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Development and maturation of natural killer cells

Theresa L Geiger[1,2] and Joseph C Sun[1,2,3]

Natural killer (NK) cells are innate lymphocytes that are critical for host protection against pathogens and cancer due to their ability to rapidly release inflammatory cytokines and kill infected or transformed cells. In the 40 years since their initial discovery, much has been learned about how this important cellular lineage develops and functions. We now know that NK cells are the founding members of an expanded family of lymphocyte known as innate lymphoid cells (ILC). Furthermore, we have recently discovered that NK cells can possess features of adaptive immunity such as antigen specificity and long-lived memory responses. Here we will review our current understanding of the molecular mechanisms driving development of NK cells from the common lymphoid progenitor (CLP) to mature NK cells, and from activated effectors to long-lived memory NK cells.

Addresses
[1] Louis V. Gerstner, Jr. Graduate School of Biomedical Sciences, New York, NY 10065, United States
[2] Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, United States
[3] Department of Immunology and Microbial Pathogenesis, Weill Cornell Medical College, New York, NY 10065, United States

Corresponding author: Sun, Joseph C (sunj@mskcc.org)

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Introduction

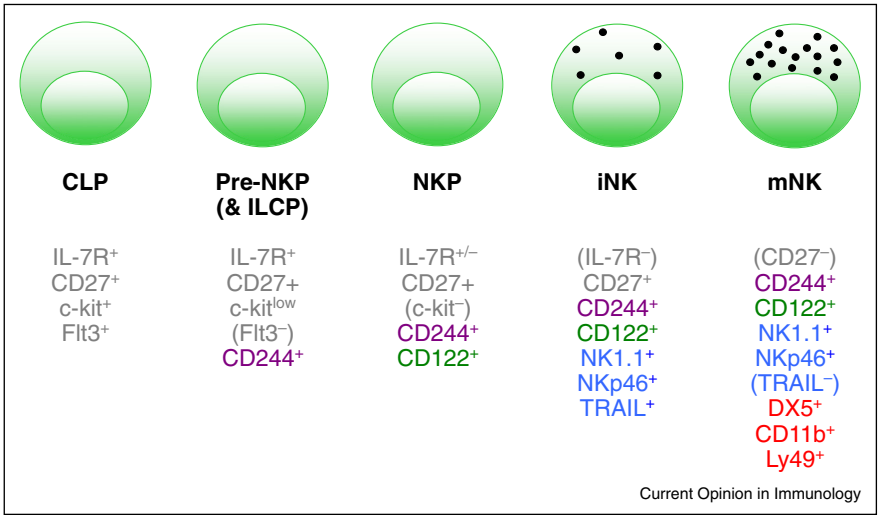
NK cells, like B and T cells, are a lymphocyte lineage derived from the CLP [1], and like B cells, are thought to develop primarily in the bone marrow [2], although other sites of development, such as the liver and thymus, have also been proposed (reviewed in [3]). However, unlike the antigen receptors of B and T cells, NK cell receptors are germ line encoded and do not require gene rearrangement by RAG recombinase [4], though recent work has suggested that RAG plays an unexpected cell-intrinsic role in NK cell development [5••]. NK cells also undergo an 'education' process during development where they acquire the ability to recognize lack of self MHC class I, or 'missing-self', a feature that facilitates their surveillance of target cells that have down-regulated MHC class I during infection or malignancy [6]. NK cells rely on both cytokines and transcription factors to promote and control their development. Cytokine signaling from interleukin (IL)-15 is critical for the development of NK cells and is required throughout their lifetime [7,8]. Transcription factors such as Nfil3 and PU.1 are necessary for development of early NK cell progenitors [9–12], whereas Id2, Tox, and others are important later in development [13–15]. Eomes and T-bet are among factors that then control the final stages of NK cell maturation [16,17]. In the periphery, the activation and differentiation of NK cells are regulated by a plethora of transcription factors mediating distinct effector functions. This review will outline current knowledge about the stages of NK cell development and the factors driving each stage.

Stages of NK cell development and differentiation

The CLP is characterized by expression of IL-7Rα (CD127), c-kit (CD117), Sca-1, and Flt-3 (CD135), as well as the lack of common lineage markers such as CD3, CD4, CD8, CD19, Ter119, Gr-1 and NK1.1 (Figure 1) [1]. From the CLP, cells develop into NK cell precursors (NKP), which are defined by expression of the IL-15 receptor β chain (CD122), and lack of common lineage markers, including the NK cell markers NK1.1 and DX5 (CD49b) (Figure 1) [2]. This NKP population has been further refined based on the co-expression of CD27 and CD244, with the majority of these cells also expressing IL-7Rα [18]. An intermediate population between the CLP and NKP termed 'pre-NKP' has also recently been defined as lineage negative, $CD244^{+}$ c-kit^{low} IL-$7R\alpha^{+}$ $Flt\text{-}3^{-}$ and $CD122^{-}$ [18,19]. However, recent work suggests that this population is heterogeneous, composed of true NK-committed precursors as well as PLZF-expressing and α4β7 integrin-expressing ILC precursors (ILCP) (Figure 1) [20••]. A precursor of this pre-NKP population also capable of producing all ILC lineages (including NK cells) has recently been identified by expression of the transcription factor Tcf-1 [21••]. From the $CD122^{+}$IL-$7R\alpha^{+/-}$ NKP stage, cells develop into immature NK (iNK) cells, which lose expression of IL-7Rα and acquire expression of NK1.1 but do not yet express CD49b (Figure 1) [2]. As immature NK cells gain expression of CD11b, CD43, Ly49 receptors, and CD49b (DX5), they also gain functional competence in cytotoxicity and production of interferon (IFN)-γ [22], and egress from the bone marrow.

The peripheral NK cell pool can be delineated by expression of CD27, with $CD27^{lo/-}$ NK cells being more cytotoxic and producing more cytokines than $CD27^{high}$

Figure 1



Stages of NK cell development.
NK cells are derived from the CLP, which differentiates into a heterogeneous pre-NKP/ILCP population distinguished from the NKP by its expression of IL-7R and lack of CD122 expression. From the NKP, cells begin to express NK cell markers NK1.1 and NKp46, and as they further mature they acquire expression of DX5 (CD49b) and CD11b while losing expression of CD27. As NK cells mature they also gain functional competence, expressing lytic molecules and cytokines such as IFN-γ. Cell surface proteins are color coded by the stage in which they are first expressed. Loss of a specific cell surface marker after a given stage is indicated by parentheses in the stage immediately following.

NK cells [23]. These mature peripheral NK cell populations have more recently been further refined into four stages of maturation, defined by sequential upregulation of CD11b expression followed by downregulation of CD27, with the most immature NK cells being $CD27^{-}CD11b^{-}$ and the most mature NK cells being $CD27^{-}CD11b^{+}$ [24]. During viral infection or pro-inflammatory cytokine exposure, mature peripheral NK cells can differentiate into effector and long-lived memory NK cells (reviewed in [25]). During the $CD8^{+}$ T cell response to viral infection, at least two different effector cell populations are thought to be generated: $KLRG1^{hi}$ short-lived effector cells (SLECs) and $KLRG1^{lo}$ memory precursor effector cells (MPECs) [26]. Recent evidence suggests that a similar paradigm exists in the resting NK cell pool, with virus-specific $KLRG1^{-}$ NK cells exhibiting a greater capacity to generate memory NK cells than their $KLRG1^{+}$ counterparts [27••]. In accordance with this finding, another recent study found that RAG expression during NK cell ontogeny was correlated with lower expression of KLRG1 and a greater memory potential [5••].

Transcriptional control of early NK cell development

Lineage commitment to either an adaptive or innate lymphocyte cell fate is determined by a complex network of transcription factors (Figure 2). For example, Notch signaling through the ligands Jagged1 and Jagged2 preferentially drives NK cell development from the CLP [28–30], whereas delta-like ligands (DLL) promote T cell development [31]. Moreover, thymocytes can be diverted into an NK cell-like fate if the Notch1-dependent transcription factor Bcl11b is ablated during T cell development [32–34], suggesting active suppression of the NK cell fate. Similarly, early B cell factor 1 (Ebf1) and Pax5 promote the B cell fate by suppressing expression of ILC and T-cell promoting transcription factors Notch1, Tcf-1, Gata3, and Id2 [35]. Even within the innate lymphocyte lineages, differential expression of specific transcription factors give rise to distinct cell fates. For example, although both NK cells and non-NK cell 'helper' ILCs require the transcription factors Id2 [13,36,37] and Nfil3 [9,10,38,39•,40•,41•,42•] for their development, only the helper ILC lineages require Gata3 for development [43••,44••,45••]. These differential requirements are consistent with recent studies indicating that ILCs are not derived from the same $CD122^{+}$ precursor as NK cells, but rather arise from an $IL\text{-}7R\alpha^{+}$, $\alpha4\beta7^{+}$, Id2-expressing precursor, referred to as the common helper innate lymphoid cell precursor or 'CHILP' (reviewed in [46]).

Nfil3 (also known as E4BP4) is a critical factor in NK cell lineage commitment. Originally identified as a circadian clock gene [47], Nfil3 is widely expressed in many hematopoietic and non-hematopoietic cells, and is expressed as early as the CLP stage in developing lymphocytes [48••]. Early studies in Nfil3-deficient mice revealed a specific loss of NK cells, whereas numbers of B cells, $CD4^{+}$, and $CD8^{+}$ T cells were normal [9–11]. Later studies revealed that Nfil3 expression is only