Transcriptional regulation of murine Natural Killer cell development, differentiation and maturation

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Abstract

Natural killer (NK) cells are innate cytotoxic effector cells that play important protective roles against certain pathogens as well as against pathogen-infected and transformed host cells. NK cells continuously arise from adult bone marrow-resident haematopoietic progenitors. Their generation can be sub-divided into three phases. The early NK cell development phase from multipotent common lymphoid progenitors occurs at least in part in common with that of additional members of a family of innate lymphoid cells (ILC), for which NK cells are the founding member. An intermediate phase of NK cell differentiation is characterized by the acquisition of IL-15 responsiveness and lineage defining properties such as the transcription of genes coding for cytotoxic effector molecules. This is followed by a late maturation phase during which NK cells lose homeostatic expansion and increase effector capacity. These three phases are regulated by multiple stage-specific but not NK cell-specific transcription factors. This review summarizes the NK cell developmental and maturation processes and their transcriptional regulation with an emphasis on data derived from genetically modified mouse models.

Keywords

NK cells, Innate Lymphoid Cells, Development, Differentiation, Maturation, Cytokine, Cytokine Receptor, Transcription factor

Abbreviations

αCLP	α4β7 integrin expressing Common Lymphoid Progenitors
BM	Bone Marrow
ChILP	Common Helper Innate Lymphoid Progenitor
CILP	Common Innate Lymphoid Progenitor
CLP	Common Lymphoid Progenitors
EILP	Early Innate Lymphoid Progenitors
Flt3	Fms like tyrosine kinase 3
Id2	Inhibitor of DNA-binding 2
IL	Interleukin
IL-7Rα	Interleukin-7 receptor alpha
ILC	Innate Lymphoid Cell
IFNγ	Interferon gamma
iNK	immature NK cell
Lin	Lineage
LTi	Lymphoid tissue inducer
Nfil3	Nuclear factor, interleukin-3 regulated
NK	Natural Killer
NKP	Natural Killer Progenitor
mNK	mature NK cell
rNKP	refined Natural Killer Progenitor
Tcf1	T cell factor 1
T_H	Helper T cell
TNFα	Tumor necrosis factor alpha
Tox	Thymocyte selection associated high-mobility group box

Introduction

Natural Killer (NK) cells play important roles for the recognition and clearance of fungal pathogens [1], host cells infected with distinct viral pathogens as well as transformed host cells. Of clinical relevance is the ability of NK cells to reject bone marrow allografts and to mediate graft versus leukemia effects [2]. NK cells detect potential target cells using a set of around 50 germ-line encoded activation and inhibition receptors that are collectively used to assess whether cells express relevant ligands at normal levels. Diseased host cells or foreign cells, that yield an excess of NK cell activation as compared inhibition signals, are killed using the directed release of cytolytic granules containing Perforin and multiple Granzyme family proteins. In addition to cell-mediated killing, NK cells are an important early source of pro-inflammatory cytokines including IFNγ and TNFα.

NK cells are the founding member of a family of innate lymphoid cells (ILC) whose development depends on the cytokines IL-7 and IL-15 but is independent of the somatic rearrangement of antigen receptors. ILCs are grouped into type 1 (NK cells, ILC1 and ILC1-like cells), type 2 (ILC2) and type 3 ILC (ILC3 and lymphoid tissue inducer (LTi) cells) [3, 4]. While the effector functions of NK cells correspond to those of CD8 T cells, the hallmark functions of ILC1, ILC2 and ILC3 cells overall correspond to those of T_H1, T_H2 and T_H17 cells, respectively. The production of the respective immunoregulatory cytokines by ILC is chiefly regulated by specific tissue-derived cytokines and this serves to amplify and direct the early phase of an immune response to infection or tissue damage.

ILCs and NK cells differ in their tissue distribution and developmental origins. NK cells are most abundant in lymphoid organs in particular the spleen and the circulation and they continuously derive from adult bone marrow (BM) progenitors. In contrast, ILC1-3 in adult mice are predominantly resident in non-lymphoid as well as lymphoid tissues whereby adult BM precursors or circulating ILCs play a minor role for their renewal [5]. Rather, ILC arise from fetal progenitors and seed non-lymphoid tissues before birth [6, 7]. Despite predominant ILC development from fetal progenitors, adult BM progenitors retain the potential to give rise to all currently known ILC subsets *in vitro* as well as *in vivo* using cell transfers into alymphoid mice [8]. Indeed adult BM progenitors have chiefly been used to elucidate the transcriptional control of early ILC development.

Early ILC development

All adaptive and innate lymphocyte lineages can be derived from adult BM resident common lymphoid progenitors (CLP), which lack markers of mature haematopoietic cell lineages (typically B220, CD4, CD8, Gr-1, Mac-1 (CD11b) and Ter-119) but express the α chain of the IL-7 receptor (CD127) and the receptor for Flt3L (FMS-like tyrosine kinase 3 ligand) (CD135) (Lin^- IL-7Rα^+ $Flt3^+$) (for further reading see [9] [10]). A recently identified integrin α4β7 expressing CLP subpopulation (αCLP) shows considerably lower B and T cell potential but can give rise to all ILC lineages [11]. No B or T cells, but all ILC subsets can derive from putative further downstream early innate lymphoid progenitors (EILP) (Lin^- IL-7Rα^- $Flt3^+$ *Tcf7*$^{gfp+}$) [12] [13] or a progenitor population termed CILP (common innate lymphocyte progenitors, also termed αLP) (Lin^- IL-7Rα^+ $Flt3^-$ α4β7$^+$) [11] [14]. ILC1-3, but not NK cells, can derive from partially overlapping populations of common helper ILC precursor (ChILP) (Lin^- IL-7Rα^+ $Flt3^{low}$ $ID2^{gfp+}$) [8] or ILC precursors (ILCP) that express PLZF (*Zbtb16*) [15] (**Fig. 1A**).

The exact relationship between the pan ILC progenitors CILP and EILP remains to be determined. EILP do not express IL-7Rα, which may prevent differentiation towards the B cell lineage. Yet IL-7Rα downregulation would need to be transient as downstream ILC1-3 progenitors as well as mature ILC2s and ILC3s express IL-7Rα and require IL-7 signaling for differentiation and/or maintenance. Even though NK cell progenitors express IL-7Rα, mature NK cells lack IL-7Rα but express IL-15Rβ (CD122). Indeed NK cell development occurs independent of IL-7 but requires IL-15 [16]. The latter is produced by radio-resistant BM stromal cells dependent on the transcription factor Irf-1 dependent fashion [17]. ILC1 also express IL-7Rα and intermediate levels of IL-15Rβ and similar to NK cells, ILC1 development depends on IL-15 but not IL-7 [8]. Thus a differential dependence on IL-15 and IL-7 distinguishes the development of NK cells/ILC1 and ILC2/ILC3, respectively.

Intermediate NK cell and ILC1 lineage differentiation

Based on the key role of IL-15 for NK cell development, the specified NK cell progenitor (NKP) was initially identified as a Lin^- IL-15Rβ^+ BM population that lacked the NK cell markers NK1.1 (CD161) and DX5 (CD49b) [18]. However only about 10% of NKP yield NK cells *in vitro* indicating that this population is heterogeneous [18]. Since then the definition of NK cell progenitors has been improved. Refined NKP cells (rNKP) (Lin^- IL-7Rα^+ $Flt3^-$ IL-15Rβ^+) yield NK cells with around 50% efficacy [19]. These analyses also identified preNKP cells that do not express IL-15Rβ (Lin^- IL-7Rα^+ $FLT3^-$ IL-15Rβ^-) but nevertheless give rise to NK cells *in vitro* with a considerable efficacy (40%) [19]. PreNK cells

appear similar to independently defined preproNKP [20]. Even though preNKP as well as rNKP cells efficiently give rise to NK cells, it is not known whether these populations also harbor ILC precursors or even multipotent progenitors that can yield more than one ILC lineage. Based on their phenotypic similarity, it is possible that there is some overlap between preNKP (Lin^- IL-7Rα^+ $Flt3^-$ IL-15Rβ^-) and CILP (Lin^- IL-7Rα^+ $Flt3^-$ $\alpha4\beta7^+$).

The acquisition of IL-15 responsiveness represents the key event of NK cell as well as ILC1 development. The IL-15 receptor is composed of IL-15 receptor α (IL-15Rα) (CD215), IL-15Rβ (CD122), and the common γ chain (CD132). The latter two chains are shared with the IL-2 receptor. Signal transduction occurs via downstream JAK1/3 and STAT5 [21] and this sustains the expression of the pro-survival Bcl2 family protein Mcl1 and blocks the pro-apoptotic Noxa and Bim [22]. The NK cell developmental defect in the absence of IL-15Rβ can be rescued by enforced Bcl2 expression [23], indicating that IL-15 mediates NK cell development by ensuring cell survival. IL-15 does not seem to commit cells towards the NK cell lineage since the abundance of NKP is unperturbed in mice lacking the common γ chain chain (CD132) [21].

Subsequent to the acquisition of IL-15Rβ, cells upregulate NK.1.1 to become immature NK cells (iNK), which is followed by the upregulation of DX5 (CD49b, VLA2) to give rise to the mature NK cell (mNK) compartment (**Fig. 1B**). Recent fate mapping studies revealed that the iNK and even the mNK populations are heterogeneous and contain ILC1 progenitors [24]. Indeed, NK cells and ILC1 share a number of markers including NKp46 and NK1.1 and, similar to NK cells, ILC1 development depends on IL-15 but not IL-7 [8]. NK cells can be distinguished from ILC1 by the differential expression of DX5 (CD49b, VLA-2) and CD49a (VLA-1), and the differential dependence on the transcription factors Eomes (Eomesodermin) and T-bet (T box transcription factor *Tbx21*) [25]. While both cell types can produce IFNγ, only NK cells acquire MHC class I-specific activating and inhibitory receptors belonging to Ly49 family and the potential to kill target cells. Despite these insights, the precise developmental steps intermediates that yield NK cell and ILC1 remain to be dissected further.

Transcription factors required for early ILC development

ILC can be derived from CLP dependent on the expression of several transcription factors. These transcription factors are not uniquely required for ILC development, as they play roles in multiple lymphoid lineages, in particular in T cells. This is compatible with the idea that the adaptive immune

system has adopted transcriptional modules established by evolutionarily older innate lymphocytes [26].

Transcription factors required for the development of all ILCs currently include the basic leucine zipper protein Nfil3 (Nuclear factor, interleukin 3 regulated, also known as E4BP4) [27] [28], the E protein antagonist Id2 (inhibitor of DNA binding 2) [29, 30], the high-mobility group (HMG) box proteins Tcf1 (T cell factor 1, encoded by the *Tcf7* gene) [12] and Tox (thymocyte selection associated high-mobility group box) [31] and the Ets family protein Ets-1 [32, 33].

CLP do not express Id2, Tcf1 or Tox but express low levels of Nfil3, yet CLP do not depend on Nfil3 expression. Nfil3 is upregulated in downstream αCLP, possibly based on IL-7 dependent signals, and these cells do depend to a significant extent on Nfil3 [11]. Nfil3 binds regulatory regions of the *Id2* and *Tox* genes and induces their expression [14, 34, 35]. In agreement with this hierarchy, downstream CILP express elevated levels of Id2 and, in addition to Nfil3, these cells depend on Id2 and in part on Tox [14, 36]. Enforced expression of Id2 in Nfil3-deficient progenitors is sufficient to restore ILC development while enforced Tox expression does so with limited efficacy [14, 35]. ChILP express particularly high levels of Id2 and this correlates with the loss of NK cell but not of ILC1 potential [8]. High level Id2 expression further depends on Ets-1 and the absence of Ets-1 drastically reduces the fitness of ChILP, which may be due to reduced Id2 expression [33]. On the other hand Id2 is not required for early NK cell specification as NKP arise normally [37]. High-level Id2 expression may thus preclude NK cell but not ILC1 development, suggesting that IL-15 responsive NK cells and ILC1 arise via two distinct developmental pathways.

Tcf1 is an additional transcription factor required for the development of all ILC [12]. Tcf1 is not expressed in CLP and αCLP but is expressed in both CILP and EILP and both of these latter pan ILC progenitor populations depend on Tcf1 expression [11, 38] (**Figure 1A**). Likewise, further downstream ChILP and pre/rNK cell populations are also severely reduced in the absence of Tcf1. Tcf1 expression depends in part on Tox [31] and is repressed by Id2 at least in mature NK cells [39]. As Id2 counteracts E protein activity, E proteins such as E2A (*Tcf3*) may promote Tcf1 expression, as seen during CD8 T cell differentiation [40]. Finally, Tcf1 is an essential target of Notch1 signaling for the specification of T cells [41] and ILC2 [42]. However, despite the fact that Tcf1 is essential for NK cell development, absence of Notch1 or RBP-Jκ, a DNA-binding protein that mediates the transcriptional output of Notch signaling, are not critical for NK cell development [43] [44]. These data suggest a

Notch independent role of Tcf1 during NK cell development. This contrasts with the requirement of Notch signaling for the development and/or expansion of ILC1, ILC2 and certain types of ILC3 [42] [44].

Tcf1 binds DNA in a sequence specific fashion and this leads to DNA bending. Like other HMG box proteins Tcf1 can thus play an architectural role, which may bring adjacent transcription factors into proximity. In addition, Tcf1 can repress target genes based on its histone deacetylase (HDAC) activity or via an association with Groucho/TLE co-repressors [45, 46]. Finally, Tcf1 is perhaps best known for its ability to mediate the nuclear response to extracellular Wnt proteins. This response is mediated via the association of the N-terminus of Tcf1 with β-catenin [47]. The specific function of Tcf1 needed for early NK cell development was addressed by complementing Tcf1-deficient mice transgenes coding for full-length or N-terminally truncated Tcf1. Unexpectedly enforced expression of a N-terminally truncated Tcf1 was sufficient to restore the preNK (but not the rNK) compartment [38], indicating that Tcf1 promotes the early steps of NK cell development independent of Wnt signals. In agreement with this conclusion, NK cell development is normal in the absence of β-catenin [42] [48]. Tcf1-dependent roles in regulating ILC development include the repression of genes associated with B cell development, such as Spib or Irf8 in αCLP [11]. Target genes that mediate the Tcf1-dependence of αCLP and/or the specification of IL-15-dependent ILC1 and NK cells have not yet been identified.

Transcription factors involved in intermediate NK cell differentiation

The key event during NK cell development is the acquisition of IL-15 responsiveness via the upregulation of the IL-15Rβ. Mouse strains lacking Tox and Id2 have normal NKP compartments, indicating that these transcription factors are not required for NK cell specification. In contrast, Ets family transcription factors play a role for NK cell specification. Fetal liver stem cells lacking Pu.1 (encoded by *Spi1*) yield NK cells inefficiently upon transfer into adult alymphoid mice whereby NKP are strongly reduced [49]. NK cells are also reduced in the absence of the Ets family protein Mef (Elf4), although the precise stage remains undefined [50]. Finally, even though NKP are normal in the absence of Ets1, rNKP cells are significantly reduced while preNKP cells are only marginally affected [32], revealing a role for Ets1 in NK cell specification. The data further highlight that the analysis of NKP may not be sufficient to properly assess NK cell specification. The developmental defects of NK cells lacking Ets family transcription factors seem to be related to the acquisition of IL-15

responsiveness. Ets1-deficient NK cells show reduced expression of IL-15R [32] and Jak3 [51], which is essential for IL-15R signaling, and residual NK cells lacking Pu.1 fail to respond to IL-15 [49].

Specific insights into NK cell differentiation are frequently based on conditional gene deletion using NKp46 controlled Cre expression (*Ncr1*^{Cre}) [52]. NKp46 is not expressed in NKP or rNKP cells but is expressed in most iNK and mNK cells [52] as well as in ILC1 and a subset of ILC3. *NCR1*^{Cre} mice thus yield insights into the NK cell intrinsic function of genes selectively following the acquisition of IL-15 responsiveness. For example, while Nfil3 is essential for the early development of ILC and NK cells, Ncr1^{Cre} mediated deletion revealed that Nfil3 is dispensable for NK cell development past the acquisition of NKp46 receptor [53]. Conversely, Id2 is not required for early NK cell specification as Id2-deficient NKP are normal [37]. However, Id2 deletion at the NKp46^{+} stage impairs NK cell development. Id2 suppresses SOCS3 thereby maintaining IL-15R signaling and NK cell survival [39]. Similarly, Runx3 is required for NK cell survival and proliferation in response to IL-15 signaling [54, 55]. Further, Eomes has been reported to regulate IL-15Rβ expression [56]. However, NKP lacking both Eomes and T-bet arise normally and express high levels of IL-15Rβ . Moreover, acute deletion of Eomes and T-bet *in vitro* does not impact L-15Rβ expression in mature NK cells [57]. Thus, even though Eomes and T-bet are essential for NK cell development downstream of NKP, their role is likely independent of IL-15Rβ expression [57, 58]. Eomes expression is promoted by Nfil3 and enforcing Eomes expression rescues NK cell development from Nfil3-deficient progenitors in vitro [34]. Tcf1 seems ensures the survival of NK lineage cells apparently independent of IL-15 signaling and the action of other transcription factors implicated in NK cell development (such as Nfil3, Id2, Tbx21 or Eomes) [38]. Tcf1 prevents excessive expression of Granzymes and this protects immature NK cells from Granzyme-mediated self-destruction [38]. Overall, a clear hierarchy among transcription factors required for the differentiation of NK cell committed cells has not yet emerged. Several transcription factors essential for NK cell differentiation at this stage ensure proper IL-15 responsiveness.

Late NK cell maturation and effector functions

Following the acquisition of lineage defining features, NK cells undergo further maturation events. Differential expression of CD27 and CD11b is commonly used to discriminate 3 consecutive stages of NK cell maturation: CD27^{+} CD11b^{-} (mature stage 1: m1), CD27^{+} CD11b^{+} (m2) and terminally mature CD27^{-} CD11b^{+} (m3) NK cells [59] [60]. During this maturational sequence, NK cells progressively lose

homeostatic expansion capacity, but increase the expression of Ly49 family receptors, upregulate genes involved in NK effector functions [60] and become prone to apoptosis [61] (**Fig. 2**).

Irf-2, Eomes and Gata3 are essential for the early transition of NK cells from the m1 to the m2 stage [57, 62, 63]. T-bet expression gradually increases with maturation and promotes the transition to the terminal mature m3 stage [64]. T-bet is essential for the induction of the transcription factor Zeb2, which then establishes the NK cell effector gene expression program in cooperation with T-bet [65]. Normal IFNγ production and cytotoxicity also depends on Mef, which directly regulates the activity of the Perforin promoter [50]. Terminal NK cell maturation further depends on Aiolos (*Ikzf1*) [66], BLIMP-1 (*Prdm1*) [67] and Klf2 [68]. The absence of Klf2 alters the expression of homing receptors such as CD62L and this may limited access to IL-15 rich niches [68].

Conversely the absence of Foxo1 in NK cells is associated with an increased abundance of terminally mature BM and spleen m3 NK cells and this is associated with increased IFNγ production and killing capacity [69]. These effects may be explained by increased expression of T-bet [69]. Similarly m3 NK cells are proportionally increased in the absence of Tcf1 cue to a NK cell intrinsic effect [38]. Conversely, enforced expression of Tcf1 prevents the emergence of terminally mature m3 NK cells. Together with the observation that Tcf1 is downregulated in m3 NK cells of wild type mice, the data actually show that Tcf1 down regulation is required for terminal NK cell differentiation (**Figure 2**). Moreover, as Tcf1 directly binds to the Granzyme B locus and suppresses its expression [38], downregulation of Tcf1 explains Granzyme B upregulation in m3 NK cells and thus the increased capacity of these cells to kill. Thus several transcription factors impact NK cell maturation positively or negatively. Changes in terminal NK cell maturation seem to be associated with corresponding changes in the NK cells' effector capacity.

NK cell stability, diversity and plasticity

Recent gene expression analyses have provided in-depth insights into the diversity of murine NK cells isolated from different tissues as well as their relatedness to other ILC populations. The data revealed that NK cells isolated from the spleen and the liver are transcriptionally indistinguishable, while those derived from the small intestine are distinct, indicating a considerable impact of the tissue environment on the transcriptional make up of NK cells [70]. Murine NK cells are generally distinct from ILC2 and ILC3, yet are relatively closely related to ILC1 [70]. These data raise the question whether NK cells

and ILC1 represent indeed distinct lineages or a spectrum of activation states. The analysis of human tonsil-derived NK cells and ILC based on single cell RNA sequencing (scRNAseq) analyses yielded slightly different conclusions. Human NK cells are more related to ILC3, while ILC1 are more related to ILC2 cells [71]. This might reflect species or tissue differences or the different technologies used (scRNA-seq versus microarray).

Despite the considerable transcriptional differences, there is significant naturally occurring plasticity among ILCs in response to alterations in the cytokine milieu [72]. In addition, disruption of transcription factor networks has provided evidence of ILC plasticity. However, there is comparably scarce evidence for plasticity involving NK cells. T cells acquire NK cell features upon the deletion of the transcription factor Bcl11b [73] or human ILC3 can differentiate into NK cells in the absence of the ligand-activated transcription factor AHR (aryl hydrocarbon receptor) [74]. Finally, enforced Eomes expression diverts ILC1 into NK-like cells [75]. Conversely, recent reports show that NK cells acquire an ILC1-like phenotype (co-expression of $DX5^+$ and $CD49a^+$) in multiple tissues as well as in tumors based on enhanced TGFβ signaling [76] [77]. This conversion may be favored since the set of genes differentially expressed in murine NK cells and ILC1 is relatively small [70].

Conclusions and Perspectives

The understanding of ILC development in general and of NK cell development in particular has considerably increased. Insights into early NK cell development downstream of multipotent CLP has improved largely thanks to efforts to identify ILC progenitors. Notwithstanding there remain considerable gaps in the understanding of NK cell developmental prior to IL-15Rβ acquisition. Additional developmental intermediates likely exist. Moreover, the cellular and molecular events that result in the dependence of ILC on IL-7 versus IL-15 are currently not understood yet represent the key determinant of NK/ILC1 versus ILC2/ILC3 lineage differentiation.

While we thought to have a relatively clear picture regarding the IL-15 dependent stages of NK cell development, recent analyses have complicated this issue. Populations that were thought to be NK cell restricted turned out to be heterogeneous and include progenitors for ILC1. The analyses will have to be refined to more precisely identify the earliest progenitor(s) with restricted NK cell or ILC1 potential and, if possible, to separate the respective developmental paths. Such refinements will be necessary to improve the understanding of NK cell lineage specification and its transcriptional control.

Finally, it will be important to define the signals and the key transcription factors that promote the acquisition of NK cell lineage specific properties as well as the final steps of NK cell maturation.

Without a doubt technological advances such as single cell RNA sequencing will help to dissect the early, intermediate and late NK cell developmental stages in an unbiased fashion. Such insights together with the further use of transcription factor reporter mouse strains, conditional knock out alleles combined with lineage or stage-specific Cre expression will provide a comprehensive road map of NK cell and ILC development and define the cell intrinsic role of transcription factors in the above processes.

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FIGURE LEGENDS

Fig, 1 Transcriptional regulation of discrete stages of the early phase of ILC/NK cell development and the intermediate phase of NK cell differentiation. NK cell development is separated into (**A**) an early phase i.e. prior to the acquisition of IL-15 responsiveness, which occurs in part in common with the development other types of ILC and (**B**) an intermediate phase of NK cell differentiation post acquisition of IL-15 responsiveness, which is specific to NK cells. Indicated are discrete developmental intermediate progenitors and their presumed precursor – product relationships. Further depicted are key cell surface markers and transcription factors used to identify specific populations. Lin^- refers to the absence of a pool of markers expressed by mature hematopoietic cells (usually a cocktail of antibodies to CD3, B220, GR-1, CD11b and TER119). Common Lymphoid Progenitor (CLP), $\alpha4\beta7$ expressing Common Lymphoid Progenitor (αCLP), early lymphoid progenitor (EILP), common innate lymphoid progenitor (CILP), common helper innate lymphocyte progenitor (ChILP), innate lymphoid cells (ILC), NK cell progenitor (NKP). Transcription factors or cytokine receptors indicated in red are limiting for the emergence of the subsequent developmental stage. and those depicted in black with brackets indicate that the respective factor is expressed but not or no longer required.

Fig. 2 Late NK cell maturation NK cells mature from a $CD27^+$ $CD11b^-$ (mature stage 1: m1) via a $CD27^+$ $CD11b^+$ (m2) to a terminally differentiated $CD27^-$ $CD11b^+$ stage (m3). During maturation the potential for homeostatic expansion diminishes and the capacity to kill target cells and the susceptibility to apoptosis increases. The promotion and inhibition of specific transitions by transcription factors is indicated by green and red symbols, respectively.

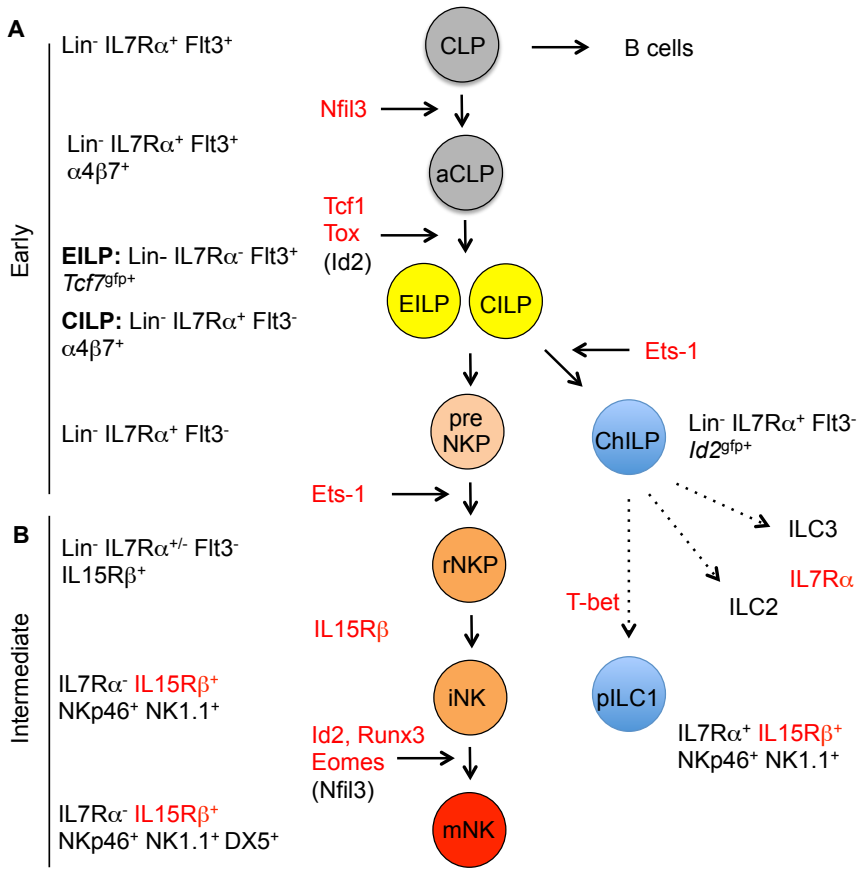
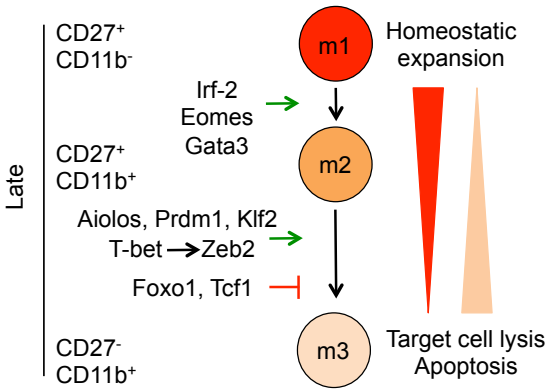

A
Lin- IL7Rα+ Flt3+
CLP
B cells
Nfil3
Lin- IL7Rα+ Flt3+
α4β7+
aCLP
Tcf1
Tox
(Id2)
EILP: Lin- IL7Rα- Flt3+
Tcf7gfp+
CILP: Lin- IL7Rα+ Flt3-
α4β7+
EILP
CILP
Ets-1
Lin- IL7Rα+ Flt3-
pre
NKP
ChILP
Lin- IL7Rα+ Flt3-
Id2gfp+
Ets-1
ILC3
IL7Rα
ILC2
T-bet
B
Lin- IL7Rα+/- Flt3-
IL15Rβ+
rNKP
IL15Rβ
IL7Rα- IL15Rβ+
NKp46+ NK1.1+
iNK
pILC1
IL7Rα+ IL15Rβ+
NKp46+ NK1.1+
Id2, Runx3
Eomes
(Nfil3)
IL7Rα- IL15Rβ+
NKp46+ NK1.1+ DX5+
mNK
Early
Intermediate

Fig. 1

Fig. 2


Late
CD27+
CD11b-
m1
Homeostatic
expansion
Irf-2
Eomes
Gata3
CD27+
CD11b+
m2
Aiolos, Prdm1, Klf2
T-bet → Zeb2
Foxo1, Tcf1
CD27-
CD11b+
m3
Target cell lysis
Apoptosis