

Diversification and Functional Specialization of Human NK Cell Subsets

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Abstract Natural killer (NK) cells are lymphocytes that participate in different facets of immunity. They can act as innate sentinels through recognition and eradication of infected or transformed target cells, so-called immunosurveillance. In addition, they can contain immune responses through the killing of other activated immune cells, so-called immunoregulation. Furthermore, they instruct and regulate immune responses by producing pro-inflammatory cytokines such as IFN- γ , either upon direct target cell recognition or by relaying cytokine cues from various cell types. Recent studies in mouse and man have uncovered infection-associated expansions of NK cell subsets with specific receptor repertoires and diverse patterns of intracellular signaling molecule expression. Moreover, distinct attributes of NK cells in tissues, including tissue-resident subsets, are being further elucidated. Findings support an emerging theme of ever-increasing diversification and functional specialization among different NK cell subsets, with a functional dichotomy between subsets involved in immunoregulation or immunosurveillance. The epigenetic landscapes and transcriptional profiles of different NK cell subsets are providing insights into the molecular regulation of effector functions. Here, we review phenotypic, functional, and developmental characteristics of a spectrum of human NK cell subsets. We also discuss the molecular underpinnings of different NK cell subsets and their potential contributions to immunity as well as disease susceptibility.

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1 Introduction

Natural killer (NK) cells were first identified as bone marrow-derived lymphocytes capable of killing allogeneic tumor cells without prior sensitization (Herberman et al. 1975; Kiessling et al. 1975). As opposed to T cell and B cell responses that are dictated by unique, somatically recombined, and clonally distributed antigen receptors, NK cell responses are controlled by a more limited repertoire of germ line-encoded receptors (Vivier et al. 2011). As such, NK cells belong to the growing family of innate lymphoid cells (ILCs) (Artis and Spits 2015).

Distinct from other ILC subsets, NK cells utilize a mechanism of cell killing that they share with cytotoxic T lymphocytes. Target cell killing relies on directed exocytosis of specialized secretory lysosomes, termed cytotoxic granules, which contain proteins such as perforin, granzymes, and Fas ligand (Bossi and Griffiths 1999; de Saint Basile et al. 2010; Voskoboinik et al. 2015). Cytotoxic granule exocytosis is induced by engagement of activating receptors that bind ligands on target cells (Bryceson et al. 2006a; Lanier 2008b; Moretta et al. 2001). Activation is counterbalanced by a number of inhibitory receptors, many of which specifically bind major histocompatibility complex (MHC) class I molecules, employing a common pathway to oppose NK cell effector functions (Long 2008). On this basis, NK cells contribute to an ongoing immunosurveillance through eradication of infected or malignant cells that downregulate expression of MHC class I molecules. Such “missing-self” recognition implies that NK cells complement T cell-mediated MHC class I-dependent immunosurveillance through so-called natural cytotoxicity (Ljunggren and Karre 1990). Moreover, expression of the low affinity Fc receptor

CD16 on NK cells facilitates antibody-dependent cellular cytotoxicity (ADCC). Dependent on B cell responses, ADCC may contribute to immunosurveillance of infected cells.

Deficiency in cell-mediated cytotoxicity, such as seen in immunodeficient patients lacking expression of perforin, is associated with potentially fatal hyperinflammatory syndromes. Such clinical responses are usually triggered by intracellular infections, e.g., herpesviruses (Meeths et al. 2014; Voskoboinik et al. 2015). Studies of mice have demonstrated that NK cells can kill other activated immune cells, thereby shaping adaptive immune responses and preventing excessive inflammation (Lang et al. 2012; Sepulveda et al. 2015; Soderquest et al. 2011; Waggoner et al. 2012). Thus, NK cell activity is increasingly appreciated as an important mechanism counterbalancing adaptive immune responses to prevent immunopathology and maintain immune homeostasis. Such natural cytotoxicity that contains immune responses represents a form of immunoregulatory NK cell activity.

Besides target cell killing, NK cells are a major source of chemokines and cytokines (Caligiuri 2008). NK cells produce interferon (IFN)- γ following interaction with susceptible target cells or upon activation by combinations of cytokines such as interleukin (IL)-2, IL-15, IL-12, and IL-18 that are released from other cells (Fauriat et al. 2010; Fehniger et al. 1999). IFN- γ enhances MHC class I expression, promotes T-helper 1 cell differentiation, protects activated T cells from NK cell killing, and has potent anti-mycobacterial, anti-viral, and growth inhibitory effects (Crouse et al. 2014; Schroder et al. 2004; Xu et al. 2014). Autosomal recessive mutations in genes encoding IFN- γ , as well as IFN- γ receptor subunits and signaling components, are associated with susceptibility to mycobacterial disease (Bustamante et al. 2014). In mice, NK cell-derived IFN- γ has also been shown to play a significant role in remodeling of the vasculature during pregnancy (Ashkar and Croy 1999). Tumor necrosis factor (TNF) is primarily released by NK cells upon interaction with susceptible target cells (Fauriat et al. 2010). TNF initiates pro-inflammatory cytokine cascades and represents an important target for treatment of several autoimmune diseases (Sedger and McDermott 2014).

While defects in perforin-mediated cytotoxicity and IFN- γ signaling compromise the function of various immune cell subtypes, a few rare cases of more selective NK cell deficiencies highlight their importance in viral immunity, particularly toward the family of herpesviruses (Orange 2013; Wood et al. 2011). Resolving a seminal case of NK cell deficiency in an adolescent presenting with multiple severe or disseminated herpesvirus infections (Biron et al. 1989), retrospective analyses identified a heterozygous mutation in *GATA2* (Mace et al. 2013). *GATA2* encodes a transcription factor required for hematopoiesis. In addition to NK cell deficiency, haploinsufficiency of *GATA-2* leads to variable deficiency in several other cell lineages including B cells, monocytes, and dendritic cells (Dickinson et al. 2014; Spinner et al. 2014). Moreover, autosomal recessive missense mutations in *MCM4*, encoding a component of the minichromosome maintenance (MCM) complex required for helicase-facilitated DNA replication, are associated with adrenal insufficiency, growth retardation, and NK cell deficiency (Gineau et al. 2012). Such patients

display a selective CD3⁻CD56^{dim} NK cell deficiency with a concomitant increase in CD3⁻CD56^{bright} NK cell numbers in peripheral blood and are susceptible to severe Epstein-Barr virus (EBV) infections. Finally, patients with homozygous missense mutations in *FCGR3A*, encoding CD16, are reported to experience severe recurrent herpesvirus and respiratory infections (de Vries et al. 1996; Jawahar et al. 1996). Intriguingly, patients with homozygous CD16 p.L48H or p.L48R mutations display defective natural cytotoxicity but normal ADCC, revealing an intriguing role for CD16 in potentiating natural cytotoxicity (Grier et al. 2012).

Here, we review phenotypic characteristics and functional capacities of an emerging spectrum of human NK cell subsets. Findings support a theme of functional diversification and specialization among NK cell subsets with distinct epigenetic and transcriptional profiles. We discuss molecular insights underpinning their development and function as well as their potential contributions to immunity and implications for disease susceptibility.

2 Phenotypic Characteristics of Different NK Cell Subsets

NK cells are found in a variety of organs. Although perforin expression and the capacity to kill target cells are the cardinal features that distinguish NK cells from other ILC subsets, NK cells in humans are usually phenotypically defined based on the expression of surface markers. The most widely used phenotypic definitions are CD3⁻CD56⁺ or CD3⁻CD16⁺ lymphocytes (Lanier et al. 1986). At least in peripheral blood, these markers largely discriminate cytotoxic T cells from other perforin-expressing lymphocyte subsets intrinsically capable of “natural killing.” In addition, low or lack of CD127 expression, a subunit of the IL-7 receptor, distinguishes NK cells from ILCs. Moreover, NKp46 has also been employed as a singular marker of NK cells, but this receptor, which is involved in triggering of natural cytotoxicity, can also be expressed by ILC3 subsets (Reynders et al. 2011). During certain viral infections, including hepatitis C virus (HCV) and human immunodeficiency virus, sizeable subsets of CD56⁻ NK cells are also observed. Notably, these NK cell definitions encompass diverse cellular subsets that can be differentiated with respect to transcription factors, receptors regulating homing and tissue localization, and surface receptors involved in target cell recognition.

2.1 *Differential Expression of Transcription Factors and Homing Receptors Among NK Cell Subsets*

In peripheral blood, NK cells constitute 5–15 % of all lymphocytes, of which 10 % generally express high levels of CD56 and are therefore often referred to as CD56^{bright} NK cells. CD56^{bright} NK cells represent the major NK cell subset in secondary lymphoid organs and correspondingly express high levels of CD62L, a

receptor required for lymphocyte homing to secondary lymphoid organs (Caligiuri 2008). Phenotypic and functional differences among peripheral blood CD56^{bright} and CD56^{dim} NK cell subsets have been described in depth (Cooper et al. 2001). In addition to CD62L, circulating CD56^{bright} NK cells can also be distinguished from CD56^{dim} NK cells on the basis of chemokine receptor expression. As opposed to CD56^{dim} NK cells, circulating CD56^{bright} NK cells express CCR7, but lack expression of CXCR1, CXCR2, and CX3CR1 (Campbell et al. 2001). CCR7 facilitates entry into secondary lymphoid organs, while CXCR1, CXCR2, and CX3CR1 are involved in migration toward sites of infection or inflammation. Both CD56^{bright} and CD56^{dim} NK cells express high levels of EOMES and T-BET, transcription factors that are key for expression of cytotoxic granule constituents (Cichocki et al. 2013).

NK cells are also found in tissues besides peripheral blood and secondary lymphoid organs (Shi et al. 2011). In these tissues, they usually express high levels of CD56 (i.e., being CD56^{bright} NK cells). Relative to other lymphocyte subsets, they are particularly abundant in the liver and female reproductive tract. In these organs, human NK cells display distinctive phenotypes (Burrows et al. 1993; Burt et al. 2009; Koopman et al. 2003). Elegant studies using surgically parabiosed animals recently demonstrated that the vast majority of murine liver and uterine NK cells express CD49a, the α_1 integrin subunit of the VLA-1 receptor that binds extracellular matrix proteins collagen and laminin. These CD49a⁺ liver and uterine NK cells are tissue-resident (Peng et al. 2013; Sojka et al. 2014). Moreover, up to 50 % of murine skin NK cells also express CD49a and are tissue-resident (Sojka et al. 2014). Subsets of human uterine and liver NK cells also express CD49a (Geiselhart et al. 1995; Marquardt et al. 2015). In addition, uterine NK cells express CD103, the α_E integrin subunit of $\alpha_E\beta_7$ that binds E-cadherin, as well as the tetraspanins CD9 and CD151 (Koopman et al. 2003). As such, tissue-resident NK cells may be distinguished from NK cells circulating through vascularized organs by expression of CD49a and/or CD103. Although further phenotypic characterizations are necessary, human liver-resident NK cells lack expression of CD62L and express low levels of EOMES and T-BET (Burt et al. 2009; Marquardt et al. 2015), corroborating reports of murine liver NK cell that also express low levels of EOMES and T-BET (Sojka et al. 2014). In contrast, at least in mice, results suggest that uterine NK cells maintain EOMES expression while expressing low levels of T-BET (Tayade et al. 2005). Tissue-resident NK cells may thus vary widely in their phenotype for adhesion receptors and transcription factors. Their chemokine receptor expression profiles have so far not been extensively studied.

Following cytomegalovirus (CMV) infection of mice, subsets of NK cells expressing the activating Ly49H receptor have been found to acquire “memory-like” or “adaptive” immune features, including robust recall responses (Sun et al. 2009). Ly49H can bind m157, a murine CMV-encoded MHC class I-like protein, representing an example of direct pathogen recognition by an NK cell receptor (Arase et al. 2002). In humans, infection with cytomegalovirus (CMV) is associated with lasting expansions of NK cell subsets expressing NKG2C or activating KIR (Beziat et al. 2013; Guma et al. 2004). Expansions of NKG2C⁺ NK

cells occur in response to acute infection or reactivation of latent CMV (Foley et al. 2012; Lopez-Verges et al. 2011); may provide protective immunity (Kuijpers et al. 2008; Sun et al. 2009); appear specific to CMV but can be augmented by co-infection by other viruses including EBV, hantavirus, and HCV (Bjorkstrom et al. 2011; Petitdemange et al. 2011; Saghafian-Hedengren et al. 2013); are long-lived; and manifest high NKG2C expression (Hendricks et al. 2014; Saghafian-Hedengren et al. 2013). However, as subsets expressing NKG2C typically are present at varying frequencies among NK cells from virtually all individuals irrespective of CMV serostatus, and CMV-associated NK cells alternatively can express other activating receptors including KIR, additional markers are needed to distinguish “memory-like” or “adaptive” NK cells. Recent findings uncover that CMV infection-associated adaptive NK cells can be distinguished from canonical CD56^{dim} NK cells on the basis of variegated allelic silencing of the intracellular signaling proteins FcεRγ, SYK, and EAT-2 (Lee et al. 2015; Schlums et al. 2015; Zhang et al. 2013). Notably, a common denominator of such adaptive NK cells is downregulation of promyelocytic leukemia zinc finger (PLZF) (Schlums et al. 2015), a transcription factor required for normal ILC development as well as invariant NKT effector programs (Constantinides et al. 2014; Kovalovsky et al. 2008; Savage et al. 2008). Although PLZF is not expressed in mouse NK cells (Constantinides et al. 2014), human circulating CD56^{bright} NK cells and canonical CD56^{dim} NK cells express PLZF (Schlums et al. 2015). Adaptive NK cells lack expression of CD62L, and otherwise display a chemokine receptor expression profile similar to canonical CD56^{dim} NK cells, albeit with increased expression of CCR5 (Zhang et al. 2013). Furthermore, adaptive NK cells express high levels of T-BET and EOMES (Schlums et al. 2015).

In addition to expansions of adaptive NK cells associated with CMV infection, a distinct phenomenon of antigen-specific NK cell memory has been described in RAG-deficient mice (O’Leary et al. 2006). Antigen-specific murine NK cells were found to be liver-derived and express CXCR6 (Paust et al. 2010). Intriguingly, the emergence of liver- and spleen-derived antigen-specific NK cell responses was also recently reported in macaques exposed to lentiviral infection or adenovirus-mediated vaccination (Reeves et al. 2015). Although the molecular underpinnings of antigen-specific NK cell memory remain unclear, blocking antibodies against NKG2A and NKG2C, but not NKp46, eliminated NK cell reactivity in macaques. The phenotype of such cells in immunocompetent macaques, let alone humans, is yet to be defined. Therefore, the phenotype and function of such memory NK cells will not be further discussed here.

On the basis of these key phenotypic differences, human NK cells can at present be broadly subdivided into four main subsets: circulating CD56^{bright} NK cells expressing CD62L, diverse canonical CD56^{dim} NK cells expressing PLZF, adaptive CD56^{dim} NK cells with low or absent PLZF, and tissue-resident CD56^{bright} NK cells with a unique pattern of adhesion receptor expression including CD49a and CD103 (Fig. 1). Potentially also influencing their anatomical distribution and function (Gregoire et al. 2007), these subsets variably express chemokine receptors (Table 1).

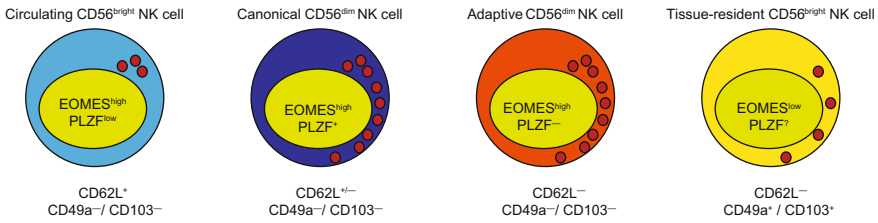


Fig. 1 Updated view of human NK cell subsets based on surface receptor expression, transcription factor expression, and granule content. Based on recent work by several groups, we propose four distinct human NK cell subsets. Circulating CD62L⁺CD49a⁻CD103⁻CD56^{bright} NK cells express high levels of EOMES and low levels of PLZF. These cells have limited granule content. Canonical CD62L^{+/-}CD49a⁻CD103⁻CD56^{dim} NK cells express high levels of EOMES and uniformly express PLZF. These cells have high granule content. Adaptive CD62L⁻CD49a⁻CD103⁻CD56^{dim} NK cells express high levels of EOMES and low-to-absent PLZF. These cells also have high granule content. Tissue-resident CD62L⁻CD49a⁺CD103⁺CD56^{bright} NK cells can express low levels of EOMES, and PLZF expression levels are not yet known. These cells have reduced granule content relative to canonical and adaptive CD56^{dim} NK cells

Table 1 Differential expression of chemokine receptors on human NK cell subsets

Chemokine receptor	CD56 ^{bright} CD62L ⁺ circulating NK cells	CD56 ^{dim} canonical NK cells	CD56 ^{dim} adaptive NK cells	Tissue-resident NK cells
CCR5	-	+	high	?
CCR7	+	-	-	?
CXCR1	-	+	+	?
CXCR2	-	+	+	?
CXCR3	-	+	+	?

2.2 Differential Expression of Receptors Involved in Target Cell Recognition

The distribution of several NK cell receptors varies among cellular subsets. In particular, NK cells display variegated expression of rapidly evolving receptors that bind MHC class I molecules (Khakoo et al. 2000; Valiante et al. 1997). Their rapid evolution and expression predominantly on NK cells signify important roles for NK cells in immunity to intracellular pathogens as well as reproductive success (Kulkarni et al. 2008; Parham and Moffett 2013). Inhibitory and activating lectin-like (Ly49 and NKG2) and immunoglobulin superfamily (KIR) receptor counterparts are encoded by the polygenic natural killer gene complex on chromosome 6 and the leukocyte receptor complex on chromosome 19, respectively. The human genome displays a large expansion of *KIR* genes but contains only a single *LY49L* pseudogene, whereas the mouse genome displays a large expansion of Ly49-encoding genes and only two *KIR* homologs. In addition, both rodents and primates encode inhibitory NKG2A and activating NKG2C lectin-like receptors

that are differentially expressed on NK cell subsets, form heterodimers with CD94, and bind the non-classical MHC class I molecule HLA-E, serving as an overall gauge of MHC class I expression (Braud et al. 1998). In contrast, KIR binds specific classical MHC class I alleles. While expression of inhibitory receptors for self-MHC class I molecules potentiates NK cell responses, a process that has been termed education (Anfossi et al. 2006; Kim et al. 2005), engagement of activating KIR and CD94/NKG2C signals for NK cell effector function.

Circulating CD56^{bright}CD62L⁺ NK cells uniformly express CD94/NKG2A, but lack expression of KIR and CD94/NKG2C. As such, they are educated by NKG2A. In contrast, canonical CD56^{dim} NK cells display variegated expression of CD94/NKG2A, CD94/NKG2C, and KIR. Interestingly, whereas NKG2A expression can be dynamically induced by IL-12 stimulation (Saez-Borderias et al. 2009), KIR expression is stably imprinted through promoter DNA methylation patterns (Chan et al. 2003). Adaptive CD56^{dim} NK cells and liver-resident NK cells infrequently express CD94/NKG2A, while CD94/NKG2A is expressed on the majority of uterine NK cells (Guma et al. 2004; Marquardt et al. 2015; Ponte et al. 1999). Whereas CD94/NKG2C is infrequently expressed on canonical CD56^{dim} NK cells, it can be observed at high frequencies on adaptive and tissue-resident NK cells (Marquardt et al. 2015; Schlums et al. 2015). CD94/NKG2C frequencies are not elevated on uterine NK cells (Bachmayer et al. 2009). Moreover, both activating and self-specific inhibitory KIRs are expressed at high frequencies on adaptive and tissue-resident NK cells (Beziat et al. 2013; Marquardt et al. 2015; Sharkey et al. 2008; Verma et al. 1997).

Besides activating CD94/NKG2C and KIR, CD16 is a potent activating receptor on human NK cells (Bryceson et al. 2006b). CD16 is expressed in low frequencies and at low levels on circulating CD56^{bright}CD62L⁺ NK cells but is present at high levels on the vast majority of canonical and adaptive CD56^{dim} NK cells. Notably, tissue-resident NK cells generally lack CD16 expression (Burrows et al. 1993; Burt et al. 2009). Contrasting CD16, CD94/NKG2C, and activating KIR, co-activating receptors are expressed on the majority of NK cells and do not initiate strong activation when triggered individually. Rather, they synergize for activation of NK cell effector functions (Bryceson et al. 2006b, 2009). These receptors include the signaling lymphocyte activation molecule (SLAM) family receptors 2B4, NTB-A, and CRACC; the lectin-like receptors NKG2D and NKp80; and the immunoglobulin superfamily receptors NKp30, NKp46, and DNAM-1. Co-activating receptors can participate in immunosurveillance by potentiating signaling through CD16, CD94/NKG2C and activating KIR or synergistically inducing immunoregulatory killing of autologous activated immune cells (Fig. 2).

In summary, individual NK cell subsets differ significantly with respect to surface receptor expression (Table 2), potentially dictating their target cell specificities. It is important to note that these cellular subsets do not necessarily represent distinct entities. Phenotypic intermediates are commonly observed (Yu et al. 2010; Schlums et al. 2015), suggesting a continuum in different NK cell differentiation axes.

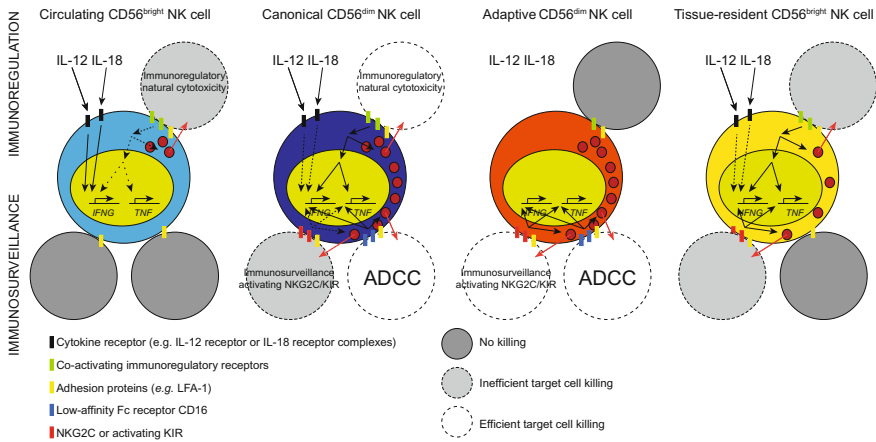


Fig. 2 Specialization of function by individual subsets of human NK cells. We propose a model whereby distinct subsets of NK cells are specialized for immunoregulatory killing of autologous, activated immune cells and/or immunosurveillance against infected or diseased cells. Circulating CD56^{bright} NK cells exhibit limited target cell-induced immunoregulation and do not mediate immunosurveillance. Canonical CD56^{dim} cells represent a functionally heterogeneous subset that mediates both target cell-induced immunoregulation and immunosurveillance. Adaptive CD56^{dim} NK cells are specialized for robust immunosurveillance, and FcεRγ⁻ and EAT-2⁻ adaptive CD56^{dim} NK cells lack immunoregulation. Tissue-resident CD56^{bright} NK cells exhibit limited target cell-induced immunoregulation and moderate immunosurveillance. However, they do produce a wide range of cytokines upon activation, involved in both immunosurveillance and tissue homeostasis

Table 2 Differential expression of key receptors involved in target cell recognition on human NK cell subsets

Receptor	CD56 ^{bright} CD62L ⁺ circulating NK cells	CD56 ^{dim} canonical NK cells	CD56 ^{dim} adaptive NK cells	Tissue-resident NK cells
NKG2A	+	±	-/(+)	+
NKG2C	-	±	±	±
Inh. KIR	-	±	+/(-)	+/(-)
Act. KIR	-	±	±	+/(-)
CD16	-/(+)	+/(-)	+	-

3 Functional Diversification and Specialization of NK Cell Subsets

Heterogeneity in NK cell functional responses has long been appreciated (Hercend et al. 1986). Single-cell flow cytometric assessments have revealed considerable heterogeneity with respect to cytotoxic activity of individual cells within a primary

NK cell population (Alter et al. 2004; Bryceson et al. 2005). Heterogeneity is not only apparent within the NK cell population from any given individual: Differing functional response patterns have also been noted between different individuals (Bryceson et al. 2010). Here, we highlight how human circulating CD56^{bright}CD62L⁺ NK cells, canonical CD56^{dim}PLZF⁺ NK cells, adaptive CD56^{dim}PLZF⁻ NK cells, and tissue-resident CD56^{bright} NK cells subsets differ with respect to key functional capacities.

3.1 Circulating CD56^{bright}CD62L⁺ NK Cells—a Cytokine-Producing Subset

Perforin expression is prerequisite for rapid induction of target cell death. With respect to cytotoxic activity, circulating CD56^{bright}CD62L⁺ NK cells express very low levels of perforin and granzymes. Compared to canonical CD56^{dim} NK cells and cytotoxic effector T cells, CD56^{bright}CD62L⁺ NK cells display tenfold and threefold lower expression levels of perforin, respectively (Chiang et al. 2013). Thus, relative to other NK cell subsets, CD56^{bright}CD62L⁺ NK cells are less cytotoxic overall. However, CD56^{bright}CD62L⁺ NK cells uniformly express co-activating receptors and degranulate upon interaction with K562 cells (a prototypical human NK cell target cell line that lacks MHC class I expression and is used for the evaluation of natural cytotoxicity) or autologous activated T cells (Chiang et al. 2013; Schlums et al. 2015). Thus, they can mediate immunoregulatory target cell killing through triggering of co-activating receptors. The paucity of CD16, as well as NKG2C and activating KIR expression, implies that circulating CD56^{bright}CD62L⁺ NK cells do not significantly contribute to ADCC or other forms of cytotoxic immunosurveillance. Of note, target cell recognition by circulating CD56^{bright}CD62L⁺ NK cells induces very little IFN- γ and TNF, likely reflecting inaccessibility of the *IFNG* locus to transcription factors induced by co-activating receptors.

In contrast to poor pro-inflammatory cytokine production in response to target cell engagement, circulating CD56^{bright}CD62L⁺ NK cells produce high levels of IFN- γ in response to combinations of cytokines such as IL-18 and IL-12 or IL-15, but less so in response to IL-12 and IL-15 (Fauriat et al. 2010; Fehniger et al. 1999). Rather, combined stimulation with IL-12 and IL-15 drives the production of IL-10, a potent anti-inflammatory cytokine (Fehniger et al. 1999). Such cytokine combinations can also induce production of TNF and GM-CSF, as well as the chemokines MIP-1 α and MIP-1 β (Fauriat et al. 2010; Fehniger et al. 1999). In summary, circulating CD56^{bright}CD62L⁺ NK cells represent a subset specialized for immunoregulatory production of both pro- and anti-inflammatory cytokines in response to distinct combinations of cytokines derived from innate as well as adaptive immune cells.

3.2 *Canonical CD56^{dim} NK Cells—a Cytotoxic Immunoregulatory Subset*

Canonical CD56^{dim} NK cells represent a phenotypically diverse subset that expresses very high levels of perforin and granzymes, excel at immunoregulatory cytotoxic activity, and readily degranulate in response to autologous, activated immune cells (Schlums et al. 2015). Due to high expression levels of CD16, canonical CD56^{dim} NK cells are strong mediators of ADCC and can also be readily stimulated through NKG2C and activating KIR, which likely contributes to early immunosurveillance of infected cells. As such, canonical CD56^{dim} NK cells efficiently participate in cytotoxic immunoregulation as well as immunosurveillance. Degranulation is potentiated by the expression of educating inhibitory receptors for self-MHC class I (Bjorkstrom et al. 2010), with increased DNAM-1 expression serving as a marker of NK cell education (Enqvist et al. 2015).

Compared to circulating CD56^{bright}CD62L⁺ NK cells, canonical CD56^{dim} NK cells produce less IFN- γ in response to combinations of cytokines such as IL-12 and IL-15 or IL-12 and IL-18 (Fauriat et al. 2010; Fehniger et al. 1999). Reduced immunoregulatory cytokine production correlates with loss of CD62L and acquisition of CD57 expression (Bjorkstrom et al. 2010; Juelke et al. 2010; Lopez-Verges et al. 2010). These surface proteins are thus deemed markers of NK cell maturation. NK cell maturation correlates with a gradual DNA hypomethylation of the *IFNG* promoter (Luetke-Eversloh et al. 2014a). Contrasting circulating CD56^{bright}CD62L⁺ NK cells, combinations of cytokines do not induce TNF production by canonical CD56^{dim} NK cells (Fauriat et al. 2010).

In summary, canonical CD56^{dim} NK cells represent a subset specialized for immunoregulatory killing of activated immune cells, but likely also contribute to immunosurveillance. Notably, murine NK cells with educating Ly49 expression fail to protect neonatal mice from fatal CMV infection, whereas transfer of NK cells lacking educating Ly49 expression can rescue infected mice (Orr et al. 2010), revealing an important role for early NK cell proliferation in response to viral infection that can be blocked by the tyrosine phosphatase SHP-1 in educated NK cells. Upon recognition of susceptible target cells, canonical CD56^{dim} NK cells also serve as an important source of IFN- γ and TNF.

3.3 *Adaptive CD56^{dim} NK Cells—A Effector Subset Trained for Immunosurveillance*

Adaptive CD56^{dim} NK cells widely differ with respect to surface receptor expression and intracellular signaling protein expression, with unique patterns among different individuals (Schlums et al. 2015). Generally defined on the basis of being CD56^{dim}PLZF⁻ NK cells, most adaptive NK cells express activating NKG2C or KIR. However, in a few individuals, these receptors are not detected on the surface

of NK cells with an adaptive phenotype. Despite diverse receptor expression, adaptive CD56^{dim} NK cells uniformly express high levels of perforin and granzymes. Nonetheless, they respond poorly to autologous, activated immune cells (Schlums et al. 2015). Loss of immunoregulatory cytotoxic activity correlates with variegated silencing of FcεRγ and EAT-2 expression, indicating a central role for NKp30, NKp46, and SLAM family receptors for NK cell-mediated killing of activated T cells (Schlums et al. 2015). These data corroborate findings in LCMV-infected mice, where NKp46 is required for immunoregulatory killing of activated T cells (Crouse et al. 2014). In contrast to the inability to mediate immunoregulatory killing, adaptive CD56^{dim} NK cells robustly respond to engagement of CD16, NKG2C, or activating KIR by degranulating and producing cytokines such as IFN-γ and TNF (Schlums et al. 2015). Upon engagement of CD16, IFN-γ and TNF production by adaptive CD56^{dim} NK cells is typically greater than that of canonical CD56^{dim} NK cells (Schlums et al. 2015; Zhang et al. 2013). DNA hypomethylation of the *IFNG* conserved noncoding region 1 has been shown to underlie increased IFN-γ production by NKG2C⁺ adaptive NK cells (Luetke-Eversloh et al. 2014b). Moreover, genome-wide DNA methylation analyses have revealed widespread DNA hypomethylation of the *IFNG* and *TNF* promoters in adaptive CD56^{dim} NK cells relative to canonical CD56^{dim} NK cell subsets (Schlums et al. 2015).

Notably, adaptive CD56^{dim} NK cells do not respond to IL-12 or IL-18 (Schlums et al. 2015). The lack of response is explained by methylation-dependent silencing of *IL12RB2* and *IL18RAP*, encoding key components of the IL-12 and IL-18 receptors (Schlums et al. 2015). As such, adaptive CD56^{dim} NK cells cannot mediate immunoregulatory cytokine production. Of note, adaptive CD56^{dim} NK cells retain responsiveness to IL-15 (Schlums and Bryceson, unpublished observations).

In summary, adaptive CD56^{dim} NK cells represent diverse subsets with target cell specificity unique to each host. Their expansion in response to CMV infection and frequent expression of rapidly evolving NKG2C and activating KIR indicate a role in immunosurveillance of persistent viral infections. Through expression of CD16, they can also mediate strong ADCC, a trigger that, at least in vitro, can expand adaptive NK cell subsets (Lee et al. 2015; Schlums et al. 2015). Upon recognition of susceptible target cells, they potently produce IFN-γ and TNF. Remarkably, they do not participate in killing of activated immune cells and therefore may not be involved in immunoregulation.

3.4 Tissue-Resident NK Cells—Diverse Roles in Tissue Homeostasis and Immunosurveillance

Studies of tissue-resident NK cells are revealing distinct attributes in different tissues. They should therefore be viewed as a spectrum of cells uniquely influenced by their microenvironments. With respect to cytotoxic function, tissue-resident NK

cells express comparatively low levels of perforin. As such, they are generally expected to be relatively poor cytotoxic mediators, as is the case for uterine and liver-derived NK cells (Burt et al. 2009; Kopcow et al. 2005). Of note, liver NK cells express high levels of TNF-related apoptosis-inducing ligand (TRAIL) and can thereby induce apoptosis of hepatocytes (Dunn et al. 2007). Mimicking engagement of activating surface receptors, PMA plus ionomycin stimulation induces high levels of IFN- γ and TNF (Marquardt et al. 2015; Sojka et al. 2014). Notably, such conditions also support production of GM-CSF. Moreover, uterine NK cells produce vascular and endothelial growth factor (VEGF) as well as placental growth factor (PLGF), supporting their role in vascular remodeling (Hanna et al. 2006).

Similar to circulating CD56^{bright}CD62L⁺ NK cells, tissue-resident CD56^{bright} NK cells also lack expression of CD16. Thus, they cannot mediate ADCC. In contrast to circulating CD56^{bright}CD62L⁺ NK cells, tissue-resident CD56^{bright} NK cells express a distinct repertoire of adhesion molecules for tissue retention and can display very high frequencies of NKG2C and KIR. As such, they may mediate non-cytotoxic immunosurveillance through production of pro-inflammatory cytokines. It is also conceivable that upon stimulation by target cells or exogenous cytokines, these tissue-resident NK cells may upregulate expression of cytotoxic granule constituents. *In vitro* culture with IL-15 potentiates uterine NK cell cytotoxicity (Cerdeira et al. 2013), supporting the notion that tissue-resident NK cells may upregulate cytotoxic activity upon tissue inflammation. To better understand their functional capacities, epigenetic investigations of resident NK cells from different tissues are warranted.

In summary, tissue-resident NK cells represent subsets with specialized roles in tissue homeostasis that involve secretion of soluble factors not usually expressed by other NK cell subsets. They may also participate in immunosurveillance of infected cells. Upon recognition of susceptible target cells, they can serve as an important early source of IFN- γ and TNF. It is not clear to what degree such recognition also triggers cytotoxic effector programs.

4 Ontological Relationships Between NK Cell Subsets

At the transcriptional level, NK cells are most closely related to cytotoxic T lymphocytes (CTL) (Bezman et al. 2012; Narni-Mancinelli et al. 2011; Sun and Lanier 2011). Given the observed phenotypic and functional similarities, it has been proposed that NK cells are related to the T cell lineage and share a common T/NK cell-committed precursor. Indeed, both T cells and NK cells can be generated from human triple-negative (CD3⁻CD4⁻CD8⁻) thymocytes isolated from fetal thymic tissue using appropriate *in vitro* conditions (Denning et al. 1991; Hori et al. 1992; Sanchez et al. 1993), and NK cells can express cytoplasmic CD3 proteins and non-functional transcripts for the T cell receptor chains (Lanier et al. 1992; Ritz et al. 1985). With respect to progenitor cells isolated from human bone marrow, a

subset of multipotent CD34⁺CD38⁺HLA-DR⁺CD45RA⁺Lin⁻ cells lacking myeloid, erythroid, and megakaryocytic potential can give rise to T cells, B cells, NK cells, and dendritic cells (Galy et al. 1995). A similar common lymphocyte progenitor (CLP) with lymphoid-restricted (T, B, and NK cell) reconstitution capability was isolated from the bone marrow of mice (Kondo et al. 1997). However, recent *in vitro* differentiation studies have demonstrated that common myeloid progenitor (CMP) and granulocytic-monocytic precursor (GMP) cells can also give rise to NK cells (Grzywacz et al. 2011). Intriguingly, a recent study employing quantitative genetic bar coding to track the clonal output of transplanted autologous hematopoietic stem cells in rhesus macaques suggested limited overlap of NK cell development with that of T, B, or myeloid cell lineages (Wu et al. 2014), supporting an alternative view that NK cells *in vivo* may rather develop from a distinct NK primitive progenitor.

Besides adaptive T and B lymphocytes, NK cells share features with other ILC subtypes, including lymphoid tissue inducer (LTI) cells that are essential for the formation and repair of secondary lymphoid tissues (Finke 2005; Scandella et al. 2008), ILC1 cells that accumulate in inflamed tissues and produce IFN- γ (Bernink et al. 2013; Vonarbourg et al. 2010), ILC2 cells that secrete T_H2-associated cytokines (Mjosberg et al. 2011; Monticelli et al. 2011; Moro et al. 2010; Neill et al. 2010), and ILC3 cells that are specialized for the secretion of IL-22 (Cella et al. 2009; Crellin et al. 2010; Satoh-Takayama et al. 2008; Takatori et al. 2009). Much of our knowledge of the developmental relationships between NK cells and ILCs stems from mouse models. In mice, expression of the Id2 and TOX transcription factors is required for both NK cell and ILC development (Aliahmad et al. 2010; Moro et al. 2010; Satoh-Takayama et al. 2008; Seehus et al. 2015; Yokota et al. 1999), suggesting that these lineages share a common Id2/TOX-dependent progenitor. Fate mapping experiments in mice have shown that PLZF expression promotes commitment to the ILC lineages, but not LTI or NK cells (Constantinides et al. 2015). Commitment of the ILC/NK cell progenitor to the ILC2 lineage requires expression of ROR α and GATA3 (Neill et al. 2010; Wong et al. 2012), while commitment to the ILC3 lineage is dependent upon the expression of ROR γ t (Sanos et al. 2009; Satoh-Takayama et al. 2008) and the aryl hydrocarbon receptor (AHR) (Kiss et al. 2011; Lee et al. 2012; Qiu et al. 2012). Expression of AHR appears necessary for the maintenance of human ILC3 lineage identity, as AHR antagonism or silencing induces *TBX21* (T-BET) and *EOMES* expression and causes ILC3s to differentiate into NK cells with a CD56^{bright} phenotype (Hughes et al. 2014). Supporting the view of fluidity in ILC and NK cell identity, experiments have shown that IL-12 and IL-15 stimulation can cause ROR γ t downregulation in ILC3s, leading to *de novo* IFN- γ production (Vonarbourg et al. 2010). Notably, the developmental relationships between human NK cells and ILCs may be regulated somewhat differently. Nonetheless, studies indicate that cues from the microenvironment, particularly inflammatory cytokines, may fundamentally alter NK cell and ILC identity and influence their differentiation programs, highlighting plasticity among innate lymphocyte subsets (Cichocki et al. 2014b).

Similar to what has been described with subsets of ILCs, inflammatory cytokines can drive circulating CD56^{bright} NK cells to differentiate. Culture of peripheral blood CD56^{bright}KIR⁻ NK cells with IL-15 and IL-12 pushes differentiation toward a more mature CD56^{dim}KIR⁺ canonical NK cell phenotype (Romagnani et al. 2007). Highlighting the plasticity of the circulating CD56^{bright} NK cell subset, CD49a⁺CD103⁺ NK cells with a tissue-resident uterine NK cell phenotype have been derived in vitro from peripheral blood CD56^{bright}CD16⁻ NK cells with a combination of hypoxia and transforming growth factor (TGF)- β (Cerdeira et al. 2013). Moreover, TGF- β produced by decidual stromal cells directly causes conversion of CD16⁺ peripheral blood NK cells into CD16⁻ NK cells. Thus, exposure to TGF- β appears to direct NK cells away from Fc γ R-mediated functional responses that could be detrimental in the uterine environment where anti-fetal antibodies can be elicited and toward tissue-specific immunoregulatory functions at the fetal–maternal boundary (Keskin et al. 2007). In addition, TGF- β suppresses expression of the key cytotoxic mediators perforin and granzyme B (Thomas and Massague 2005). Interestingly, mouse models have established that a combination of IL-15 and TGF- β induces the development of CD103-expressing skin-resident T cells (Mackay et al. 2013). Thus, in infectious settings, it is possible that inflammatory cues, including TGF- β imprinting, direct NK cell subsets for tissue residency. Such mechanisms would allow for a diversity of NK cells specialized for targeting distinct infections at a variety of anatomical sites.

Relative to circulating CD56^{bright} NK cells, the phenotypes of tissue-resident and mature adaptive NK cells appear to be more phenotypically stable. The ontological relationships between canonical NK cells, adaptive NK cells, and tissue-resident NK cells are beginning to be revealed. In mice, *Nfil3*, which encodes the basic leucine zipper transcription factor E4BP4, is requisite for canonical NK cell development. *Nfil3*^{-/-} mice lack splenic NK cells and exhibit a developmental block in early bone marrow NK cell development downstream of IL-15 signaling and upstream of Id2 and EOMES (Gascoyne et al. 2009; Kamizono et al. 2009; Male et al. 2014). Supporting the view that NK cells and ILCs share a common progenitor, *Nfil3*^{-/-} mice are also deficient in all ILC subsets (Geiger et al. 2014; Seillet et al. 2014). Although *Nfil3* is required for early NK cell and ILC development in the bone marrow, the expansion and maintenance of Ly49H⁺ memory NK cells in response to mouse CMV infection are unperturbed in *Nfil3*^{-/-} mice (Firth et al. 2013). Interestingly, CD49a⁺DX5⁻ tissue-resident NK cells in the mouse liver, which express significantly less EOMES relative to canonical splenic NK cells, are also present at normal frequencies in *Nfil3*^{-/-} mice (Sojka et al. 2014). Mouse fate mapping experiments indicate that such EOMES-independent, TRAIL-expressing NK cells represent an independent lineage of ILC that require IL-15 and T-BET for their development (Daussy et al. 2014). Thus, whereas a developmental relationship from canonical to adaptive CD56^{dim} NK cells is suggested by the observed continuum of cellular intermediates (Schlums et al. 2015), mouse experiments suggest that liver-resident NK cells and circulating adaptive NK cells may arise from distinct precursors or along alternative developmental pathways compared to canonical NK cells and ILCs.

The epigenetic diversification in response to CMV infection (Schlums et al. 2015) adds another layer of complexity onto the NK cell developmental paradigm and suggests a broad continuum of differentiation states with intriguing stochastic elements. At the epigenetic level, differentiation of adaptive NK cells appears to parallel that of effector CD8⁺ T cells (Schlums et al. 2015). Moreover, a similar diversification process has been reported in analyses of the differentiation patterns of individual CD8⁺ T cells. Exhibiting remarkable diversification, a single CD8⁺ T cell can give rise to phenotypically and functionally diverse progeny representing both short- and long-lived subsets in response to viral infection (Stemberger et al. 2007). How this capacity for diversification generates reproducible immune responses geared toward a specific pathogen has been a major focus of CD8⁺ T cell research. Recently, *in vivo* fate mapping of single CD8⁺ T cell progeny combined with mathematical modeling has shown that robust acute immunity requires the initial recruitment of multiple precursors whose cell fate is specified within a probabilistic framework. The stochastic processes built into this framework create a division of labor that is necessary to guarantee robust immunological outcomes. Both intrinsic stochastic variation and extrinsic signals, such as local cytokine levels, could contribute to population diversification (Buchholz et al. 2013; Gerlach et al. 2013). While engagement of NKG2C or activating KIR likely promotes adaptive NK cell expansion, the expression of these receptors does not appear to be a prerequisite for adaptive NK cell differentiation (Schlums et al. 2015). The full extent of NK cell diversification in response to viral infections and the mechanisms that drive this process are exciting topics for future investigation.

5 Molecular Insights to NK Cell Specialization

The molecular underpinnings of phenotypic and functional heterogeneity among NK cell subsets are of considerable interest. One of the major distinguishing features of circulating CD56^{bright}CD62L⁺ NK cells is remarkably robust IFN- γ production relative to canonical CD56^{dim} NK cells in response to IL-12 stimulation, which can be enhanced by the addition of IL-15 or IL-18 (Fehniger et al. 1999). Signal transducer and activator of transcription 4 (STAT4) is a critical mediator of IL-12-stimulated inflammatory immune responses, including IFN- γ production by NK cells and CD4⁺ T cells polarized toward the T_H1 phenotype (Kaplan et al. 1996). STAT4 promotes transcriptional activation of its target genes primarily through the recruitment of chromatin remodeling enzymes to key regulatory elements (Cichocki et al. 2014a; O'Sullivan et al. 2004; Zhang and Boothby 2006). In mouse T_H1 cells, the switch (Swi)–sucrose non-fermenter (SNF) component, Brahma-related gene 1 (Brg1), is recruited to regulatory sites within the *Ifng* locus in a Stat4-dependent manner, leading to rapid nucleosome remodeling and transcriptional competence (Zhang and Boothby 2006). A similar mechanism is likely responsible for IL-12-driven IFN- γ production by circulating CD56^{bright}CD62L⁺ NK cells.

Differentiation of NK cells to the CD56^{dim} phenotype is associated with a reduced responsiveness to exogenous inflammatory cytokines. This is particularly evident in adaptive CD56^{dim} NK cells, which exhibit methylation-dependent silencing of *IL12RB2* and *IL18RAP* and lack responsiveness to IL-12 and IL-18 stimulation (Schlums et al. 2015). The loss of cytokine responsiveness by adaptive NK cells strongly correlates with silencing of PLZF (Schlums et al. 2015). PLZF was initially described as a transcription factor that is central for directing the innate-like effector differentiation program of the NKT cell lineage (Kovalovsky et al. 2008; Savage et al. 2008). Subsequently, it was shown that PLZF induction upon TCR cross-linking is also necessary for the acquisition of innate, NKT-like properties by $\gamma\delta$ T cells (Kreslavsky et al. 2009). Functionally, PLZF-deficient murine NKT cells are incapable of adequate IFN- γ responses to IL-12 and IL-18 due to dramatically reduced levels of IL-12 and IL-18 receptor expression (Gleimer et al. 2012). Thus, it is tempting to speculate that, similar to murine T cell subsets, expression of PLZF directs the innate effector and immunoregulatory programs of human canonical CD56^{dim} NK cells, and its silencing in adaptive CD56^{dim} NK cells allows for specialization toward immunosurveillance. Of note, whereas PLZF appears to promote *IL12RB2* and *IL18RAP* transcription in humans, *Il12rb1* and *Il18r1* are targeted in mice (Gleimer et al. 2012). Besides IL-12 and IL-18 signaling, PLZF also controls CD7 and CD161 expression, with lack of PLZF expression strongly associated with DNA methylation-dependent silencing of the *CD7* and *KLRB1* genes (Schlums et al. 2015). It is also noteworthy that a single PLZF-deficient individual has been described with an NK cell phenotype consistent with a predominantly adaptive compartment (Eidson et al. 2011). Thus, it is tempting to speculate that PLZF might be required to maintain canonical, immunoregulatory NK cells, with adaptive NK cells presiding in the absence of PLZF.

Considerable efforts have been made toward identifying the factors necessary for the expansion of adaptive NK cells following CMV infection. NK cells from *Il12rb2*^{-/-} and *Stat4*^{-/-} mice exhibit defects in the generation of memory responses, demonstrating an important role for inflammatory cytokines in driving the maturation program that leads to NK cell memory (Sun et al. 2012). IL-12 and STAT4 promote NK cell memory, at least in part, by inducing expression of microRNA-155 (miR-155). Stimulation of mouse NK cells with IL-12 and IL-18 promotes STAT4 binding to a conserved noncoding sequence upstream of the miR-155 transcriptional start site and drives high miR-155 expression. Ly49H⁺ NK cells from *miR-155*^{-/-} mice exhibit defective expansion and maintenance relative to wild-type counterparts in mixed bone marrow chimeras during MCMV infection. Mechanistically, miR-155 was shown to regulate NK cell memory responses by targeting mRNA transcripts encoding the pro-apoptotic protein Noxa and suppressor of cytokine signaling 1 (SOCS1) (Zawislak et al. 2013). Whether miR-155 also targets Noxa and SOCS1 to promote the expansion and maintenance of human adaptive NK cells requires further study. miR-155 is also induced by IL-12 and IL-18 stimulation in human NK cells (Trotta et al. 2012), and, relative to canonical NK cells, the *MIR155* locus is hypomethylated in adaptive NK cells (Schlums et al. 2015). However, in contrast to mouse studies (Zawislak et al. 2013), miR-155 was shown to enhance IFN- γ

production in human NK cells by targeting SH2-containing inositol phosphatase 1 (SHIP1) (Trotta et al. 2012).

Apart from the induction of miR-155, inflammatory cytokine signaling promotes virus-specific NK cell memory responses in mice through induction of the BTB-ZF transcription factor *Zbtb32*. In an adoptive transfer model, Ly49H⁺ NK cells from wild-type, but not *Zbtb32*^{-/-} mice, protected mice from lethal MCMV infection. While *Zbtb32* was shown to be dispensable for NK cell development, function, and homeostasis, it promoted the proliferative burst of Ly49H⁺ cells in response to MCMV infection by antagonizing B lymphocyte-induced maturation protein 1 (Blimp1) (Beaulieu et al. 2014). ZBTB32 likely plays a similar role in promoting the expansion or survival of human adaptive NK cells, as the promoter of *ZBTB32* is dramatically hypomethylated in adaptive NK cells relative to canonical NK cells (Schlums et al. 2015). Given the striking differential expression pattern of transcripts encoding an array of transcription factors belonging to the BTB-ZF family in adaptive NK cells (Schlums et al. 2015), it is likely that BTB-ZF proteins control many aspects of adaptive NK cell biology.

Similar to CMV-induced adaptive NK cells, tissue-resident NK cells exhibit functional specialization. Supporting a role for uterine NK cells in promoting trophoblast invasion and vascular growth, these cells produce significant levels of chemokines and angiogenic factors (Hanna et al. 2006). As mentioned above, uterine NK cells display hallmarks of TGF- β imprinting, and both TGF- β and hypoxia can skew conventional NK cells toward a uterine NK cell phenotype (Cerdeira et al. 2013; Keskin et al. 2007), including expression of CD49a. TGF- β signals through both SMAD-dependent (i.e., canonical) and SMAD-independent (i.e., non-canonical) signaling pathways in a context-dependent fashion (Shi and Massague 2003; Zhang 2009). Determining which TGF- β signaling components contribute to tissue-resident NK cell specialization and how this occurs at a molecular level are exciting topics for further research.

6 Relevance of NK Cell Diversification to Human Health and Disease

Given the different roles NK cells play in a variety of physiological contexts, it is not surprising that paucity, dysfunction, or aberrant function of NK cells are associated with a variety of severe pathological conditions. Primary NK cell deficiency is remarkably rare, possibly highlighting important molecular redundancy as well as cellular plasticity underlying the pathways for NK cell development (Cichocki et al. 2014b). However, recent studies are uncovering major inter-individual variation in the frequency and distribution of different NK cell subsets with distinct functional attributes. Hence, distinct NK cell subsets may be uniquely involved in various immune reactions and related disease susceptibilities.

The rapid evolution of germ line-encoded receptors in response to various viral immune evasion strategies as well as susceptibility to severe herpesvirus infections

observed in rare individuals with NK cell deficiency underscores the role of NK cells in immunity to a variety of viral infections (Jost and Altfeld 2013; Lanier 2008a; Orange 2013). High frequencies of NKG2C-expressing NK cell subsets are strongly associated with latent CMV infection. Remarkably, elevated titers of anti-CMV IgG antibodies were found in individuals homozygous for *KLRC2* deletions, i.e., lacking NKG2C (Goodier et al. 2014). Thus, mounting evidence suggests that the expansion of NK cell and T cell subsets expressing NKG2C is directly involved in controlling CMV infection.

Tissue-resident NK cells can also exhibit high expression frequencies of rapidly evolving activating receptors. Interactions between maternal activating KIR and respective fetal MHC class I ligands are associated with reduced risk of pregnancy disorders as well as increased fetal growth and birth weight (Hiby et al. 2010, 2014). Asoprisnil, a pharmacological progesterone receptor modulator with both agonistic and antagonistic activity, suppresses endometrial bleeding and diminishes uterine NK cell numbers (Wilkens et al. 2013). Thus, uterine NK cells regulate the homeostasis of the cycling endometrium as well as placentation. While tissue-resident NK cells contribute to tissue homeostasis in healthy individuals, a loss of balance due to excessive pro-inflammatory cytokines or insufficient anti-inflammatory cytokines such as TGF- β and IL-10 can promote pathology and disease (Dunn et al. 2007; Murphy et al. 2005). An interesting topic for future research is how the cytokine milieu may modulate potentially pathogenic NK cell responses in tissue.

Immunoregulatory function is a hallmark of canonical NK cells that, in mice, can result in weaker germinal center responses and diminished immune memory (Rydzynski et al. 2015). In contrast, adaptive NK cells have a remarkably diminished immunoregulatory killing capacity (Schlums et al. 2015). With the differing frequencies of immunoregulatory versus adaptive CD56^{dim} NK cells among individuals, it is tempting to speculate on the possible implications for immunity and immune pathology. In European and North American populations where CMV seropositivity is approximately 70, 30 % of healthy adults display sizeable subsets of adaptive NK cells in peripheral blood. In 5–10 % of individuals from European populations, adaptive cells are the predominating peripheral blood CD56^{dim} NK cell population. Animal models suggest the differentiation of adaptive NK cell subsets is driven by inflammation. The frequency of individuals with predominating adaptive CD56^{dim} NK cell populations may therefore be higher in other populations with higher infectious burdens. Speculatively, high frequencies of adaptive CD56^{dim} NK cell may predispose to stronger immune responses if adaptive T and B cell responses are less restrained. Supporting this notion, CMV seropositivity is associated with better antibody responses to influenza vaccination in young individuals (Furman et al. 2015). Conversely, a reduced capacity to kill activated immune cells may also predispose to immune pathology, either in the form of hyperinflammatory syndromes upon acute infection or in the longer term development of autoimmunity. Thus, it will be interesting to determine whether high frequencies of adaptive NK cells are associated with inflammatory or autoimmune diseases.

NK cells that reconstitute during hematopoietic cell transplantation (HCT) can mediate graft-versus-leukemia effects. Several groups have reported an association between CMV reactivation early post-transplant and lower rates of relapse, particularly in individuals with acute myeloid leukemia (AML) (Elmaagacli et al. 2011; Green et al. 2013; Ito et al. 2013). One possible explanation for these findings is that cross-reactive T cells expanding in response to CMV are responsible for this phenomenon. However, retrospective analyses of transplant recipients that received adoptive transfer of CMV-specific T cells showed no benefit of this therapy on cumulative relapse rates (Thomson et al. 2012). A second possibility is that subsets of adaptive NK cells that expand in response to CMV reactivation mediate the protective effect. We have shown that heterogeneous subsets of adaptive NK cells expand in response to CMV reactivation in transplant recipients, and these populations are maintained at stable frequencies for at least one year post-transplant (Foley et al. 2012; Schlums et al. 2015). It is possible that adaptive NK cells with enhanced survival and functional specialization toward immunosurveillance promote relapse protection after HCT either directly through enhanced cytotoxicity and inflammatory cytokine production or indirectly through less killing of activated, autologous immune cells. Exploring this hypothesis is tempting.

7 Concluding Remarks

Our knowledge with respect to the diversification and functional specialization among human NK cell subsets is increasing rapidly. Several of these processes resemble what has been termed “trained” immunity in other immune cell subsets (Netea et al. 2015). Much work still remains to understand how past pathogen encounters shape NK cell subsets and impact their functional capacities, metabolism, and longevity. With respect to adaptive NK cell differentiation, epigenetic diversification implies a number of potential branching points (Schlums et al. 2015). The finer differences among adaptive NK cell subsets that are potentially specialized for recall responses to distinct pathogens remain to be dissected. Major questions also pertain to mechanisms that underlie antigen-specific NK cell memory (Reeves et al. 2015). Whether this phenomena represent a new form of true innate memory, potentially derived through somatic hypermutation, remains to be resolved.

Moreover, the developmental relationships between ILCs in general, and different NK cell subsets in particular, is an interesting topic. The anatomical sites of NK cell differentiation are unclear. To what extent tissue-resident NK cells traffic into tissues versus developing in situ is not definitively known. Thus, major questions remain with respect to the ontogeny of different NK cell subsets, their branching points, and plasticity. Genetic insights from human immunodeficiency patients promise to shed light on some of these issues. With an increasing realization that a number of infections can dramatically shape the NK cell repertoire,

the consequences of epigenetic differences among individuals are of high interest. Such inter-individual variability can be utilized to understand better how different NK cell subsets contribute to physiological and pathological processes.

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