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Subsets of human natural killer cells and their regulatory effects

Binqing Fu,[1,2] Zhigang Tian[1,2] and Haiming Wei[1,2]

[1]*Institute of Immunology, School of Life Sciences, University of Science and Technology of China, Hefei, and* [2]*Hefei National Laboratory for Physical Sciences at Microscale, Hefei, China*

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Correspondence: Haiming Wei and Zhigang Tian, Institute of Immunology, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China.
Emails: ustcwhm@ustc.edu.cn; tzg@ustc.edu.cn
Senior author: Haiming Wei

Summary

Human natural killer (NK) cells have distinct functions as $NK^{tolerant}$, $NK^{cytotoxic}$ and $NK^{regulatory}$ cells and can be divided into different subsets based on the relative expression of the surface markers CD27 and CD11b. $CD27^+$ NK cells, which are abundant cytokine producers, are numerically in the minority in human peripheral blood but constitute the large population of NK cells in cord blood, spleen, tonsil and decidua tissues. Recent data suggest that these NK cells may have immunoregulatory properties under certain conditions. In this review, we will focus on these new NK cell subsets and discuss how regulatory NK cells may serve as rheostats or sentinels in controlling inflammation and maintaining immune homeostasis in various organs.

Keywords: cell differentiation; human natural killer cells.

Introduction

For a long time, natural killer (NK) cells were regarded only as killers but now they are thought not only to have key roles in innate immunity but also to have important functions that shape and influence adaptive immune responses and play immunoregulatory roles. However, NK cells are not a homogeneous cell population and the diversity of NK cells has been demonstrated by the diversity of NK cell receptors and functions. In human peripheral blood, the $CD56^+$ $CD3^-$ NK cell subpopulations can be defined on the basis of the relative expression of the markers CD16 and CD56. $CD56^{dim}$ $CD16^+$ NK cells are found predominantly in the peripheral blood and can spontaneously lyse targeted tumour cells, yet $CD56^{bright}$ $CD16^-$ NK cells are found mostly in the lymphoid organs and can produce abundant amounts of cytokines but have little ability to kill tumour cell targets.[1–3] Recent studies have also reported that CD27 of the tumour necrosis factor receptor family is an important marker for distinguishing between NK cell subsets.[4,5] The surface density of CD27 and CD11b divides both human and murine NK cells into four subsets and denotes their level of maturation.[6,7]

The local microenvironment and unique cellular interactions provide important signals to shape the properties of NK cells. In the microenvironment of a pathological process, NK cells persistently and progressively access local inflammatory factors to induce programmed differentiation and proliferation, ultimately generating $NK^{tolerant}$, $NK^{cytotoxic}$ and $NK^{regulatory}$ cells. Moreover, recent research highlights the fact that natural killer cells act not only as killers towards tumour or virus-infected cells, but also as regulatory cells to affect the adaptive immune response.[8] Here, we review the recent advances mainly concerning human regulatory NK cells and present some data obtained in our laboratory. We will focus on the new NK cell subsets and discuss how regulatory NK cells may be involved in controlling inflammation and maintaining immune homeostasis in different organs.

Human NK subsets divided in phenotype and function

In 1983, Lewis Lanier was the first to divide NK cells into subsets.[9] Now, it is widely accepted that human mature NK cells have two subsets: $CD56^{dim}$ NK and $CD56^{bright}$ NK.[1,10] However, mouse NK cells do not express the CD56 antigen; hence, translating the biological information in mouse NK cells to human NK cells is problematic. Meanwhile, the development of mouse NK cells has been widely studied using the precursors. The integrin CD11b

(Mac-1) has been regarded as a mature marker of both mouse and human NK cells.[11,12] CD27 has been indicated as a marker to divide mature NK cells into two subsets.[4] NK cells from CD27-deficient mice show normal NK cell differentiation but impaired function upon stimulation.[13] Subsequently, the heterogeneity of mature murine NK cells was ultimately represented by four subsets on the basis of CD27 and CD11b.[7] These new NK subsets have quickly attracted much attention because human NK cells have also been shown to express CD27, making comparative interpretations of the functionality of the subsets more straightforward.[4,5] In the mouse, NK cells can be divided into $CD27^{lo}$ $CD11b^{lo}$, $CD27^{hi}$ $CD11b^{lo}$, $CD27^{hi}$ $CD11b^{hi}$ and $CD27^{lo}$ $CD11b^{hi}$ stages. The differentiation of NK cells has been shown to proceed from $CD27^{hi}$ $CD11b^{lo}$ through $CD27^{hi}$ $CD11b^{hi}$ to $CD27^{lo}$ $CD11b^{hi}$.[7] In humans, it has been indicated that approximately 6% of peripheral blood NK cells express CD27, 14% of $CD27^+$ NK cells exist in bone marrow, and > 30% of $CD27^+$ NK cells exist in the spleen and tonsils.[5] Our group has characterized four novel populations defined by CD11b and CD27, which can represent the distinct stages of human NK cells from different tissues. More than 90% of NK cells from peripheral blood are of the $CD11b^+$ $CD27^-$ population, whereas NK cells from cord blood have populations that are 80% $CD11b^+$ $CD27^-$ and 20% $CD11b^+$ $CD27^+$. Compared with these two types of NK cells, decidual NK cells are more immature, having nearly 60% $CD11b^-$ $CD27^-$ NK cells and > 20% $CD27^+$ NK cells. The NK cells from tumour-infiltrating tissues also showed large populations of the $CD11b^-$ $CD27^-$ subset,[14] indicating the heterogeneity of NK cells (Fig. 1). Each population could be characterized by unique functional and phenotypic attributes: $CD11b^-$ $CD27^+$ and $CD11b^+$ $CD27^+$ NK cells show the best ability to secrete cytokines, $CD11b^+$ $CD27^-$ NK cells exhibit high cytolytic function, and $CD11b^-$ $CD27^-$ NK cells display an immature phenotype, expressing high percentages of NKG2A.[15]

Affected by various microenvironments and signals, NK cells can be divided into three functional subsets: $NK^{tolerant}$ (NK cells with dominant inhibitory signals), $NK^{cytotoxic}$ (NK cells with dominant activating signals, target cells with a high expression of pressure stimulus-induced ligand) and $NK^{regulatory}$ (NK cells with dominant activating signals, target cells with a high expression of inflammatory molecules) (Fig. 2). From the phenotype, the $NK^{cytotoxic}$ subset is mainly $CD56^{dim}$ NK cells or $CD11b^+$ $CD27^-$ NK cells defined on the basis of the relative expression of the markers CD11b and CD27. The $NK^{tolerant}$ subset is mainly $CD56^{bright}$ NK cells or $CD27^-$ $CD11b^-$ NK cells. The $NK^{regulatory}$ subset is mainly $CD56^{bright}$ NK cells or $CD27^+$ NK cells. Furthermore, these different NK subsets exist in a variety of tissues or organs, reflecting their functional diversity.[16] For example, liver NK cells can mediate immune tolerance or immune injury,[17–19] decidual NK cells can mediate maternal–fetal immune regulation or vascular remodelling,[20] and tumour-infiltrating NK (TINK) cells can mediate tumour immune escape or direct killing.[21]

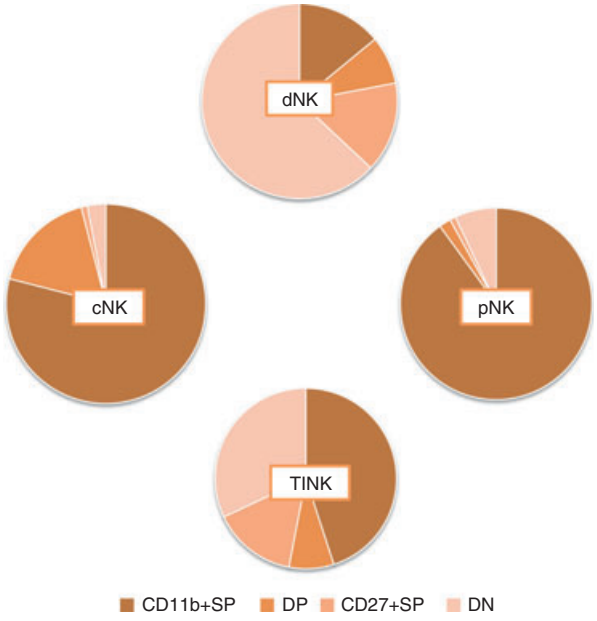

Figure 1. Four natural killer (NK) subsets defined by CD11b and CD27 in humans. Human NK cells can be divided into four subsets on the basis of the relative expressions of the markers CD11b and CD27, including $CD11b^+$ $CD27^-$ ($CD11b^+$ SP), $CD11b^+$ $CD27^+$ (DP), $CD11b^-$ $CD27^+$ ($CD27^+$ SP) and $CD11b^-$ $CD27^-$ (DN). More than 90% of NK cells from peripheral blood (pNK) are of the $CD11b^+$ $CD27^-$ population, whereas NK cells from cord blood (cNK) have 80% $CD11b^+$ $CD27^-$ and 20% $CD11b^+$ $CD27^+$ subset. Decidual NK cells (dNK) are nearly 60% $CD11b^-$ $CD27^-$ and > 20% $CD27^+$ subset. NK cells from tumour-infiltrating tissues (TINK) also show a large population of the $CD11b^-CD27^-$ subset.

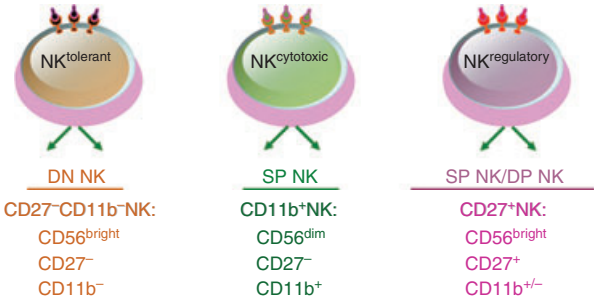

Figure 2. Human natural killer (NK) subsets presented according to phenotype and function. Human NK cells can be divided into three functional subsets: $NK^{tolerant}$, which is mainly $CD56^{bright}$ NK cells or $CD27^-$ $CD11b^-$ NK cells; $NK^{cytotoxic}$, which is mainly $CD56^{dim}$ NK cells or $CD11b^+$ $CD27^-$ NK cells; $NK^{regulatory}$, which is mainly $CD56^{bright}$ NK cells or $CD27^+$ NK cells.

The main checkpoint in the differentiation of NK subsets

The differentiation of NK cells depends on extrinsic regulation within the physiological microenvironment and the pathological microenvironment in addition to intrinsic regulation by various transcription factors.

Under the effect of early haematopoietic growth factors, such as FLT-3 ligand and c-kit ligand, $CD34^+$ haematopoietic stem cells (HSCs) up-regulate the expression of interleukin-2 (IL-2)/IL-15Rβ (CD122) and gradually differentiate into the $CD34^+$ $CD122^+$ $CD56^-$ NK precursor cells.[22] Via the CD122 molecule, these NK precursor cells obtain the ability to respond to IL-15, which is produced mainly by bone marrow stromal cells and plays a key role in the ultimate expression of CD56 to promote the formation of mature $CD3^-$ $CD56^+$ NK cells.[23–25] However, several observations also suggested that bone marrow is not the only important site for NK cell development. One clue is that NK cells can also develop from other secondary lymphoid tissue such as the lymph nodes and tonsils.[26] Most of these haematopoietic precursor cells become $CD56^{bright}$ NK cell subsets when stimulated by IL-15 or IL-2 or activated lymph node T cells.[27,28] In human intestinal mucosa, $CD34^+$ $CD45RA^+$ NK precursor cells expressing CD38, CD33, IL-2Rα and IL-7Rα, with the abundant expression of Id2, PU.1 and SpiB1, may differentiate into $CD56^+$ c-kit^{dim} cells during *in vitro* culture.[29,30] In addition to bone marrow, lymph nodes and the small intestine, NK cells can also develop in the liver, spleen and thymus.[31]

The main checkpoints that lead to the generation of different NK subsets appear to depend on the pathological microenvironment, local-specific chemokines and cytokines, as well as unique cellular interactions. Natural killer cells express a variety of chemokine receptors, which are affected by the local tissue microenvironment. $CD56^{dim}$ $CD16^+$ NK cells at a resting state highly express CXCR1, CXCR2, CXCR3, CXCR4 and CX3CR1, whereas $CD56^{bright}$ $CD16^-$ NK cells highly express CCR5 and CCR7. These receptors interact with their corresponding chemokines and regulate the migration of NK cells to various tissues, thereby playing different biological functions.[32] For example, during pregnancy, human $CD56^{bright}$ $CD16^-$ NK cells in peripheral blood can be recruited by chemokine CXCL12 and migrate to the uterus.[33] In B16 metastatic melanoma, CX3CR1 plays an important role for $DX5^+$ $CD3^-$ cells accumulating in the lung.[34] Moreover, CXCL16, constitutively presented by the liver endothelium, plays an important role in maintaining the $CXCR6^+$ NK subset in the liver.[35]

Cytokines from accessory cells in the microenvironment have been revealed to have an important impact on the maturation and function of NK cells. In patients with systemic lupus erythematosus, interferon-α (IFN-α) produced by plasmacytoid dendritic cells mediate the activation-induced cell death of NK cells.[36] In persistent hepatitis B virus liver infection, transforming growth factor-β_1 (TGF-β_1) exhibits an important role in reducing the expression of NKG2D/DAP10 and 2B4/SAP to impair NK cell function and induce tolerant NK cells.[37] It has been indicated that $CD56^{bright}$ NK cells are present in human lymph nodes and are co-stimulated by $CD4^+$ T-cell-derived IL-2 to secrete IFN-γ.[28] In the tumour microenvironment, regulatory T cells can effectively suppress NK cell-mediated tumour rejection via a TGF-β-dependent mechanism.[38,39] Interfering with such a negative impact, tumour-infiltrating NK cells induce a substantial $CD11b^-$ $CD27^-$ NK cell population that exhibits profound defects in degranulation and IFN-γ production in humans.[14] Moreover, in the pathological microenvironment of cancer, monocytes have been shown to mediate the terminal differentiation of peripheral NK cells and to sustain their transition from the $CD11b^+$ $CD27^+$ to $CD11b^+$ $CD27^-$ stage.[40] Interestingly, another study has further reported that members of the commensal microbiota are necessary for the priming of NK cells by mononuclear phagocytes.[41] Mature neutrophils have recently been shown to be required both in the bone marrow and in the periphery for proper NK cell development, and neutrophil deficiency impairs the maturation of $CD11b^+$ $CD27^+$ NK to $CD11b^+$ $CD27^-$ NK in mice. The role of neutrophils as key regulators of NK cell functions was confirmed in patients with severe congenital neutropenia and autoimmune neutropenia.[42] Hence, the pathological microenvironment including specific cytokines, chemokines and several immune responses shapes NK cells, emphasizing the plasticity and the adaptive nature of these innate immune cells.

The differentiation and maturation of NK cells are accompanied by the intrinsic signals from transcription factors. Recent studies in mice have afforded great progress in our understanding of the transcription factors involved in NK cell development.[3] For example, PU.1, E4pb4, Ikaros and Ets-1 are involved in the generation of NK precursor cells.[43–46] Although Id2 is expressed in pre-pro-NK cells, its activity is required later during NK development.[47] T-bet expression is required for the maintenance and homeostasis of immature NK cells, whereas the induction of Ly49 receptors and DX5 requires cooperation with Eomes.[48] Later, GATA-3 plays an important role in NK cell expression of the mature marker CD11b and IFN-γ production.[49] The final maturation of NK cells involves the reduction of CD27, and the proliferative potential requires Blimp-1.[50] These transcription factors provide important intrinsic signals that impact the differentiation of NK cells and shape the cytotoxicity or immunoregulatory effects of NK cell activation.

In summary, the physiological microenvironment provides conditions for the development and differentiation of NK cells, and the pathological microenvironment induces NK cell activation, programmed proliferation and function polarization, whereas transcription factors mediate intrinsic signals for NK cell maturation and function (Fig. 3). Although several cytokines, such as type I IFN, IL-2, IL-12, IL-15, IL-18 and insulin-like growth factor-1, are potent activators of the NK cell effector function,[51–53] very limited information is available to demonstrate the key threshold required to induce regulatory NK cells. Nevertheless, several cytokines may have impacts on the generation of regulatory NK cells. Transforming growth factor-β has key impact on NK cells and promotes the conversion of $CD16^+$ peripheral blood NK cells into $CD56^{bright}$ NK cells.[54] Evidence has also shown that IL-7 is necessary for promoting the survival of the regulatory $CD56^{bright}$ NK cell subset.[55] The way of inducing regulatory NK cells and the mechanism involved remain to be explored further.

Regulatory NK cells in organs

In the first trimester of pregnancy, nearly 70% of human decidual lymphocytes are NK cells with a $CD56^{bright}$ $CD16^-$ phenotype, making deciduas a typical model to use when researching regulatory NK cell subsets. These accumulated NK cells may migrate from the peripheral blood through a CXCR4- and CXCL12-dependent mechanism[33] or may develop *in situ* from $CD34^+$ haematopoietic precursors[56] or endometrial NK cells.[57] We and others have provided evidence that human decidual NK cells comprise a large population of the $CD27^+$ $CD11b^-$ and $CD27^-$ $CD11b^-$ subset, express the activation markers CD69 and killer cell immunoglobulin-like receptors and are granulated but of low cytotoxicity.[15,58] Decidual NK cells, capable of producing IL-22, have been found to resemble the unique early developmental stages of human NK cell differentiation.[59] Multiple tetraspanin family members, such as CD9 and CD151, have also been found to be exclusively expressed on decidual NK cells but not on peripheral blood NK cells. Two secreted proteins, galectin-1 and progestogen-associated protein 14, which are known to have immunomodulatory functions, are selectively expressed in decidual NK cells.[58] These characteristics make decidual NK cells a unique subset of NK cells with immunomodulatory potential, sharing the properties of, but not identical to, peripheral blood NK cells.

Decidual NK cells exist at the unique maternal–fetal interface, whereby a pregnant mother recognizes her semi-allogeneic fetus, and her immune system has to retain tolerance and not reject the fetus. Recent studies have characterized that decidual NK cells play a key role in this adaptation. Croy and colleagues reported landmark research in which decreased NK cells in mouse deciduas led to the disordered adaption of blood vessels in the uterine mucosa. Decidual NK cell-derived IFN-γ is required for vascular modifications to occur during pregnancy, and it is now evident that NK cell depletion or disruption of the IFN-γ signal in mice results in altered vascular remodelling.[60–62] Human decidual NK cells have also been

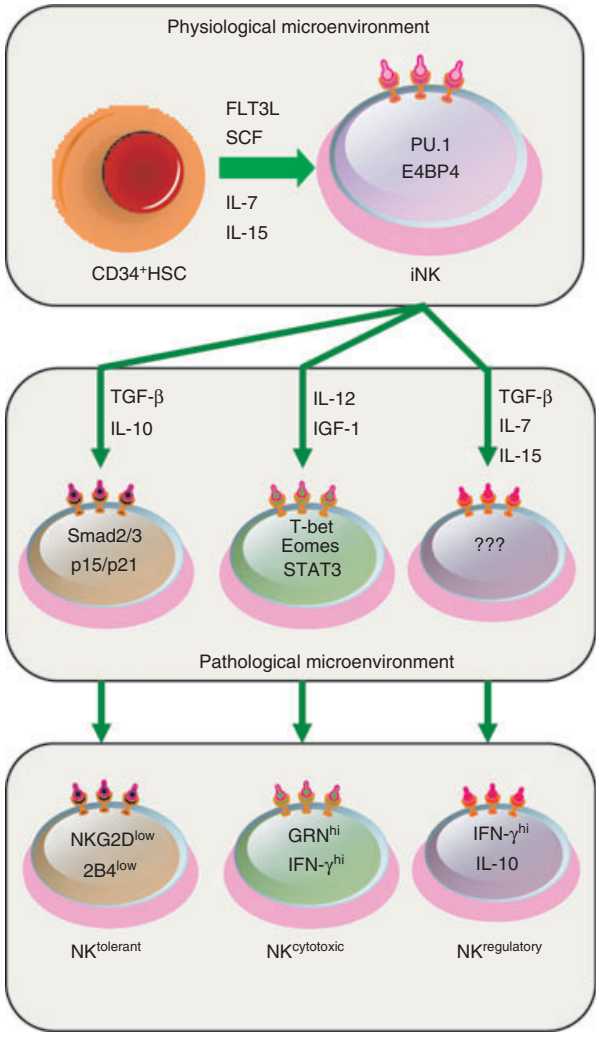

Figure 3. The programmed differentiation of natural killer (NK) cells and the generation of $NK^{tolerant}$, $NK^{cytotoxic}$ and $NK^{regulatory}$ cells. The programmed differentiation of NK cells can be divided into three steps. First, NK cells predominantly develop from $CD34^+$ haematopoietic stem cells (HSCs) in the physiological microenvironment of bone marrow or lymph nodes, producing immature NK (iNK) cells. Second, under the effect of chemokines, NK cells are recruited into different pathological microenvironments, such as the uterus and brain, and then develop under the control of specific cytokines and transcription factors. Third, these differentiated NK cells may act as $NK^{tolerant}$, $NK^{cytotoxic}$ or $NK^{regulatory}$ cells. IFN-γ, interferon-γ; IGF-1, insulin-like growth factor 1; IL-7, interleukin-7; SCF, stem cell factor; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β. ??? refers to unknown transcription factors that provide important intrinsic signals to impact the differentiation of regulatory NK cells.

shown to control trophoblast invasion and vascular remodelling through their ability to secrete an array of angiogenesis-regulating molecules, cytokines and chemokines, such as vascular endothelial growth factor, IL-8, IFN-inducible protein-10 and placental growth factor.[63] Hence, in addition to simply killing cells, a new paradigm of NK cell function has emerged through the pregnancy model, whereby these cells also promote the regulation of tissue homeostasis.

Moreover, invasion from allogenic fetal cells or spiral arteries may cause inflammation at the maternal–fetal interface. Indeed, the prevention of strong inflammatory responses is essential to ensure normal pregnancy.[64] Our group recently showed that CD56^{bright} CD27^{+} decidual NK cells function as key regulatory cells at the maternal–fetal interface by suppressing T helper type 17-mediated local inflammation via IFN-γ-dependent pathways. This NK cell-mediated regulatory response is lost in women with recurrent spontaneous abortions, resulting in a prominent T helper type 17 response, extensive local inflammation and eventual loss of maternal–fetal tolerance.[65] These findings provided evidence that decidual NK cells act as sentinel cells to control local inflammation, which is clearly critical for maintaining tolerance at the fetal–maternal interface. A recent study also demonstrates that resident decidual NK cells have close contact with particular myelomonocytic CD14^{+} cells, which results in the induction of regulatory T cells.[66] Interestingly, seminal studies of human maternal and fetal genotypes have suggested that the interactions between fetal HLA-C molecules and killer cell immunoglobulin-like receptors on uterine NK cells are important for reproductive success, showing that the regulation of NK cell activation is crucial for normal placentation and hence a successful pregnancy.[67] Hence, both mouse and human studies suggest that decidual NK cells act as key regulatory cells at the maternal–fetal interface by regulating trophoblast invasion and vascular remodelling, promoting tolerogenic DCs and monocytes and suppressing T helper type 17-mediated local inflammation (Fig. 4).

In other organs, regulatory NK cells can also inhibit immune reactions. In the central nervous system (CNS), depletion of NK cells from Lewis rats, SJL mice and C57BL/6 mice exacerbated demyelination in different experimental autoimmune encephalomyelitis models.[68–70] Importantly, the effects of NK cells on CNS pathology are dependent on the activity of CNS-resident but not peripheral NK cells,[69,71] demonstrating that CNS-specific

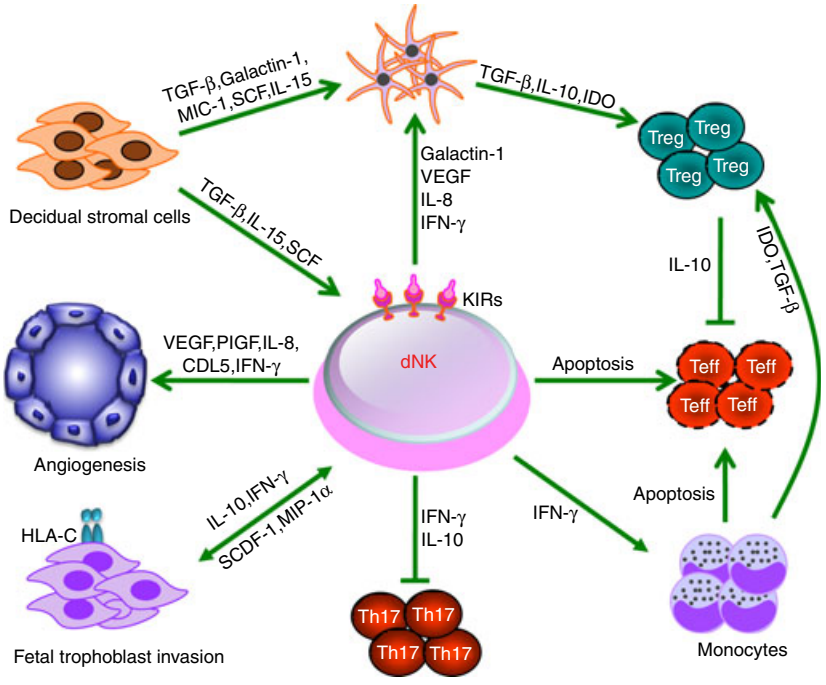

Figure 4. Key pathways of the regulatory decidual natural killer (dNK) cells involved in immune tolerance during the first trimester of human pregnancy. Decidual NK cells with a CD56^{bright} CD16^{-} phenotype can control trophoblast invasion and vascular remodelling, inhibit inflammatory T helper type 17 cells, promote the generation of indoleamine 2,3-dioxygenase (IDO) -producing monocytes and regulatory T cells and induce the apoptosis of effector T cells. Meanwhile, dNK cells are maintained and educated by the decidual microenvironment including stromal cells, trophoblast cells and hormones such as progesterone. IFN-γ, interferon-γ; IL, interleukin; MIC-1, macrophage inhibitory cytokine-1; MIP-1α, macrophage inflammatory protein-1α; PlGF, placental growth factor; SCDF-1, stromal cell-derived factor-1; SCF, stem cell factor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

NK cells control inflammation during experimental autoimmune encephalomyelitis in mice. In humans, the administration of daclizumab, a humanized monoclonal antibody against the IL-2 receptor α-chain (CD25), consistently reduces CNS lesions and inflammation in multiple sclerosis patients.[72–74] Daclizumab therapy was associated with a significant expansion of regulatory $CD56^{bright}$ NK cells *in vivo* and a gradual decline in circulating $CD4^+$ and $CD8^+$ T cells, providing supporting evidence for the existence of an immunoregulatory pathway through which activated $CD56^{bright}$ NK cells inhibit T-cell survival.[75] Defined by the differential expression of a combination of CD27 and CD11b, analysis of NK cell subsets indicated that the immature subset was dominant in the liver and that the immature $CD27^+$ $CD11b^-$ hepatic NK cell subset was protective against liver metastasis,[76] indicating that the liver maintains a special local immune tolerogenic microenvironment and educates $NK^{tolerant}$ cells.[77]

Concluding remarks

Herein, we review the NK subsets and the regulatory effect of NK cells, and provide examples of how these cells may serve as rheostats or sentinels in controlling inflammation and in maintaining immune homeostasis in different organs. We also discuss the three differentiated functions of NK cells in different microenvironments: $NK^{tolerant}$, $NK^{cytotoxic}$ and $NK^{regulatory}$ cells. It is interesting that regulatory $CD56^{bright}$ $CD16^-$ NK cells predominate in extensive disease models, such as in deciduas during pregnancy,[65] rheumatoid arthritis joints,[78] the CNS after daclizumab treatment[75] and patients with hepatitis B virus after pegylated IFN-α therapy.[79] However, many aspects of regulatory NK cells remain to be unveiled. The persisting questions include the following. Which subpopulation of NK cells plays the key role as regulatory NK cells? What is the relationship between $CD56^{bright}$ NK and $CD27^+$ NK cells? How does the organ-specific pathological microenvironment direct NK cells into different directions? Which transcription factors are involved in the regulatory effect of NK cells? Additionally, few studies have been undertaken to explore regulatory NK cells in humans. Although many observations and the mechanisms involved remain to be explored, the regulatory ability of NK cells deserves further attention, as the improved understanding of regulatory NK cells may pave the way for new immunotherapeutic approaches for alleviating or preventing many diseases.

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Disclosures

All authors declare no competing financial interests.

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