

Subsets of human natural killer cells and their regulatory effects

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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

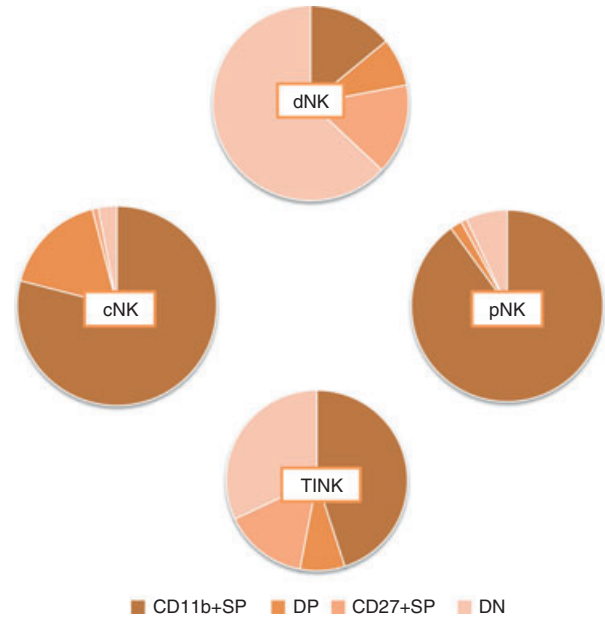


Figure 1. Four natural killer (NK) subsets defined by CD11b and CD27 in humans. Human NK cells can be divided into four subsets on the basis of the relative expressions of the markers CD11b and CD27, including CD11b⁺ CD27⁻ (CD11b⁺ SP), CD11b⁺ CD27⁺ (DP), CD11b⁻ CD27⁺ (CD27⁺ SP) and CD11b⁻ CD27⁻ (DN). 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.

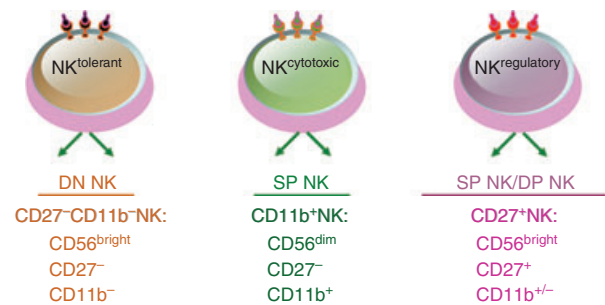


Figure 2. Human natural killer (NK) subsets presented according to phenotype and function. Human NK cells can be divided into three functional subsets: NK^{tolerant}, which is mainly CD56^{bright} NK cells or CD27⁻ CD11b⁻ NK cells; NK^{cytotoxic}, which is mainly CD56^{dim} NK cells or CD11b⁺ CD27⁻ NK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

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

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-15R β (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-2R α and IL-7R α , 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 mechanism³³ or may develop *in situ* from CD34⁺ haematopoietic precursors⁵⁶ 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 progesterone-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

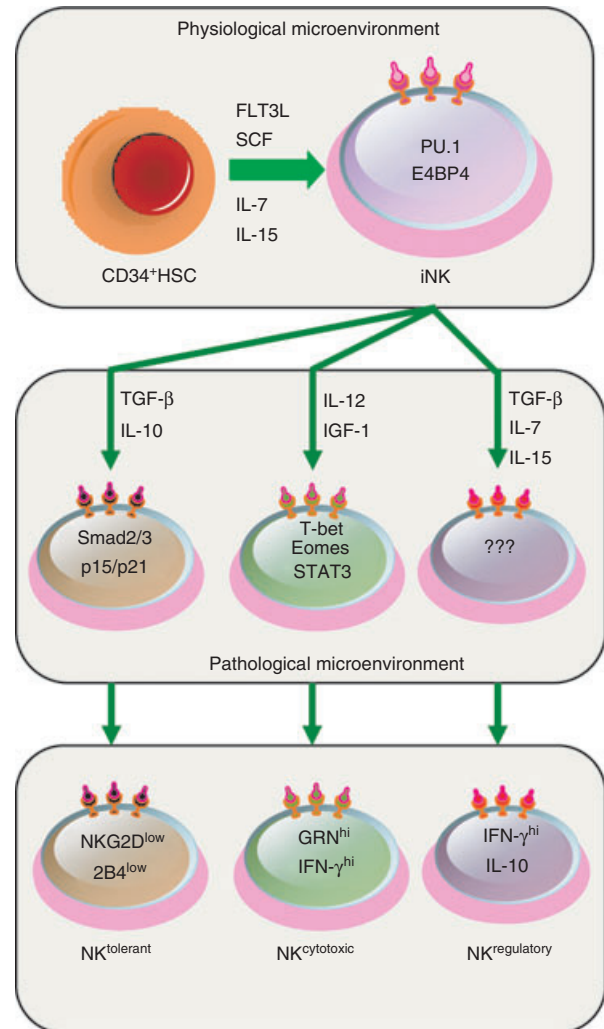


Figure 3. The programmed differentiation of natural killer (NK) cells and the generation of NK^{tolerant}, NK^{cytotoxic} and NK^{regulatory} cells. The programmed differentiation of NK cells can be divided into three steps. First, NK cells predominantly develop from CD34⁺ haematopoietic stem cells (HSCs) 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.

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 remodeling.^{60–62} Human decidual NK cells have also been

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 treatment⁷⁵ 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.

References

- Caligiuri MA. Human natural killer cells. *Blood* 2008; **112**:461–9.
- Flodstrom-Tullberg M, Bryceson YT, Shi FD, Hoglund P, Ljunggren HG. Natural killer cells in human autoimmunity. *Curr Opin Immunol* 2009; **21**:634–40.
- Narni-Mancinelli E, Ugolini S, Vivier E. Tuning the threshold of natural killer cell responses. *Curr Opin Immunol* 2013; **25**:53–8.
- Hayakawa Y, Smyth MJ. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *J Immunol* 2006; **176**:1517–24.
- Vossen MT, Matmati M, Hertoghs KM *et al.* CD27 defines phenotypically and functionally different human NK cell subsets. *J Immunol* 2008; **180**:3739–45.
- Hayakawa Y, Huntington ND, Nutt SL, Smyth MJ. Functional subsets of mouse natural killer cells. *Immunol Rev* 2006; **214**:47–55.
- Chiosso L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. Maturation of mouse NK cells is a 4-stage developmental program. *Blood* 2009; **113**:5488–96.
- Zhang C, Zhang J, Tian Z. The regulatory effect of natural killer cells: do “NK-reg cells” exist? *Cell Mol Immunol* 2006; **3**:241–54.
- Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF. Subpopulations of human natural-killer cells defined by expression of the Leu-7 (Hnk-1) and Leu-11 (Nk-15) antigens. *J Immunol* 1983; **131**:1789–96.
- Poli A, Michel T, Thérèse M, André E, Hentges F, Zimmer J. CD56 (bright) natural-killer (NK) cells: an important NK cell subset. *Immunology* 2009; **126**:458–65.
- Kim S, Izuka K, Kang HS, Dokun A, French AR, Greco S, Yokoyama WM. *In vivo* developmental stages in murine natural killer cell maturation. *Nat Immunol* 2002; **3**:523–8.
- Freud AG, Yokohama A, Becknell B, Lee MT, Mao HC, Ferketich AK, Caligiuri MA. Evidence for discrete stages of human natural killer cell differentiation *in vivo*. *J Exp Med* 2006; **203**:1033–43.
- De Colvenaer V, Taveirne S, Delforche M *et al.* CD27-deficient mice show normal NK-cell differentiation but impaired function upon stimulation. *Immunol Cell Biol* 2011; **89**:803–11.
- Jin J, Fu B, Mei X, Yue T, Sun R, Tian Z, Wei H. CD11b⁻ CD27⁻ NK cells are associated with the progression of lung carcinoma. *PLoS ONE* 2013; **8**:e61024.
- Fu B, Wang F, Sun R, Ling B, Tian Z, Wei H. CD11b and CD27 reflect distinct population and functional specialization in human natural killer cells. *Immunology* 2011; **133**:350–9.
- Shi FD, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011; **11**:658–71.
- Tian Z, Chen Y, Gao B. Natural killer cells in liver disease. *Hepatology* 2013; **57**:1654–62.
- Sun HY, Sun C, Tian ZG, Xiao WH. NK cells in immunotolerant organs. *Cell Mol Immunol* 2013; **10**:202–12.
- Peng H, Jiang X, Chen Y *et al.* Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. *J Clin Invest* 2013; **123**:1444–56.
- Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nat Med* 2013; **19**:548–56.
- Man YG, Stojadinovic A, Mason J *et al.* Tumor-infiltrating immune cells promoting tumor invasion and metastasis: existing theories. *J Cancer* 2013; **4**:84–95.
- McKenna HJ, Stocking KL, Miller RE *et al.* Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* 2000; **95**:3489–97.
- Farag SS, Caligiuri MA. Human natural killer cell development and biology. *Blood Rev* 2006; **20**:123–37.
- Miller JS, Alley KA, Mcglave P. Differentiation of natural-killer (Nk) cells from human primitive marrow progenitors in a stroma-based long-term culture system – identification of a Cd34⁺ Nk progenitor. *Blood* 1994; **83**:2594–601.
- Mrozek E, Anderson P, Caligiuri MA. Role of interleukin-15 in the development of human CD56⁺ natural killer cells from CD34⁺ hematopoietic progenitor cells. *Blood* 1996; **87**:2632–40.

- 26 Freud AG, Caligiuri MA. Human natural killer cell development. *Immunol Rev* 2006; **214**:56–72.
- 27 Freud AG, Becknell B, Roychowdhury S *et al*. A human CD34⁺ subset resides in lymph nodes and differentiates into CD56^{bright} natural killer cells. *Immunity* 2005; **22**:295–304.
- 28 Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Facchetti F, Colonna M, Caligiuri MA. CD56^{bright} natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 2003; **101**:3052–7.
- 29 Lynch L, O'Donoghue D, Dean J, O'Sullivan J, O'Farrelly C, Golden-Mason L. Detection and characterization of hemopoietic stem cells in the adult human small intestine. *J Immunol* 2006; **176**:5199–204.
- 30 Chinen H, Matsuoka K, Sato T *et al*. Lamina propria c-kit⁺ immune precursors reside in human adult intestine and differentiate into natural killer cells. *Gastroenterology* 2007; **133**:559–73.
- 31 Huntington ND, Vossenrich CAJ, Di Santo JP. Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nat Rev Immunol* 2007; **7**:703–14.
- 32 Bernardini G, Gismondi A, Santoni A. Chemokines and NK cells: regulators of development, trafficking and functions. *Immunol Lett* 2012; **145**:39–46.
- 33 Hanna J, Wald O, Goldman-Wohl D *et al*. CXCL12 expression by invasive trophoblasts induces the specific migration of CD16⁺ human natural killer cells. *Blood* 2003; **102**:1569–77.
- 34 Yu YR, Fong AM, Combadiere C, Gao JL, Murphy PM, Patel DD. Defective antitumor responses in CX3CR1-deficient mice. *Int J Cancer* 2007; **121**:316–22.
- 35 Paust S, Gill HS, Wang BZ *et al*. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol* 2010; **11**:1127–35.
- 36 Huang Z, Fu B, Zheng SG, Li X, Sun R, Tian Z, Wei H. Involvement of CD226⁺ NK cells in immunopathogenesis of systemic lupus erythematosus. *J Immunol* 2011; **186**:3421–31.
- 37 Sun C, Fu B, Gao Y, Liao X, Sun R, Tian Z, Wei H. TGF- β 1 down-regulation of NKG2D/DAP10 and 2B4/SAP expression on human NK cells contributes to HBV persistence. *PLoS Pathog* 2012; **8**:e1002594.
- 38 Smyth MJ, Teng MW, Swann J, Kyriassoudis K, Godfrey DI, Hayakawa Y. CD4⁺CD25⁺ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. *J Immunol* 2006; **176**:1582–7.
- 39 Pedroza-Pacheco I, Madrigal A, Saudemont A. Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy. *Cell Mol Immunol* 2013; **10**:222–9.
- 40 Soderquest K, Powell N, Luci C *et al*. Monocytes control natural killer cell differentiation to effector phenotypes. *Blood* 2011; **117**:4511–18.
- 41 Ganai SC, Sanos SL, Kallfass C *et al*. Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity* 2012; **37**:171–86.
- 42 Jaeger BN, Donadieu J, Cognet C *et al*. Neutrophil depletion impairs natural killer cell maturation, function, and homeostasis. *J Exp Med* 2012; **209**:565–80.
- 43 Gascoyne DM, Long E, Veiga-Fernandes H *et al*. The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol* 2009; **10**:1118–24.
- 44 Colucci F, Samson SI, DeKoter RP, Lantz O, Singh H, Di Santo JP. Differential requirement for the transcription factor PU.1 in the generation of natural killer cells versus B and T cells. *Blood* 2001; **97**:2625–32.
- 45 Ramirez K, Chandler KJ, Spaulding C, Zandi S, Sigvardsson M, Graves BJ, Kee BL. Gene deregulation and chronic activation in natural killer cells deficient in the transcription factor ETS1. *Immunity* 2012; **36**:921–32.
- 46 Boggs SS, Trevisan M, Patrene K, Georgopoulos K. Lack of natural killer cell precursors in fetal liver of Ikaros knockout mutant mice. *Nat Immunol* 1998; **16**:137–45.
- 47 Boos MD, Yokota Y, Eberl G, Kee BL. Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity. *J Exp Med* 2007; **204**:1119–30.
- 48 Gordon SM, Chaix J, Rupp LJ, Wu J, Madera S, Sun JC, Lindsten T, Reiner SL. The transcription factors t-bet and eomes control key checkpoints of natural killer cell maturation. *Immunity* 2012; **36**:55–67.
- 49 Samson SI, Richard O, Tavian M *et al*. GATA-3 promotes maturation, IFN- γ production, and liver-specific homing of NK cells. *Immunity* 2003; **19**:701–11.
- 50 Kallies A, Carotta S, Huntington ND, Bernard NJ, Tarlinton DM, Smyth MJ, Nutt SL. A role for Blimp1 in the transcriptional network controlling natural killer cell maturation. *Blood* 2011; **117**:1869–79.
- 51 Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "l'union fait la force". *Blood* 2005; **106**:2252–8.
- 52 Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; **9**:503–10.
- 53 Ni F, Sun R, Fu B, Wang F, Guo C, Tian Z, Wei H. IGF-1 promotes the development and cytotoxic activity of human NK cells. *Nat Commun* 2013; **4**:1479.
- 54 Keskin DB, Allan DS, Rybalov B, Andzelm MM, Stern JN, Kopcow HD, Koopman LA, Strominger JL. TGF- β promotes conversion of CD16⁺ peripheral blood NK cells into CD16⁻ NK cells with similarities to decidual NK cells. *Proc Natl Acad Sci USA* 2007; **104**:3378–83.
- 55 Michaud A, Dardari R, Charrier E, Cordeiro P, Herblot S, Duval M. IL-7 enhances survival of human CD56^{bright} NK cells. *J Immunother* 2010; **33**:382–90.
- 56 Vacca P, Vitale C, Montaldo E *et al*. CD34⁺ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. *Proc Natl Acad Sci USA* 2011; **108**:2402–7.
- 57 Manaster I, Mizrahi S, Goldman-Wohl D *et al*. Endometrial NK cells are special immature cells that await pregnancy. *J Immunol* 2008; **181**:1869–76.
- 58 Koopman LA, Kopcow HD, Rybalov B *et al*. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003; **198**:1201–12.
- 59 Male V, Hughes T, McClory S, Colucci F, Caligiuri MA, Moffett A. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. *J Immunol* 2010; **185**:3913–18.
- 60 Ashkar AA, Di Santo JP, Croy BA. Interferon- γ contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000; **192**:259–70.
- 61 Croy BA, He H, Esadeg S *et al*. Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. *Reproduction* 2003; **126**:149–60.
- 62 Zhang JH, Chen ZL, Smith GN, Croy BA. Natural killer cell-triggered vascular transformation: maternal care before birth? *Cell Mol Immunol* 2011; **8**:1–11.
- 63 Hanna J, Goldman-Wohl D, Hamani Y *et al*. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006; **12**:1065–74.
- 64 Sargent IL, Borzychowski AM, Redman CWG. NK cells and human pregnancy – an inflammatory view. *Trends Immunol* 2006; **27**:399–404.
- 65 Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, Wei H. Natural killer cells promote immune tolerance by regulating inflammatory T_H17 cells at the human maternal-fetal interface. *Proc Natl Acad Sci USA* 2013; **110**:E231–40.
- 66 Vacca P, Cantoni C, Vitale M *et al*. Crosstalk between decidual NK and CD14⁺ myelomonocytic cells results in induction of Tregs and immunosuppression. *Proc Natl Acad Sci USA* 2010; **107**:11918–23.
- 67 Sharkey AM, Gardner L, Hiby S *et al*. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition gestational age. *J Immunol* 2008; **181**:39–46.
- 68 Xu W, Fazekas G, Hara H, Tabira T. Mechanism of natural killer (NK) cell regulatory role in experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2005; **163**:24–30.
- 69 Zhang BN, Yamamura T, Kondo T, Fujiwara M, Tabira T. Regulation of experimental autoimmune encephalomyelitis by natural killer (NK) cells. *J Exp Med* 1997; **186**:1677–87.
- 70 Matsumoto Y, Kohyama K, Aikawa Y, Shin T, Kawazoe Y, Suzuki Y, Tanuma N. Role of natural killer cells and TCR $\gamma\delta$ T cells in acute autoimmune encephalomyelitis. *Eur J Immunol* 1998; **28**:1681–8.
- 71 Hao J, Liu R, Piao W *et al*. Central nervous system (CNS)-resident natural killer cells suppress Th17 responses and CNS autoimmune pathology. *J Exp Med* 2010; **207**:1907–21.
- 72 Rose JW, Watt HE, White AT, Carlson NG. Treatment of multiple sclerosis with an anti-interleukin-2 receptor monoclonal antibody. *Ann Neurol* 2004; **56**:864–7.
- 73 Rose JW, Burns JB, Bjorklund J, Klein J, Watt HE, Carlson NG. Daclizumab phase II trial in relapsing and remitting multiple sclerosis – MRI and clinical results. *Neurology* 2007; **69**:785–9.
- 74 Bielekova B, Howard T, Packer AN *et al*. Effect of anti-CD25 antibody daclizumab in the inhibition of inflammation and stabilization of disease progression in multiple sclerosis. *Arch Neurol-Chicago* 2009; **66**:483–9.
- 75 Bielekova B, Catalfamo M, Reichert-Scrivner S *et al*. Regulatory CD56^{bright} natural killer cells mediate immunomodulatory effects of IL-2R α -targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci USA* 2006; **103**:5941–6.
- 76 Ballas ZK, Buchta CM, Rosean TR, Heusel JW, Shey MR. Role of NK cell subsets in organ-specific murine melanoma metastasis. *PLoS ONE* 2013; **8**:e65599.
- 77 Li FL, Tian ZG. The liver works as a school to educate regulatory immune cells. *Cell Mol Immunol* 2013; **10**:292–302.
- 78 Pridgeon C, Lennon GP, Thompson RN, Christmas SE, Moots RJ. Natural killer cells in the synovial fluid of rheumatoid arthritis patients exhibit a CD56^{bright}, CD94^{bright}, CD158^{negative} phenotype. *Rheumatology* 2003; **42**:870–8.
- 79 Micco L, Peppia D, Loggi E *et al*. Differential boosting of innate and adaptive antiviral responses during pegylated-interferon- α therapy of chronic hepatitis B. *J Hepatol* 2013; **58**:225–33.