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Natural Killer Cells

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Preface

Since their identification 40 years ago, natural killer (NK) cells write a never-ending story of excitement and intrigue for immunologists and clinicians due to their unique fundamental properties and therapeutic promise for translational medicine. This is in part due to the diversity of NK cell biology that has been and continues to be unraveled. To celebrate the 40th anniversary of the discovery of NK cells, we asked a group of NK cell experts to provide a series of comprehensive reviews on the recent advances in NK cell development and differentiation, NK cell acquisition of functional properties, models for analysis of NK cells in mice, and applications of NK cells in clinical medicine.

Joe Sun introduces the topic of NK cell development via the action of specific transcription factors, while Cyril Seillet, Gabriella Belz, and Nick Huntington continue in this arena and further elaborate how diverse NK cell subsets are maintained in peripheral tissues. Frank Cichocki (Yenan T. Bryceson) discusses the functional diversification of NK cell subsets and their implications for human pathophysiology. Nadir Kadri (Petter Hoglund) examines the important role for NK cell responsiveness in dictating the biological responses of these innate effectors, while Deborah W. Hendricks (Lewis Lanier) focuses on the 'adaptive features' of NK cells in providing long-lasting memory responses to certain types of antigenic stimulation. Two chapters discuss mouse models for studying the impact of NK cells in vivo: Florence Deauvieau (Eric Vivier) discusses models for NK cell deficiency in the mouse, while Yan Li and James Di Santo focus on 'humanized' mice as tools to assess human NK cell biology. Concerning NK cells in the clinic, Mariella Della Chiesa (Alessandro Moretta) focuses on the role for NK cells in conditioning haplo-identical bone marrow transplantation, while Frank Cichocki (Jeffrey Miler) continues in this topic and further examines the utility of adoptive NK cell transfer for treating human disease. Finally, Camille Guillerey and Mark J. Smyth discuss the critical and persistent role for NK cells in immunity against cancer.

Together, these reviews provide a timely and concise picture of the evolution of NK cells as essential actors in immunity and as potent arms against human disease.

In 40 years, NK cells have come a long way from their initial description of ‘spontaneous killers’ (for some simply an experimental artifact) to a bona fide subset of lymphoid cells with a complementary mode of action in immune defense to an important mediator of immune reactivity in health and disease. Still our knowledge of NK cell biology, while impressive, only represents the tip of the iceberg. What does the future hold for NK cells? Only time will tell…

Eric Vivier
James Di Santo
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Transcriptional Control of NK Cells

Joseph C. Sun

Abstract Natural killer (NK) cells are innate lymphocytes that survey the environment and protect the host from infected and cancerous cells. As their name implies, NK cells represent an early line of defense during pathogen invasion by directly killing infected cells and secreting inflammatory cytokines. Although the function of NK cells was first described more than four decades ago, the development of this cytotoxic lineage is not well understood. In recent years, we have begun to identify specific transcription factors that control each stage of development and maturation, from ontogeny of the NK cell progenitor to the effector functions of activated NK cells in peripheral organs. This chapter highlights the transcription factors that are unique to NK cells, or shared between NK cells and other hematopoietic cell lineages, but govern the biology of this cytolytic lymphocyte.

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1 Introduction

The phrase "natural killer" was first coined in 1975, and in the 40 years since their discovery, NK cells have been demonstrated to function as a component of innate immunity to protect the host against infectious disease, rapidly secreting perforin and granzymes to lyse infected cells, and pro-inflammatory cytokines such as IFN-gamma and TNF-α to alert additional host defenses. Furthermore, NK cells survey the host landscape for "stressed" or transformed cells that may lead to cancer. Although NK cells respond rapidly and without antigen specificity during pathogen invasion or cellular transformation as part of the innate immune response, NK cells have also been recently described to possess features of adaptive immunity, including antigen specificity, clonal proliferation, and long-lived memory similar to T and B cells (Sun and Lanier 2011b; Vivier et al. 2011). The combination of innate and adaptive characteristics places NK cells at the boundary between these two immune compartments.

NK cells arise from a common lymphoid progenitor (CLP) shared by T and B cells (Kondo et al. 1997). However, the receptors used for recognition of infected or transformed cells found on NK cells are germ line-encoded and do not involve gene rearrangement mediated by the RAG recombinase like with T and B cell receptors (Lanier/Kumar JI 1986). Thus, genetic deletion of either RAG-1 or RAG-2 results in complete loss of T and B cells (Mombaerts et al. 1992; Shinkai et al. 1992), whereas NK cells are present in normal numbers. Interestingly, recent evidence suggests that RAG is expressed in a subset of developing NK cells and its endonuclease activity is required for the function and fitness of the mature peripheral NK cell pool (Karo et al. 2014). Possessing both activating and inhibitory receptors (Ly49 family in mice and KIR family in humans), NK cells are "educated" during development such that they can recognize the loss of MHC class I on potential target cells (i.e., "missing self"), which can occur on infected or transformed host cells (Orr and Lanier 2010).

NK cells are thought to primarily develop in the bone marrow. However, fetal thymus and liver contain bipotent T/NK progenitor cells that possess the ability to develop into NK cells (Carlyle et al. 1997; Douagi et al. 2002; Ikawa et al. 1999; Sanchez et al. 1994; Spits et al. 1998). Similar to T and B cells, NK cells require the common gamma chain of the IL-2 receptor complex for their development. Removal of the common gamma chain, and the ability to sense IL-15, results in a near complete loss of NK cells under steady-state conditions (Di Santo 2006; Ma et al. 2006). IL-15 is thought to be required during the entire life span of NK cells (Di Santo 2006; Yokoyama et al. 2004). Although NK cells develop and mature in the bone marrow, they continue to mature in peripheral tissues and undergo "tuning" of functional competence dependent upon specific environmental cues including MHC class I (Orr and Lanier 2010; Sun 2010). Other sites of development (liver, lymph node, thymus, and salivary glands) have also been proposed;

however, whether the cells described at these sites represent unique NK cell subsets or distinct innate lymphoid cell (ILC) lineages remain to be determined.

A recent paradigm has arisen where shared transcription factors regulate in parallel the development of helper CD4+ T cell and ILC subsets, enabling them to possess aligned effector function. The ILC family consists of at least three members, and these innate lymphocytes preferentially reside at mucosal surfaces throughout the body, providing barrier immunity and protection (reviewed in Artis and Spits 2015; Spits and Cupedo 2012). ILC subsets constitute an early source of distinct cytokines, whereas T helper subsets direct later immune responses using a parallel set of cytokines (Gasteiger and Rudensky 2014). A similar innate and adaptive relationship may exist in NK cells and CD8+ T cells, as these represent the cytotoxic lymphocytes among cells of the immune system. Beyond their killing ability, CD8+ T cells and NK cells share many additional similarities, including shared cell surface receptors and the robust production of IFN-γ (reviewed in Sun and Lanier 2011b).

NK cells also share many similarities with the newly described type 1 ILCs (ILC1), including identical cell surface markers (NK1.1 and NKp46) and the ability to rapidly produce large amounts of IFN-γ upon activation by pro-inflammatory cytokines IL-12 and IL-18 (Artis and Spits 2015; Spits and Cupedo 2012). Cytotoxic potential appears to be the distinguishing feature between NK cells and ILC1, along with a small number of specific surfaces markers and transcription factors described to be differentially expressed between the two populations in many but not all mouse tissues (Bernink et al. 2013; Fuchs et al. 2013; Klose et al. 2014). Lineage-tracing studies have suggested that NK cells and ILC1 originate from distinct precursors (Constantinides et al. 2014; Klose et al. 2014); however, recent data from the Immunological Genome Project found that these two populations possessed overlapping gene-expression patterns (Robinette et al. 2015). Thus, the distinction between NK cells and ILC1 remains controversial and complicated at the current time, and it is possible that ILC1 may represent a developmental stage of NK cells rather than a distinct lineage.

Transcription factors govern the development of NK cells, starting from the earliest progenitor. Although many of these DNA-binding and chromatin-modifying proteins are shared with other cells of the immune system or even with non-hematopoietic cells, some are unique to the NK cell lineage. Transcription factors Id2, E proteins, STAT5, IRF-2, Tox, Ets1, and Nfil3 are among proteins known to drive early stages of NK cell development. Additional transcription factors, including T-bet, Eomes, and Blimp-1, play specific roles at distinct stages of NK cell development and maturation. Lastly, STAT1, STAT4, Zbtb32, and AhR are among transcription factors that regulate different effector functions of mature NK cells in the periphery. The remainder of this chapter will focus on these and other transcription factors that specifically promote NK cell development and function, and describe the shared transcriptional regulation between NK cells and other lymphocyte lineages.

2 Generation of an NK Cell from a Common Lymphoid Progenitor

The classic NK cell is considered the founding member of the ILC family, and the third lineage of lymphocytes to originate from the CLP, along with T and B cells. The signals and transcriptional regulation that allows a CLP to become an NK cell rather than a T cell, B cell, or ILC are beginning to be elucidated. During hematopoiesis, the self-renewing hematopoietic stem cell (HSC) gives rise to a multipotent progenitor (MPP), which can then become a CLP (or a common myeloid progenitor, CMP) (Fig. 1). Although additional stages may exist between the MPP and CLP (a lymphoid-primed multipotent progenitor, LMPP) has been described (reviewed in De Obaldia and Bhandoola 2015), these early stages of lymphoid differentiation are not well understood, but likely require cytokines and other signals from the bone marrow environment. In addition, Ikaros, PU.1, E2A, Stat5, Bcl11a, Hoxa9, Lyl-1, and Satb1 represent some of the transcription factors that have been reported to play a cell-intrinsic role in lymphoid lineage specification from the HSC and MPP (reviewed in De Obaldia and Bhandoola 2015).

From the IL-7Rα (CD127), c-kit (CD117), and common gamma chain (CD132)-expressing CLP, NK cell development and maturation consists of at least three additional stages in the bone marrow (reviewed in Di Santo 2006; Yokoyama et al. 2004). In brief, the NK progenitor (NKP) begins as a lineage marker (CD3/CD4/CD8/CD19/Ter119/Gr-1/NK1.1)-negative, CD122-expressing cell. As the NKP transitions to the immature NK cell (iNK) stage, lineage-specific receptors NKG2D, NK1.1, CD94, TRAIL, and NKp46 are sequentially expressed. As the iNK transitions to the mature NK cell (mNK), CD49b (DX5), CD16, and Ly49 s are expressed. During the mNK stage, TRAIL and CD27 begin to be downregulated, while maturation markers CD11b, KLRG1, CD43, and Ly6C become upregulated, albeit at low levels (Fig. 1). Differentiation to mNK stage in the bone marrow is accompanied by the acquisition of functional competence, including cytokine secretion (IFN-γ) and cytolytic (perforin and granzymes) capability, and the appropriate chemokine receptors that permit egress into the periphery (reviewed in Di Santo 2006; Yokoyama et al. 2004) (Fig. 1). NK cells exiting the bone marrow continue to mature and gain functional competence as they undergo homeostatic proliferation to fill the peripheral niche and specific organ sites. Several distinct stages of maturation occur in the periphery and have been described throughout the literature using co-expression of the markers KLRG1, CD27, and CD11b (Chiossone et al. 2009; Hayakawa and Smyth 2006; Huntington et al. 2007b), or DX5 and TRAIL (Gordon et al. 2012; Kim et al. 2002; Takeda et al. 2005) (Fig. 1).

There is genome-wide transcriptome evidence that some HSC may be lymphoid-biased (Ng et al. 2009) and that DNA-binding helix-loop-helix **E proteins** (e.g., E2A and its isoform E47) can promote the development of LMPP and CLP by repressing myeloid potential while initiating V(D)J recombination (Borghesi et al. 2005; Dias et al. 2008; Quong et al. 2002; Schlissel et al. 1991). Consistent with evidence for antigen receptor gene recombination in early lymphoid precursors,

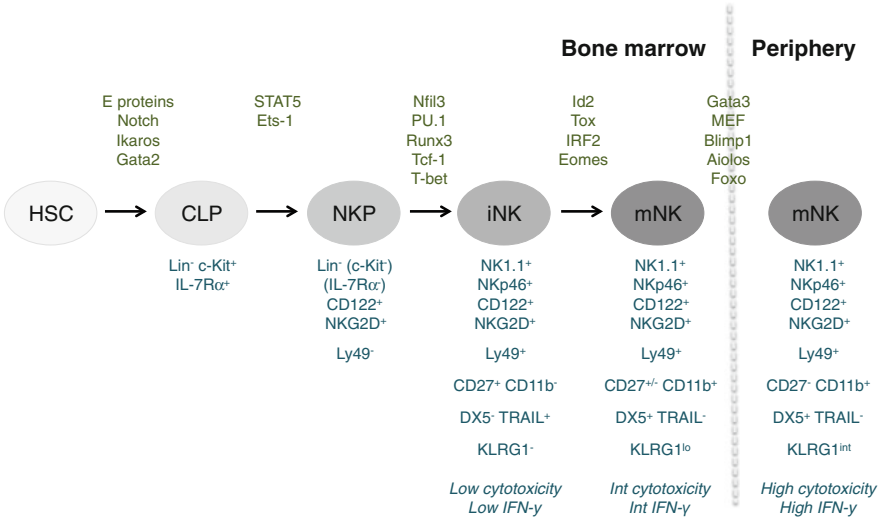

Fig. 1 Transcription factors critical for NK cell development. Mature NK cells are derived from a hematopoietic stem cell (HSC), which gives rise to a multipotent progenitor (MPP) with lymphoid and myeloid potential, which can generate a common lymphoid progenitor (CLP) capable of generating T, B, NK, and ILC lineages. From the CLP, developing NK cells transition through several stages marked by expression of specific markers followed by acquisition of function, beginning with the NK cell progenitor (NKP), the immature NK cell (iNK), followed by the mature NK cell (mNK). The mNK in the bone marrow is the final stage of development before NK cells egress to the periphery, where they undergo further maturation. (*Note* Additional stages exist between the HSC and mNK that are not described in this diagram.) Specific transcription factors regulate developmental progression at each stage during NK cell development, and these are highlighted at the stage where they are believed to exert activity. (*Note* initial expression of these factors may occur at an early stage, and the placement of some of these factors are a best approximation based on the limited data.) Loss of a specific surface receptor from the previous stage is denoted with parentheses. Surface receptors commonly used to phenotype NK cell stages/maturity are clustered together

RAG expression has also been documented to occur in LMPP and CLP populations using reporter mice (Igarashi et al. 2002; Karo et al. 2014; Yokota et al. 2003). However, prolonged E protein activity (along with downstream transcription factors EBF1 and Pax5) drives development toward the B cell lineage rather than T and NK cells (reviewed in Busslinger 2004; Nutt and Kee 2007). Thus, suppression of E2A by its antagonist Id2 is required for the development of NK cells (Boos et al. 2007). It is not well understood whether a balance of E and Id protein activity determines whether the CLP becomes a B cell instead of a T or NK cell, and whether this balance impacts adaptive versus innate lymphocyte lineage choice.

In lineage choice beyond the CLP, it is thought that lineage-defining transcription factors drive specific lineages, while actively suppressing myeloid differentiation. Although the highly conserved **Notch** signaling pathway is essential even in early hematopoiesis for derivation of the HSC from embryonic sites such as the yolk sac (Kumano et al. 2003), specific Notch signaling is required for development of T cells

and can enhance NK cell development. The Notch target Hes1 is a transcriptional repressor that inhibits the myeloid lineage inducer C/EBPα in T cell progenitors (De Obaldia and Bhandoola 2015), but a role for Hes1 in maintaining NK cell identity has not been described. The Notch ligands Jagged1 and Jagged2 preferentially drive in vitro differentiation of cultured human and mouse lymphoid precursors into NK cells (DeHart et al. 2005; Jaleco et al. 2001; Lehar et al. 2005), whereas Notch Delta-like ligands (DLL) are highly expressed on thymic epithelial cells and promote αβ T cell development (reviewed in Maillard et al. 2005). Of note, DLL-expressing stromal cells or cells without Notch ligand can also permit development of NK cells, albeit at lower frequency (Lehar et al. 2005; Schmitt et al. 2004), suggesting that although Notch signals can boost NK cell development, it is not a requirement. Pax5-deficient pro-B cells can be re-differentiated into NK cells with transient Notch signaling (Carotta et al. 2006). Similarly, thymocytes (as late as the DN2 or DN3 stage) deficient in the T cell lineage-promoting factor Bcl11b (a target of Notch1 signaling) can be reprogrammed into NK cells when cultured in the presence of Notch ligands (Ikawa et al. 2010; Li et al. 2010a, b). These results suggest that in the absence of transcription factors that specify the B and T cell lineages, perhaps a "default" pathway results in the production of NK cells. Furthermore, these lineage-specifying factors may have to be continuously expressed for the developing B or T cell to avoid reverting to a cell type with natural killer abilities in the presence of Notch ligands. Although Notch signaling does not appear to be an absolute requirement for the development of conventional or even thymic-derived NK cells (Di Santo 2006; Vosshenrich et al. 2006), it has been recently implicated in other innate lymphocyte lineages, including the development of ILC2, LTi, and ILC3 (Cherrier et al. 2012; Lee et al. 2012; Mielke et al. 2013; Possot et al. 2011; Rankin et al. 2013; Wong et al. 2012; Yang et al. 2013).

Additional transcription factors induced by Notch1 signaling include **Tcf-1** (encoded by the *Tcf7* gene) and the Th2-promoting **Gata3** (Ho et al. 2009; Tindemans et al. 2014; Weber et al. 2011). Both have been described to be important for the development of T cells and various ILC subsets (reviewed in De Obaldia and Bhandoola 2015; Ho et al. 2009; Tindemans et al. 2014), but largely dispensable for conventional NK cell development. However, there is evidence that Tcf-1 may regulate Ly49 receptor usage in mouse NK cells (Held et al. 1999), directly binding to the *Klra1* (Ly49A) gene and promoting its expression (Kunz and Held 2001), even if overall NK cell numbers and maturation are not grossly perturbed. The lack of a major NK cell phenotype in Tcf-1-deficient mice may be attributable to a redundant function of Lef-1 in NK cell development (Held et al. 2003). Similarly, no major defect in NK cell numbers was observed in Gata3-deficient mice; however, Gata3 was found to regulate T-bet expression, IFN-γ production, and liver-specific homing of NK cells in mice (Samson et al. 2003), and drive expression of the CD94-NKG2A receptor in human NK cells (Marusina et al. 2005). In addition, there is a population of thymic NK cells that are thought to require Gata3 for their development (Vosshenrich et al. 2006); however, the function of this NK cell subset remains unknown. Thus, the overall influence of Tcf-1 and Gata3 on NK cell development is minor. Like Gata3, PLZF is another