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Review

Human natural killer cell development in secondary lymphoid tissues

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ABSTRACT

For nearly a decade it has been appreciated that critical steps in human natural killer (NK) cell development likely occur outside of the bone marrow and potentially necessitate distinct microenvironments within extramedullary tissues. The latter include the liver and gravid uterus as well as secondary lymphoid tissues such as tonsils and lymph nodes. For as yet unknown reasons these tissues are naturally enriched with NK cell developmental intermediates (NKDI) that span a maturation continuum starting from an oligopotent $CD34^+CD45RA^+$ hematopoietic precursor cell to a cytolytic mature NK cell. Indeed despite the detection of NKDI within the aforementioned tissues, relatively little is known about how, why, and when these tissues may be most suited to support NK cell maturation and how this process fits in with other components of the human immune system. With the discovery of other innate lymphoid subsets whose immunophenotypes overlap with those of NKDI, there is also need to revisit and potentially re-characterize the basic immunophenotypes of the stages of the human NK cell developmental pathway *in vivo*. In this review, we provide an overview of human NK cell development in secondary lymphoid tissues and discuss the many questions that remain to be answered in this exciting field.

1. Introduction

The natural killer (NK) cell is the prototypic innate lymphoid cell (ILC) whose functional capabilities have evolved to provide direct antimicrobial and antitumor protection as well as indirect immunomodulaton through the production of soluble chemokines and cytokines. NK cells are important if not necessary in a variety of clinical settings, and gaining a comprehensive understanding of how these cells are derived in humans can facilitate the development of targeted therapies to boost NK cell effector function for patients with cancer and/or immune deficiency, can allow us to better evaluate the downstream effects of therapeutic interventions, and can also provide insights into how NK cells undergo malignant transformation.

Though it was discovered more than twenty years ago that human NK cells can be derived *in vitro* from purified $CD34^+$ hematopoietic precursor cells (HPC), the *in vivo* NK cell developmental pathway has remained somewhat of a mystery in relative comparison to the pathways for B cell and T cell development [1]. For decades it was generally accepted that NK cells develop exclusively within the bone marrow (BM) similar to most other leukocyte populations other than T cells [2,3]. BM ablation results in NK cell deficiency in mouse models, and human NK cells may be derived *in vitro* from BM-derived $CD34^+$ HPC on BM-derived stroma or in BM stroma-derived cytokines [4–9]. Human BM has also recently been shown to contain $CD34^-$ NK cell developmental intermediates (NKDI) [10]. Interestingly, NK cell precursors are normally detected in the circulation, and recent data indicate that specific $CD34^+$ NK cell precursors are selectively enriched in extramedullary tissues where unique subsets of mature NK cells reside, suggesting that the latter may derive locally *in situ* [11]. As the body has evolved to generate millions of NK cells that are self-tolerant yet capable of substantial tissue destruction and immunomodulation, the notion that NK cells may develop in multiple microenvironments carries important and interesting implications regarding how this process may be regulated *in vivo*.

Within the last ten years, numerous studies from multiple groups have demonstrated that human NKDI are naturally and selectively enriched in extramedullary tissues including the thymus, peripheral lymph nodes, gastrointestinal track, tonsils, gravid uterus, and liver. Interestingly, these NKDI are not only enriched in

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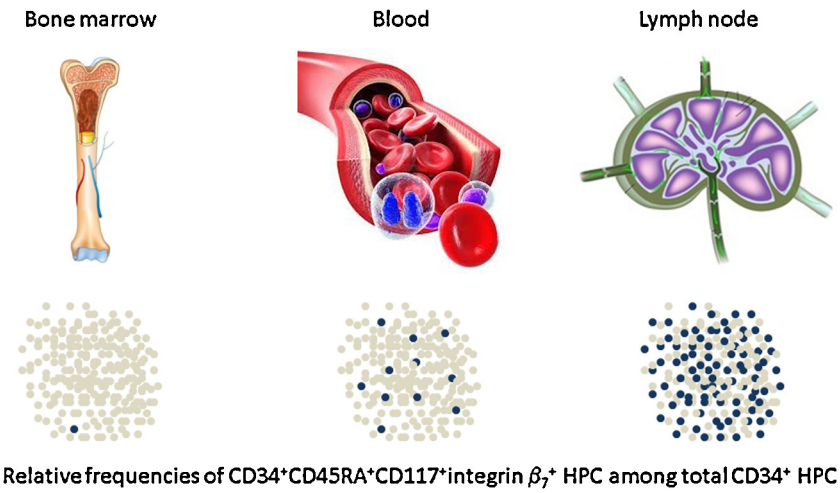

Fig. 1. Human IL-15 responsive $CD34^+$ NK cell precursors (stage 2 cells) are naturally relatively enriched in secondary lymphoid tissues. Schematic representation of the relative frequencies of human stage 2 NK cell precursors ($CD34^+CD45RA^+CD117^+$integrin β_7^+) among total $CD34^+$ HPC in bone marrow, blood, and lymph node. Stage 2 NK cell precursors are extremely rare in the body (<0.1% of total mononuclear cells in all hematolymphoid tissues). However, it is notable that among total $CD34^+$ HPC, NK cell precursors represent <1% and <10% in the bone marrow and blood, respectively, whereas in secondary lymphoid tissues, such as lymph nodes (as depicted in the figure), stage 2 $CD34^+$ NK cell precursors represent close to half of all $CD34^+$ HPC, of which >95% are $CD34^+CD45RA^+$ [11]. Bone marrow, blood, and lymph node illustrations with licensure for publication were purchased from iStockphoto and Fotosearch.

the aforementioned tissues, they may also provide some immune function *en route* to their full development into cytolytic NK cells. Here we provide an overview of NKDI and their development in human secondary lymphoid tissues (SLT).

2. Human natural killer cell developmental intermediates (NKDI) in SLT

To date there are no known surface antigens that are entirely specific to the human NK cell lineage. By convention, NK cells are minimally defined as lineage $(Lin)^-CD56^+$ lymphocytes, with "lineage" markers often including CD3 (for T cells), CD19 or CD20 (for B cells), and CD14 (for monocytes). Other pan-NK cell markers, such as NKp46, are typically selectively expressed on normal blood NK cells, but in humans even this marker is rarely expressed on some reactive or neoplastic T cells as well as non-NK ILCs in SLT [12–16]. In human peripheral blood, most NK cells show dim or moderate CD56 expression as well as bright coexpression of CD16 [17]. These $CD56^{dim}$ NK cells are thought to represent the most mature NK cell population in humans and are capable of robust natural cytotoxicity and target-induced cytokine production [18,19]. In contrast, a small population of NK cells in the blood shows bright CD56 expression and low or undetectable coexpression of CD16. These $CD56^{bright}$ NK cells show relatively higher capacity for *ex vivo* proliferation and monokine-induced cytokine stimulation yet relatively lower capacity for natural cytotoxicity in comparison to the $CD56^{dim}$ NK subset [20]. A recent study also shows that peripheral $CD56^{dim}$ NK cells produce low levels of IFN-γ earlier than $CD56^{bright}$ NK cells [21]. Whereas $CD56^{bright}$ NK cells normally only constitute up to 10% of the circulating NK cell pool in healthy individuals, they are naturally enriched and comprise the major mature NK cell population in SLT where they likely provide immunomodulatory cytokines in the setting of infection [22]. Interestingly, multiple lines of evidence suggest that $CD56^{bright}$ NK cells not only represent a functionally distinct lymphocyte population, they are also the direct precursors to $CD56^{dim}$ NK cells: (1) the $CD56^{bright}$ NK subset is the major NK cell population that is derived early *in vitro* when $CD34^+$ HPC are cultured in NK development supportive conditions, whereas $CD56^{dim}$ NK cells develop later over time; (2) likewise, $CD56^{bright}$ NK cells appear to accumulate earlier in the blood following bone marrow or stem cell transplantation, whereas $CD56^{dim}$ NK cells accumulate at later time points; (3) $CD56^{bright}$ NK cells are relatively more abundant in fetal and post-natal blood in contrast to adult peripheral blood; (4) $CD56^{bright}$ NK cells have longer telomeres compared to $CD56^{dim}$ NK cells, suggesting that $CD56^{dim}$ NK cells have undergone more rounds of proliferation; (5) NK cells with immunophenotypic and functional features intermediate between those of the $CD56^{bright}$ and $CD56^{dim}$ subsets have been described in adult human blood; and (6) purified $CD56^{bright}$ NK cells can develop into $CD56^{dim}$ NK cells in *in vitro* and *in vivo* experimental systems [23–29]. These data provide substantial evidence for a linear relationship between the two major blood NK cell subsets; however, the possibility that $CD56^{bright}$ and $CD56^{dim}$ NK cells represent distinct terminal maturation pathways in the NK cell lineage (akin to $CD4^+$ and $CD8^+$ T cells) has not been formally disproven. Moreover, it should be noted that purified $CD56^{dim}$ NK cells that are activated *in vitro* can adopt a $CD56^{bright}$-like immunophenotype with loss of CD16 and upregulation of CD56 suggesting that at least some $CD56^{bright}$ cells may be activated NK cells [30,31]. This transition of $CD56^{dim}$ phenotype to $CD56^{bright}$ phenotype may also occur *in vivo* [32]. As discussed below, *ex vivo* analyses of NKDI in SLT directly link the $CD56^{bright}$ NK cell population to less mature developmental populations, whereas there is as yet no known direct link between these populations and the $CD56^{dim}$ NK cell subset. Therefore, the current prevailing view is that $CD56^{bright}$ NK cells are the direct precursors to $CD56^{dim}$ NK cells in a linear maturation model.

As mentioned above, *in vitro*-derived NK cells are predominantly $CD56^{bright}$, at least during the first few weeks of cultures and especially in the absence of stromal feeder cells [11,33–35]. Although multiple growth factors likely facilitate the development of NK cells, interleukin-15 (IL-15), which is produced by mesenchymal stromal cells as well as hematopoietic cells such as dendritic cells, is considered to be the most important NK cell homeostatic cytokine known to date. IL-15 promotes the proliferation and survival of mature NK cells and is also capable of inducing the differentiation and maturation of $CD34^+$ HPC into $CD56^{bright}$ NK cells in stroma-free medium [36–38]. IL-15 is typically provided in soluble form *in vitro*, yet *in vivo* it is presented *in trans* as a membrane bound ligand in association with the IL-15 receptor alpha chain (IL-15Rα) [39,40]. In order to respond to IL-15, recipient cells must express the common gamma chain (CD132) as well as the IL-2Rβ (CD122) [41,42]. As such, human NK cell precursors have been