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Phenotype and functions of conventional and non-conventional NK cells

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Abstract

Here we focus on the phenotypic and functional diversity of NK cells. We give an overview of the phenotype and developmental pathways of conventional and tissue-resident NK cells. We also discuss the potential complementary functions of conventional NK cells and tissue-resident NK cells in a variety of tissues.

Introduction

The innate immune system is comprised of a variety of cell types that utilize different methods to furnish rapid protection against pathogens. Natural killer (NK) cells are cytotoxic innate lymphocytes that provide crucial defenses against viral pathogens and tumor cells. NK cells have the ability to recognize and induce apoptosis in target cells through the release of perforin and granzymes, or engagement of target cell death receptors such as Fas by Fas-L on the NK cell surface [1]. Activated NK cells also produce pro-inflammatory cytokines such as IFN- γ that stimulate further innate and adaptive immune responses [2].

For decades after their discovery, circulating conventional (c)NK cells were thought to be the only innate effector lymphocytes in the body. Within the last few years, however, many new subsets of innate effector cells have been discovered, and classified as innate lymphoid cells (ILCs) [3,4]. ILCs display great diversity in phenotype and function, and appear to represent the innate analog of T helper cells [5]. ILCs are classified into three groups -- ILC1, ILC2, and ILC3 -- based on the cytokines they produce and the transcription factors required for their development [6–8]. cNK cells are considered to be the prototypical ILC1 subset, and several distinct lineages of NK cells have recently been discovered in various tissues in humans and mice [5]. These unique NK cell populations have alternatively been called ILC1 [9] and tissue-resident (tr)NK cells [10]. This is merely a difference in

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nomenclature, as all NK cells ultimately belong to the ILC1 group [6,7,11]. However, the common ILC precursor (ILCP or CHILP) does not generate cNK cells [12,13].

Current research indicates that there are multiple unique lineages of NK cells: circulating cNK cells, thymic NK cells, trNK cells of the liver and skin, uterine (u)NK cells, submandibular gland (SMG) trNK cells, and kidney trNK cells [14–22]. Each of these NK cell populations possesses unique phenotypic characteristics and appears to arise from a distinct developmental pathway. Of particular interest are the NK cells that reside in mucosal tissues, since these tissues are diverse in structure and function, and also provide an interface with the external environment [23]. NK cells in the respiratory tract, urogenital tract, salivary glands, as well as other mucosal tissues function to counter potential invading organisms, while at the same time limiting inflammatory damage to these delicate tissues. In this review, we discuss the phenotypic and functional diversity of NK cells with a focus on tissue-resident NK cells in mucosal tissues. Markers expressed by the different subsets of NK cells are described in Table 1. We do not discuss the intestine as it has been covered extensively in other reviews [24–26].

Conventional NK Cells

Conventional NK cells undergo most of their development in the bone marrow. They are derived from the common lymphoid progenitor (CLP), which is defined as Lin[−]Sca-1^{low}CD117^{low}CD127⁺CD135⁺ [27]. After the CLP, the earliest known NK cell precursor is the pre-pro A, which are Lin[−]ID2⁺Sca-1⁺CD117^{low}CD127⁺CD135[−]CD122[−]. Pre-pro A NK cells are very similar to another early NK precursor called the pre-pro B stage, which lack CD117 expression, but otherwise have the same surface receptor profile. After the pre-pro stages, NK cell precursors progress to the refined (r)NKP stage, which is defined as Lin[−]CD27⁺CD244⁺CD117^{low}CD127⁺CD135[−]CD122⁺. These rNKP then develop into immature (i)NK cells, which acquire NK1.1 expression and further develop into mature (m)NK cells [28,29]. DX5 (CD49b), a late maturation marker, is expressed just before the mNK cells exit the bone marrow [30]. Nkp46 is also a marker of mature NK cells, and it is expressed before DX5 in the bone marrow [31,32], though this notion has been challenged by a recent study [33]. Later stages of NK cell maturity can be assessed by expression of CD11b and CD27, in a 4-stage developmental process. The least mature NK cells, which have not yet expressed DX5, are CD11b^{low}CD27^{low}. This is followed by CD11b^{low}CD27^{high}, CD11b^{high}CD27^{high}, and finally CD11b^{high}CD27^{low} stages [34]. The most mature DX5⁺CD11b^{high}CD27^{low} NK cells also express KLRG1 and are CD43^{high} at steady state [35,36].

The development of cNK cells requires the coordinated activity of several transcription factors and cytokines. NFIL3 (nuclear factor, IL-3 regulated, also called E4BP4) is critical at the progenitor stage [37] for cNK cell differentiation and development [38–41]. Although NFIL3 was recently shown to be required for the development of all helper innate lymphoid cells [42–45], trNK cells appear to develop independently of this transcription factor [15,21,22,46]. Id2 (inhibitor of DNA binding 2), T-bet (Tbx21), and Eomes (eomesodermin) are all required at different stages for cNK cell development and maintenance [47–51]. IL-15 signaling through the IL-15R α is also necessary for the development and maintenance

of mature cNK cells [52–54]. The spleen harbors mostly cNK cells, though very few ILC1 and/or trNK cells can be found in this organ [55]. Beside the spleen, cNK cells can be found in significant number in the blood, liver, kidney, and mucosal tissues (see below).

Non-conventional NK Cells

Thymic NK cells

The thymus contains a unique lineage of NK cells, separate from cNK. Thymic NK cells have a unique surface phenotype in that they are CD127⁺CD69^{high}Ly49^{low}CD11b^{low}, as opposed to cNK cells, which are CD127⁺CD69⁺Ly49⁺CD11b^{high}. Thymic NK cells are less cytotoxic than their splenic cNK cell counterparts. However, thymic NK cells produce more IFN- γ than splenic cNK cells, as well as more TNF- α and GM-CSF [56,57]. Interestingly, a small percentage of thymic NK cells traffic to lymph nodes, but not to other organs [56,57]. Thymic NK cells require the transcription factor GATA-3 for development, as well as the cytokine IL-7. GATA-3 is generally more closely associated with T cell development than NK cell development. However, thymic NK cells do not develop from RORC-expressing precursors, and develop in the absence of Notch signaling, indicating that these NK cells are not derived from a committed T cell progenitor [58]. Upon further investigation, it was shown that thymic NK cells develop from a double-negative (CD4⁺CD8⁺) thymocyte precursor [59], and that their development is mostly unimpaired in the absence of NFIL3 [55]. Despite their NFIL3 independence and cytokine production profile, thymic NK cell affiliation to the ILC1 family has not been decisively confirmed.

Liver NK cells

The murine liver is home to both circulating cNK cells and trNK cells [18,50]. The liver trNK cells are CD49a⁺DX5⁺, whereas cNK cells are CD49a⁺DX5⁺. Liver trNK cells also constitutively express the programmed cell death receptor ligand TRAIL (TNF-related apoptosis-inducing ligand) [60,61]. Moreover, liver trNK cells develop in the absence of NFIL3 and do not express Eomes, though they do require T-bet for development and maintenance [15]. In the bone marrow, early T-bet expression is restricted, which allows for the development of Eomes⁺ cNK cells. In the liver, T-bet is expressed early during trNK cell development, which suppresses Eomes expression and allows for the development of Eomes⁺TRAIL⁺DX5⁺CD49a⁺ NK cells [51]. Altogether, these data indicate that liver trNK cells arise from a different developmental pathway than bone marrow-derived cNK cells [33]. There has been a call to classify the liver trNK cells as ILC1, although there is still some debate over the nomenclature [9,14,15].

Similarly to splenic NK cells, conventional liver NK cells produce IFN- γ and perform cytotoxic functions via release of perforin and granzymes [62]. Liver trNK cells also produce IFN- γ and can degranulate, as evidenced by CD107a expression [15,18]. It is agreed that liver trNK cells constitutively express TRAIL [10,51] and produce TNF- α at levels not observed with cNK cells during *in vitro* stimulation assays [15]. TNF- α has been shown to promote the recruitment of neutrophils [63], which in turn may participate in the immune response. Although it is not yet known how trNK cells contribute to pathogen

control in the liver, the effector molecules and cytokines produced by cNK cells and trNK cells suggest the two subsets perform complementary effector functions.

Lung NK cells

NK cells make up roughly 10% of the total lung lymphocytes [19]. These lung NK cells are predominantly CD11b^{high}CD27^{low}, and express higher levels of DX5, CD122, Ly49s, and CD43 than splenic NK cells, suggesting a more mature phenotype. Current evidence suggests that lung NK cells are derived from the same early precursors as bone marrow-derived cNK cells, which precludes them from being a distinct lineage. However, the lung environment shapes these cNK progenitors into a mature NK cell subset with a unique surface receptor phenotype [64].

The respiratory tract is especially vulnerable to viral, bacterial, and fungal pathogens. Aging appears to have a detrimental effect on the ability of lung NK cells to combat influenza virus infection. In aged mice versus young mice, lung NK cells showed impaired proliferation and cytotoxic responses during influenza virus infection [65]. While lung NK cells have been shown to respond to influenza virus infection, both directly and indirectly, the benefits of this response are in contention. Though some studies have shown that NK cell depletion results in higher viral titers and greater severity of infection [65], others have shown that *IL-15*^{-/-} mice experience better protection against influenza-mediated lung injury, due mainly to the lack of NK cells [66]. NK cell production of IFN- γ was also demonstrated to cause acute lung injury during the early stages of respiratory syncytial virus (RVS) infection [67]. It is possible that NK cell activity, up to a certain threshold, helps to control viral infections, while it results in inflammation and tissue damage when NK cells are over-activated.

Although ILC2 have been well characterized in the lung [68], the presence of trNK cells and/or ILC1 in this organ has not been documented. Interestingly, lung NK cells were shown to produce IL-22, both *in vitro* and *in vivo* during influenza virus infection. However, this NK cell-produced IL-22 does not appear to be crucial to the immune defense against the virus [69]. Regardless of the phenotype of these IL-22-producing cells, the potential presence of ILC1 and/or trNK cells in this organ needs to be re-examined.

Skin NK cells

When NK cells were first discovered in the skin, they were shown to express all common NK cell markers, except for lower levels of DX5 than cNK cells from the spleen. They were also shown to be mature, CD11b^{high}CD27^{high} in phenotype [70]. Subsequent research has revealed that much like the liver, the NK cells of the skin can be divided into two populations: circulating cNK cells, which are CD49a⁻DX5⁺, and trNK cells which are CD49a⁺DX5⁻. The trNK cells of the skin share other phenotypic similarities with liver trNK cells, including constitutive CD69 expression and a lack of Eomes expression. Furthermore, skin trNK cells remain present in NFIL3-deficient mice, but still rely on T-bet for development, indicating a probable developmental link to liver trNK cells [15]. The recent finding that liver trNK cells develop a memory-like phenotype in response to skin contact

hypersensitivity lends support to the potential developmental connection between these two NK cell populations [18].

Studies in humans have shown that skin NK cells are capable of lysing melanoma cells in culture [71]. Most studies of skin NK cells have focused on their potential role in autoimmune diseases that affect the skin. For instance, NK cells have been linked to the progression of inflammatory skin diseases like atopic dermatitis, psoriasis, alopecia areata, and pemphigus vulgaris [72–78]. The evidence from these studies is mostly correlative linking NK cell trafficking and increased NK cell activity to the sites of inflammation, and true mechanistic studies are so far lacking.

Uterine NK cells

The murine uterus is also home to several NK cell subsets [15,21]. Uterine (u)NK cells were found in 2-week old mice in an early study [79], and have been shown to proliferate rapidly after pregnancy [80]. Similarly to the liver, CD49a⁺DX5⁺ cNK cells and NFIL3 independent CD49a⁺DX5⁺ trNK cells are found in the uterus, indicating there are at least two NK cell populations in this organ. Interestingly, a recent study suggests that the CD49a⁺DX5⁺ population can be further subdivided into CD49a⁺Eomes⁺ and CD49⁺Eomes⁺ uNK cells [21].

Multiple studies have shown that under normal physiological conditions, uNK cells are protective toward the implanting fetus, and help to promote a normal pregnancy. In order for normal pregnancy to proceed, placental trophoblasts must invade the uterine tissues, and these invading extravillous trophoblast cells must be tolerated by the maternal immune system [81]. Trophoblast invasion is mediated by uNK cell production of VEGF-C, which maintains uNK cell non-cytotoxicity toward the invading fetal trophoblast cells and promotes vascularization of the placenta through upregulation of TAP-1 on extravillous trophoblast cells [82]. In humans, uNK cells were also shown to be protective against several viruses, including HIV-1 [83] and HCMV [84]. It is unclear at this point how the different subsets of uterine NK cells coordinate their activities to contribute to the anti-pathogen response.

Kidney NK cells

Victorini et al. recently identified both CD49a⁺DX5⁺ and NFIL3-independent CD49a⁺DX5⁺ NK cells in the kidney, indicating the presence of cNK and trNK cells in this organ [22]. Interestingly, the authors also demonstrated that ischemic acute kidney injury was promoted by kidney trNK cells and not cNK cells as previously thought [22].

Pancreatic NK cells

Pancreatic NK cells have been studied in the type 1 diabetes-prone NOD mice [85]. NK cells were identified in the exocrine pancreas of 4–5 week old mice, and in both the exocrine and endocrine pancreas of 9–10 week old mice, indicating a progressive infiltration of NK cells into the organ. Pancreatic NK cells were also observed in B6, BALB/c, and C3H mice, none of which exhibit pancreatic pathology or diabetes symptoms [85]. However,

information on the development of these NK cells is lacking and additional studies are required to determine whether this organ harbors trNK cells.

Salivary gland NK cells

The murine SMG contains a resident population of NK cells with a unique maturation phenotype compared to cNK cells, being mostly CD27^{low}CD11b^{high}, yet lacking KLRG1 expression [16]. A recent study found that the NK cells of the salivary gland develop entirely independently of NFIL3, yet still express Eomes and T-bet, indicating a potentially novel developmental pathway [46]. We also find that SMG NK cells express Eomes and T-bet, but we find a significant reduction in frequency and number of NK cells in *NFIL3*^{-/-} mice compared to wild-type (Erick and Brossay, unpublished data). This difference could be potentially explained by the influence of parameters such as housing, inflammation, and infection on the development of NFIL3-independent NK cells [3]. Similarly to the liver and the uterus, our data indicate that the SMG contains two populations of NK cells, with the majority being NFIL3-dependent cNK cells, and the minority NFIL3-independent tissue-resident NK cells. Notably, total SMG NK cells have a unique surface receptor phenotype in C57BL/6 mice, indicating that like the cNK cells of the skin, the phenotype of cNK cells that take up residence in the SMG is molded by tissue signals.

The SMG is a site of persistence for murine cytomegalovirus (MCMV) in an immunocompetent host. NK cells are crucial components of the early immune response to MCMV, and work to clear the virus in a matter of days from all infected organs except the SMG, where the virus remains active for several weeks [86]. SMG NK cells express KLRG1 during MCMV infection, albeit at a lower magnitude than cNK cells in the spleen. CD69 expression is also increased on SMG NK cells during infection, indicating that these NK cells are capable of becoming activated in response to MCMV [87]. However, *in vivo* during the peak of infection, IFN- γ production by SMG NK cells is severely impaired when compared to splenic cNK cells [16,46]. This hyporesponsive phenotype was also observed *ex vivo* when SMG NK cells were stimulated with antibodies against NK cell activating receptors, IL-12/IL-18, or PMA/Ionomycin [16]. MCMV infection is known to induce severe salivary gland dysfunction, and SMG NK cells are crucial for limiting this secretory dysfunction [88,89]. Interestingly, a recent study indicated that TRAIL⁺ SMG NK cells induce apoptosis of CD4⁺ T cells in the SMG during MCMV infection [90]. Since CD4⁺ T cells are crucial for eventual clearance of MCMV in the SMG [91], this TRAIL-mediated killing of CD4⁺ T cells could contribute to the persistence of MCMV in the SMG, but also help to lessen inflammatory damage to this organ. CD4⁺ T cells have also been shown to produce IL-10 in the SMG during MCMV infection [92], indicating that the immunoregulatory relationships between CD4⁺ T cells, cNK, and NFIL3-independent NK cells in this organ and presumably other organs are quite complex (Figure 1).

Concluding Remarks

Our understanding of innate immunology has come a long way in the 40 years since conventional NK cells were first discovered in mice [93,94] and humans [95]. The recent discovery and characterization of many new classes of innate lymphoid cells, including several distinct populations of tissue-resident NK cells, and of NK cell memory potential

[96] shows that the lymphocytes of the innate immune system display diversity of phenotype and function akin to the adaptive immune system. cNK cells have long been viewed as an innate immune counterpart of CD8⁺ and CD4⁺ T cells in function [1]. Recent discoveries have revealed that NK cells in different tissues display a great capacity for phenotypic plasticity in order to serve their roles in protecting against infection, limiting inflammatory tissue damage, and in some cases in remodeling tissues [97,98]. In the lung and SMG, populations of bone marrow-derived cNK cells take up residence and are remodeled by local signals to develop tissue-specific phenotypes. The thymus, liver, skin, uterus, SMG, and kidney also contain populations of tissue-resident NK cells that belong to lineages distinct from cNK. These trNK cell populations have unique phenotypes and developmental pathways that set them apart from cNK cells. In tissues where cNK and trNK cells are present, their functions may complement each other to promote optimal tissue health.

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Highlights

- Several tissues contain conventional and tissue-resident NK cell populations
- Tissue-resident NK cells have unique phenotypes and developmental pathways
- The functions of conventional and tissue-resident NK cells appear to be complementary

Figure 1. Relationship between cNK, NFIL3-independent NK, and CD4⁺ T cells in the salivary glands

cNK and CD4⁺ T cells both produce IFN- γ in response to MCMV infection of SMG acinar epithelial cells. CD4⁺ T cells produce IL-10, which may dampen the cNK inflammatory response. At the same time, NFIL3-independent NK cells kill CD4⁺ T cells through TRAIL-mediated apoptosis during viral infection, presumably to limit organ damage.

Table 1

Phenotypic characteristics of cNK cells and tissue-resident NK cell subsets.

	cNK*	Thymic NK	Liver trNK	Skin trNK	Uterine trNK	SMG trNK	Kidney trNK
NK1.1	+	+	+	+	+	+	+
DX5	+	+	-	-	-	+/-	-
CD49a	-	?	+	+	+	+	+
CD127	-	+	-	-	-	-	?
TRAIL	-	?	+	?	?	+/-	+
Eomes	+	?	-	-	+/-	+	?
IFN- γ	++	+	+	?	+	+/-	?
TNF- α	+/-	+	+	?	?	-	?
Granzymes	+	+	+	?	+	?	?

* cNK cells are prominent in the spleen and blood, and are also found in the liver, skin, uterus, SMG, and kidney.