Developmental programming of natural killer and innate lymphoid cells

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Short title: NK and ILC Developmental Pathways

Highlights:

ILCs include NK cells, nuocytes/NH cells, LTi cells and NKp46+ IL-22+ cells.
ILC rapidly produce cytokines (IL-5, -13, -17A, -22, IFN-γ) required for immunity to infection.
Transcription factors program ILC differentiation from lymphoid precursors.
ILC and Th responses are coordinated during innate and adaptive immunity.

Abstract

In recent years we have witnessed a blooming interest in innate lymphoid cell (ILC) biology thanks to the discovery of novel lineages of ILC that are phenotypically and functionally distinct from NK cells. While the importance of these novel ILC subsets as essential functional components of the early immune responses are now clearly established, many questions remain as to how early ILC developmental fates are determined and how specific effector functions associated with individual ILC subsets are achieved. As the founding member of the ILC family, properties of NK cells have defining attributes that characterize this group of innate effectors. Analysing their developmental rules may provide clues to principles that guide ILC development in general.

Introduction

ILC represent an expanding family of innate effector cells that have critical roles in the generation and maintenance of immunity, especially at mucosal surfaces. Natural Killer (NK) cells can be considered as the founding member of the ILC family, having immunological characteristics that include a lymphoid developmental origin, absence of clonally rearranging antigen receptors, and an activation profile that includes T-bet (Tbx21)-regulated prompt cytokine production (IFN-γ) but generally does not result in long-lasting immunological memory. We know much about the molecular determinants that guide NK cell development and generate these potent innate effector cells in both mouse and man (reviewed in [1–3]). The second ILC family member identified was the Lymphoid Tissue-inducer (LTi) cell that expresses the retinoic acid receptor-related orphan receptor γt (RORγt) and has a determinant role in lymphoid tissue formation during fetal and adult life. In adult mice, RORγt-expressing LTi cells can also promptly produce IL-17 and IL-22 following activation with cytokines such as IL-1β and IL-23 suggesting that LTi cells have additional roles in immune defense (reviewed in [4]). More recently, other IL-22-producing RORγt$^+$ ILC that express natural cytotoxicity receptors have been described in mucosal tissues from mice and man (reviewed in [5]). Finally, novel ILC subsets that have the capacity to produce high levels of the Th2 cytokines IL-5 and IL-13 were discovered and variably named natural helper cells, nuocytes or innate helper cells (reviewed in [6]). All of these different ILC subsets have common characteristics of lymphoid morphology, independence from the recombinase machinery, and a common developmental dependence for the transcriptional repressor Id2 and for γ_c cytokines (reviewed in [7]). Recently, a standardized nomenclature was proposed for describing these functionally different ILC subsets: group 1 ILC include NK and other ILC that produce IFNγ and are dependent on T-bet for their development and function; group 2 ILC comprise ILC that produce type 2 cytokines (IL-4, IL-5, IL-9 and IL-13) and are dependent on Gata3 and RORα for their development and function; and group 3 ILC subtypes that produce IL-17 and/or IL-22 and depend on RORγt for their development and function (reviewed in [••8]). This framework clusters cells based primarily on functional characteristics but also on signature transcription factors that drive their cytokine production. Still, functional plasticity is characterized by

modifications in cytokine secretion profiles that can result from changes in transcription factor expression. As such, an inter-conversion between different ILC subsets may occur during immune responses or following ILC activation. Nevertheless, this ILC nomenclature may be useful to help understand and define the general principles that guide the development of ILC subsets from hematopoietic precursors.

While these new ILC subsets have generated considerable interest and excitement within the scientific community, remarkable advances in our understanding of the development and functional differentiation of NK cells have as well been recently reported. In this review, we will discuss our current knowledge on the development of NK cells from early hematopoietic precursors in the mouse, focusing on the transcription factors that orchestrate this process and discuss how these developmental pathways may also provide insights in to the developmental biology of other ILC subsets.

A Refined Model for Bone Marrow NK cell Development in the mouse

During hematopoiesis hematopoietic stem cells give rise to progressively more restricted precursor populations that generate lymphoid restricted common lymphoid progenitor (CLP) as well as precursors with restricted myeloid potential (CMP). In the mouse, CLP possess precursor potential for T, B, and NK cell at the single cell level [9], and can give rise to ILC2 at the population [••10,11] and single cell level (Klein Wolterink and Di Santo, submitted). CLP are lineage marker (Lin^-) negative and express of low levels of c-Kit (CD117), Sca1 (Ly6A/E), IL7Rα (CD127) and Flk2/Flt3 (CD135). A decade ago, we reported identification of the earliest murine NK cell committed precursor (NKP) residing within a Lin^- population expressing the IL2/15Rβ chain (CD122)[12]. The developmental potential of this population was limited to NK cells, but only gave rise to NK cells in vitro with a frequency of one in ten, indicating that the NKP subset was not functionally homogeneous. The fact that only 10% of phenotypically defined NKP had true NK precursor potential meant that mutations strongly affecting NKP development could go unnoticed, while normal NKP numbers might still be present in mice lacking almost all phenotypically defined NKPs. As such, it was difficult to reach definitive conclusions regarding factors that influence NKP homeostasis. While the identification of NKP was an important advance, the pathway between CLP and NKP was still poorly defined.

Recently, two reports have further refined the phenotypic definition of NKP and also provided evidence for an additional NK committed precursor populations that is upstream of NKP. Fathman et al. used in vitro assays and in vivo adoptive transfer experiments to identify NKP [••13]. They found that all NK cell potential resided in a $Lin^-CD27^+CD244^+$ fraction that included several $CD127^+$ cell subsets. As expected, this included $CD135^+$ CLP, but also included two additional NK cell restricted subsets. Both of these subsets lacked CD135, but they differentially expressed CD122: the $CD122^-$ subset was designated a pre-NKP, while the second $CD122^+$ subset likely corresponds to a refined definition of our previously identified NKP subset, termed refined NKP (rNKP), that also was shown to contain $CD122^+CD127^+$ cells [12–14]. Together, this report would suggest a model where the transition from CLP to NKP that involves sequential down-regulation of CD135, up-regulation of CD122, followed by down-regulation of CD127 (Figure 1). This refined model of early NK cell development should provide a means to further dissect the transcriptional program that accompanies NK cell specification and commitment in the mouse. Along these lines, analysis of Ets-1 mutant mice demonstrated a clear defect in pre-NKP and rNKP cells [••15], while analysis of Rag2 x γ_c DKO mice showed a decrease in the $CD122^+CD127^+$ NKP subset but not in its immediate $CD122^-CD127^+$ precursor (Brauner, Vosshenrich and Di Santo; unpublished results).

In a second report, Carotta et al. used an Id2 reporter mouse to identify early NK cell precursor cells in adult bone marrow. They also identified NK cell potential (using in vitro assays on OP9 stromal cells) amongst Lin^-CD127^+ cell populations that lacked CD135 (i.e. were not CLP) but differentially expressed CD117 and Sca1 [••16]. Both populations lacked CD122 expression but expressed low levels of NKG2D, previously identified on a subset of NKP [14,••16] and were therefore different from previously identified NKP; these cells were referred to as pre-pro NK cells and likely correspond to the pre-NKP described by Fathman [16]. Interestingly, these pre-pro NK cells also appear to phenotypically overlap with a recently identified $CD127^+Sca1^{hi}$ ILC2 precursor population that also expresses CD25 and T1/ST2 [17]. Whether the pre-pro NK cell populations described by Carotta et al. harbors ILC2 potential or whether the putative ILC2 precursor described by Halim et al. also contains NK cell potential has not been studied [••16,••17]. Potentially, these populations might represent an interface between ILC1 and ILC2 development.

Figure 1

Transcriptional Regulation of NK Cell Development

General considerations

During the lineage restriction of hematopoietic precursor cells undergo dramatic alterations at both proteomic and transcriptomic levels. Alternative lineage-potentials are progressively restrained until desired cell fates are completed. These changes occur within a dynamic cellular context that varies with respect to topography (cell niche, microenvironmental positioning) as well as activation state (resting/steady-state versus infectious/inflammatory milieu). Transcription factor repertoires consist of lineage-determining factors ('master regulators') that need to be co-expressed with transcriptional modifiers (such as NFκB, or Foxo1) to orchestrate transcriptional programs through cooperative binding with other lineage-determining transcription factors to regulatory promoters/enhancers and activate transcription via recruitment of chromatin remodeling proteins [18,19]. Epigenetic regulation represents an additional layer that controls TF access to their binding sites. The loci of master regulators like *Pax5* or *Ebf1* have been shown to carry both activating and repressive marks in HSC before lineage-specification takes place, that resolve into either positive marks upon specification along the corresponding lineage or into repressive marks upon specification into a different lineage [20,21]. In a similar fashion, bivalent epigenetic marks are found as well in more differentiated Th cells that may help explain the observed plasticity in Th subset differentiation [••22].

Transcription factors driving generation of NK cell precursors (NKP)

While NKP represent a lymphoid precursor stage that is distinct from T and B cell precursors and has demonstrated NK cell potential, it is not yet shown whether NKP lack potential for other ILC lineages. Similarly, pre-NKP or pre-pro NKP have likewise been shown to give rise to NK cells, but their capacity to generate other ILC subsets has not been excluded. Nevertheless, several TF have been identified that are critical for the development of multiple ILC subsets (including NK cells) but do not overtly affect T or B cell precursor activity. The prime example is that of the transcriptional repressor Id2

that acts (like all Id proteins) to counteract E box TF (E2A, E2-2, HEB) activity. In the absence of Id2, very few NK cells develop and residual cells display maturation defects [23,24]. Importantly, ILC2 and ILC3 subsets (LTi cells, IL-22-producing NKp46^{+} cells) are equally dependent on Id2 activity for their development [7,8]. This would place Id2 at an early stage of ILC development, potentially involved in the generation of a common ILC precursor. While earlier analysis of NKP in Id2$^{-/-}$ mice suggested a normal development of NKP [24], a further analysis of rNKP as well as pre-NKP/pre-pro NKP cells in these mice should also be performed. This would provide interesting information on the role of Id2 repressor activity in early ILC precursor development.

Thymocyte selection-associated high mobility group box (Tox) is a second example of a TF that predominantly affects ILC but not T and B cell commitment. Tox deficiency ablates NK cell development and in addition, strongly reduces generation of group ILC3, especially LTi cells, resulting in decreased lymph node and Peyer's patch formation [25]. Whether Tox is required for the generation of ILC2 or other ILC3 subsets (IL-22-producing NKp46^{+} cells) is not known. Moreover, the mechanism by which Tox promotes the development of NK and LTi cells is poorly understood and the analysis of pre-NKP/pre-pro NKP cells and rNKP in Tox$^{-/-}$ mice has not been reported.

Several TF affect NK cell generation through a reduction in multiple lymphoid precursor populations; these may include CLP or their downstream committed progenitors for the B, T, NK and possibly other ILC lineages. This group of TF includes Ikaros and Gfi1 that compromise CLP and more restricted lymphoid precursors ([26,27], Klein Wolterink and Di Santo; unpublished). Another TF that may fall into this category is Ets-1 that has roles in B and T cell lineage development, and also in NK commitment, as recently shown by Ramirez et al. [••15]. Interestingly, Ets-1-deficiency did not affect CLP numbers but resulted in the reduction of NK cell committed pre-NKP and rNKP populations that ultimately translated to a 5-10 fold reduction in mature NK cells [••15]. Ets-1 targets in mature NK cells include *Idb2* (coding for Id2), *Tbx21* (T-bet) and *Il2rb* (CD122), although these targets were not confirmed in NKP. Low levels of Ets-1 are already detectable in LMPP and CLP [28] and therefore precede that of Id2 [••15]. This observation suggests that Ets-1 might be upstream of Id2 in the NK lineage-defining program. Still, the NK cell deficiency in Id2$^{-/-}$ mice is stronger than that of Ets-1$^{-/-}$ mice that might be due to the fact that Id2 levels are reduced but not ablated in absence of Ets-1. Alternatively, other Ets-family members may compensate in the absence of Ets-1

to maintain Id2 levels. Since Ets-1 controls CD122 mRNA expression in NK cells [••15] and regulates CD127 expression in peripheral T cells [29], Ets-1 may have additional roles in the development of other ILC groups.

Transcription factors driving immature NK cells development from NKP

Beyond the TF that determine the initial steps in NKP and (possibly) other ILC precursor generation, several TF have been identified that play critical roles in the development of 'immature' NK cells in the bone marrow and other tissues. Immature NK cells can be defined as $CD3^{-}CD122^{+}NK1.1^{+}DX5^{-}$ and are thought to represent a transient developmental intermediate that can further differentiate giving rise to mature NK cells that circulate in the blood and are detected throughout the organism.

One critical TF require for iNK generation is Nfil3/E4bp4, a transcriptional activator/repressor that was initially identified as protein binding the ATF site in the adenovirus E4 promoter [30]. While Nfil3 is strongly expressed in mature NK cells and their precursors, it is not an NK cell-specific TF, and is expressed in T, B and dendritic cell (DC) subsets, where it regulates production of Th2 cytokines IL-5, IL-10, IL-13 in T cells, IL-12 production from macrophages and TLR-3 induced IL-12 production from $CD8\alpha^{+}$ cDC and is involved in the regulation of Ig germ-line epsilon expression affecting subsequent class switch to IgE (reviewed in [31]). In the absence of Nfil3, NK cell homeostasis is strongly reduced and NK cell differentiation is blocked at the iNK cell stage [••32–34]. While downstream targets of Nfil3 are not clearly defined, residual levels of Gata3 and Id2 were reduced in Nfil3-deficient lymphoid precursors, suggesting that Nfil3 may regulate these TFs. Whether Nfil3 KO mice have defects in the generation of other ILC subsets has not been reported, although preliminary data indicated normal LN development [35] and presence of IL-22^{+} $NKp46^{+}$ intestinal ILC3 (Xu, Serafini and Di Santo, unpublished) ruling out a role for Nfil3 in controlling differentiation of all ILC3 subsets. These observations would further suggest that Nfil3 does not regulate Id2 in a global fashion but would rather play a more selective role in development of NK cells and perhaps other IFN-γ-producing ILC1.

The TF Tbx21 (encoding T-bet) was initially described as a 'master regulator' of Th1 differentiation and also shown to control IFN-γ production in various T cell subsets. The initial analysis of NK cell differentiation in T-bet KO mice found several defects [36],

although most appeared related to the homeostasis of mature NK cells that may be related to regulation of receptors for S1P5 [37]. Tbx21 is a member of a larger family of TF that also includes the broadly expressed Eomesodermin [38]. Functional redundancy between T-box TF has been demonstrated in several cell types and T-bet/Eomes are co-expressed in $CD8^+$ T cells where they have overlapping/redundant roles [39,40]. Recently, an interesting study of the specific versus redundant role of T-bet and Eomes in NK cell differentiation was reported [••41]. While T-bet was expressed throughout NK cell development (in iNK and mNK in newborn and adult mice), Eomes expression was more restricted and was clearly absent in $DX5^-$ iNK cells that could be found throughout the body but were conspicuously enriched in the liver [••41]. Previous studies have characterized these $DX5^-$ hepatic NK cells and using adoptive transfer experiments showed that they could give rise to functionally mature NK cells (with up-regulation of DX5 and acquisition of MHC class I-specific Ly49 receptors and effector functions) [42]. In the absence of Eomes, NK cell development was blocked at the $DX5^-$ iNK cell stage, clearly indicating a role for Eomes in terminal NK cell maturation. The residual Eomes- iNK cells expressed T-bet and the combined deletion of T-bet and Eomes ablated development of all NK cells beyond the NKP stage [••41]. Further analysis of $Tbx21^{-/-}$ mice demonstrated a defect in the generation of iNK cells, placing T-bet as a critical regulator of the NKP -> iNK transition (Figure 2). The transcriptional targets of T-bet at this stage are not known but may include the NK cell activating receptors *Ncr1* and *Cd161* (encoding NKp46 and NK1.1, respectively) [••41] that may further complicate the unambiguous identification of T-bet-deficient iNK cells.

The TF Gata3 has multiple stage-dependent roles in T-cell development and has also been shown to control multiple aspects of NK cell subset differentiation and more recently, ILC2 differentiation ([43–••47], RKW submitted). Gata3 is a zinc-finger TF that binds a consensus (A/T)GATA(A/G) motif and can deliver activating or repressive transcriptional signals. The critical targets of Gata3 in early lymphoid precursors are not fully defined. In immature thymocytes, Gata3 binds hundreds of distinct loci for which the functional implications are not clear [••22]. Interestingly, one predominant Gata3 target is the retinoic acid receptor RORα that has multiple roles in hematopoietic and non-hematopoietic lineages. The similar loss of ILC2 in Gata3 and RORα deficiencies [••10, ••17, ••46, ••47] suggest that these two TF operate in a sequential fashion (Gata3 -> RORα).

The effects of Gata3 ablation on NK cell development include an ablation of hepatic and CD127$^+$ thymic NK cells and a defect in BM and splenic NK cell maturation; the latter involves a reduction in CD11b and CD43 expression on mature NK cells and a decrease in their IFN-γ producing capacity [43,44]. The loss of IFN-γ was unexpected and indicated that Gata3 could regulate T-bet in NK cells [43], although a role for Gata3-mediated control of Eomes expression can not be ruled out (see below). The selective impact of Gata3 deletion on thymic versus BM NK cell homeostasis suggests that these two developmental pathways are distinct. Concerning thymic NK cell development, we hypothesize that Gata3 is upregulated in lymphoid precursors that enter the thymic microenvironment; this may involve a Notch-dependent signal, although this does not appear to be essential [•48]. Gata3-expressing lymphoid intrathymic precursors primarily generate T cells, but can give rise to NK cells and ILC2 [••10,44,49]. Gata3$^+$ NK cells that develop within the thymus differ phenotypically and functionally from mature splenic NK cells (higher IFN-γ, CD16$^-$, few Ly49 receptors); these different attributes may be transiently imposed by the thymic microenvironment. Along these lines, adoptive transfer of thymic NK cells to Rag/γ_c recipients allows for their further maturation with acquisition of Ly49 receptor repertoires and loss of CD127 (Vosshenrich, Di Santo; unpublished results).

Concerning hepatic NK cells, it is tempting to speculate that functions of Gata3, Eomes and T-bet may again be intertwined. In the absence of T-bet, hepatic NK cells, especially the immature DX5$^-$ subset, are decreased in number. The DX5$^+$ subset is also affected but possibly 'rescued' due to Eomes expression [••41] either through local generation of DX5$^+$ NK cells in the liver or from influx of DX5$^+$ NK cells from the bone marrow. In contrast, Gata3 deficiency strongly reduces all hepatic NK cell subsets. While the mechanism behind this defect is not understood, one possibility is that Gata3 controls not only T-bet but also Eomes expression in hepatic NK cells. Further experiments are required to validate this hypothesis.

Transcription factors driving NK cell maturation

Several TF have been found to impact on the differentiation at later stages of NK cell differentiation. Of these, Eomes appears as a critical factor required for completion of NK cell maturation following DX5 acquisition (stage 4; Figure 1). Interestingly, NK

cells lacking Eomes, T-bet or bearing temporal deletions in both genes retained significant functional capacities [••41] indicating that Eomes/T-bet-independent regulation of NK cell cytotoxicity and cytokine production. In contrast, inducible deletion of Eomes in $DX5^+$ mature NK cells resulted in the appearance of $DX5^-$ NK cells that co-expressed TRAIL; this demonstrates that Eomes is actively required in order to maintain the mature NK cell phenotype. Moreover, these $DX5^-$ NK cells preferentially accumulated in the liver, suggesting that this tissue provides an environment that favors homeostasis of this NK cell subset.

Helios, another zinc-finger TF and member of the Ikaros family is abundantly expressed in immature $CD11b^-$ NK cells [50]. Helios over-expression has been associated with hyper-activated mature NK cells mice carrying 'Noe' mutations in the Ncr1 locus that ablate NKp46 cell surface expression [50]. The proposed link between the absence of cell surface NKp46 availability and the up-regulation of Helios transcript that lead to NK cell hyperactivity remains unclear.

Additional TF have been implicated in the final differentiation of NK cells. In addition to its role in the transition from NKP -> iNK cells, Ets-1 also regulates homeostasis of mature NK cells. In the absence of Ets-1, mature NK cells have an activated phenotype with enhanced Helios expression and a hyper-responsiveness to IL-15 [••15]. Runx3 is highly expressed throughout NK cell development and targets of Runx3 include NKp46 and possibly CD122 and members of the Ly49 family [51,52].

Blimp1 (encoded at *Prdm1*) acts as transcriptional repressor that regulates the terminal differentiation of B and T cells (reviewed in [53,54]). In NK cells, Blimp1 regulates the frequency of terminally differentiated $CD27^-CD11b^+$ NK cells. Like Ets-1-deficient NK cells, Blimp1-deficient NK cells are hyper-responsive to IL-15 stimulation. Blimp1-deficiency might also impact on NK trafficking as Blimp1 was shown to regulate chemokine receptor expression in T cells (reviewed in [54]). While Blimp1-knockdown in human NK cells resulted in increased IFN-γ production, murine NK cells lacking Blimp1 do not show alterations in IFN-γ production [55,56]. The reason for this species-specific difference is not known but could be related to the presence of Hobit (homolog of Blimp1 in T cells, Znf683) a related TF that is highly expressed in NKT cells [••57]. While Hobit is apparently not expressed in resting wild-type NK cells, its expression (up-regulation?) in the absence Blimp1 could contribute to the attenuated phenotype of

Blimp1-deficient NK cells. As such, Hobit might sustain IFN-γ production and cytotoxicity in Prdm1$^{-/-}$ NK cells.

An extended model for NK cell and other ILC subset development

We would like to propose a model that takes into account these different observations thus far discussed. It is now documented that CLP can give rise to B, T, NK and ILC2 in vivo; whether CLP are precursors for ILC3 subsets needs to be shown. In contrast, dedicated precursors for ILC subsets that are downstream of CLP are less clearly defined in adult mice. Ample evidence exists for the presence of a NKP that has NK lineage potential, but the existence of a 'common ILC precursor' (ILCp) that can subsequently give rise at the clonal level to ILC1, ILC2 and ILC3 subsets has been proposed [••8] but not clearly documented. Up-regulation of Id2 in CLP would provide a mechanism to restrict CLP to the non-T, non-B cell fate and would be necessary for subsequent ILC subset differentiation (Figure 2). If such an ILCp exists, it would then undergo additional transcriptional changes to selectively differentiate towards individual ILC sublineages. Previous studies have identified Notch signals as important for ILC2 and lymphoid-tissue inducer (LTi, an ILC3 subset) cell generation [••58–••60] from multipotent lymphoid precursors. In contrast, NK cells do not apparently require Notch signals [•48,61] to develop from CLP. Thus Notch signals could provide an early mechanism to initiate ILC subset differentiation from ILCp. In this model, ILC2 and ILC3 (at least the LTi cell subset) would more closely resemble T cells in their requirements for Notch. Potential downstream targets for Notch in early T cells include Tcf1, Bcl11b and Gata3; the latter could have a dominant role in the initiation of ILC2 specification and its completion via RORα. A similar mechanism could be operative in the generation of RORγt^{+} ILC3 subsets. Still, sustained Notch signaling appears detrimental for ILC3 homeostasis as it favors T cell development at the expense of ILC3 precursors [••60].

In contrast, these same factors (Notch, Gata3, RORα, RORγt) have little or no apparent impact on 'mainstream' NK cell development in the bone marrow. In contrast, two TF have critical roles at the earliest stages: Nfil3 and T-bet. While these two TF have no obvious ability to interact physically or functionally, their coincident expression and critical roles in the generation of iNK from NKP are clearly demonstrated. Up-regulation of T-bet and Nfil3 in CLP and/or ILCp are likely to be critical in the early stages of

commitment to the ILC1 lineage as NKP are generated. What mechanisms might control Nfil3 and/or T-bet expression? Although clear answers are not yet available, there are several hypotheses. First, as yet unidentified TF(s) may link Id2 expression (and loss of E-box activity) with up-regulation of Nfil3 or T-bet. These might be identified through comparison of TF profiles between CLP and pre-NKP. Alternatively, the absence of Notch signaling in ILC precursors may be associated with default expression of Nfil3 or T-bet that can then be stably reinforced by extrinsic signals (cytokines).

In the not so distant past, the transcriptional map of NK cell development consisted of a straight line between the hematopoietic stem cell and the mature NK cell with no defining directional markers. We now have a detailed phenotypic model for the emergence of mature NK cells from hematopoietic precursors that proposes a step-by-step process of commitment to the NK lineage, education to self MHC, and maturation and acquisition of NK cell effector functions. Several key TF that guide this process have been revealed and provide a framework for a better understanding of NK cell differentiation in particular, and of ILC development in general.

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Figure Legends

Figure 1. Stages of NK cell development from CLP in the mouse

In the mouse, refined NK cell precursors (rNKP) represent the earliest precursor committed to the NK lineage (stage 1). Precursor potential of pre-NKP for other ILC lineage remains to be verified. The iNK and mNK nomenclature is based on absence or presence of DX5 expression. Current knowledge allows for their further subdivision according to the markers indicated on the left as indicated by the ”stages”. Of note, rNKP comprise $CD127^+$ and $CD127^-$ cells. It should also be noted that only a fraction of iNK and mNK cells express CD94 in adult mice. Similarly, any given Ly49 is expressed at a characteristic frequency in mature NK cells, which is dependent on the genetic background (“Ly49 repertoire”).

Figure 2. Schematic view of NK development in the bone marrow and liver and comparison with ILC2 and ILC3 development in the mouse

Transcription factors and their potential targets are indicated. $DX5^-$ and $DX5^+$ NK cell subsets are present in hepatic tissue, although their origin remains a matter of debate (dotted arrows). The impact of Nfil3/E4bp4 on iNK cells has been documented, but the potential effects of this TF on NKP is unclear and should be revisited. Runx3 and Gata3 control of Eomes expression has been demonstrated in T cells but their role in NK cells is still speculative. A similar comment applies to the putative role of Notch for Gata3 induction in ILC2.

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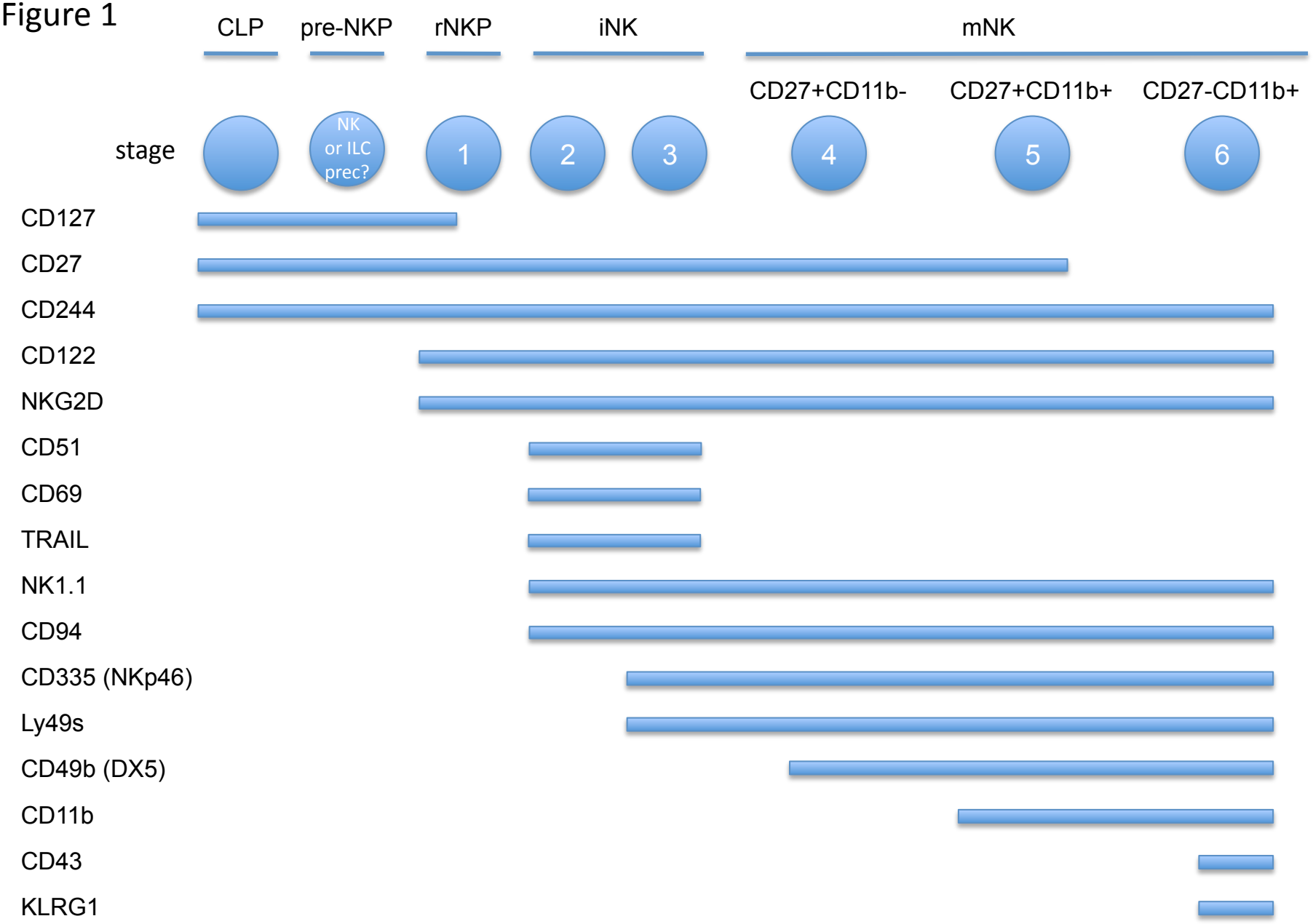
Figure 1


CLP
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stage
NK or ILC prec?
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CD27
CD244
CD122
NKG2D
CD51
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CD94
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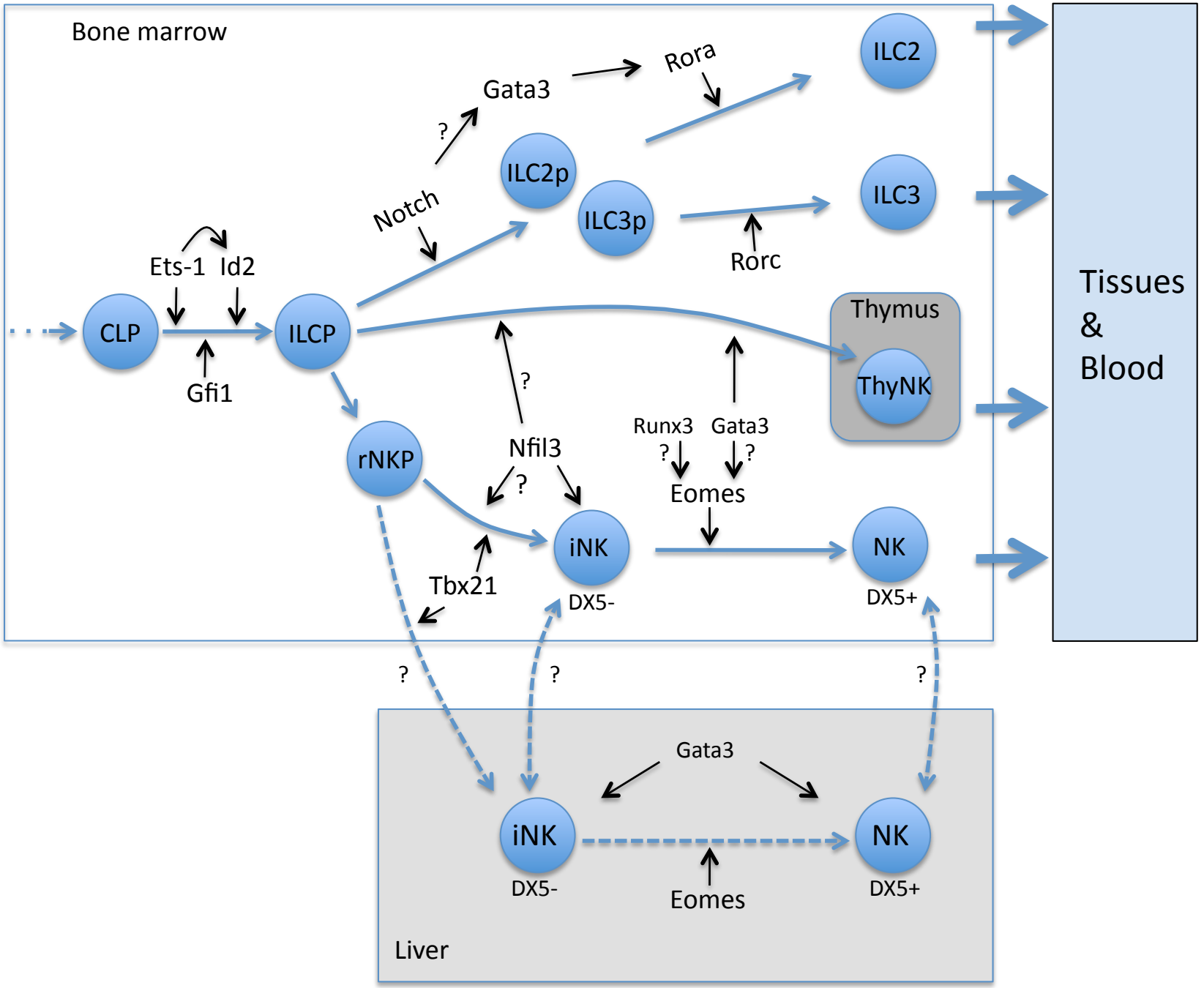
CD49b (DX5)
CD11b
CD43
KLRG1

Figure 2


Bone marrow
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