T., Ohara, O., Taniguchi, M., 2017. Alternative pathway for the development of Vα14 + NKT cells directly from CD4–CD8– thymocytes that bypasses the CD4 + CD8 + stage. Nat. Immunol. 18, 274–282. https://doi.org/10.1038/ni.3668.

De Calisto, J., Wang, N., Wang, G., Yigit, B., Engel, P., Terhorst, C., 2014. SAP-dependent and -independent regulation of innate T cell development involving SLAMF receptors. Front. Immunol. 5, 186. https://doi.org/10.3389/fimmu.2014.00186.

Dellabona, P., Padovan, E., Casorati, G., Brockhaus, M., Lanzavecchia, A., 1994. An invariant Vα24-JαQ/Vβ11 T cell receptor is expressed in all individuals by clonally expanded CD4-8- T cells. J. Exp. Med. 180, 1171–1176. https://doi.org/10.1084/jem.180.3.1171.

Dickgreber, N., Farrand, K.J., van Panhuys, N., Knight, D.A., McKee, S.J., Chong, M.L., Miranda-Hernandez, S., Baxter, A.G., Locksley, R.M., Le Gros, G., Hermans, I.F., 2012. Immature murine NKT cells pass through a stage of developmentally programmed innate IL-4 secretion. J. Leukoc. Biol. 92, 999–1009. https://doi.org/10.1189/jlb.0512242.

Dose, M., Sleckman, B.P., Han, J., Bredemeyer, A.L., Bendelac, A., Gounari, F., 2009. Intrathymic proliferation wave essential for Vα14 + natural killer T cell development depends on c-Myc. Proc. Natl. Acad. Sci. U. S. A. 106, 8641–8646. https://doi.org/10.1073/pnas.0812255106.

Dougan, S.K., Rava, P., Hussain, M.M., Blumberg, R.S., 2007. MTP regulated by an alternate promoter is essential for NKT cell development. J. Exp. Med. 204, 533–545. https://doi.org/10.1084/jem.20062006.

Drees, C., Vahl, J.C., Bortoluzzi, S., Heger, K.D., Fischer, J.C., Wunderlich, F.T., Peschel, C., Schmidt-Supprian, M., 2017. Roquin paralogs differentially regulate functional NKT cell subsets. J. Immunol. 198, 2747–2759. https://doi.org/10.4049/jimmunol.1601732.

Drennan, M.B., Govindarajan, S., Verheugen, E., Coquet, J.M., Staal, J., McGuire, C., Taghon, T., Leclercq, G., Beyaert, R., van Loo, G., Lambrecht, B.N., Elewaut, D., 2016. NKT sublineage specification and survival requires the ubiquitin-modifying enzyme TNFAIP3/A20. J. Exp. Med. 213, 1973–1981. https://doi.org/10.1084/jem.20151065.

Eberl, G., Fehling, H.J., von Boehmer, H., MacDonald, H.R., 1999a. Absolute requirement for the pre-T cell receptor α chain during NK1.1 + TCRαβ cell development. Eur. J. Immunol. 29, 1966–1971. https://doi.org/10.1002/(SICI)1521-4141(199906)29:06<1966::AID-IMMU1966>3.0.CO;2-G.

Eberl, G., Lowin-Kropf, B., MacDonald, H.R., 1999b. Cutting edge: NKT cell development is selectively impaired in Fyn-deficient mice. J. Immunol. 163, 4091–4094.

Egawa, T., Eberl, G., Taniuchi, I., Benlagha, K., Geissmann, F., Hennighausen, L., Bendelac, A., Littman, D.R., 2005. Genetic evidence supporting selection of the Vα14i NKT cell lineage from double-positive thymocyte precursors. Immunity 22, 705–716. https://doi.org/10.1016/j.immuni.2005.03.011.

Eidson, M., Wahlstrom, J., Beaulieu, A.M., Zaidi, B., Carsons, S.E., Crow, P.K., Yuan, J., Wolchok, J.D., Horsthemke, B., Wieczorek, D., Sant'Angelo, D.B., 2011. Altered development of NKT cells, γδ T cells, CD8 T cells and NK cells in a PLZF deficient patient. PLoS One 6, e24441. https://doi.org/10.1371/journal.pone.0024441.

Elewaut, D., Brossay, L., Santee, S.M., Naidenko, O.V., Burdin, N., De Winter, H., Matsuda, J., Ware, C.F., Cheroutre, H., Kronenberg, M., 2000. Membrane lymphotoxin is required for the development of different subpopulations of NK T cells. J. Immunol. 165, 671–679. https://doi.org/10.4049/jimmunol.165.2.671.

Elewaut, D., Lawton, A.P., Nagarajan, N.A., Maverakis, E., Khurana, A., Honing, S., Benedict, C.A., Sercarz, E., Bakke, O., Kronenberg, M., Prigozy, T.I., 2003a. The adaptor protein AP-3 is required for CD1d-mediated antigen presentation of glycosphingolipids and development of Vα14i NKT cells. J. Exp. Med. 198, 1133–1146. https://doi.org/10.1084/jem.20030143.

Elewaut, D., Shaikh, R.B., Hammond, K.J.L., De Winter, H., Leishman, A.J., Sidobre, S., Turovskaya, O., Prigozy, T.I., Ma, L., Banks, T.A., Lo, D., Ware, C.F., Cheroutre, H., Kronenberg, M., 2003b. NIK-dependent RelB activation defines a unique signaling pathway for the development of Vα14i NKT cells. J. Exp. Med. 197, 1623–1633. https://doi.org/10.1084/jem.20030141.

Enders, A., Stankovic, S., Teh, C., Uldrich, A.P., Yabas, M., Juelich, T., Altin, J.A., Frankenreiter, S., Bergmann, H., Roots, C.M., Kyparissoudis, K., Goodnow, C.C., Godfrey, D.I., 2012. ZBTB7B (Th-POK) regulates the development of IL-17-producing CD1d-restricted mouse NKT cells. J. Immunol. 189, 5240–5249. https://doi.org/10.4049/jimmunol.1201486.

Engel, I., Hammond, K., Sullivan, B.A., He, X., Taniuchi, I., Kappes, D., Kronenberg, M., 2010. Co-receptor choice by Vα14i NKT cells is driven by Th-POK expression rather than avoidance of CD8-mediated negative selection. J. Exp. Med. 207, 1015–1029. https://doi.org/10.1084/jem.20090557.

Engel, I., Seumois, G., Chavez, L., Samaniego-Castruita, D., White, B., Chawla, A., Mock, D., Vijayanand, P., Kronenberg, M., 2016. Innate-like functions of natural killer T cell subsets result from highly divergent gene programs. Nat. Immunol. 17, 728–739. https://doi.org/10.1038/ni.3437.

Exley, M., Garcia, J., Balk, S.P., Porcelli, S., 1997. Requirements for CD1d recognition by human invariant Vα24 + CD4-CD8- T cells. J. Exp. Med. 186, 109–120. https://doi.org/10.1084/jem.186.1.109.

Fedeli, M., Napolitano, A., Wong, M.P., Marcais, A., de Lalla, C., Colucci, F., Merkenschlager, M., Dellabona, P., Casorati, G., 2009. Dicer-dependent microRNA pathway controls invariant NKT cell development. J. Immunol. 183, 2506–2512. https://doi.org/10.4049/jimmunol.0901361.

Fedeli, M., Riba, M., Garcia Manteiga, J.M., Tian, L., Viganò, V., Rossetti, G., Pagani, M., Xiao, C., Liston, A., Stupka, E., Cittaro, D., Abrignani, S., Provero, P., Dellabona, P., Casorati, G., 2016. miR-17 ~ 92 family clusters control iNKT cell ontogenesis via modulation of TGF-β signaling. Proc. Natl. Acad. Sci. U. S. A. 113, E8286–E8295. https://doi.org/10.1073/pnas.1612024114.

Felices, M., Berg, L.J., 2008. The Tec kinases Itk and Rlk regulate NKT cell maturation, cytokine production, and survival. J. Immunol. 180, 3007–3018. https://doi.org/10.4049/JIMMUNOL.180.5.3007.

Finlay, D.K., Kelly, A.P., Clarke, R., Sinclair, L.V., Deak, M., Alessi, D.R., Cantrell, D.A., 2010. Temporal differences in the dependency on phosphoinositide-dependent kinase 1 distinguish the development of invariant Vα14 NKT cells and conventional T cells. J. Immunol. 185, 5973–5982. https://doi.org/10.4049/jimmunol.1000827.

Föhse, L., Reinhardt, A., Oberdörfer, L., Schmitz, S., Förster, R., Malissen, B., Prinz, I., 2013. Differential postselection proliferation dynamics of αβ T cells, Foxp3 + regulatory T cells, and invariant NKT cells monitored by genetic pulse labeling. J. Immunol. 191, 2384–2392. https://doi.org/10.4049/jimmunol.1301359.

Fowlkes, B.J., Kruisbeek, a M., Ton-That, H., Weston, M., Coligan, J.E., Schwartz, R.H., Pardoll, D.M., 1987. A novel population of T-cell receptor αβ-bearing thymocytes which predominantly expresses a single Vβ gene family. Nature 329, 251–254. https://doi.org/10.1038/329251a0.

Franki, A.S., Van Beneden, K., Dewint, P., Hammond, K.J.L., Lambrecht, S., Leclercq, G., Kronenberg, M., Deforce, D., Elewaut, D., 2006. A unique lymphotoxin αβ-dependent pathway regulates thymic emigration of Vα14 invariant natural killer T cells. Proc. Natl. Acad. Sci. U. S. A. 103, 9160–9165. https://doi.org/10.1073/pnas.0508892103.

Gadola, S.D., Silk, J.D., Jeans, A., Illarionov, P.A., Salio, M., Besra, G.S., Dwek, R., Butters, T.D., Platt, F.M., Cerundolo, V., 2006. Impaired selection of invariant natural killer T cells in diverse mouse models of glycosphingolipid lysosomal storage diseases. J. Exp. Med. 203, 2293–2303. https://doi.org/10.1084/jem.20060921.

Gadue, P., Morton, N., Stein, P.L., 1999. The Src family tyrosine kinase Fyn regulates natural killer T cell development. J. Exp. Med. 190, 1189–1196. https://doi.org/10.1084/jem.190.8.1189.

Gadue, P., Yin, L., Jain, S., Stein, P.L., 2004. Restoration of NK T cell development in fyn-mutant mice by a TCR reveals a requirement for Fyn during early NK T cell ontogeny. J. Immunol. 172, 6093–6100. https://doi.org/10.4049/jimmunol.172.10.6093.

Gapin, L., 2016. Development of invariant natural killer T cells. Curr. Opin. Immunol. 39, 68–74. https://doi.org/10.1016/j.coi.2016.01.001.

Gapin, L., Matsuda, J.L., Surh, C.D., Kronenberg, M., 2001. NKT cells derive from double-positive thymocytes that are positively selected by CD1d. Nat. Immunol. 2, 971–978. https://doi.org/10.1038/ni710.

Gordy, L.E., Bezbradica, J.S., Flyak, A.I., Spencer, C.T., Dunkle, A., Sun, J., Stanic, A.K., Boothby, M.R., He, Y.-W., Zhao, Z., Van Kaer, L., Joyce, S., 2011. IL-15 regulates homeostasis and terminal maturation of NKT cells. J. Immunol. 187, 6335–6345. https://doi.org/10.4049/jimmunol.1003965.

Greenaway, H.Y., Ng, B., Price, D.A., Douek, D.C., Davenport, M.P., Venturi, V., 2013. NKT and MAIT invariant TCRα sequences can be produced efficiently by VJ gene recombination. Immunobiology 218, 213–224. https://doi.org/10.1016/j.imbio.2012.04.003.

Griewank, K., Borowski, C., Rietdijk, S., Wang, N., Julien, A., Wei, D.G., Mamchak, A.A., Terhorst, C., Bendelac, A., 2007. Homotypic interactions mediated by Slamf1 and Slamf6 receptors control NKT cell lineage development. Immunity 27, 751–762. https://doi.org/10.1016/j.immuni.2007.08.020.

Gumperz, J.E., Miyake, S., Yamamura, T., Brenner, M.B., 2002. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J. Exp. Med. 195, 625–636. https://doi.org/10.1084/jem.20011786.

Hager, E., Hawwari, A., Matsuda, J.L., Krangel, M.S., Gapin, L., 2007. Multiple constraints at the level of TCR rearrangement impact Vα14i NKT cell development. J. Immunol. 179, 2228–2234. https://doi.org/10.4049/jimmunol.179.4.2228.

Hayakawa, K., Lin, B.T., Hardy, R., 1992. Murine thymic CD4 + T cell subsets: a subset (Thy0) that secretes diverse cytokines and overexpresses the Vβ8 T cell receptor gene family. J. Exp. Med. 178, 269–274. https://doi.org/10.1084/jem.176.1.269.

Henao-Mejia, J., Williams, A., Goff, L.A., Staron, M., Licona-Limó, P., Kaech, S.M., Nakayama, M., Rinn, J.L., Flavell, R.A., Licona-Limón, P., Kaech, S.M., Nakayama, M., Rinn, J.L., Flavell, R.A., 2013. The microRNA miR-181 is a critical cellular metabolic rheostat essential for NKT cell ontogenesis and lymphocyte development and homeostasis. Immunity 38, 984–997. https://doi.org/10.1016/j.immuni.2013.02.021.

Honey, K., Benlagha, K., Beers, C., Forbush, K., Teyton, L., Kleijmeer, M.J., Rudensky, A.Y., Bendelac, A., 2002. Thymocyte expression of cathepsin L is essential for NKT cell development. Nat. Immunol. 3, 1069–1074. https://doi.org/10.1038/ni844.

Hu, T., Wang, H., Simmons, A., Bajaña, S., Zhao, Y., Kovats, S., Sun, X.-H., Alberola-Ila, J., 2013. Increased level of E protein activity during invariant NKT development promotes differentiation of invariant NKT2 and invariant NKT17 subsets. J. Immunol. 191, 5065–5073. https://doi.org/10.4049/jimmunol.1301546.

Huang, B., Gomez-Rodriguez, J., Preite, S., Garrett, L.J., Harper, U.L., Schwartzberg, P.L., 2016. CRISPR-mediated triple knockout of SLAMF1, SLAMF5 and SLAMF6 supports positive signaling roles in NKT cell development. PLoS One 11, 1–19. https://doi.org/10.1371/journal.pone.0156072.

Kageyama, R., Cannons, J.L., Zhao, F., Yusuf, I., Lao, C., Locci, M., Schwartzberg, P.L., Crotty, S., 2012. The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. Immunity 36, 986–1002. https://doi.org/10.1016/j.immuni.2012.05.016.

Kain, L., Webb, B., Anderson, B.L., Deng, S., Holt, M., Constanzo, A., Zhao, M., Self, K., Teyton, A., Everett, C., Kronenberg, M., Zajonc, D.M., Bendelac, A., Savage, P., Teyton, L., 2014. The identification of the endogenous ligands of natural killer t cells reveals the presence of mammalian α-Linked glycosylceramides. Immunity 41, 543–554. https://doi.org/10.1016/j.immuni.2014.08.017.

Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., Koseki, H., Taniguchi, M., 1997. CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science 278, 1626–1629. https://doi.org/10.1126/science.278.5343.1626.

Kim, P.J., Pai, S.-Y., Brigl, M., Besra, G.S., Gumperz, J., Ho, I.-C., 2006. GATA-3 regulates the development and function of invariant NKT cells. J. Immunol. 177, 6650–6659. https://doi.org/10.4049/JIMMUNOL.177.10.6650.

Kisielow, J., Tortola, L., Weber, J., Karjalainen, K., Kopf, M., 2011. Evidence for the divergence of innate and adaptive T-cell precursors before commitment to the αβ and γδ lineages. Blood 118, 6591–6600. https://doi.org/10.1182/blood-2011-05-352732.

Kjer-Nielsen, L., Borg, N.A., Pellicci, D.G., Beddoe, T., Kostenko, L., Clements, C.S., Williamson, N.A., Smyth, M.J., Besra, G.S., Reid, H.H., Bharadwaj, M., Godfrey, D.I., Rossjohn, J., McCluskey, J., 2006. A structural basis for selection and cross-species reactivity of the semi-invariant NKT cell receptor in CD1d/glycolipid recognition. J. Exp. Med. 203, 661–673. https://doi.org/10.1084/jem.20051777.

Koseki, H., Imai, K., Nakayama, F., Sado, T., Moriwaki, K., Taniguchi, M., 1990. Homogenous junctional sequence of the V14+ T-cell antigen receptor α chain expanded in unprimed mice. Proc. Natl. Acad. Sci. U. S. A. 87, 5248–5252. https://doi.org/10.1073/pnas.87.14.5248.

Koseki, H., Asano, H., Inaba, T., Miyashita, N., Moriwaki, K., Lindahl, K.F., Mizutani, Y., Imai, K., Taniguchi, M., 1991. Dominant expression of a distinctive Vα14+ T-cell antigen receptor α chain in mice. Proc. Natl. Acad. Sci. U. S. A. 88, 7518–7522. https://doi.org/10.1073/pnas.88.17.751.

Kovalovsky, D., Uche, O.U., Eladad, S., Hobbs, R.M., Yi, W., Alonzo, E., Chua, K., Eidson, M., Kim, H.-J., Im, J.S., Pandolfi, P.P., Sant'Angelo, D.B., 2008. The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. Nat. Immunol. 9, 1055–1064. https://doi.org/10.1038/ni.1641.

Kovalovsky, D., Alonzo, E.S., Uche, O.U., Eidson, M., Nichols, K.E., Sant'Angelo, D.B., 2010. PLZF induces the spontaneous acquisition of memory/effector functions in T cells independently of NKT cell-related signals. J. Immunol. 184, 6746–6755. https://doi.org/10.4049/jimmunol.1000776.

Kumar, A., Gordy, L.E., Bezbradica, J.S., Stanic, A.K., Hill, T.M., Boothby, M.R., Van Kaer, L., Joyce, S., 2017. NF-κB protects NKT cells from tumor necrosis factor receptor 1-induced death. Sci. Rep. 7, 15594. https://doi.org/10.1038/s41598-017-15461-y.

Kunisaki, Y., Tanaka, Y., Sanui, T., Inayoshi, A., Noda, M., Nakayama, T., Harada, M., Taniguchi, M., Sasazuki, T., Fukui, Y., 2006. DOCK2 is required in T cell precursors for development of Vα14 NK T cells. J. Immunol. 176, 4640–4645. https://doi.org/10.4049/jimmunol.176.8.4640.

Lacorazza, H.D., Miyazaki, Y., Di Cristofano, A., Deblasio, A., Hedvat, C., Zhang, J., Cordon-Cardo, C., Mao, S., Pandolfi, P.P., Nimer, S.D., 2002. The ETS protein MEF plays a critical role in perforin gene expression and the development of natural killer and NK-T cells. Immunity 17, 437–449. https://doi.org/10.1016/S1074-7613(02)00422-3.

Lantz, O., Bendelac, A., 1994. An invariant T cell receptor α chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4-8- T cells in mice and humans. J. Exp. Med. 180, 1097–1106. https://doi.org/10.1084/jem.180.3.1097.

Lawson, V.J., Maurice, D., Silk, J.D., Cerundolo, V., Weston, K., 2009. Aberrant selection and function of invariant NKT cells in the absence of AP-1 transcription factor Fra-2. J. Immunol. 183, 2575–2584. https://doi.org/10.4049/jimmunol.0803577.

Lazarevic, V., Zullo, A.J., Schweitzer, M.N., Staton, T.L., Gallo, E.M., Crabtree, G.R., Glimcher, L.H., 2009. The gene encoding early growth response 2, a target of the transcription factor NFAT, is required for the development and maturation of natural killer T cells. Nat. Immunol. 10, 306–313. https://doi.org/10.1038/ni.1696.

Lee, P.T., Benlagha, K., Teyton, L., Bendelac, A., 2002. Distinct functional lineages of human Vα24 natural killer T cells. J. Exp. Med. 195, 637–641. https://doi.org/10.1084/jem.20011908.

Lee, A.J., Zhou, X., Chang, M., Hunzeker, J., Bonneau, R.H., Zhou, D., Sun, S.-C., 2010. Regulation of natural killer T-cell development by deubiquitinase CYLD. EMBO J. 29, 1600–1612. https://doi.org/10.1038/emboj.2010.31.

Lee, Y.J., Holzapfel, K.L., Zhu, J., Jameson, S.C., Hogquist, K.A., 2013. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. Nat. Immunol. 14, 1146–1154. https://doi.org/10.1038/ni.2731.

Lee, Y.J., Starrett, G.J., Lee, S.T., Yang, R., Henzler, C.M., Jameson, S.C., Hogquist, K.A., 2016. Lineage-specific effector signatures of invariant NKT cells are shared amongst γδ t, innate lymphoid, and Th cells. J. Immunol. 197, 1460–1470. https://doi.org/10.4049/jimmunol.1600643.

Li, K., Seo, K.-H., Gao, T., Zheng, Q., Qi, R.-Q., Wang, H., Weiland, M., Dong, Z., Mi, Q.-S., Zhou, L., 2011a. Invariant NKT cell development and function in microRNA-223 knockout mice. Int. Immunopharmacol. 11, 561–568. https://doi.org/10.1016/j.intimp.2010.11.004.

Li, S., Symonds, A.L.J., Zhu, B., Liu, M., Raymond, M.V., Miao, T., Wang, P., 2011b. Early growth response gene-2 (Egr-2) regulates the development of B and T cells. PLoS One 6, e18498. https://doi.org/10.1371/journal.pone.0018498.

Li, J., Wu, D., Jiang, N., Zhuang, Y., 2013. Combined deletion of Id2 and Id3 genes reveals multiple roles for E proteins in invariant NKT cell development and expansion. J. Immunol. 191, 5052–5064. https://doi.org/10.4049/jimmunol.1301252.

Lin, H., Nieda, M., Hutton, J.F., Rozenkov, V., Nicol, A.J., 2006. Comparative gene expression analysis of NKT cell subpopulations. J. Leukoc. Biol. 80, 164–173. https://doi.org/10.1189/jlb.0705421.

Liu, X., Yin, S., Cao, W., Fan, W., Yu, L., Yin, L., Wang, L., Wang, J., 2014. Runt-related transcription factor 3 is involved in the altered phenotype and function in ThPok-deficient invariant natural killer T cells. Cell. Mol. Immunol. 11, 232–244. https://doi.org/10.1038/cmi.2014.3.

Liu, Q., Zhang, X., Yin, C., Chen, X., Zhang, Z., Brown, S., Xie, H., Zhou, L., Mi, Q.-S., Liu, Q., Zhang, X., Yin, C., Chen, X., Zhang, Z., Brown, S., Xie, H., Zhou, L., Mi, Q.-S., 2017. HDAC4 is expressed on multiple T cell lineages but dispensable for their development and. function. Oncotarget 8, 17562–17572. https://doi.org/10.18632/oncotarget.15077.

Mackay, L.K., Minnich, M., Kragten, N.A.M., Liao, Y., Nota, B., Seillet, C., Zaid, A., Man, K., Preston, S., Freestone, D., Braun, A., Wynne-Jones, E., Behr, F.M., Stark, R., Pellicci, D.G., Godfrey, D.I., Belz, G.T., Pellegrini, M., Gebhardt, T., Busslinger, M., Shi, W., Carbone, F.R., van Lier, R.A.W., Kallies, A., van Gisbergen, K.P.J.M., 2016. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. Science 352, 459–463. https://doi.org/10.1126/SCIENCE.AAD2035.

Mao, A.P., Constantinides, M.G., Mathew, R., Zuo, Z., Chen, X., Weirauch, M.T., Bendelac, A., 2016. Multiple layers of transcriptional regulation by PLZF in NKT-cell development. Proc. Natl. Acad. Sci. U. S. A. 113, 7602–7607. https://doi.org/10.1073/pnas.1601504113.

Matsuda, J.L., Zhang, Q., Ndonye, R., Richardson, S.K., Howell, A.R., Gapin, L., 2006. T-bet concomitantly controls migration, survival, and effector functions during the development of Vα14i NKT cells. Blood 107, 2797–2805. https://doi.org/10.1182/blood-2005-08-3103.

McNab, F.W., Pellicci, D.G., Field, K., Besra, G., Smyth, M.J., Godfrey, D.I., Berzins, S.P., 2007. Peripheral NK1.1 NKT cells are mature and functionally distinct from their thymic counterparts. J. Immunol. 179, 6630–6637. https://doi.org/10.4049/JIMMUNOL.179.10.6630.

Michel, M.-L., Mendes-da-Cruz, D., Keller, A.C., Lochner, M., Schneider, E., Dy, M., Eberl, G., Leite-de-Moraes, M.C., 2008. Critical role of ROR-γt in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation. Proc. Natl. Acad. Sci. U. S. A. 105, 19845–19850. https://doi.org/10.1073/pnas.0806472105.

Michel, M.-L., Lenoir, C., Massot, B., Diem, S., Pasquier, B., Sawa, S., Rignault-Bricard, R., Lehuen, A., Eberl, G., Veillette, A., Leite-de-Moraes, M., Latour, S., 2016. SLAM-associated protein favors the development of iNKT2 over iNKT17 cells. Eur. J. Immunol. 46, 2162–2174. https://doi.org/10.1002/eji.201646313.

Minagawa, M., Watanabe, H., Miyaji, C., Tomiyama, K., Shimura, H., Ito, A., Ito, M., Domen, J., Weissman, I.L., Kawai, K., 2002. Enforced expression of Bcl-2 restores the number of NK cells, but does not rescue the impaired development of NKT cells or intraepithelial lymphocytes, in IL-2/IL-15 receptor beta-chain-deficient mice. J. Immunol. 169, 4153–4160. https://doi.org/10.4049/JIMMUNOL.169.8.4153.

Moran, A.E., Holzapfel, K.L., Xing, Y., Cunningham, N.R., Maltzman, J.S., Punt, J., Hogquist, K.A., 2011. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. J. Exp. Med. 208, 1279–1289. https://doi.org/10.1084/jem.20110308.

Mycko, M.P., Ferrero, I., Wilson, A., Jiang, W., Bianchi, T., Trumpp, A., MacDonald, H.R., 2009. Selective requirement for c-Myc at an early stage of Vα14i NKT cell development. J. Immunol. 182, 4641–4648. https://doi.org/10.4049/jimmunol.0803394.

Nichols, K.E., Hom, J., Gong, S.-Y., Ganguly, A., Ma, C.S., Cannons, J.L., Tangye, S.G., Schwartzberg, P.L., Koretzky, G.A., Stein, P.L., 2005. Regulation of NKT cell development by SAP, the protein defective in XLP. Nat. Med. 11, 340–345. https://doi.org/10.1038/nm1189.

Niemeyer, M., Darmoise, A., Mollenkopf, H.-J., Hahnke, K., Hurwitz, R., Besra, G.S., Schaible, U.E., Kaufmann, S.H.E., 2008. Natural killer T-cell characterization through gene expression profiling: an account of versatility bridging T helper type 1 (Th1), Th2 and Th17 immune responses. Immunology 123, 45–56. https://doi.org/10.1111/j.1365-2567.2007.02701.x.

Northrup, D., Yagi, R., Cui, K., Proctor, W.R., Wang, C., Placek, K., Pohl, L.R., Wang, R., Ge, K., Zhu, J., Zhao, K., 2017. Histone demethylases UTX and JMJD3 are required for NKT cell development in mice. Cell Biosci. 7, 25–38. https://doi.org/10.1186/s13578-017-0152-8.

Nowyhed, H.N., Chandra, S., Kiosses, W., Marcovecchio, P., Andary, F., Zhao, M., Fitzgerald, M.L., Kronenberg, M., Hedrick, C.C., 2017. ATP binding cassette transporter ABCA7 regulates NKT cell development and function by controlling CD1d expression and lipid raft content. Sci. Rep. 7, 40273–40284. https://doi.org/10.1038/srep40273.

Nunez-Cruz, S., Yeo, W.C.J., Rothman, J., Ojha, P., Bassiri, H., Juntilla, M., Davidson, D., Veillette, A., Koretzky, G.A., Nichols, K.E., 2008. Differential requirement for the SAP-Fyn interaction during NK T cell development and function. J. Immunol. 181, 2311–2320. https://doi.org/10.4049/JIMMUNOL.181.4.2311.

O'Hagan, K.L., Zhao, J., Pryshchep, O., Wang, C.-R., Phee, H., 2015. Pak2 controls acquisition of NKT cell fate by regulating expression of the transcription factors PLZF and Egr2. J. Immunol. 195, 5272–5284. https://doi.org/10.4049/jimmunol.1501367.

Oh, S.J., Ahn, S., Jin, Y.-H., Ishifune, C., Kim, J.H., Yasutomo, K., Chung, D.H., 2015.

Notch 1 and Notch 2 synergistically regulate the differentiation and function of invariant NKT cells. J. Leukoc. Biol. 98, 781–789. https://doi.org/10.1189/jlb.1A0914-459RR.

Ou, X., Huo, J., Huang, Y., Li, Y.-F., Xu, S., Lam, K.-P., 2018. Transcription factor YY1 is essential for iNKT cell development. Cell. Mol. Immunol. 1–10. https://doi.org/10.1038/s41423-018-0002-6.

Park, S.H., Benlagha, K., Lee, D., Balish, E., Bendelac, A., 2000. Unaltered phenotype, tissue distribution and function of Vα14(+) NKT cells in germ-free mice. Eur. J. Immunol. 30, 620–625. https://doi.org/10.1002/1521-4141(200002)30:2<620::AID-IMMU620>3.0.CO;2-4.

Park, H., Tsang, M., Iritani, B.M., Bevan, M.J., 2014. Metabolic regulator Fnip1 is crucial for iNKT lymphocyte development. Proc. Natl. Acad. Sci. U. S. A. 111, 7066–7071. https://doi.org/10.1073/pnas.1406473111.

Pasquier, B., Yin, L., Fondanèche, M.-C., Relouzat, F., Bloch-Queyrat, C., Lambert, N., Fischer, A., de Saint-Basile, G., Latour, S., 2005. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. J. Exp. Med. 201, 695–701. https://doi.org/10.1084/jem.20042432.

Pei, B., Zhao, M., Miller, B.C., Véla, J.L., Bruinsma, M.W., Virgin, H.W., Kronenberg, M., 2015. Invariant NKT cells require autophagy to coordinate proliferation and survival signals during differentiation. J. Immunol. 194, 5872–5884. https://doi.org/10.4049/jimmunol.1402154.

Pellicci, D.G., Hammond, K.J.L., Uldrich, A.P., Baxter, A.G., Smyth, M.J., Godfrey, D.I., 2002. A natural killer T (NKT) cell developmental pathway involving a thymus-dependent NK1.1-CD4+ CD1d-dependent precursor stage. J. Exp. Med. 195, 835–844. https://doi.org/10.1084/jem.20011544.

Pellicci, D.G., Uldrich, A.P., Kyparissoudis, K., Crowe, N.Y., Brooks, A.G., Hammond, K.J.L., Sidobre, S., Kronenberg, M., Smyth, M.J., Godfrey, D.I., 2003. Intrathymic NKT cell development is blocked by the presence of α-galactosylceramide. Eur. J. Immunol. 33, 1816–1823. https://doi.org/10.1002/eji.200323894.

Plati, T., Visigalli, I., Capotondo, A., Buono, M., Naldini, L., Cosma, M.P., Biffi, A., 2009. Development and maturation of invariant NKT cells in the presence of lysosomal engulfment. Eur. J. Immunol. 39, 2748–2754. https://doi.org/10.1002/eji.200939639.

Pobezinsky, L.A., Etzensperger, R., Jeurling, S., Alag, A., Kadakia, T., McCaughtry, T.M., Kimura, M.Y., Sharrow, S.O., Guinter, T.I., Feigenbaum, L., Singer, A., 2015. Let-7 microRNAs target the lineage-specific transcription factor PLZF to regulate terminal NKT cell differentiation and effector function. Nat. Immunol. 16, 517–524. https://doi.org/10.1038/ni.3146.

Popovic, Z.V., Rabionet, M., Jennemann, R., Krunic, D., Sandhoff, R., Gröne, H.J., Porubsky, S., 2017. Glucosylceramide synthase is involved in development of invariant natural killer T cells. Front. Immunol. 8, 1–16. https://doi.org/10.3389/fimmu.2017.00848.

Porubsky, S., Speak, A.O., Luckow, B., Cerundolo, V., Platt, F.M., Gröne, H.-J., 2007. Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. Proc. Natl. Acad. Sci. 104, 5977–5982. https://doi.org/10.1073/pnas.95.14.7945.

Prevot, N., Pyaram, K., Bischoff, E., Sen, J.M., Powell, J.D., Chang, C.-H., 2015. Mammalian target of rapamycin complex 2 regulates invariant NKT cell development and function independent of promyelocytic leukemia zinc-finger. J. Immunol. 194, 223–230. https://doi.org/10.4049/jimmunol.1401985.

Pyaram, K., Sen, J.M., Chang, C.-H., 2017. Temporal regulation of Wnt/β-catenin signaling is important for invariant NKT cell development and terminal maturation. Mol. Immunol. 85, 47–56. https://doi.org/10.1016/J.MOLIMM.2017.01.025.

Qi, Q., Huang, W., Bai, Y., Balmus, G., Weiss, R.S., August, A., 2012. A unique role for ITK in survival of invariant NKT cells associated with the p53-dependent pathway in mice. J. Immunol. 188, 3611–3619. https://doi.org/10.4049/jimmunol.1102475.

Sagiv, Y., Hudspeth, K., Mattner, J., Schrantz, N., Stern, R.K., Zhou, D., Savage, P.B., Teyton, L., Bendelac, A., 2006. Cutting edge: impaired glycosphingolipid trafficking and NKT cell development in mice lacking Niemann-Pick type C1 protein. J. Immunol. 177, 26–30. https://doi.org/10.4049/jimmunol.177.1.26.

Saito, S., Kawamura, T., Higuchi, M., Kobayashi, T., Yoshita-Takahashi, M., Yamazaki, M., Abe, M., Sakimura, K., Kanda, Y., Kawamura, H., Jiang, S., Naito, M., Yoshizaki, T., Takahashi, M., Fujii, M., 2015. RASAL3, a novel hematopoietic RasGAP protein, regulates the number and functions of NKT cells. Eur. J. Immunol. 45, 1512–1523. https://doi.org/10.1002/eji.201444977.

Salio, M., Puleston, D.J., Mathan, T.S.M., Shepherd, D., Stranks, A.J., Adamopoulou, E., Veerapen, N., Besra, G.S., Hollander, G.A., Simon, A.K., Cerundolo, V., 2014. Essential role for autophagy during invariant NKT cell development. Proc. Natl. Acad. Sci. U. S. A. 111, E5678–87. https://doi.org/10.1073/pnas.1413935112.

Savage, A.K., Constantinides, M.G., Han, J., Picard, D., Martin, E., Li, B., Lantz, O., Bendelac, A., 2008. The transcription factor PLZF directs the effector program of the NKT cell lineage. Immunity 29, 391–403. https://doi.org/10.1016/j.immuni.2008.07.011.

Savage, A.K., Constantinides, M.G., Bendelac, A., 2011. Promyelocytic leukemia zinc finger turns on the effector T cell program without requirement for agonist TCR signaling. J. Immunol. 186, 5801–5806. https://doi.org/10.4049/jimmunol.1100119.

Schrantz, N., Sagiv, Y., Liu, Y., Savage, P.B., Bendelac, A., Teyton, L., 2007. The Niemann-Pick type C2 protein loads isoglobotrihexosylceramide onto CD1d molecules and contributes to the thymic selection of NKT cells. J. Exp. Med. Artic. JEM 00, 841–852. https://doi.org/10.1084/jem.20061562.

Schümann, J., Pittoni, P., Tonti, E., Macdonald, H.R., Dellabona, P., Casorati, G., 2005. Targeted expression of human CD1d in transgenic mice reveals independent roles for thymocytes and thymic APCs in positive and negative selection of Vα14i NKT cells. J. Immunol. 175, 7303–7310. https://doi.org/10.4049/jimmunol.175.11.7303.

Schümann, J., Mycko, M.P., Dellabona, P., Casorati, G., Macdonald, H.R., 2006. Cutting edge: influence of the TCR Vβ domain on the selection of semi-invariant NKT cells by endogenous ligands. J. Immunol. 176, 2064–2068. https://doi.org/10.4049/jimmunol.176.4.2064.

Seiler, M.P., Mathew, R., Liszewski, M.K., Spooner, C., Barr, K., Meng, F., Singh, H., Bendelac, A., 2012. Elevated and sustained expression of the transcription factors Egr1 and Egr2 controls NKT lineage differentiation in response to TCR signaling. Nat. Immunol. 13, 264–271. https://doi.org/10.1038/ni.2230.

Seo, K.-H., Zhou, L., Meng, D., Xu, J., Dong, Z., Mi, Q.-S., 2010. Loss of microRNAs in thymus perturbs invariant NKT cell development and function. Cell. Mol. Immunol. 7, 447–453. https://doi.org/10.1038/cmi.2010.49.

Sharma, A., Berga-Bolaños, R., Sen, J.M., 2014. T cell factor-1 controls the lifetime of CD4+ CD8+ thymocytes in vivo and distal T cell receptor α-chain rearrangement required for NKT cell development. PLoS One 9, e115803. https://doi.org/10.1371/journal.pone.0115803.

Shen, S., Chen, Y., Gorentla, B.K., Lu, J., Stone, J.C., Zhong, X.-P., 2011a. Critical roles of RasGRP1 for invariant NKT cell development. J. Immunol. 187, 4467–4473. https://doi.org/10.4049/jimmunol.1003798.

Shen, S., Wu, J., Srivatsan, S., Gorentla, B.K., Shin, J., Xu, L., Zhong, X.-P., 2011b. Tight regulation of diacylglycerol-mediated signaling is critical for proper invariant NKT cell development. J. Immunol. 187, 2122–2129. https://doi.org/10.4049/jimmunol.1100495.

Shimamura, M., Ohteki, T., Beutner, U., MacDonald, H.R., 1997. Lack of directed Vα14-Jα281 rearrangements in NK1+ T cells. Eur. J. Biochem. 27, 1576–1579. https://doi.org/10.1002/eji.1830270638.

Sillé, F.C.M., Martin, C., Jayaraman, P., Rothchild, A., Besra, G.S., Behar, S.M., Boes, M., 2011. Critical role for invariant chain in CD1d-mediated selection and maturation of Vα14-invariant NKT cells. Immunol. Lett. 139, 33–41. https://doi.org/10.1016/j.imlet.2011.04.012.

Sintes, J., Cuenca, M., Romero, X., Bastos, R., Terhorst, C., Angulo, A., Engel, P., 2013. Cutting edge: Ly9 (CD229), a SLAM family receptor, negatively regulates the development of thymic innate memory-like CD8+ T and invariant NKT cells. J. Immunol. 190, 21–26. https://doi.org/10.4049/jimmunol.1202435.

Sivakumar, V., Hammond, K.J.L., Howells, N., Pfeffer, K., Weih, F., 2003. Differential requirement for Rel/nuclear factor kappa B family members in natural killer T cell development. J. Exp. Med. 197, 1613–1621. https://doi.org/10.1084/jem.20022234.

Sklarz, T., Guan, P., Gohil, M., Cotton, R.M., Ge, M.Q., Haczku, A., Das, R., Jordan, M.S., 2017. mTORC2 regulates multiple aspects of NKT-cell development and function. Eur. J. Immunol. 47, 516–526. https://doi.org/10.1002/eji.201646343.

Spada, F.M., Grant, E.P., Peters, P.J., Sugita, M., Melián, A., Leslie, D.S., Lee, H.K., van Donselaar, E., Hanson, D.A., Krensky, A.M., Majdic, O., Porcelli, S.A., Morita, C.T., Brenner, M.B., 2000. Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. J. Exp. Med. 191, 937–948. https://doi.org/10.1084/JEM.191.6.937.

Stanic, A.K., Bezbradica, J.S., Park, J.-J., Van Kaer, L., Boothby, M.R., Joyce, S., 2004a. Cutting edge: the ontogeny and function of Vα14Jα18 natural T lymphocytes require signal processing by protein kinase Cθ and NF-κB. J. Immunol. 172, 4667–4671. https://doi.org/10.4049/jimmunol.172.8.4667.

Stanic, A.K., Bezbradica, J.S., Park, J.J., Matsuki, N., Mora, A.L., Van Kaer, L., Boothby, M.R., Joyce, S., 2004b. NF-κB controls cell fate specification, survival, and molecular differentiation of immunoregulatory natural T lymphocytes. J. Immunol. 172, 2265–2273. https://doi.org/10.4049/jimmunol.172.4.2265.

Sundararaj, S., Zhang, J., Krovi, S.H., Bedel, R., Tuttle, K.D., Veerapen, N., Besra, G.S., Khandokar, Y., Praveena, T., Le Nours, J., Matsuda, J.L., Rossjohn, J., Gapin, L., 2018. Differing roles of CD1d2 and CD1d1 proteins in type I natural killer T cell development and function. Proc. Natl. Acad. Sci. U. S. A. 115, E1204–E1213. https://doi.org/10.1073/pnas.1716669115.

Tani-ichi, S., Shimba, A., Wagatsuma, K., Miyachi, H., Kitano, S., Imai, K., Hara, T., Ikuta, K., 2013. Interleukin-7 receptor controls development and maturation of late stages of thymocyte subpopulations. Proc. Natl. Acad. Sci. U. S. A. 110, 612–617. https://doi.org/10.1073/pnas.1219242110.

Thapa, P., Das, J., McWilliams, D., Shapiro, M., Sundsbak, R., Nelson-Holte, M., Tangen, S., Anderson, J., Desiderio, S., Hiebert, S., Sant'Angelo, D.B., Shapiro, V.S., 2013. The transcriptional repressor NKAP is required for the development of iNKT cells. Nat. Commun. 4, 1–11. https://doi.org/10.1038/ncomms2580.

Thapa, P., Chen, M.W., McWilliams, D.C., Belmonte, P., Constans, M., SantAngelo, D.B., Shapiro, V.S., 2016. NKAP regulates invariant NKT cell proliferation and differentiation into ROR- γt-expressing NKT17 cells. J. Immunol. 196, 4987–4998. https://doi.org/10.4049/jimmunol.1501653.

Thapa, P., Romero Arocha, S., Chung, J.Y., Sant'Angelo, D.B., Shapiro, V.S., 2017. Histone deacetylase 3 is required for iNKT cell development. Sci. Rep. 7, 5784–5797. https://doi.org/10.1038/s41598-017-06102-5.

Townsend, M.J., Weinmann, A.S., Matsuda, J.L., Salomon, R., Farnham, P.J., Biron, C.A., Gapin, L., Glimcher, L.H., 2004. T-bet regulates the terminal maturation and homeostasis of NK and Vα14i NKT cells. Immunity 20, 477–494. https://doi.org/10.1016/S1074-7613(04)00076-7.

Vallabhapurapu, S., Powolny-Budnicka, I., Riemann, M., Schmid, R.M., Paxian, S., Pfeffer, K., Körner, H., Weih, F., 2008. Rel/NF-κB family member RelA regulates NK1.1− to NK1.1+ transition as well as IL-15-induced expansion of NKT cells. Eur. J. Immunol. 38, 3508–3519. https://doi.org/10.1002/eji.200737830.

Verykokakis, M., Krishnamoorthy, V., Iavarone, A., Lasorella, A., Sigvardsson, M., Kee, B.L., 2013. Essential functions for ID proteins at multiple checkpoints in invariant NKT cell development. J. Immunol. 191, 5973–5983. https://doi.org/10.4049/jimmunol.1301521.

Walunas, T.L., Wang, B., Wang, C.-R., Leiden, J.M., 2000. Cutting edge: the Ets1 transcription factor is required for the development of NK T cells in mice. J. Immunol. 164, 2857–2860. https://doi.org/10.4049/jimmunol.164.6.2857.

Wang, L., Carr, T., Xiong, Y., Wildt, K.F., Zhu, J., Feigenbaum, L., Bendelac, A., Bosselut, R., 2010. The sequential activity of Gata3 and Thpok is required for the differentiation of CD1d-restricted CD4+ NKT cells. Eur. J. Immunol. 40, 2385–2390. https://doi.org/10.1002/eji.201040534.

Wei, D.G., Curran, S.A., Savage, P.B., Teyton, L., Bendelac, A., 2006. Mechanisms imposing the Vβ bias of Vα14 natural killer T cells and consequences for microbial glycolipid recognition. J. Exp. Med. 203, 1197–1207. https://doi.org/10.1084/jem.20060418.

Wei, J., Yang, K., Chi, H., 2014. Cutting edge: discrete functions of mTOR signaling in invariant NKT cell development and NKT17 fate decision. J. Immunol. 193, 4297–4301. https://doi.org/10.4049/jimmunol.1402042.

White, A.J., Jenkinson, W.E., Cowan, J.E., Parnell, S.M., Bacon, A., Jones, N.D., Jenkinson, E.J., Anderson, G., 2014. An essential role for medullary thymic epithelial cells during the intrathymic development of invariant NKT cells. J. Immunol. 192, 2659–2666. https://doi.org/10.4049/jimmunol.1303057.

Williams, K.L., Zullo, A.J., Kaplan, M.H., Brutkiewicz, R.R., Deppmann, C.D., Vinson, C., Taparowsky, E.J., 2003. BATF transgenic mice reveal a role for activator protein-1 in NKT cell development. J. Immunol. 170, 2417–2426. https://doi.org/10.4049/JIMMUNOL.170.5.2417.

Williams, J.A., Lumsden, J.M., Yu, X., Feigenbaum, L., Chang, J., Steinberg, S.M., Hodes, R.J., 2008. Regulation of thymic NKT cell development by the B7-CD28 costimulatory pathway. J. Immunol. 181, 907–917. https://doi.org/10.4049/jimmunol.181.2.907.

Yu, S., Cantorna, M.T., 2008. The vitamin D receptor is required for iNKT cell development. Proc. Natl. Acad. Sci. U. S. A. 105, 5207–5212. https://doi.org/10.1073/pnas.0711558105.

Yue, X., Izcue, A., Borggrefe, T., 2011. Essential role of Mediator subunit Med1 in invariant natural killer T-cell development. Proc. Natl. Acad. Sci. U. S. A. 108, 17105–17110. https://doi.org/10.1073/pnas.1109095108.

Zhang, L., Tschumi, B.O., Corgnac, S., Rüegg, M.A., Hall, M.N., Mach, J.-P., Romero, P., Donda, A., 2014. Mammalian target of rapamycin complex 1 orchestrates invariant NKT cell differentiation and effector function. J. Immunol. 193, 1759–1765. https://doi.org/10.4049/jimmunol.1400769.

Zhang, X., Gu, J., Zhou, L., Mi, Q., 2016. TIM-4 is expressed on invariant NKT cells but dispensable for their development and. function. Oncotarget 1, 1–13. https://doi.org/10.18632/oncotarget.12153.

Zheng, X., Zhang, H., Yin, L., Wang, C.-R., Liu, Y., Zheng, P., 2008. Modulation of NKT cell development by B7-CD28 interaction: an expanding horizon for costimulation. PLoS One 3, e2703. https://doi.org/10.1371/journal.pone.0002703.

Zheng, Q., Zhou, L., Mi, Q.-S., 2012. MicroRNA miR-150 is involved in Vα14 invariant NKT cell development and function. J. Immunol. 188, 2118–2126. https://doi.org/10.4049/jimmunol.1103342. (Baltimore, Md 1950).

Zhou, L., Seo, K.-H., He, H.-Z., Pacholczyk, R., Meng, D.-M., Li, C.-G., Xu, J., She, J.-X., Dong, Z., Mi, Q.-S., 2009. Tie2cre-induced inactivation of the miRNA-processing enzyme Dicer disrupts invariant NKT cell development. Proc. Natl. Acad. Sci. U. S. A. 106, 10266–10271. https://doi.org/10.1073/pnas.0811119106.

Zullo, A.J., Benlagha, K., Bendelac, A., Taparowsky, E.J., 2007. Sensitivity of NK1.1-negative NKT cells to transgenic BATF defines a role for activator protein-1 in the expansion and maturation of immature NKT cells in the thymus. J. Immunol. 178, 58–66. https://doi.org/10.4049/jimmunol.178.1.58.