Early Cellular Pathways of Mouse Natural Killer Cell Development

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Lineage commitment • Hematopoietic progenitors •
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Abstract

Natural killer (NK) cells are large granular lymphocytes that are components of the innate immune system. These cells are key players in the defense against viral and other microbial infections and cancer and have an important function during pregnancy, autoimmunity and allergy. Furthermore, NK cells play important roles in hematopoietic stem cell (HSC) transplantation by providing the graft versus leukemia effect and preventing the development of graft versus host disease. Thus, understanding the developmental pathway(s) from multipotent HSCs to the NK cell lineage-restricted progenitors is of significant clinical value. However, despite extensive progress in the delineation of mature blood cell development, including the B- and T-cell lineages, the early stages of NK cell lineage commitment and development have been less well established and characterized. Here, I review the progress made thus far in dissecting the developmental stages, from HSCs in the bone marrow to the lineage-committed NK cells in mouse.

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Introduction

Due to the short half-life of the majority of blood cell lineages, during steady state, millions of different mature blood cells are replenished every second in man [1]. The integrity of this process is strictly dependent on the existence of a rare population of hematopoietic stem cells (HSCs) with extensive self-renewing properties as well as the ability to commit and generate all blood cell lineages to persistently support hematopoiesis throughout the life of an individual [2].

Natural killer (NK) cells are lymphocytes primarily participating in the innate immunity but also having important roles in regulating the adaptive immune responses. Diverse NK cell functions include an early defense that can directly eliminate infected and transformed cells by immediate cytotoxic activity, but also via cytokine (tumor necrosis factor and interferon- γ) and chemokine production, with the latter serving to generate and sustain an inflammatory environment and influence adaptive immunity [3]. Thus, NK cells prime B- and T-cell responses, and cross-talk between NK cells and dendritic cells is required to generate efficient immune response [4].

After bone marrow transplantation, donor NK cells play a protective role against alloreactive T cells initiating

the graft versus host disease [5], and mismatched NK cells provide the graft versus leukemia effect in acute myeloid leukemia patients. Also, by producing hematopoietic growth factors such as granulocyte/macrophage colony-stimulating factor, macrophage colony-stimulating factor and interleukin (IL)-3, NK cells are involved in modulating hematopoiesis [3]. Excitingly, NK cells were recently demonstrated to also have the capacity for memory-like responses, a property previously thought to be limited to adaptive immunity [6].

A current prevailing model suggests that bone marrow in the adult is the primary site of NK cell development in steady state [7]. However, NK cells have also been shown to arise at the sites that were traditionally thought to be occupied only by the adaptive immune cells [8], such as the thymus [9] and lymph nodes [10]. NK cells are also found in the gut-associated lymphoid tissues and are specialized in IL-22 production [11, 12]. Further, the subset of NK cells in the uterine mucosa plays an important function during fetal development by regulating the trophoblast invasion and development of the placenta [13]. Characterization and understanding of the functional diversity of NK cell subsets and how these distinct effector cells develop is important to fully realize the potential of NK cell-based therapies [14].

Lineage commitment is the process by which a multipotent stem or progenitor cell becomes increasingly restricted in its lineage fate choices, to ultimately develop into a fully committed progenitor of a single cell lineage. Decades of extensive research provided the evidence that the lineage commitment of HSCs occurs as a stepwise process; however, the cellular pathways and restriction sites, to generate unipotent progenitors of the lymphoid lineage, remain the subject of debate and investigation [15, 16].

Turning on lineage-specific gene programs leads to lineage specification, while repression of lineage-inappropriate gene programs and concomitant restriction of alternative lineage differentiation potential drives the lineage commitment [17, 18]. Such transcriptional events are coordinated by networks of transcription factors and their associated chromatin remodeling factors, presumably under the guidance of extrinsic signals from the local environment.

On the road map of NK lineage commitment and development, several questions remain. Are all diverse mature NK cell subsets generated from the same NK lineage-restricted progenitor (NKP)? What is the relationship between NKPs and other lymphoid progenitors? What is the direct predecessor cell upstream of NKP? Where and when do NK cells develop and differentiate? What is the

relationship between the thymic and bone marrow NK developmental pathways? From the perspective of these outstanding questions, I have here attempted to review the current knowledge and controversies in the field, with a focus on mouse NK cell development.

NK Cell Development in Adult Mice

From HSCs to Immature NK Cells – The Bone Marrow-Dependent Pathway

All the blood cell types are derived from HSCs that reside in bone marrow in the adult individual [15]. HSCs have unique properties of self-renewal required to maintain the blood cell production over the lifetime of the host.

A considerable amount of data supports the hierarchical model of hematopoiesis in which mature blood cells are generated from HSCs in an ordered fashion through a stepwise loss of self-renewal and lineage potentials. This process of HSC differentiation starts with the loss of the self-renewing ability and generation of multipotent progenitors (MPPs), while the developmental stages downstream of MPPs are less defined. The prevailing model of HSC commitment established by the Weissman group predicts that HSCs and MPPs gradually lose their self-renewing ability and sustain pluripotentiality, whereas downstream progenitors exhibit lineage restriction which occurs in a stepwise manner [15].

A number of recent improvements in methodology, used for the prospective purification of candidate progenitors, as well as assays evaluating their lineage potential, allow an alternative road map for blood development to be drawn. The classical model of hematopoiesis proposed a first branching point – a strict lineage commitment/restriction into lymphoid and myeloerythroid lineages – which was supported by identification of the common lymphoid (CLPs) [19] and the common myeloid progenitors [20]. The CLPs can only generate lymphoid cells (T, B and NK cells) [19], while the common myeloid progenitors can produce myeloid and erythroid/megakaryocyte cells [20]. However, identification of lymphoid-primed MPPs (LMPPs) that lack erythroid/megakaryocyte potential but retain both lymphoid and myeloid potential [21] suggested an alternative hematopoietic hierarchy (fig. 1). Based on this finding, a revised model of hematopoiesis proposes the existence of lymphoid-myeloid progenitors as an intermediate stage in T, B, NK cell and myeloid development [21].

Fig. 1. Schematic drawing of the early stages of lymphopoiesis in the adult mouse. Solid arrows indicate established pathways, while the fragmented arrows indicate suggested pathways. HSC = Hematopoietic stem cell; MPP = multipotent progenitor; LMPP = lymphoid primed multipotent progenitor; CLP = common lymphoid

progenitor; ETP = early thymic progenitor; NKP = NK cell progenitor; NK = NK cell; tNK = thymic NK cell; T = T cell; B = B cell. The red/bottom area indicates the bone marrow pathway; the blue/top area indicates the thymus-dependent pathway.

The most immature hematopoietic progenitors in adult mouse bone marrow have a lineage negative (Lin^-), stem cell antigen 1 positive (SCA-1^+), tyrosine kinase receptor CKIT high (KIT^{high}) phenotype (so-called LSK cells) [22]. The LSK compartment is very heterogeneous and can be further sub-fractionated into different stem and progenitor populations based on multiple additional markers. Self-renewing long-term reconstituting HSCs in adult mice are contained within a small fraction of LSK cells that lack the expression of fms-tyrosine kinase receptor FLT3 [21, 23], do not express CD34 molecule [24], but express Slam family receptor CD150 ($\text{LSK FLT3}^- \text{CD34}^- \text{CD150}^+$) [25]. Single $\text{LSK FLT3}^- \text{CD34}^- \text{CD150}^+$ HSCs can permanently sustain efficient production of all blood lineages after transplantation into lethally irradiated recipient mice [24, 25].

The compartment of MPPs downstream of HSCs is composed of cells that have little or no self-renewing ability and maintain multilineage potential. The MPPs are heterogeneous: both the $\text{LSK FLT3}^- \text{CD34}^+ \text{CD150}^+$ cells [21] as well as the $\text{LSK FLT3}^{\text{low/+}} \text{CCR9}^- \text{VCAM-1}^+$ cells [26] are progenitors that retain both lymphoid and myeloerythroid/megakaryocyte potential. The fraction of LSK cells that expresses high levels of FLT3 ($\text{LSK FLT3}^{\text{high}}$)

represents LMPP, that has both lymphoid (B, T, NK cells) and myeloid potentials but has little or no ability to generate the erythroid or megakaryocyte lineages and expresses high levels of transcripts for multiple lymphoid genes [16, 21, 27]. Thus, the LMPP population is the earliest lineage-restricted lymphomyeloid progenitor identified in mouse hematopoiesis [16].

Downstream of the LSK compartment (HSCs, MPPs and LMPPs), there are several progenitors that possess NK cell lineage potential, including the CLPs, defined based on the expression of low levels of SCA-1 and KIT and high levels of IL-7 receptor α chain ($\text{IL-7R}\alpha$) and FLT3 ($\text{Lin}^- \text{SCA-1}^{\text{low}} \text{KIT}^{\text{low}} \text{IL-7R}\alpha^+ \text{FLT3}^+$) [19, 28]. Transplantation studies have demonstrated that CLPs are generated in the bone marrow from LMPPs [28, 29]. Although CLPs have been shown to generate B, T and NK cells, recent studies have shown that a fraction of CLPs still maintains myeloid potential [30, 31]. Further purification of CLPs based on LY6D expression suggests the branching point between the $\text{Lin}^- \text{SCA-1}^{\text{low}} \text{KIT}^{\text{low}} \text{IL-7R}\alpha^+ \text{FLT3}^+ \text{LY6D}^+$ population that has primarily B-cell potential with little or no ability to generate NK and T cells, whereas the $\text{Lin}^- \text{SCA-1}^{\text{low}} \text{KIT}^{\text{low}} \text{IL-7R}\alpha^+ \text{FLT3}^+ \text{LY6D}^-$ CLPs produced B, T and NK cells [32].

Fig. 2. Schematic model for NK cell development in the adult mouse. Stages of developing NK cells in bone marrow are defined based on the expression of cell surface markers. For each sequential maturation stage, differentially expressed markers are listed. Solid arrows indicate established pathways, while the fragmented arrows indicate suggested pathways. iNK = Immature NK cell; mNK = mature NK cell; tNK = thymic NK cell; NK-22 = interleukin 22 producing NK cell. White granules illustrate cytokine production, black granules illustrate lytic activity, and increased

number of granules represents increased functional activity of developing NK cells. Stages A–F and I–V were proposed by Di Santo [7] and Yokoyama et al. [39]. The red/middle left area indicates bone marrow, the blue/top area represents the thymus, the green/middle right area indicates the periphery, and the purple/bottom area represents the gut. The intestinal NKp46⁺ cells represent a heterogeneous population of innate lymphoid cells, distinct from conventional NK cells.

The acquisition of IL-2R β (CD122) that together with IL-15R α and the common γ chain is a part of the IL-15 receptor complex, marks the commitment to the NK lineage, and CD122 continues to be expressed through all the NK cell developmental stages [7, 33]. A candidate NKP has been identified in adult mouse bone marrow as lacking the expression of lineage-specific as well as mature NK cell markers and expressing IL-2R β (CD122; Lin⁻CD122⁺NK1.1⁻DX5⁻) [33]; however, the lineage potential of this NKP has not been extensively studied, and its *in vivo* reconstituting ability was not evaluated. We have recently demonstrated that the Lin⁻CD122⁺NK1.1⁻DX5⁻ cells, in addition to having a robust NK cell potential, sustain the ability to generate T cells *in vivo*, as well as *in vitro* at the single cell level, thus representing a bipotent NK/T-cell progenitor [34]. Therefore,

the identity of the progenitor restricted to NK cell lineage is yet to be established. The CLPs are negative for CD122 and the hierarchical relation between early lymphoid progenitors as well as the progenitor directly upstream of NKPs remains unclear. The fact that the fraction of NKPs expresses IL-7R α , KIT and FLT3 [33–36] suggests that they are downstream of CLPs.

The bone marrow is the main site of NK cell development as the ablation of the bone marrow environment is associated with dramatic defects in NK cell compartment and function [37]. Essentially, all the stages of NK cell development take place in the bone marrow, including the generation of NKPs, differentiation and maturation of developing NK cells, NK cell education and the emergence of fully competent functional NK cells [7]. NKPs were also found in the other mouse tissues including the

thymus, spleen and lymph nodes [7, 33, 38], suggesting that NKPs and immature NK cells are not unique to the bone marrow and that other sites can support NK cell differentiation. Alternatively, NKPs and immature NK cells generated in the bone marrow might seed the other tissues through the circulation.

In contrast to the early stages of NK cell development, the more terminal steps of NK cell maturation are better understood. Based on the studies from the laboratories of Di Santo [7] and Yokoyama et al. [39], a model of NK cell development downstream of NKPs has been established in adult mouse bone marrow. In this model, the differentiation of NKPs to immature NK cells involves progressive acquisition of NK cell receptors starting with CD161c (NKR1C, a family of C-type lectin receptors) recognized by the anti-NK1.1 antibody in C57BL/6 and C57BL/10 mouse strains, a marker that can also be detected on NK T cells and subsets of $\gamma\delta$ T cells [40, 41]. The natural cytotoxicity receptor NKp46 (NCR1) is restricted to the NK lineage and starts to be expressed on immature NK cells downstream of NKPs [42]. The NKp46 is considered to be the most specific NK cell lineage marker. The differentiation of immature NK cells into functionally mature NK cells involves sequential acquisition of multiple cell surface receptors such as CD94/NKG2A/C/E and Ly49 (recognizing major histocompatibility complex class I) and DX5/CD49b, with the final maturation being associated with increased expression of CD11b/Mac-1 and CD43 molecules [7, 43]. Mature NK cells leave the bone marrow and migrate to the periphery and are primarily found in the spleen, blood, lungs and lymph nodes, as well as some of them recirculating through the bone marrow (fig. 2) [44].

The Thymic NK Cell Developmental Pathway

A distinct population of NK cells that has reduced cytotoxic activity and is highly efficient in secreting cytokines has been recently identified in the thymus of adult mice [9]. In contrast to the conventional bone marrow-dependent NK cells, thymic NK (tNK) cells lack the expression of Ly49, Mac-1 and CD43 receptors but express IL-7R α [9]. The cells with the tNK cell phenotype are also present in the lymph nodes, but not in the spleen or bone marrow and do not develop in athymic mice (Foxn1^{-/-}) [9]. The origin of tNK cells remains controversial, and the developmental relationship between bone marrow and tNK pathways is not known. Although tNK cells depend on the thymic environment, it is not clear whether they are generated from the progenitors that seed the thymus or whether they are derived from the bone marrow NK

cells and undergo final maturation in the thymus. In support of the first possibility, previous studies have shown that the early thymic progenitors (ETPs) can generate both T and NK cells in vitro [45]. We have recently shown that tNK cells and bone marrow-dependent NK cells can be generated from both NKPs as well as from ETPs at the single cell level [34], and their generation is not restricted by Notch signaling. Recent studies using Notch-1-deficient and RBP(CSL)-deficient bone marrow chimeras demonstrated that loss of Notch signaling did not alter the generation of tNK cells, suggesting that tNK cells can develop independently of T-cell progenitors [46]. However, further studies are needed to determine to what degree NKPs and ETPs contribute to generate NK cells in the thymus in vivo.

A distinct population of NKPs has also been identified in the mouse lymph nodes [10]. These progenitors resemble double-negative (DN) thymocytes with LinCD44⁺CD25⁻ (DN1) and Lin⁻CD44⁺CD25^{lo} (pre-DN2) phenotypes and express very low levels of IL-7R α [10]. However, both IL-7R⁺ and IL-7R⁻ DN progenitors in the lymph nodes have been shown to generate NK cells in vitro [10]. It remains to be determined how much these lymph node NK progenitors contribute to the different NK cell compartments as well as their origin. The lymph node NK progenitors are undetectable in athymic mice, suggesting that they might develop from thymic progenitors migrating and seeding the lymph nodes [9, 10].

Recent studies identified a population of NK-like lymphoid cells in the intestine that do not express NK1.1 receptor, but are positive for NKp46 and produce IL-22 [11, 12]. These cells were named 'natural cytotoxicity receptor 22' or NK-22 based on the expression of NK cell lineage markers and the ability to produce IL-22 [11, 47]. However, further characterization of intestinal NKp46⁺ cell subsets suggests that they are different from conventional NK cells and represent distinct innate cell lineage. It has been recently postulated that the innate lymphoid cells include: (1) NK cells, (2) lymphoid tissue inducer cells, and (3) cells that produce ILs: IL-5, IL-13, IL-17 and/or IL-22 [48]. The different subsets of innate lymphoid cells are developmentally related and depend on the expression of transcription factor Id2 and cytokine signaling through the common γ chain and IL-2R [47, 48]. Further studies are needed to establish direct progenitors, as well as signals governing their development, to resolve the controversy in the field.

NK Cell Development in the Mouse Embryo

The relationship between fetal and adult NK cell development is poorly understood. Multiple fetal tissues (liver, thymus, spleen) contain hematopoietic progenitors that have the potential to generate NK cells; however, the biological importance of fetal NK cell development is not clear.

The liver is the main site of hematopoiesis during fetal development [49]. The HSCs in the embryo originate within the intra-embryonic sites, i.e. in the aorta-gonadomesonephros region derived from the paraaortic splanchnopleura and in the placenta [49, 50], as well as in the extra-embryonic site – in the yolk sac [51]. The fetal liver starts to be colonized by hematopoietic progenitors between days E10 and E12, and from day E12 to E15, the number of HSCs increases exponentially [52]. The fetal spleen is colonized by HSCs and hematopoietic progenitors from day E13.5 and is considered to be a transient hematopoietic organ as it becomes an adult immune organ [52].

Using recombination activating gene 1 (RAG1) expression as a marker for the most primitive lymphoid progenitors, Yokota et al. [53] provided a detailed chart of the emergence and expansion of cells giving rise to the cells of the immune system. The first RAG1-enhanced green fluorescence protein (EGFP)-expressing cells were found in the embryo proper at day E10.5, at day E11 in the fetal liver and at day E12.5 within the thymus [53]. These RAG-1⁺EGFP⁺CD45⁺ progenitors have the ability to generate B and T cells *in vitro*; however, their NK cell lineage potential was not investigated. The fetal equivalent of CLP that has been identified in the liver in 14.5-day-old fetus retains, in addition to T, B and NK cell lymphoid potential, significant myeloid potential [54].

The NKPs have not yet been identified in the fetal liver; however, hematopoietic progenitors with combined NK/T/dendritic cell and NK/T-cell potentials have been found in different embryonic sites. The Lin⁻KIT⁺IL-7R⁺ progenitor cells able to generate NK/T/dendritic cells have been identified in the fetal blood at day E10.5 [55] and found to persist in circulation until day E15. Bipotent NK/T-cell progenitors have been located to multiple sites during embryonic development, including the fetal thymus at day E13 as NK1.1⁺KIT⁺CD44⁺CD25⁻ cells [56, 57], at day E 14.5 as CD44⁺CD25⁻FcγR⁻ [58] and CD4⁻CD8TCR⁻Fcγ⁺ thymocytes [59], as well as in the fetal liver at day E15 as B220^{low}KIT⁺CD19⁻ cells [60]. It remains unclear whether these bipotent NK/T-cell progenitors are directly upstream of NKPs. In most of the studies, the

lineage potential of these progenitor cells was only evaluated *in vitro*; thus, their physiological contribution to NK and other lymphoid lineages in the developing embryo is not known.

Previous studies have shown that the NK1.1⁺ mature NK cells are detected first in the fetal thymus at day E14, and then at day E16 in the fetal liver as well as in the spleen [61]. Studies in adult mice suggested that commitment to the NK lineage coincides with the upregulation of the surface expression of the IL-2Rβ CD122 [33]. To determine the time and the sites when different NK cell populations arise during development, we performed detailed flow cytometric analyses of different mouse fetal tissues. Cells negative for mature lineage markers and expressing CD122 (Lin⁻CD122⁺) arise first in the fetal liver at day E13.5, while the CD3⁻NK1.1⁺DX5⁺ NK cells can be first detected in the fetal liver at day E14.5 and in the spleen at day E15.5. Mature NK cells with lytic activity are first found in the fetal liver at day E16.5 and in the spleen at day E17.5 [C. Stolz, Y. Tang and E. Sitnicka, unpubl. obs.]. The fetal equivalent of bone marrow Lin⁻CD122⁺NK1.1DX5⁻ NKPs arises first in the fetal liver at day E13.5 and the numbers of NKPs expand between days E14.5 and E17.5 [Y. Tang, C. Stolz and E. Sitnicka, unpubl. obs.]. These Lin⁻CD122⁺NK1.1⁻DX5⁻ cells can also be found in the fetal thymus, spleen, blood and bone marrow [C. Stolz, Y. Tang and E. Sitnicka, unpubl. obs.]. It is currently unclear whether the fetal NKPs first expand in the fetal liver and then migrate to the other tissues through the circulation or whether they arise independently in the different embryonic sites. Likewise, it remains to be determined what contribution to the generation of the mature NK cell compartments these differentially located NKPs make.

Conclusion

Recent progress in understanding NK cell biology has identified multiple NK cell populations, each with distinct properties that appear to be generated as the result of distinct developmental programs that are regulated by alternative intrinsic and extrinsic mechanisms. Further studies are needed to understand the relationship between these diverse NK progenitors, their origin as well as their contributions to the mature NK cell compartments. Uncovering how different microenvironments/tissues support the development of NK cell subsets will help to identify critical extrinsic factors supporting NK cell lymphopoiesis and to understand how they affect de-

veloping NK cells. Identifying the cellular NK developmental pathway(s) and its regulatory networks will further drive the therapeutic application of NK cells and establish the molecular basis for diseases that result from altered NK cell development including syndromes manifesting as NK cell deficiencies and NK cell malignancies.

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References

- Ogawa M: Differentiation and proliferation of hematopoietic stem cells. *Blood* 1993;81:2844–2853.
- Orkin SH, Zon LI: Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* 2008;132:631–644.
- Trinchieri G: Biology of natural killer cells. *Adv Immunol* 1989;47:187–376.
- Andoniou CE, Coudert JD, Degli-Esposti MA: Killers and beyond: NK-cell mediated control of immune responses. *Eur J Immunol* 2008;38:2938–2942.
- Ruggeri L, Capanni M, Tosti A, et al: Innate immunity against hematological malignancies. *Cytotherapy* 2002;4:343–346.
- Cooper MA, Colonna M, Yokoyama WM: Hidden talents of natural killers: NK cells in innate and adaptive immunity. *EMBO Rep* 2009;10:1103–1110.
- Di Santo JP: Natural killer cell developmental pathways: a question of balance. *Annu Rev Immunol* 2006;24:257–286.
- Huntington ND, Vosshenrich CA, Di Santo JP: Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nat Rev Immunol* 2007;7:703–714.
- Vosshenrich CA, Garcia-Ojeda ME, Samson-Villegier SI, et al: A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. *Nat Immunol* 2006;7:1217–1224.
- Veinotte LL, Halim TY, Takei F: Unique subset of natural killer cells develops from progenitors in lymph node. *Blood* 2008;111:4201–4208.
- Colonna M: Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* 2009;31:15–23.
- Di Santo JP, Vosshenrich CA, Satoh-Takayama N: A 'natural' way to provide innate mucosal immunity. *Curr Opin Immunol* 2010;22:435–441.
- Manaster I, Mandelboim O: The unique properties of uterine NK cells. *Am J Reprod Immunol* 2010;63:434–444.
- Ljunggren HG, Malmberg KJ: Prospects for the use of NK cells in immunotherapy of human cancer. *Nat Rev Immunol* 2007;7:329–339.
- Reya T, Morrison SJ, Clarke MF, Weissman IL: Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105–111.
- Luc S, Buza-Vidas N, Jacobsen SE: Biological and molecular evidence for existence of lymphoid-primed multipotent progenitors. *Ann NY Acad Sci* 2007;1106:89–94.
- Hu M, Krause D, Greaves M, et al: Multilineage gene expression precedes commitment in the hemopoietic system. *Genes Dev* 1997;11:774–785.
- Boos MD, Ramirez K, Kee BL: Extrinsic and intrinsic regulation of early natural killer cell development. *Immunol Res* 2008;40:193–207.
- Kondo M, Weissman IL, Akashi K: Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 1997;91:661–672.
- Akashi K, Traver D, Miyamoto T, Weissman IL: A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 2000;404:193–197.
- Adolfsson J, Mansson R, Buza-Vidas N, et al: Identification of Flt3+ lymphomyeloid stem cells lacking erythro-megakaryocytic potential: a revised road map for adult blood lineage commitment. *Cell* 2005;121:295–306.
- Morrison SJ, Weissman IL: The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1994;1:661–673.
- Christensen JL, Weissman IL: Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc Natl Acad Sci USA* 2001;98:14541–14546.
- Osawa M, Hanada K, Hamada H, Nakauchi H: Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996;273:242–245.
- Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ: SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 2005;121:1109–1121.
- Lai AY, Lin SM, Kondo M: Heterogeneity of Flt3-expressing multipotent progenitors in mouse bone marrow. *J Immunol* 2005;175:5016–5023.
- Ng SY, Yoshida T, Zhang J, Georgopoulos K: Genome-wide lineage-specific transcriptional networks underscore Ikaros-dependent lymphoid priming in hematopoietic stem cells. *Immunity* 2009;30:493–507.
- Serwold T, Ehrlich LI, Weissman IL: Reductive isolation from bone marrow and blood implicates common lymphoid progenitors as the major source of thymopoiesis. *Blood* 2009;113:807–815.
- Adolfsson J, Borge OJ, Bryder D, et al: Up-regulation of Flt3 expression within the bone marrow Lin(–)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* 2001;15:659–669.
- Rumfelt LL, Zhou Y, Rowley BM, Shinton SA, Hardy RR: Lineage specification and plasticity in CD19– early B cell precursors. *J Exp Med* 2006;203:675–687.
- Mansson R, Zandi S, Anderson K, et al: B-lineage commitment prior to surface expression of B220 and CD19 on hematopoietic progenitor cells. *Blood* 2008;112:1048–1055.
- Inlay MA, Bhattacharya D, Sahoo D, et al: Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. *Genes Dev* 2009;23:2376–2381.
- Rosmaraki EE, Douagi I, Roth C, Colucci F, Cumano A, Di Santo JP: Identification of committed NK cell progenitors in adult murine bone marrow. *Eur J Immunol* 2001;31:1900–1909.
- Nozad Charoudeh H, Tang Y, Cheng M, Cilio CM, Jacobsen SE, Sitnicka E: Identification of a NK/T cell restricted progenitor in adult bone marrow contributing to bone marrow and thymic-dependent NK cells. *Blood* 2010;116:183–192.
- Vosshenrich CA, Ranson T, Samson SI, et al: Roles for common cytokine receptor gamma-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo. *J Immunol* 2005;174:1213–1221.
- Di Santo JP, Vosshenrich CA: Bone marrow versus thymic pathways of natural killer cell development. *Immunol Rev* 2006;214:35–46.

- 37 Haller O, Wigzell H: Suppression of natural killer cell activity with radioactive strontium: effector cells are marrow dependent. *J Immunol* 1977;118:1503–1506.
- 38 Ikawa T, Fujimoto S, Kawamoto H, Katsura Y, Yokota Y: Commitment to natural killer cells requires the helix-loop-helix inhibitor Id2. *Proc Natl Acad Sci USA* 2001;98:5164–5169.
- 39 Yokoyama WM, Kim S, French AR: The dynamic life of natural killer cells. *Annu Rev Immunol* 2004;22:405–429.
- 40 Godfrey DI, Stankovic S, Baxter AG: Raising the NK T cell family. *Nat Immunol* 2010;11:197–206.
- 41 Stewart CA, Walzer T, Robbins SH, Malissen B, Vivier E, Prinz I: Germ-line and rearranged Tcrd transcription distinguish bona fide NK cells and NK-like gammadelta T cells. *Eur J Immunol* 2007;37:1442–1452.
- 42 Walzer T, Blery M, Chaix J, et al: Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46. *Proc Natl Acad Sci USA* 2007;104:3384–3389.
- 43 Kim S, Iizuka K, Kang HS, et al: In vivo developmental stages in murine natural killer cell maturation. *Nat Immunol* 2002;3:523–528.
- 44 Gregoire C, Chasson L, Luci C, et al: The trafficking of natural killer cells. *Immunol Rev* 2007;220:169–182.
- 45 Schmitt TM, Ciofani M, Petrie HT, Zuniga-Pflucker JC: Maintenance of T cell specification and differentiation requires recurrent notch receptor-ligand interactions. *J Exp Med* 2004;200:469–479.
- 46 Ribeiro VS, Hasan M, Wilson A, et al: Cutting edge: thymic NK cells develop independently from T cell precursors. *J Immunol* 2010;185:4993–4997.
- 47 Satoh-Takayama N, Lesjean-Pottier S, Vieira P, et al: IL-7 and IL-15 independently program the differentiation of intestinal CD3–NKp46+ cell subsets from Id2-dependent precursors. *J Exp Med* 2010;207:273–280.
- 48 Spits H, Di Santo JP: The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;12:21–27.
- 49 Dzierzak E, Speck NA: Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nat Immunol* 2008;9:129–136.
- 50 Cumano A, Godin I: Ontogeny of the hematopoietic system. *Annu Rev Immunol* 2007;25:745–785.
- 51 Lux CT, Yoshimoto M, McGrath K, Conway SJ, Palis J, Yoder MC: All primitive and definitive hematopoietic progenitor cells emerging before E10 in the mouse embryo are products of the yolk sac. *Blood* 2008;111:3435–3438.
- 52 Christensen JL, Wright DE, Wagers AJ, Weissman IL: Circulation and chemotaxis of fetal hematopoietic stem cells. *PLoS Biol* 2004;2:E75.
- 53 Yokota T, Huang J, Tavian M, et al: Tracing the first waves of lymphopoiesis in mice. *Development* 2006;133:2041–2051.
- 54 Mebius RE, Miyamoto T, Christensen J, et al: The fetal liver counterpart of adult common lymphoid progenitors gives rise to all lymphoid lineages, CD45+CD4+CD3 cells, as well as macrophages. *J Immunol* 2001;166:6593–6601.
- 55 Ikawa T, Masuda K, Lu M, Minato N, Katsura Y, Kawamoto H: Identification of the earliest prethymic T-cell progenitors in murine fetal blood. *Blood* 2004;103:530–537.
- 56 Carlyle JR, Michie AM, Furlonger C, et al: Identification of a novel developmental stage marking lineage commitment of progenitor thymocytes. *J Exp Med* 1997;186:173–182.
- 57 Michie AM, Carlyle JR, Schmitt TM, et al: Clonal characterization of a bipotent T cell and NK cell progenitor in the mouse fetal thymus. *J Immunol* 2000;164:1730–1733.
- 58 Ikawa T, Kawamoto H, Fujimoto S, Katsura Y: Commitment of common T/natural killer (NK) progenitors to unipotent T and NK progenitors in the murine fetal thymus revealed by a single progenitor assay. *J Exp Med* 1999;190:1617–1626.
- 59 Rodewald HR, Moingeon P, Lucich JL, Dosiou C, Lopez P, Reinherz EL: A population of early fetal thymocytes expressing Fc gamma RII/III contains precursors of T lymphocytes and natural killer cells. *Cell* 1992;69:139–150.
- 60 Douagi I, Colucci F, Di Santo JP, Cumano A: Identification of the earliest prethymic bipotent T/NK progenitor in murine fetal liver. *Blood* 2002;99:463–471.
- 61 Koo GC, Peppard JR, Hatzfeld A: Ontogeny of Nk-1+ natural killer cells. 1. Promotion of Nk-1+ cells in fetal, baby, and old mice. *J Immunol* 1982;129:867–871.