REVIEW

Recent advances in iNKT cell development [version 1; peer review: 3 approved]

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Abstract

Recent studies suggest that murine invariant natural killer T (iNKT) cell development culminates in three terminally differentiated iNKT cell subsets denoted as NKT1, 2, and 17 cells. Although these studies corroborate the significance of the subset division model, less is known about the factors driving subset commitment in iNKT cell progenitors. In this review, we discuss the latest findings in iNKT cell development, focusing in particular on how T-cell receptor signal strength steers iNKT cell progenitors toward specific subsets and how early progenitor cells can be identified. In addition, we will discuss the essential factors for their sustenance and functionality. A picture is emerging wherein the majority of thymic iNKT cells are mature effector cells retained in the organ rather than developing precursors.

Keywords

invariant natural killer T cells, subsets, development, T cell receptor signalling, thymus, CD1d, lipid, thymus, agonist selection

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Introduction

Identified by their T-cell receptor (TCR) specificity for lipids, invariant natural killer T (iNKT) cells are innate-like $\alpha\beta$ T cells capable of releasing cytokines almost instantly upon stimulation without the need for prior activation^{1,2}. Like conventional $\alpha\beta$ T cells, iNKT cells arise from common lymphoid progenitors and run through their developmental program in the thymus. At the double-positive (DP) stage, their developmental programs bifurcate: While conventional $\alpha\beta$ T cells get positively and negatively selected by thymic epithelial cells presenting peptide antigens by classical class I and II major histocompatibility complex (MHC) molecules^{3,4}, iNKT cell progenitors are selected by other DP thymocytes presenting lipid antigens by CD1d, a non-classical MHC-like molecule⁵⁻⁸. Strong TCR signaling is required at this stage (referred to as agonist selection)⁹ for upregulation of *Egr2*^{10,11} and *PLZF*^{12,13}, the latter of which is a master regulator of iNKT cell development. This consequently commits the DP $\alpha\beta$ T-cell progenitor with the “right” TCR rearrangement to the iNKT cell pathway^{14,15}. In addition to the strong TCR stimulation, auxiliary co-stimulatory signals are required by engaging CD80/CD86¹⁶ and via homotypic interactions between signaling lymphocyte activation molecule family (SLAMF) receptors, *Slamf1* and *Slamf6*¹⁷. Following selection, iNKT cells complete their developmental program in the thymus and can egress to peripheral tissues. However, a substantial number are retained in the thymus, ending up as terminally differentiated functional subsets in this organ.

Despite the latest insights in the field of iNKT cell biology, the development of iNKT cell subsets and their differentiation pathways remain puzzling^{14,15,18-21}. In this review, we will consider the contemporary understanding of iNKT cell subset development and in parallel we will discuss factors required for their maintenance and proper function. Moreover, we will focus on TCR signal strength involvement in iNKT cell lineage commitment and stability.

The developmental map of iNKT cells

The initial studies investigating iNKT cell development postulated that all iNKT cells execute the same developmental program divided into four stages (S0–S3). According to this model, iNKT cells progress from the most immature stage S0 (CD24⁺CD44⁺NK1.1⁺) to their final mature stage S3 (CD24⁺CD44⁺NK1.1⁺) by losing CD24 expression and subsequently upregulating first CD44 (in stage S2) and lastly natural killer NK1.1 (in stage S3)^{22,23}. Although this holds true for some iNKT cells, the latest data demonstrate that this model does not apply to all iNKT cells. For instance, this model fails to incorporate interleukin-17 (IL-17)-producing iNKT cells²⁴⁻²⁶, it does not account for iNKT cells that produce high levels of IL-4 but never express NK1.1, and it cannot be employed with mouse strains that do not express NK1.1²⁷. Therefore, a new functional classification of iNKT cells into three terminally differentiated subsets, which is based on the expression pattern of characteristic cytokines and transcription factors, was proposed^{28,29}. In this model, all iNKT cells arise from a common progenitor designated as NKT0 cells (*Egr2*^{hi}CD24⁺) and further differentiate into NKT1, NKT2, or NKT17

cell subsets. NKT1 cells (*PLZF*^{lo}*Tbet*⁺) produce interferon gamma (IFN γ) and low levels of IL-4 upon stimulation. In addition, they are the only subset expressing NK cell signature proteins like NK1.1, NKG2D, Nkp46, and a cytotoxic gene expression program³⁰⁻³². NKT2 cells express the highest levels of *PLZF* and IL-4. Lastly, NKT17 (*PLZF*^{int}*ROR γ t*⁺) cells produce IL-17. Of note, only NKT2 cells are shown to actively produce and secrete IL-4 under steady-state conditions, an essential process for CD8 innate-like T-cell generation in the thymus and periphery^{28,33-38}. In this model, NKT1 cells, IL-4–producing NKT2 cells, and NKT17 cells are considered terminally differentiated cells which generally do not give rise to any of the other subsets^{24,25,28}. Subsequently, three independent groups performed transcriptome analysis of thymic-derived iNKT cell subsets and congruently observed distinct gene expression patterns for each subset³⁰⁻³². Only NKT1 cells pass through all the stages of development S0–S3 defined by the original model. In contrast, NKT2 and NKT17 cells finish their maturation as terminally differentiated effector cells at stage 2. Taken together, these data widely validate the foundations of the NKT1/2/17 concept (Figure 1).

Two additional subsets—NKT10 and NKT follicular helper (NKT_{FH}) cells—have recently been proposed as an extension to the iNKT cell subset family; IL-10–producing E4BP4⁺ NKT10 cells were resident in the adipose tissue³⁹⁻⁴¹, and Bcl-6⁺IL-21⁺ NKT_{FH} cells were found in germinal centers^{42,43}. Notably, these two functional subsets have been described only in the periphery and are not present in the thymus.

The complexity of iNKT cell subsets

Although current data indicate that NKT1, 2, and 17 cells are terminally differentiated functional iNKT cell subsets, the latest data bring a further level of complexity and reflect advances in the field. For instance, NKT1 cells segregate into CD4⁺ and CD4[−] fractions. CD4[−] NKT1 cells were shown to display a more NK-like phenotype with preferential expression of NK cell signature receptors and soluble cytotoxic mediators (for example, granzyme a, b, and perforin)³⁰. In contrast, CD4⁺ NKT1 cells express higher levels of NK cell-unrelated genes like IL-4 and CD81^{30,44}. In light of these findings, new questions arise about iNKT cell subset development and function. For example, are both NKT1 fractions distinct terminally differentiated cell subsets with divergent functions or do they represent intermediate versus fully matured stages of the NKT1 cell subset? Along the same line, a recent study described an alternative iNKT cell developmental pathway where a small CD4[−] NKT1 population can arise from double-negative (DN) stage thymocytes⁴⁵. However, this pathway seems to contribute in only a minor way to the mature CD4[−] NKT1 cell pool.

Even though less is known about NKT17 cell development⁴⁶, a recent study provided valuable insight into NKT17 biology⁴⁷. By evaluating the expression pattern of the NKT17 characteristic genes CD138⁴⁸ and CCR6²⁶, the investigators suggested the final steps of the NKT17 developmental pathway from *ROR γ t*⁺ NKT17 committed progenitors which progressively gain CD138 followed by CCR6 to become CD138⁺CCR6⁺ DP mature NKT17 cells⁴⁷.

Figure 1. Invariant natural killer T (iNKT) cell development in the thymus. In the cortex of the thymus (left), double-positive (DP) iNKT progenitors are positively selected by other DP thymocytes presenting lipids via CD1d. This results in survival and lineage commitment; only those rare DP thymocytes bearing T-cell receptors (TCRs) (invariant V β 14 chain paired with a limited set of beta chains) with the right specificity are selected and committed to the iNKT cell lineage. This step requires strong TCR signaling in combination with co-stimulatory signals via homotypic interactions between SLAM (signaling lymphocyte activation molecule) family members. Signaling leads to upregulation of Egr1 and Egr2, which are needed for PLZF induction and stable expression. Immediate post-selection iNKT cells are Egr2^{hi}CD24^{hi}CD44^{CD69}^{hi} and are designated as NKT0 cells. Subsequently, NKT0 cells downregulate CD24 and transition into CCR7⁺ multi-potent NKT cell progenitors (NKTp). At that stage, NKTp cells can egress from the thymus or continue their differentiation into one of the effector subsets (iNKT1, 2, or 17). Mature NKT cells are CCR7⁻ and reside in the medulla region as terminally differentiated tissue-resident NKT cell subsets.

Expectedly, the PLZF^{hi} iNKT cells display the highest level of heterogeneity^{28,30} since this cell fraction encompasses both mature NKT2 and immature NKT progenitor cells^{28,49} (Figure 1). Yet, in a 2016 study, Engel *et al.* performed a single-cell RNA-sequencing analysis on each of the iNKT cell subsets from thymic origin³¹. In addition to including NKT1, 2, and 17 cells, this study included the most immature NKT0 cells which are still CD24^{hi} and have recently undergone selection. Despite that, authors still detected some NKT2 cells with transcripts of characteristic genes known to be highly expressed in recently selected cells (for example, *Itm2a*, *Ccr9*, and *Ldhb*)^{31,50,51}. In line with that, principal component analysis of NKT0, 1, 2, and 17 cells showed that each of these fractions segregated as different subsets with only marginal overlap between NKT0 and NKT2 cells^{31,32}. Taken

together, these data argue for a missing link in the development of the terminally differentiated iNKT cell subsets from the CD24^{hi} NKT0 stage. As mentioned above, there are several reported genes with a shared expression pattern between NKT0 and some CD24-PLZF^{hi} cells. Hence, they might be suitable as markers for iNKT cell subset progenitors “hiding” within the PLZF^{hi} iNKT cell fraction. Such a candidate is CCR7, which was shown to be expressed on both cell fractions at RNA^{30,31} and protein^{30,52} levels. Indeed, a recent study by Wang *et al.* described CCR7 as a characteristic marker for multi-potent iNKT cell progenitors (NKTp)⁵² (Figure 1). In that study, the authors took advantage of the KN2 mouse model in which IL-4-secreting cells can be identified by human CD2 (hCD2) expression⁵³. Those experiments demonstrated that the small fraction of CCR7⁺ iNKT cells

did not produce IL-4 yet could give rise to all iNKT effector subsets in the thymus and periphery.

CCR7⁺ cells (amongst NKT2) represent an undifferentiated precursor that emigrates from the thymus

Although previous studies had shown first that NK1.1⁻ iNKT^{22,23} cells and later that “NKT2-like” cells³⁰ can emigrate into the periphery, these reports did not investigate CCR7 expression on recent thymic emigrants (RTEs). Interestingly, Wang *et al.* also found that CCR7⁺ iNKT cells, despite representing only about 5% of total thymic iNKT cells, were prominent amongst RTEs, a process stringently dependent on Klf2 and S1PR1⁵² (Figure 1). This suggests that undifferentiated NKTp cells exit the thymus and can initiate or complete (or both) differentiation in peripheral tissues. Indeed, 5 days after adoptive transfer, CCR7⁺ NKTp cells differentiated into all three effector subsets whether they had been transferred directly into the thymus or intravenously into the periphery. Moreover, parabiosis experiments showed that more than 99% of all thymic iNKT cells were tissue-resident as opposed to arriving from circulation⁵². Thus, only a small fraction of “developing” NKTp cells in the thymus are constantly replenishing the pool of iNKT cells in the thymus and periphery.

Overall, these data raise the logical questions: What are the precise signals specifying iNKT cell differentiation, and what are the checkpoints for subset commitment? Moreover, does commitment occur at the DP stage in the cortex or at a later developmental stage post-selection as the undifferentiated CCR7⁺ cell encounters a new cellular milieu in the thymic

medulla? In fact, recent evidence suggests a combination of the two. These new findings are discussed in the following section.

TCR signaling: strength and context

It has long been known that TCR signaling is critical for iNKT cell development, as recognition of CD1d:lipid ligands is required for positive selection⁵⁻⁸. However, recent data suggest a critical role for TCR signaling in subset differentiation as well. Of interest in this regard is the observation that mature iNKT cell subsets exhibit different levels of TCR on their surface^{30,54} where NKT1 cells are low, NKT17 cells are intermediate, and NKT2 cells display the highest level of TCR expression. This also appears to correlate with their ongoing TCR signal strength under steady-state conditions^{54,55}. Overall, this suggests a potential requirement for different TCR signal intensities in the development of each (Figure 2). In fact, two independent groups recently addressed this question by exploiting the SKG mouse model, in which TCR signaling is weakened because of a hypo-morphic ZAP70 allele^{54,56}. Hence, both studies showed that weakened TCR signaling led to abrogation in NKT2 and, to a lesser extent, NKT17 cell development while not reducing NKT1 cell development. In addition, genome-wide analysis of chromatin accessibility between NKT2 cells from SKG and wild-type control mice showed that gene regions coding MAPK/ERK and Notch pathway regulators were less accessible in the SKG mouse. Moreover, a recent study showed that a deficiency in TRAF3-interacting protein which facilitates MEK/ERK signaling at the trans-Golgi network led to a decrease in IL-4-producing NKT2 cells⁵⁷. This places TCR strength signaling as a possible modulator of MAPK/ERK and Notch signaling, which might influence

Figure 2. Model of T-cell receptor (TCR) signaling in the cortex for invariant natural killer T (iNKT) differentiation. In this model, TCR binding strength (avidity) during the cortical selection process steers stage 0 iNKT cells to specific cell fates. For iNKT cells, which express a semi-invariant TCR alpha chain, TCR beta chain usage can influence TCR binding avidity. For example, Vβ2 and Vβ7 chains tend to confer higher binding avidity. TCRs with high avidity drive NKT2 differentiation preferentially, TCRs with intermediate binding avidity drive NKT17 differentiation, and those with low binding avidity drive commitment to the NKT1 pathway. NKT progenitor (NKTp) cells finish their differentiation in the medulla.

iNKT cell subset development. Additionally, the Src homology2 domain-containing phosphatase 1 (Shp1)-deficient mouse showed an increase of NKT2 and NKT17 cells⁵⁸. Although the authors did not find direct evidence that this is due to altered TCR signaling, Shp1 was previously identified as a negative regulator of TCR signaling by targeting ZAP-70⁵⁹.

One critical gene target of TCR signaling in iNKT cells is PLZF¹¹. Indeed, PLZF expression in iNKT subsets mirrors TCR levels and signaling; NKT2 expresses the highest level, NKT17 expresses intermediate, and NKT1 expresses the lowest level²⁸ (Figure 1). An interesting recent study showed that, like that of TCR, the quantity of PLZF expressed by a given cell is a critical factor in iNKT subset differentiation. In that study, a hypomorphic allele of PLZF was found to strongly reduce NKT2 and NKT17 numbers while relatively sparing NKT1 cells⁶⁰. Collectively, these findings corroborate the idea that the quantity of TCR signaling is a critical factor in iNKT cell subset differentiation.

Since it is known that iNKT cell selection happens in the cortex⁶¹, it seems likely that the interaction of stage 0 iNKT cells with CD1d/lipid-presenting DP thymocytes is the stage at which signal strength is critical (Figure 2). In this regard, it is curious that cells immediately following the CCR7⁺ stage do not yet show signs of differentiation to distinct subsets and

express a uniformly high level of TCR⁵². Furthermore, the CCR7⁺ iNKT cell population has a transcriptome that does not yet resemble any of the differentiated subsets³². Thus, it remains an open question whether CCR7⁺ cells are already “committed” to a specific cell subset or are multi-potent at the single-cell level. Future studies will need to address this by, for example, using single-cell adoptive transfer assays of cell fate or single-cell epigenomics analysis of CCR7⁺ cells (Figure 2).

Another (non-mutually exclusive) possibility is that TCR signaling is critical during and following the CCR7⁺ stage (Figure 3). CCR7 is a chemokine receptor that facilitates the movement of iNKT cells from the cortex to the medulla and is crucial for proper maturation and maintenance of resident iNKT cells in the thymus⁵². It is possible that NKT cells require continued interaction with CD1d-expressing antigen-presenting cells in the medulla, particularly to maintain expression of survival factors, including bcl2 family members⁶⁰ (Figure 3). Of note, medulla-derived factors have already been shown to play an essential role in iNKT cell subset homeostasis. For instance, it has been shown that IL-15 is required for terminal maturation and survival of NKT1 cells⁶² and that IL-25 is implicated in NKT2 cell development and effector functions^{24,63}. A new study by Wang *et al.* sought to discern whether steady-state IL-4 production by mature NKT2 cells is TCR-CD1d interaction-dependent⁵⁵. Indeed, this work showed that

Figure 3. Model of T-cell receptor (TCR) signaling in the medulla for invariant natural killer T (iNKT) subset activation and survival.

According to this non-mutually exclusive model, subset commitment decisions are made or reinforced (or both) in the thymic medulla. At the CCR7⁺ stage, developing natural killer cell progenitors (NKTp) may still be uncommitted to any particular subset (as shown). Alternatively, signaling at the double-positive (DP) stage may have altered their epigenomes such that they have a propensity to differentiate into one of the three major subsets (as depicted in Figure 2). Either way, following migration into the medulla, NKTp cells experience different strengths of TCR signaling—based on their TCR:CD1d/lipid binding avidity with distinct antigen-presenting cells (APCs) they stochastically encounter in the medulla—which governs their subset commitment or survival or both. Cells with the highest binding avidity survive as NKT2 cells, those with intermediate affinity survive as NKT17 cells, and only NKT1 cells survive without continued TCR stimulation. In the medulla, NKT2 cells require TCR-CD1d interaction with macrophages in order to produce interleukin-4 (IL-4) in the steady state, and NKT1 cells need IL-15 produced by medullary thymic epithelial cells (mTECs) for their proper differentiation and survival. It is unknown what cell types present lipids to NKT17 cells, although it is reported that they encounter intermediate TCR signaling in the steady state, which is crucial for their differentiation.

intrathymic transfer of NKT2 cells into CD1d-deficient recipient mice resulted in a loss of IL-4 production within 9 days after transfer⁵⁵. Moreover, using inducible knockout (KO) models, the authors identified the CD1d-presenting cell subset as macrophages. At this point, it is unknown whether the medullary macrophages that activate NKT2 cells present the same or distinct self-lipid ligands compared with the cortical DP thymocytes that initially select NKT cells (Figure 2 and Figure 3).

Although NKT cells are often assumed to be mono-specific given the semi-invariant nature of their TCR alpha chain, evidence suggests that the particular TCR beta chain used can influence recognition^{64–67}. In this context, it is interesting that the different NKT subsets have reproducible differences in TCR beta usage⁵⁴. Furthermore, retrogenic experiments showed that TCR:CD1d/lipid binding avidity positively correlated with selection efficiency. These avidity differences were conferred by different TCR beta chains. In addition, a longer half-life of binding favored the development of PLZF^{hi} NKT2 cells over the other subsets⁶⁸. Further deep sequencing of the TCR alpha and beta repertoires of iNKT subsets could provide insight in the future.

As previously mentioned, SLAM family receptors (SFRs) play a crucial role in iNKT cell development¹⁷. This family includes six members that can convey both activating or inhibitory signals depending on the adaptor proteins that are recruited upon receptor engagement⁶⁹. Lu *et al.* recently addressed the role of SLAM receptor proteins (SRPs) in iNKT cell development by generating a KO mouse strain for all six members of this family⁷⁰. They showed that loss of SFRs led to higher TCR signaling in developing iNKT cells, as judged by higher Nur77 and Egr2 levels, and to reduced numbers of all mature iNKT cells. In addition, they reported that the number of CD24⁺ immature iNKT cells was not altered. Considering these findings, the authors concluded that the initial positive selection of iNKT cells was unaffected in SFR-deficient mice but that the observed iNKT cell loss was due to augmented TCR signal strength driving apoptosis. The authors also conclude that inhibitory signals provided by SFRs attenuate TCR signal strength after positive selection to promote NKT cell development, as opposed to previous studies proposing that SFRs contributes with a positive signal that complements TCR signaling to support NKT cell development⁷¹. Interestingly, however, Lu *et al.* provided evidence that V β usage was altered, whereby V β 7 clones were preferentially enriched in SFR-deficient mice⁷⁰. Of note, it was reported that V β 7 usage confers higher avidity binding to CD1d and is expressed mostly by NKT2 cells^{28,54,66}. As mentioned above, NKT2 cells are thought to experience the strongest TCR signaling in the steady state^{54,55}. Hence, this raises the possibility that mainly clones with high-avidity V β chain rearrangements, which result in stronger TCR signaling, are able to survive in the absence of SFR co-stimulation. Strikingly, a previous study showed that in conditions of limited endogenous ligand concentrations, thymic selection favors V β 7⁺ iNKT cells over iNKT cells expressing other V β chains⁶⁶. Thus, although Lu *et al.* show that iNKT cells from SFR-deficient mice have

higher ongoing TCR signal strength in comparison with wild-type mice, this effect might be due to selective survival of high-avidity V β 7-expressing NKT2 cells and not to lack of SFR-mediated inhibition. Therefore, further studies are needed to understand the precise role of SFR signaling in NKT cell development or subset differentiation or both.

Concluding remarks

A couple of technical points regarding the development of iNKT cells can be gleaned from the studies we discussed here. First is that the paradigm of “staging” NKT cell development using CD44 and NK1.1 is relevant only to NKT1 lineage cells in B6 (C57BL/6) mice and thus should be employed with caution. Second, a high level of PLZF expression is not sufficient to distinguish functionally differentiated NKT2 cells from multi-potent NKTp cells. Therefore, CCR7, PD1, or other markers are needed to discriminate between developing and differentiating iNKT subsets. In addition, NKT2 cells were shown to actively produce and secrete high levels of IL-4 whereas multi-potent NKTp cells did not. Furthermore, most studies investigating cytokine production in iNKT cells use PMA/ionomycin as a stimulus, which might be misleading in this regard. Hence, reporter mouse models currently represent the best way to identify cells actively producing IL-4. In the future, it will be interesting and important to determine when NKT1 or NKT17 or both actively produce cytokine in uninfected animals.

Despite the recent progress in the field of iNKT cell biology, many questions remain unanswered. For instance, assuming surface TCR levels dictate TCR signaling quantity, what drives the high TCR expression on NKT2 and 17 cells? Is it a signal received during selection owing to specific V β chain usage or rather an environmental consequence such as high presence of endogenous cognate ligand? Moreover, what are the relevant self-lipid ligands in the thymus and do they differ between cortical and medullary antigen-presenting cells? How diverse are they and is this diversity detected? In this context, a recent study showed that non-agonist CD1d-associated lipids could alter lipid presentation and impact iNKT development in a complex way⁷². Thus, many interesting questions remain open about the complex biology induced by the T-cell recognition of lipids.

Abbreviations

DP, double-positive; iNKT, invariant natural killer T; MHC, major histocompatibility complex; NK, natural killer; NKT_{FH}, invariant natural killer T follicular helper; NKTp, multi-potent invariant natural killer T-cell progenitor; RTE, recent thymic emigrant; SFR, SLAM family receptor; Shp1, Src homology2 domain-containing phosphatase 1

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References

1. **F** Stetson DB, Mohrs M, Reinhardt RL, *et al.*: **Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function.** *J Exp Med.* 2003; **198**(7): 1069–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
2. Crowe NY, Uldrich AP, Kyriakoudis K, *et al.*: **Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells.** *J Immunol.* 2003; **171**(18): 4020–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Starr TK, Jameson SC, Hogquist KA: **Positive and negative selection of T cells.** *Annu Rev Immunol.* 2003; **21**: 139–76.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Palmer E: **Negative selection—clearing out the bad apples from the T-cell repertoire.** *Nat Rev Immunol.* 2003; **3**(5): 383–91.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Bendelac A, Savage PB, Teyton L: **The biology of NKT cells.** *Annu Rev Immunol.* 2007; **25**: 297–336.
[PubMed Abstract](#) | [Publisher Full Text](#)
6. Godfrey DI, Stankovic S, Baxter AG: **Raising the NKT cell family.** *Nat Immunol.* 2010; **11**(3): 197–206.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Kronenberg M, Gapin L: **The unconventional lifestyle of NKT cells.** *Nat Rev Immunol.* 2002; **2**(8): 557–68.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Bendelac A: **Positive selection of mouse NK1+ T cells by CD1-expressing cortical thymocytes.** *J Exp Med.* 1995; **182**(6): 2091–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Stritesky GL, Jameson SC, Hogquist KA: **Selection of self-reactive T cells in the thymus.** *Annu Rev Immunol.* 2012; **30**: 95–114.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Lazarevic V, Zullo AJ, Schweitzer MN, *et al.*: **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.* 2009; **10**(3): 306–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. **F** Seiler MP, Mathew R, Liszewski MK, *et al.*: **Elevated and sustained expression of the transcription factors Egr1 and Egr2 controls NKT lineage differentiation in response to TCR signaling.** *Nat Immunol.* 2012; **13**(3): 264–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
12. Kovalovsky D, Uche OU, Eladad S, *et al.*: **The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions.** *Nat Immunol.* 2008; **9**(9): 1055–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. **F** Savage AK, Constantinides MG, Han J, *et al.*: **The transcription factor PLZF directs the effector program of the NKT cell lineage.** *Immunity.* 2008; **29**(3): 391–403.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
14. **F** Verykokakis M, Kee BL: **Transcriptional and epigenetic regulation of innate-like T lymphocyte development.** *Curr Opin Immunol.* 2018; **51**: 39–45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
15. **F** Gapin L: **Development of invariant natural killer T cells.** *Curr Opin Immunol.* 2016; **39**: 68–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
16. Chung Y, Nurieva R, Esashi E, *et al.*: **A critical role of costimulation during intrathymic development of invariant NK T cells.** *J Immunol.* 2008; **180**(4): 2276–83.
[PubMed Abstract](#) | [Publisher Full Text](#)
17. **F** Griewank K, Borowski C, Rietdijk S, *et al.*: **Homotypic interactions mediated by Slamf1 and Slamf6 receptors control NKT cell lineage development.** *Immunity.* 2007; **27**(5): 751–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
18. **F** Krovi SH, Gapin L: **Invariant Natural Killer T Cell Subsets—More Than Just Developmental Intermediates.** *Front Immunol.* 2018; **9**: 1393.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
19. Wang H, Hogquist KA: **How Lipid-Specific T Cells Become Effectors: The Differentiation of iNKT Subsets.** *Front Immunol.* 2018; **9**: 1450.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. **F** Dashtsoodol N, Bortoluzzi S, Schmidt-Suppran M: **T Cell Receptor Expression Timing and Signal Strength in the Functional Differentiation of Invariant Natural Killer T Cells.** *Front Immunol.* 2019; **10**: 841.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
21. **F** Bennis SB: **Unraveling Natural Killer T-Cells Development.** *Front Immunol.* 2017; **8**: 1950.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
22. **F** Benlagha K, Kyin T, Beavis A, *et al.*: **A thymic precursor to the NK T cell lineage.** *Science.* 2002; **296**(5567): 553–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
23. Pellicci DG, Hammond KJ, Uldrich AP, *et al.*: **A natural killer T (NKT) cell developmental pathway involving a thymus-dependent NK1.1-CD4⁺ CD1d-dependent precursor stage.** *J Exp Med.* 2002; **195**(7): 835–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Watarai H, Sekine-Kondo E, Shigeura T, *et al.*: **Development and function of invariant natural killer T cells producing T_H2- and T_H17-cytokines.** *PLoS Biol.* 2012; **10**(2): e1001255.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Michel ML, Mendes-da-Cruz D, Keller AC, *et al.*: **Critical role of ROR- γ t in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation.** *Proc Natl Acad Sci U S A.* 2008; **105**(50): 19845–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Doisne JM, Becourt C, Amniai L, *et al.*: **Skin and peripheral lymph node invariant NKT cells are mainly retinoic acid receptor-related orphan receptor (gamma)⁺ and respond preferentially under inflammatory conditions.** *J Immunol.* 2009; **183**(3): 2142–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
27. Carlyle JR, Mesci A, Ljutic B, *et al.*: **Molecular and genetic basis for strain-dependent NK1.1 alloreactivity of mouse NK cells.** *J Immunol.* 2006; **176**(12): 7511–24.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Lee YJ, Holzappel KL, Zhu J, *et al.*: **Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells.** *Nat Immunol.* 2013; **14**(11): 1146–54.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Constantinides MG, Bendelac A: **Transcriptional regulation of the NKT cell lineage.** *Curr Opin Immunol.* 2013; **25**(2): 161–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Georgiev H, Ravens I, Benarafa C, *et al.*: **Distinct gene expression patterns correlate with developmental and functional traits of iNKT subsets.** *Nat Commun.* 2016; **7**: 13116.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. **F** Engel I, Seumois G, Chavez L, *et al.*: **Innate-like functions of natural killer T cell subsets result from highly divergent gene programs.** *Nat Immunol.* 2016; **17**(6): 728–39.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
32. Lee YJ, Starrett GJ, Lee ST, *et al.*: **Lineage-Specific Effector Signatures of Invariant NKT Cells Are Shared amongst $\gamma\delta$ T, Innate Lymphoid, and Th Cells.** *J Immunol.* 2016; **197**(4): 1460–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Weinreich MA, Odumade OA, Jameson SC, *et al.*: **T cells expressing the transcription factor PLZF regulate the development of memory-like CD8⁺ T cells.** *Nat Immunol.* 2010; **11**(8): 709–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Kurzweil V, LaRoche A, Oliver PM: **Increased peripheral IL-4 leads to an expanded virtual memory CD8⁺ population.** *J Immunol.* 2014; **192**(12): 5643–51.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
35. Lee YJ, Jameson SC, Hogquist KA: **Alternative memory in the CD8 T cell lineage.** *Trends Immunol.* 2011; **32**(2): 50–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Carly SA, Koretzky GA, Jordan MS, *et al.*: **Interleukin-4 regulates eomesodermin in CD8⁺ T cell development and differentiation.** *PLoS One.* 2014; **9**(9): e106659.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Jameson SC, Lee YJ, Hogquist KA: **Innate memory T cells.** *Adv Immunol.* 2015; **126**: 173–213.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Georgiev H, Ravens I, Shibuya A, *et al.*: **CD155/CD226-interaction impacts on the generation of innate CD8⁺ thymocytes by regulating iNKT-cell differentiation.** *Eur J Immunol.* 2016; **46**(4): 993–1003.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. **F** Lynch L, Michelet X, Zhang S, *et al.*: **Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of T_{reg} cells and macrophages in adipose tissue.** *Nat Immunol.* 2015; **16**(1): 85–95.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
40. Lynch L, Nowak M, Varghese B, *et al.*: **Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production.** *Immunity.* 2012; **37**(3): 574–87.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. **F** Sag D, Krause P, Hedrick CC, *et al.*: **IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset.** *J Clin Invest.* 2014; **124**(9): 3725–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
42. Chang PP, Barral P, Fitch J, *et al.*: **Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses.** *Nat Immunol.* 2011; **13**(1): 35–43.
[PubMed Abstract](#) | [Publisher Full Text](#)
43. King IL, Fortier A, Tighe M, *et al.*: **Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner.** *Nat Immunol.* 2011; **13**(1): 44–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Crowe NY, Coquet JM, Berzins SP, *et al.*: **Differential antitumor immunity**

- mediated by NKT cell subsets *in vivo*. *J Exp Med*. 2005; **202**(9): 1279–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. **F** Dashtsoodol N, Shigeura T, Aihara M, *et al.*: **Alternative pathway for the development of V_α14⁺ NKT cells directly from CD4⁺CD8⁺ thymocytes that bypasses the CD4⁺CD8⁺ stage.** *Nat Immunol*. 2017; **18**(3): 274–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 46. Tsagaratou A: **Unveiling the regulation of NKT17 cell differentiation and function.** *Mol Immunol*. 2019; **105**: 55–61.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. **F** Kilbi J, Li S, Amable L, *et al.*: **Characterization of the developmental landscape of murine ROR_γ⁺ iNKT cells.** *Int Immunol*. 2020; **32**(2): 105–116.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 48. Dai H, Rahman A, Saxena A, *et al.*: **Syndecan-1 identifies and controls the frequency of IL-17-producing naïve natural killer T (NKT17) cells in mice.** *Eur J Immunol*. 2015; **45**(11): 3045–51.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 49. **F** Kwon DI, Lee YJ: **Lineage Differentiation Program of Invariant Natural Killer T Cells.** *Immune Netw*. 2017; **17**(6): 365–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 50. Kirchner J, Bevan MJ: **ITM2A is induced during thymocyte selection and T cell activation and causes downregulation of CD8 when overexpressed in CD4⁺CD8⁺ double positive thymocytes.** *J Exp Med*. 1999; **190**(2): 217–28.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 51. Uehara S, Hayes SM, Li L, *et al.*: **Premature expression of chemokine receptor CCR9 impairs T cell development.** *J Immunol*. 2006; **176**(1): 75–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
 52. **F** Wang H, Hogquist KA: **CCR7 defines a precursor for murine iNKT cells in thymus and periphery.** *eLife*. 2018; **7**: pii: e34793.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 53. **F** Mohrs K, Wakil AE, Killeen N, *et al.*: **A two-step process for cytokine production revealed by IL-4 dual-reporter mice.** *Immunity*. 2005; **23**(4): 419–29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 54. **F** Tuttle KD, Krovi SH, Zhang J, *et al.*: **TCR signal strength controls thymic differentiation of iNKT cell subsets.** *Nat Commun*. 2018; **9**(1): 2650.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 55. Wang H, Breed ER, Lee YJ, *et al.*: **Myeloid cells activate iNKT cells to produce IL-4 in the thymic medulla.** *Proc Natl Acad Sci U S A*. 2019; **116**(44): 22262–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. **F** Zhao M, Svensson MND, Venken K, *et al.*: **Altered thymic differentiation and modulation of arthritis by invariant NKT cells expressing mutant ZAP70.** *Nat Commun*. 2018; **9**(1): 2627.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 57. **F** Zhang X, Wang K, Zhao W, *et al.*: **TRAF3IP3 at the trans-Golgi network regulates NKT2 maturation via the MEK/ERK signaling pathway.** *Cell Mol Immunol*. 2019; **16**: 1114.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 58. **F** Cruz Tleugabulova M, Zhao M, Lau I, *et al.*: **The Protein Phosphatase Shp1 Regulates Invariant NKT Cell Effector Differentiation Independently of TCR and Siam Signaling.** *J Immunol*. 2019; **202**(8): 2276–86.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 59. Plas DR, Johnson R, Pingel JT, *et al.*: **Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling.** *Science*. 1996; **272**(5265): 1173–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 60. **F** Park JY, DiPalma DT, Kwon J, *et al.*: **Quantitative Difference in PLZF Protein Expression Determines iNKT Lineage Fate and Controls Innate CD8 T Cell Generation.** *Cell Rep*. 2019; **27**(9): 2548–2557.e4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 61. Benlagha K, Wei DG, Veiga J, *et al.*: **Characterization of the early stages of thymic NKT cell development.** *J Exp Med*. 2005; **202**(4): 485–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 62. Gordy LE, Bezbradica JS, Flyak AI, *et al.*: **IL-15 regulates homeostasis and terminal maturation of NKT cells.** *J Immunol*. 2011; **187**(12): 6335–45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 63. **F** Miller CN, Proekt I, von Moltke J, *et al.*: **Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development.** *Nature*. 2018; **559**(7715): 627–31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 64. Cameron G, Pellicci DG, Uldrich AP, *et al.*: **Antigen Specificity of Type I NKT Cells Is Governed by TCR β-Chain Diversity.** *J Immunol*. 2015; **195**(10): 4604–14.
[PubMed Abstract](#) | [Publisher Full Text](#)
 65. **F** Clancy-Thompson E, Chen GZ, Tyler PM, *et al.*: **Monoclonal Invariant NKT (iNKT) Cell Mice Reveal a Role for Both Tissue of Origin and the TCR in Development of iNKT Functional Subsets.** *J Immunol*. 2017; **199**(1): 159–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 66. Schümann J, Mycko MP, Dellabona P, *et al.*: **Cutting edge: influence of the TCR Vβeta domain on the selection of semi-invariant NKT cells by endogenous ligands.** *J Immunol*. 2006; **176**(4): 2064–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 67. Mallevaey T, Clarke AJ, Scott-Browne JP, *et al.*: **A molecular basis for NKT cell recognition of CD1d-self-antigen.** *Immunity*. 2011; **34**(3): 315–26.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 68. **F** Cruz Tleugabulova M, Escalante NK, Deng S, *et al.*: **Discrete TCR Binding Kinetics Control Invariant NKT Cell Selection and Central Priming.** *J Immunol*. 2016; **197**(10): 3959–69.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 69. Veillette A: **NK cell regulation by SLAM family receptors and SAP-related adapters.** *Immunol Rev*. 2006; **214**: 22–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
 70. **F** Lu Y, Zhong MC, Qian J, *et al.*: **SLAM receptors foster iNKT cell development by reducing TCR signal strength after positive selection.** *Nat Immunol*. 2019; **20**(4): 447–57.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 71. **F** Dutta M, Kraus ZJ, Gomez-Rodriguez J, *et al.*: **A role for Ly108 in the induction of promyelocytic zinc finger transcription factor in developing thymocytes.** *J Immunol*. 2013; **190**(5): 2121–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 72. **F** Melum E, Jiang X, Baker KD, *et al.*: **Control of CD1d-restricted antigen presentation and inflammation by sphingomyelin.** *Nat Immunol*. 2019; **20**(12): 1644–55.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)

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