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Review

Tissue-resident natural killer cells and their potential diversity

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ABSTRACT

Conventional NK cells are well characterized in the mouse spleen and circulate in the blood. Less well described are NK cells found in organs such as the liver, thymus, and uterus. Recently we identified a tissue-resident NK (trNK) cell population in the liver, suggesting a potential diversity of trNK cells in other organs. In this review we compare and contrast the similarities and differences among the subpopulations of NK and innate lymphoid cells to the trNK cells in the liver.

1. Introduction

Several immune cell lineages migrate throughout the body *via* the circulatory system in search of detrimental insults provoked by pathogenic events, such as invading microorganisms or developing tumors. Once detected, the circulating immune cells stop and respond in secondary lymphoid organs such as the lymph nodes and spleen. What follows subsequently is an orchestrated host immune response, which controls the pathological process by recruiting relevant immune cells to the damaged tissue. In contrast to the well-studied circulating immune cells are tissue-resident immune cells, which already reside in selected organs where they appear to be armed and ready to rapidly respond. However, less is known about the properties of tissue-resident immune cells that seem to be closely related to their counterparts which re-circulate.

Conventional natural killer (cNK) cells are constituents of the innate arm of the immune system [1]. First described on the basis of their inherent capacity to directly kill tumor cells without prior sensitization, NK cells are now known to participate in a wide variety of immune responses, such as viral infections, stem cell transplantation, and pregnancy. In addition, they can respond to pro-inflammatory cytokines by producing interferon-γ (IFN-γ), their signature cytokine, which can impact adaptive immunity. Although classically studied in the mouse spleen, NK cells are also found in organs, such as the thymus and liver [1]. In the thymus, NK cells have been described which are phenotypically different from cNK cells [2]. In the liver, we recently showed that there are two populations of NK cells, one that resembles splenic cNK cells and that recirculates and another that is tissue-resident [3].

In this review we will discuss the developmental, phenotypic, and functional relationships between the splenic cNK, thymic NK cells, and tissue-resident NK (trNK) cells in the liver. We will highlight features of cNK cells that are relevant to understanding the other NK cell subpopulations and we will also describe NK cells found in other organs, such as the uterus, which may include trNK cells. Finally, we will discuss how these NK cells relate not only to one another but to the larger family of innate lymphoid cells (ILCs) [4,5].

2. Developmental requirements of cNK cells

The bone marrow (BM) is the site of splenic cNK development and maturation. In the BM, the developmental stages are characterized by acquisition and loss of cytokine receptors, NK cell receptors, and integrins [6–8]. One of the late maturation markers, DX5 (α2 integrin), is expressed prior to exit out of the BM and is one of the markers of mature splenic cNK cells. Out in the periphery, mature splenic cNK cells can be further distinguished by a loss of CD27 expression [6,9]. Thus, the maturation status of splenic cNK cells is closely related to the expression of defined developmental markers.

The family of cytokines, which uses the common receptor gamma chain (γc), a component of receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21, has been classically defined as growth and survival factors for many immune cells spanning many cell lineages [10]. More specifically for NK cells, splenic cNK cells require IL-15 and its cognate receptor, IL-15R, for development [11–15]. In mice deficient in IL-15 or any chain of the trimeric IL-15R (α, β, γ) chains, splenic cNK cells are absent. While the exact stage of developmental arrest has not been clearly characterized, it is likely that immature NK cells at a very early stage of lineage

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commitment are affected because IL-2/15Rβ (CD122) is expressed even before other markers associated with NK cells in the BM. Interestingly, cNK cells can develop from precursors lacking expression of IL-15Rα, indicating that trans-presentation of IL-15 from a non-NK cell is sufficient for cNK cell development [16,17]. Thus, IL-15 and its receptor are critical for cNK cell development.

The development of cNK cells requires certain transcription factors [18], in particular NFIL3 (nuclear factor, IL-3 regulated; also known as E4BP4), to date described as the NK cell-specification factor [19]. Mice deficient in NFIL3 have essentially no splenic cNK cells though other organs were not thoroughly examined [20–22]. The transcription factor Id2 (inhibitor of DNA binding 2) also is essential for the development and maturation of splenic cNK cells [23]. More specifically, Id2-deficient mice have a defect in mature splenic cNK cells while a normal immature cNK population is maintained in the BM, emphasizing that Id2 plays a later role in cNK cell differentiation [24]. Id2 in turn is regulated by the E protein, E2A. Tbet (Tbx21) and eomesodermin (Eomes), related t-box transcription factors, play more intricate roles in NK cell development [25,26]. In the absence of Tbet, splenic NK cells display an immature phenotype, and a subpopulation of NK cells in the liver is absent, consistent with redundant and cooperative roles of Tbet and Eomes in NK cell development. Importantly, the Tbet and Eomes studies suggest that NK cell subsets in different tissues may be distinguishable from cNK cells based on the differential transcription factor requirements for their development in the various anatomical locations.

3. Thymic NK cells

Among the lymphoid tissues, the thymus has NK cells with surface marker phenotypes resembling immature cNK cells [2]. Specifically, as compared to splenic cNK cells that are Ly49^{hi} CD11b^{hi}, thymic NK cells are Ly49^{low} CD11b^{low}, like immature BM cNK cells. However, thymic NK cells are CD127^{+} CD69^{high} unlike resting splenic cNK cells and developing cNK cells in the BM. While the thymic NK cells have not been rigorously tested for selective tissue localization, their relatively unique phenotype as compared to cNK cells suggests they may be trNK cells in the thymus. On the other hand, cells with a thymic NK cell phenotype are enriched in LNs and are absent in GATA3-deficient mice while Id2 was dispensable [2]. Moreover, peripheral thymic NK cells require a thymus for development and can develop *in vivo* and *in vitro* from double negative (CD4^{-}CD8^{-}) 1 (DN1) subset of immature thymocytes [27], indicating that they do not develop directly in the BM, unlike cNK cells. Moreover, unlike immature cNK cells, thymic NK cells have cytotoxic capability and the ability to produce IFN-γ [2]. Thus, the thymus is both a site for development of thymic NK cell maturation and may be a "home" for thymic NK cells.

4. Tissue-resident NK (trNK) cells

In the liver, a population of NK cells appeared to be similar to immature cNK cells because they express similar surface markers such as NK1.1 and NKp46 and low levels of CD11b [3,6,8]. Moreover, they are DX5^{-}and display high levels of TNF-related apoptosis-inducing ligand (TRAIL). This immature phenotype gave the impression that organs such as the liver contained a subpopulation of NK cells that was not fully differentiated. Our recent studies, however, indicate that these liver NK cells appeared to be distinct from immature BM cNK cells [3].

Detailed phenotypic analysis revealed that DX5 and CD49a are mutually exclusively expressed on liver NK cells, *i.e.*, there are CD49a^{+} DX5^{-} and CD49a^{-}DX5^{+} NK cells in the liver [3]. There were no NK cells which were double-positive or double-negative for CD49a and DX5. Adoptive transfer of DX5^{-} liver NK cells into

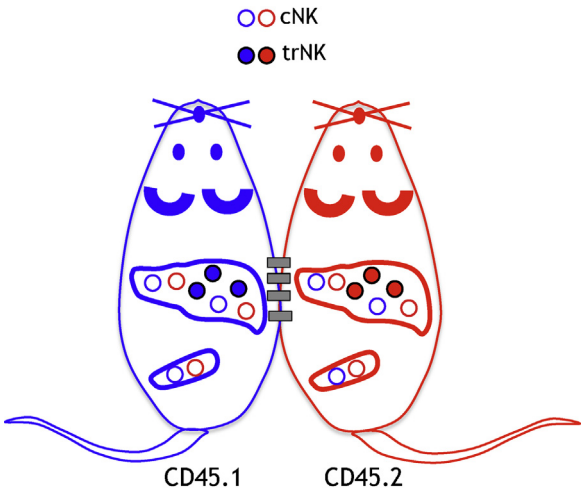

Fig. 1. trNK cells in the liver do not migrate. Congenically marked animals were surgically parabiosed and on day 14 post-surgery the livers and spleens were analyzed for cNK cells and trNK cells, as schematically shown.

congenically distinct hosts indicated selective migration of the cells to the liver. However, DX5^{+} liver NK cells could be found in both the liver and spleen after adoptive transfer. Based on other markers, CD49a^{+} DX5^{-} were unlike splenic cNK cells whereas CD49a^{-}DX5^{+} were very similar to splenic cNK cells. In parabiotic mice, the host liver contained CD49a^{+} DX5^{-} NK cells primarily of host origin as well as CD49a^{-}DX5^{+} NK cells derived from both the host and other parabiont (Fig. 1). Thus, the CD49a^{+} DX5^{-} NK cells in the liver are non-circulating trNK cells whereas the CD49a^{-}DX5^{+} NK cells are circulating cNK cells.

The liver receives blood from two afferent vessels, the hepatic artery which supplies oxygenated blood and the portal vein which supplies nutrient-rich blood from the intestines and spleen [28]. The blood mixes in the sinusoidal space, a low pressure, highly fenestrated vascular system. Immune cells such as NKT cells patrol sinusoidal endothelial cells by crawling in the sinusoidal space [29]. Selective sampling of the thoracic aorta (which ultimately feeds the hepatic artery), portal vein, and vena cava (which receives blood from the hepatic vein) showed only CD49a^{-}DX5^{+} NK cells resembling cNK cells in the spleen [3]. However, ligation of the vasculature of the liver before excision, then flushing of the excised liver produced both CD49a^{+} DX5^{-} and CD49a^{-} DX5^{+} NK cells, similar to single cell suspensions from homogenized livers. Thus, the liver trNK cells selectively reside in the sinusoids.

The localization of liver trNK cells to the sinusoids highlights the difficulty in identifying NK cells in liver tissue sections. Examination of the liver parenchyma revealed essentially no NK cells though the tissue sections did not preserve sinusoidal blood [30]. Moreover, these findings are reminiscent of early electron microscopy studies of the liver sinusoids. The rat liver was found to contain "pit cells," now known to be NK cells, in the sinusoidal space [31,32]. These studies provide micro-anatomical relationships to sinusoidal cells. Usually adjacent to sinusoidal endothelial cells, pit cells were often found next to Kupffer cells, the macrophage of the liver. Although liver trNK cells have not been formally examined as pit cells, it seems likely that they are related, if not equivalent.

The discovery of trNK cells in the liver suggests that other organs may also contain subpopulations of tissue-resident cells as well as circulating cNK cells resembling those in the spleen. For example, NK cells are normally present in the non-pregnant uterus [33–35] but have been mostly studied after they expand at the site of embryo implantation during pregnancy [36,37]. Some uterine NK (uNK) cells are phenotypically different from splenic cNK cells in that they lack the expression of the maturation marker DX5 [34].