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REVIEW

NK cells in immunotolerant organs

Haoyu Sun, Cheng Sun, Zhigang Tian and Weihua Xiao

Organs such as the liver, uterus and lung possess hallmark immunotolerant features, making these organs important for sustaining self-homeostasis. These organs contain a relatively large amount of negative regulatory immune cells, which are believed to take part in the regulation of immune responses. Because natural killer cells constitute a large proportion of all lymphocytes in these organs, increasing attention has been given to the roles that these cells play in maintaining immunotolerance. Here, we review the distribution, differentiation, phenotypic features and functional features of natural killer cells in these immunotolerant organs, in addition to the influence of local microenvironments on these cells and how these factors contribute to organ-specific diseases.
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INTRODUCTION

By definition, non-reactivity to an antigen resulting from previous exposure to the same antigen is termed immunotolerance.[1] One important aspect of immunotolerance is non-reactivity to self-antigens, as autoimmune diseases may develop in the absence of such regulation. Immunotolerance is also inducible to non-self-antigens.[1,2] Therefore, immunotolerance is an important feature in protecting an organism from autoimmune damage and for the maintenance of the organism's homeostasis. In the search for new therapeutics, researchers studying immunotolerance may be able to reprogram the immune system to resolve conditions that threaten human health.[2–4] The intestine is generally recognized as an immune-privileged organ, in which immunotolerant mechanisms constantly face but do not overreact to microbial antigens and pathogens acquired from food.[5] The liver is exposed to the food and foreign antigens that reach the intestines and thus exhibits defensive functions to these environmental challenges.[6] For these reasons, the risk of hyperimmune activation in the liver is greater than the risk in any other organ in the body. Therefore, specialized immunotolerant mechanisms are required to avoid overactivation of immune responses in the liver.[7] The lung is in danger of environmentally acquired infections and attacks from pathogens and bacteria due to its constant exposure to airborne pathogens.[8,9] Due to the importance of immune regulation in pregnancy, it is not surprising that the uterus is the most immunotolerant organ in the body.[10] These organs rely on microorganisms and their own organ-specific immunotolerant features to maintain moderate levels of immune responses and thus to sustain adequate growth and survival.

Negative regulatory immune cells (such as dendritic cells (DCs), regulatory T cells, T_H17 cells, M2 macrophages, natural killer (NK) cells, natural killer T (NKT) cells and neutrophils) in these immunotolerant organs play important roles in directing immune responses.[11] Furthermore, these immune cells are generally considered to play an important role in the inhibition of autoimmune diseases and the maintenance of immune homeostasis. Among these innate immune cells, NK cells are rarely reviewed with respect to their regulatory functions. NK cells are one of three major lymphocytes with specific functional features. It has been generally recognized that NK cells play important roles in immunodefense, immunosurveillance and immunohomeostasis.[12] Furthermore, because NK cells constitute a large percentage of all lymphocytes in immunotolerant organs, it is becoming more accepted that NK cells play an important role in sustaining an immunotolerant status. Because NK cells possess several functional subsets, the expression arrays of activating receptors and inhibitory receptors may differ for each specialized condition;[13,14] therefore, it is likely that NK cells play dual roles both in immunoactivation and immunotolerance. The proportion of NK cells in each specific organ relative to other organs is as follows: uterus>lung>liver>peripheral blood mononuclear cells>spleen>bone marrow>lymph node>thymus.[8] Additionally, the local microenvironments in each of these organs influence the biological

Institute of Immunology, School of Life Sciences, University of Science and Technology of China, Hefei, China
Correspondence: Dr WH Xiao, School of Life Sciences, University of Science and Technology of China, Hefei, China.
E-mail: xiaow@ustc.edu.cn
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processes of the NK cells, including migration, homing, differentiation and activation. NK cells have been shown to offer special potential for immunotherapy.[15–17] In this review, we will focus on addressing the regulatory roles of NK cells in immunotolerant organs, including the liver, lung and uterus.

Differentiation and subsets of human NK cells

Early studies suggested that NK cells possess only one subset denoted by the absence of CD3 and the presence of CD56.[18,19] Years later, it was discovered that the surface markers on murine NK cells and human NK cells are distinct and that these cells exhibit limited homology between them. It was later shown that human NK cells possess different subsets of NK cells. Human NK cells are collectively classified by the expression of CD56 and the lack of CD3.[20] This population is further differentiated based on the relative surface expression of CD56 and the low-affinity F*c* receptor CD16.[19] Two subpopulations of NK cells are now widely accepted. First is the $CD3^-CD56^{bright}CD16^-$ population, which is the main NK cell subset in peripheral lymphoid organs. This population bears homing receptors such as CCR7 and CXCR3 and the adhesion molecule CD62L; it produces a large amount of cytokines, such as tumor-necrosis factor, granulocyte macrophage colony-stimulating factor, interferon-γ (IFN-γ), IL-10 and IL-13 after stimulation with pre-inflammatory cytokines; and it undergoes cytotoxicity after prolonged stimulation.[20,21] Second is the $CD3^-CD56^{dim}CD16^+$ population that is related to the major histocompatibility complex (MHC)-unrestricted cytotoxicity (antibody-dependent cellular cytotoxicity), with high expression of killer cell immunoglobulin-like receptors (KIRs) and low expression of the CD94/NKG2 receptors; this population is rich in granzymes and perforins, and it kills target cells after specific recognition.[19,22,23] Moreover, the $CD3^-CD56^{dim}CD16^+$ population lacks the expression of CD27, while the $CD3^-CD56^{bright}CD16^-$ population expresses CD27.[24] Recent research has suggested a role for the T-cell immunoglobulin- and mucin domain-containing (Tim)-3 receptor as a maturation marker on NK cells because the Tim-3 protein is expressed on essentially all mature $CD3^-CD56^{dim}CD16^+$ NK cells and may be induced on $CD3^-CD56^{bright}CD16^-$ NK cells after stimulation with IL-15 or IL-12 and IL-18 *in vitro*.[25] The two NK subsets also express shared and distinct chemokines; shared chemokines include CC chemokine receptor 2 (CCR2), CXCR1, CXCR3 and CXCR4, while only $CD3^-CD56^{bright}$ NK cells express CCR5 and CCR7 and only $CD3^-CD56^{dim}$ NK cells express CX3C-chemokine receptor 1, SIP5 and ChemR23.[8] $CD3^-CD56^{bright}CD16^-$ NK cells may eventually differentiate into $CD3^-CD56^{dim}CD16^+$ NK cells and may be further differentiated and modified over time.[26,27]

Differentiation and subsets of murine NK cells

Murine NK cells share many features with human NK cells; nevertheless, murine NK cells lack the expression of the hallmark human NK cell marker CD56 and other surface markers, making it difficult to compare these cells with human NK cell subsets. Murine NK cells are subdivided into different groups based on CD11b, CD27, CD127 and B220 expression.[28] In mice, integrin CD11b is used as a marker for mature NK cells.[29] The distinct stages of differentiation are further subdivided based on the expression of CD27.[24] Therefore, murine NK cells can be subdivided into four subsets: $CD11b^{low}CD27^{low}$, $CD11b^{low}CD27^{high}$, $CD11b^{high}CD27^{high}$ and $CD11b^{high}CD27^{low}$, with the maturation process beginning at the $CD11b^{low}CD27^{low}$ stage and progressing to the $CD11b^{low}CD27^{high}$, $CD11b^{high}CD27^{high}$ and finally $CD11b^{high}CD27^{low}$ stages.[30] Immature $CD11b^-$ NK cells undergo a $CD27^+$ stage before reaching full developmental maturity at the $CD11b^+CD27^-$ stage.[30] The $CD27^+$ subset predominates in the lymph node, while the $CD27^-$ subset localizes mainly to the blood and spleen.[24] Different repertoires of activating receptors, inhibitory receptors and chemokines are expressed by the $CD11b^{high}CD27^{high}$ and $CD11b^{high}CD27^{low}$ subsets, with $CD11b^{high}CD27^{high}$ NK cells producing the largest number of cytokines and having the strongest cytotoxicity.[24] Interestingly, a new subset of NK cells with a regulatory function was identified in a study by Ehlers *et al.*[31] using two mouse models for type 1 diabetes mellitus; this new immunoregulatory NK cell subset, characterized by the surface markers CD117 (also known as c-Kit) and programmed death-ligand 1, suppresses autoimmunity by downregulating antigen-specific $CD8^+$ T cells, suggesting a direct link between innate and adaptive immunity. Although the markers expressed on human and murine NK cells vary, it has been suggested that the murine $CD11b^{high}CD27^{low}$ NK cell subset resembles the human $CD56^{dim}CD16^+$ NK cell subset;[32] additionally, the natural cytotoxicity receptor NKp46 is present on both human and murine NK cells.[33] In addition, unlike human NK cells, murine NK cells do not express CCR7, and the $CD27^+$ subset expresses higher levels of CXCR3, a homing receptor for lymph nodes, suggesting an explanation for the observed distribution of the two murine NK cell subsets. Despite this potential explanation, CD62L is expressed on both the $CD27^+$ and $CD27^-$ subsets.[19]

Homing and migration of NK cells

NK cells are widely distributed throughout the body, including but not limited to healthy skin, the liver, lungs, and uterus.[32] Bone marrow-derived NK cell precursors undergo a complex maturation process that ultimately determines their effector functions [8,34,35] and their distribution to specific organs under the influence of the varied expression of chemokine receptors and adhesion molecules.[8] The allocation of NK cells is a dynamic rather than stationary process because these cells constantly recirculate between different organs.[8] The varied expression of chemokine receptors such as CCR2, CCR5, CXCR3 and CX3C-chemokine receptor 1 drives NK cells to different sites under inflammatory conditions.[8,36] Chemokines produced by organ-specific cells play a critical role in the migration of NK cells, leading to different NK cell distributions in these organs. For example, Kupffer cell-derived CCL2 attracts NK cells expressing CCR2 to the liver during murine cytomegalovirus infection.[37,38] The migration of NK cells from the red pulp to the white pulp depends on the synergic effect of both

CXCR3 and CCR5 after treatment with poly(I:C) injection or murine cytomegalovirus infection.[39] In a study by Beider *et al.*,[40] the authors found that the migration of cells to the site of inflammation depends on the downregulation of CXCR4 and upregulation of CXCR3, which may be accomplished by the induction of IL-2, leading to the inhibition of the migration of NK and NKT cells to the bone marrow and spleen. In addition to chemokine receptors, G protein-coupled receptors and sphingosine 1-phosphate (S1P) receptors also participate in lymphocyte mobilization. One member of the S1P family, S1P5, which is expressed on both murine and human NK cells, has been shown to regulate NK cell trafficking *in vivo* by a FTY720-resistant mechanism.[41] NK cell recruitment, under the influence of varied chemokine expression mediated by organ-specific cells, suggests that microenvironments may have a substantial effect on the migration of NK cells under different physiological and pathological conditions.

NK CELLS IN THE LIVER

The liver is located in the middle of the gastrointestinal tract and the systemic circulation. Although this organ is in constant contact with food-derived antigens, bacterial products, and environmental toxins from the gut, no severe inflammation is induced, partly due to selective responses to the antigens that prevent inappropriate immune activation.[42] The liver is an immune organ with a large proportion of innate immune cells such as NK cells, macrophages, NKT cells and γδT cells.[43,44] In humans, 30%–50% of intrahepatic lymphocytes are NK cells,[45] while in mice, NK cells constitute approximately 10%–15%. Furthermore, the number of NK cells varies widely in different liver disease models, suggesting different roles for these cells under different pathological conditions.[38,46] For example, NK cell accumulation is observed in the murine liver after viral infection [38,47] or poly(I:C) treatment.[28] Previous research also suggests that hepatic NK cells not only protect the host from invading microorganisms and tumor transformation but also participate in liver injury and repair.[44]

Phenotypes and functions of hepatic NK cells

The liver acts as an innate immunity-dominant organ, not only because it is largely composed of innate immune cells, but also due to its specific properties and functions. NK cells provide a first line of defense against invading pathogens, viral infections and tumors. In humans, hepatic NK cells constitute 20%–30% of the total lymphocytes, and similarly to NK cells in the peripheral blood, hepatic NK cells are defined by the surface marker phenotype $CD3^-CD56^+$.[48] However, human hepatic NK cells lack the expression of CD16, differing from NK cells in the peripheral blood (which are largely $CD56^{dim}CD16^+$).[49,50] In mice, hepatic NK cells constitute 5%–10% of the total lymphocytes, and these cells are defined by the surface marker phenotype $CD3^-NK1.1^+$ in C57BL/6 mouse strains or $CD3^-DX5^+$. In a study by Cordon *et al.*,[51] the authors identified two transcription factors, T-bet and Eomes, as sequential, genetically separable checkpoints of NK cell maturation. Eomes is essential for NK cell maturation, while T-bet is required for the developmental stability of immature NK cells.[52] The microenvironment maintained by the liver appears to be non-permissive for Eomes induction, leading to the stability of $Eomes^-$ NK cells in the liver.[51] While approximately half of all NK cells in the liver are $Eomes^+$, indicating that hepatic NK cells are likely either NK cells that originally resided in the liver or conventional NK cells circulated from the bone marrow.[52] The liver is the site of NK cell generation when life begins. During the early stage of the life cycle, hepatic NK cells possess all the typical characteristics of classical NK cells, but still have their own unique phenotypes and functions. In embryonic and newborn mice, hepatic NK cells lack the expression of molecules associated with NK cell maturation, such as membrane-bound CD11b, the Ly49 receptor, DX5, and the intracellular transcription factor Eomes, but highly express the effector molecule TRAIL. This expression pattern occurs not only in the liver but also in other organs such as the spleen.[51,53] Notably, as development progresses, this specific subset is replaced by phenotypically and functionally mature NK cells in the spleen but remains in the liver. Even in the later stages of the life cycle, approximately half of the hepatic NK cells remain as $TRAIL^+DX5^-CD11b^{low}Eomos^-$.[52] The inhibitory receptor NKG2A is another hallmark hepatic NK cell marker that interacts with murine MHC class I-related protein Qa-1 (HLA-E in humans).

The activation of NK cells is based on the imbalance between the effects of inhibitory and stimulatory receptors expressed on NK cells and their interactions with corresponding ligands expressed on target cells.[14,54,55] Stimulatory receptors include NKG2D and members of the well-known natural cytotoxic receptor family, including NKp30, NKp44 and NKp46. Meanwhile, inhibitory receptors include members of the KIR family, Ly-49A and CD94/NKG2 receptors that recognize MHC class I molecules and subsequently inactivate NK cell functions.[54] NK cells kill infected or transformed target cells, such as virus-infected hepatocytes, either directly or by secreting the pro-inflammatory cytokine IFN-γ.[48] Compared with NK cells in the peripheral blood and the spleen, hepatic NK cells possess a higher number of granules and express higher levels of TRAIL, perforin, granzyme B and other molecules, in turn mediating greater cytotoxicity against tumor cells.[56] Accumulating evidence supports the anti-tumor functions of hepatic NK cells.[57] Clinical evidence indicates that NK cells provide innate immunity against primary and metastatic liver tumors; indeed, the number of hepatic NK cells increases in patients with carcinomas, and moreover, the progression of carcinoma is associated with the decrease in the activity of hepatic NK cells.[44] It is likely that these findings will provide a breakthrough for the therapeutic cure of carcinoma, as tumor development may be inhibited by enhancing NK cell functions.

NK cells in liver immunotolerance

Liver immunotolerance includes both local and systemic tolerance to self and foreign antigens. Liver resident cells,

including liver dendritic cells, liver sinusoidal endothelial cells, Kupffer cells and hepatic stellate cells, mediate immunosuppression by producing anti-inflammatory cytokines, such as transforming growth factor-beta (TGF-β) and IL-10, and by expressing inhibitory ligands for T-cell activation. The liver has evolved specialized mechanisms to avoid overactivation toward harmless antigens to maintain its own immunotolerance. The liver microenvironment may modulate NK cell function through the expression of activating receptors and inhibitory receptors and the secretion of inflammatory cytokines.[58] NK cells maintain their own tolerance by interacting with other cells. For example, upon the interaction of NK cells with dendritic cells, DCs start to induce tolerogenic regulatory T cells, and this ability is dependent on the engagement of NKG2A expressed on NK cells, leading to the maintenance of immunotolerance.[59,60] Kupffer cells, acting as liver tissue macrophages, secrete large amounts of immunosuppressive cytokines, such as IL-10, under LPS stimulation, leading to the inactivation of NK cells.[61] Kupffer cell-derived IL-10 downregulates not only NK cells but also other immune cells during inflammation to avoid excessive liver injury. Despite this system of immunotolerance, NK cells also clear pathogens effectively as a primary line of defense.[7] The number of hepatic NK cells increases during pathogen infection, likely due to the recruitment of NK cells to the site of infection and/or the proliferation of residing NK cells. The expression of NKG2A contributes to liver immunotolerance.[58] A specific population of functionally hyporesponsive NK cells resides in the liver with high expression of the inhibitory receptor NKG2A and the absence of the MHC class I-binding Ly49 receptor. These NK cells display a suppressed IFN-γ response under IL-12/IL-18 stimulation. Adoptively transferred splenic NK cells displayed phenotypical and functional changes after migrating to the liver, suggesting that the liver microenvironment modifies NK cells over time.[62]

Hepatic NK cells in liver diseases

The liver is the major site of viral replication after a viral infection; virus-induced hepatitis is one such infection and is one of the most threatening diseases in the world.[63] Patients infected with virus-induced hepatitis are at an increased risk of developing liver fibrosis, cirrhosis and hepatocellular carcinoma.

Hepatitis B virus (HBV) infects more than 350 million people worldwide and contributes to over 1 million deaths annually due to chronic liver damage, including cirrhosis and hepatocellular carcinoma.[64] NK cells are believed to be one of the major players in the control of viral infections. Infections with HBV lead to persistence of the infection and impaired NK cell functions. HBV suppresses plasmacytoid DC-induced IFN-γ production by NK cells, demonstrating that the virus modulates plasmacytoid DC–NK cell crosstalk, contributing to HBV persistence.[65] Studies have demonstrated that high levels of TGF-β1 are associated with reduced NKG2D/DAP10 and CD244/SH2D1A expression, functional impairment of NK cells and consequently, the development of persistent HBV infection.[66] HMBOX1 may also negatively regulate NK cell functions by suppressing the NKG2D/DAP10 signaling pathway.[67] The injection of poly(I:C) recruits and activates NK cells in the liver and induces mild liver injury in wild-type mice.[68] In contrast to wild-type mice, HBsAg transgenic mice, a mimic of healthy human chronic HBsAg carriers, are oversensitive to poly(I:C)-induced liver injury in an NK cell- and IFN-γ-dependent manner.[69] IL-15 may play an important role in regulating immune responses against HBV, and overexpression of IL-15 in the liver suppresses HBV replication *via* an IFN-β-dependent manner.[70] Another possible treatment for HBV is RNA interference of virus-specific genes. Transfection with HBx-siRNAs increases the expression of dsRNA-dependent protein kinase R, suggesting that HBx-siRNAs greatly promote protein kinase R activation, which leads to greater production of type I interferon, and further contribute to HBV inhibition.[71] 3p-HBx-siRNA can both inhibit HBV replication and trigger innate immunity in a RIG-I-dependent manner, synergistically benefitting the reversal of HBV-induced immune tolerance.[72] A triple shRNA (shRae1-shMult1-shH60) against all murine NKG2D ligands would effectively alleviate NK cell-mediated hepatitis in a poly(I:C)/D-GalN-induced model and NKG2D-dependent acute hepatitis in a Con-A-induced model; moreover, this triple shRNA-expressing vector is far more effective than a double or single shRNA-expressing vector, demonstrating another potential method to treat liver diseases.[73]

Meanwhile, hepatitis C virus (HCV) causes chronic infections in approximately 2% of the world's population. NK cells kill HCV-infected hepatocytes *via* perforin and granzymes or by producing cytokines such as IFN-γ and tumor-necrosis factor, leading to the activation of adaptive immune responses and the suppression of virus replication.[63] In a study by Kramer *et al.*,[74] the authors defined a specific NK cell subset based on relatively high expression of NKp46, termed NKp46^{high} NK cells, which may be involved in the suppression of HCV replication and HCV-associated liver damage. Hepatic NK cells may upregulate NKp30 and contribute to innate resistance in HCV infections.[75] In chronically infected HCV patients, increased expression of NKG2A suggests a role for NK cells in sustaining chronic viral infections by inhibiting anti-viral T-cell responses and facilitating pathogen persistence.[60,76] In a CCl_4-induced liver injury and regeneration model, the activation of NK cells inhibits liver regeneration *via* a tumor necrosis factor-α-dependent mechanism.[77]

NK CELLS IN THE UTERUS

For a long time, the immunology of reproductive immunotolerance was explained by the 'Th1/Th2' hypothesis of Tom Wegmann. He first proposed that fetal survival depends on the avoidance of maternal T-cell rejection, accomplished by a bias toward a Th2 response and the inhibition of Th1 response.[78] However, years later, it was found that the major immune interactions in the decidua contributing to immunotolerance during pregnancy are between trophoblast cells and maternal NK cells, rather than T cells. Approximately 30%–40% of decidual stromal cells are leukocytes in early pregnancy,

with decidual natural killer (dNK) cells constituting the major portion. NK cells start to accumulate in the decidua basalis, the trophoblast layer, after fertilization and implantation. Trophoblast invasion and spiral artery remodeling are the two major processes that establish a successful pregnancy,[79] and studies have shown that dNK cells play an important role in both of these processes.[80] In humans, more than 80% of decidual lymphocytes are $CD56^{bright}CD16^{-}$ NK cells, and these cells interact with stromal cells and invade trophoblast cells directly.[81,82] dNK cells may either be attracted by locally produced chemokines and thus migrate from the peripheral blood or develop *in situ*.[28]

Phenotypes and functions of dNK cells

In humans, the decidua is largely occupied by $CD56^{bright}CD16^{-}$ NK cells, which share a phenotype with the minor proportion of peripheral natural killer (pNK) cells. However, dNK cells are distinguishable from both subsets of pNK cells because they are less cytotoxic,[83] they are poor at killing target cells, and they express higher levels of cytokines, chemokines, and angiogenic factors compared to pNK cells.[84] dNK cells were previously suggested to be a unique NK cell subset that overexpresses several genes, such as granzyme A, C-type lectin-like receptors, NKG2C and NKG2E.[84] These cells resemble classic NK cells in a variety of ways; they express classic NK cell-activating receptors such as NKG2D, NKp30, NKp44 and NKp46,[81] and induce cytotoxicity against target cells after stimulation with IL-12/IL-15.[85] However, dNK cells possess many unique phenotypes and functions that are distinct from those of classic NK cells; these cells are also different from pNK cells in the expression of CD94, CD103, KIR receptors, CXCR3 and CCR7, among others. These cells also lack cytotoxic activity compared to pNK cells, but they retain the ability to secrete cytokines. These differences between dNK cells and pNK cells are induced by the specific decidual microenvironment. Successful implantation is ensured by the binding of dNK cells to MHC class I molecules *via* the expression of receptors specific for the MHC class I molecules HLA-E and HLA-G. Higher secretions of granulocyte macrophage colony-stimulating factor, macrophage inflammatory protein, colony stimulating factor 1, leukemia inhibitory factor, vascular endothelial growth factor C, angiopoietin-2 and placental growth factor are observed in dNK cells.[86] The unique functions of these cells are regulated by estrogen and progesterone.[81,87] In mice, dNK cells only appear during pregnancy with an activated, $B220^{+}CD11c^{+}$ phenotype and interact with *Dolichos biflorus* agglutinin.[88] It is believed that these recruited or resident NK cells are highly specialized for pregnancy-related functions.

NK cells in uterus immunotolerance

During early pregnancy, the maternal/fetal interface provides precise immune-regulatory mechanisms, protecting the fetus from attacks induced by the maternal immune system. Inflammatory responses may result in embryo loss during pregnancy, and therefore, the suppression of such responses is necessary to ensure a normal pregnancy.[82,89] A variety of mechanisms have been developed by the immune system to ensure a healthy pregnancy. For example, a Th2-biased response and the avoidance of Th1 cytotoxicity,[90] the inhibition of complement activation[91] and the expression of Fas ligand by trophoblast cells[92] are all essential for maternal–fetal tolerance. Eighteen years ago, Tom Wegmann[78] proposed that the survival of a fetus depends on the inhibition of a Th1 response by a Th2 response. For a long time, this hypothesis was well accepted and useful to studies on productive immunity. However, even though the process of pregnancy is biased toward a Th2 response, there is no strong evidence proving that such an inclination is necessary for a successful pregnancy.[93] Years later, it was found that the major immune interactions in the decidua contributing to immunotolerance during pregnancy occur between trophoblast cells and maternal NK cells rather than T cells. Trophoblast cells are considered to play a critical role during the process. These cells are located on top of the maternal/fetal interface and thus contact the maternal immune system directly. Trophoblast cells do not express antigens that induce T-cell graft rejection (such as HLA-A, HLA-B and HLA-D), but rather express special antigens such as HLA-C, HLA-E and HLA-G, which interact with decidual NK cells.[94] HLA-G-expressing trophoblast cells[95] are in direct contact with lymphocytes that largely exist in the uterus and thus inhibit maternal immune rejection during the early stages of pregnancy. The interaction between KIRs on NK cells and fetal HLA-C molecules is important for a successful pregnancy in humans, as this interaction determines the threshold for NK cell activation.[96] Hanna *et al.*[81] demonstrated that dNK cells may regulate trophoblast invasion and vascular remodeling by secreting a variety of vascular-regulating molecules. Maternal dNK cells control trophoblast invasion, while the invading trophoblast cells regulate the migration of immune cells, including $CD56^{+}$ NK cells, to deciduas by secreting chemokines such as stromal cell-derived factor and macrophage inflammatory protein-1.[94] Blois *et al.*[97] have found in their research that galectin-1 (Gal-1), an immunoregulatory glycan-binding protein, plays an important role in fetomaternal tolerance. Decreased expression or deficiency of Gal-1 leads to fetal loss, while treatment with Gal-1 restores tolerance. Gal-1 and progesterone work synergically to maintain tolerance during pregnancy. Decidual NK cells possess unique properties and promote immunotolerance and successful pregnancy through IFN-γ-secreting $CD56^{bright}CD27^{+}$ NK cells, which dampens the inflammatory response of T_H17 cells; this specific regulatory response is lost in patients with recurrent spontaneous abortions, suggesting a pivotal role for dNKs in maternal–fetal immunotolerance.[98] An appropriate balance between inhibitory (Stat3, programmed death-ligand 1 and TGF-β1) and stimulatory (CD80 and CD86) signals is also important in establishing immune privilege.[99–103] Immune cells such as regulatory T cells, DCs and macrophages all take part in the mechanisms underlying immunotolerance at the maternal/fetal interface. Murine NK cell and DC crosstalk is

modulated by trophoblast cells *in vitro* and promotes a tolerogenic microenvironment. Furthermore, the proliferation of uterine cells is also influenced by the interaction between NK cells and dendritic cells, which is modulated by trophoblast-induced signals, suggesting an important role for this interaction involving NK cells, DCs and trophoblasts in murine maternal–fetal immunotolerance.[104]

Decidual NK cells in uterine diseases

Pregnancy is a classic and ideal condition to study immunotolerance, during which the fetus will not be attacked or rejected by the maternal immune system but rather successfully accepted by the mother. During the process, precise regulation by the maternal immune system is critical for a normal pregnancy and fetal development.[81,105] The probability of failure is relatively high after implantation, reaching approximately 25%–40%. Although some of these failures are due to mutations or abnormalities in a gene, most are due to uncertain reasons.[82,85] Pregnancy may also be influenced by a variety of environmental factors such as infection, smoking, nutrition or stress. These factors not only influence pregnancy in a detrimental way but also endanger the fetus during development. Immunoregulatory mechanisms such as Gal-1, regulatory T cells and $CD56^{bright}Gal\text{-}1^{+}CD16^{-}$ NK cells are important for regulating stress-induced effects.[85,97,106] dNK cell-derived IFN-γ is necessary for vascular modifications. In a study by Croy *et al.*,[107] the authors demonstrated that mice lacking dNK cells or elements of the IFN-γ pathway show several abnormalities, including inadequate modification of the spiral arteries and reduced decidual cellularity.[107]

When two or more consecutive pregnancy losses occur before the twentieth week of gestation, this condition is identified as recurrent spontaneous miscarriage (RSM). Recurrent spontaneous miscarriages only occur in 1%–5% of women of reproductive age. It has been suggested that RSM may be caused by an imbalance of KIRs toward an activating status of NK cells.[108] TLR3 contributes to unexplained recurrent spontaneous miscarriage, as the expression of TLR3 in dNK cells is significantly higher in unexplained recurrent spontaneous miscarriage patients, indicating the possible role of TLR3 in the activation of dNK cells during early pregnancy.[109] In a study by Karami *et al.*,[110] the authors found that the percentage of $CD56^{dim}$ cells and the level of cytotoxicity of peripheral blood NK cells in recurrent spontaneous abortion patients and patients with *in vitro* fertility failure are significantly higher than in control patients, suggesting that $CD56^{dim}$ cells and NK cytotoxicity may be important factors contributing to recurrent spontaneous abortion and *in vitro* fertility.[110]

Preeclampsia is a hypertensive disorder characterized by increased blood pressure and proteinuria occurring at 20 weeks of gestation. Preeclampsia occurs in approximately 4% of all pregnancies and is one of the major causes of morbidity and mortality during early pregnancy.[111] Lower expression of vascular endothelial growth factor-C[112] and a lower percentage of Gal-1-expressing NK cells[113] in women are possibly related to preeclampsia. Preeclampsia patients carry abnormal natural cytotoxic receptors on peripheral blood $CD56^{+}$ NK cells during pregnancy. Lower expression of NKp46 may contribute to the type 1 shift of NK cells and may lead to preeclampsia, indicating the potential role of NKp46 in identifying the onset of preeclampsia.[111]

NK CELLS IN THE LUNG

The respiratory tract and lung are the two major components constituting the respiratory system, and the lung is the primary site of gas exchange. The lung acts as an important immunotolerant organ that, similarly to the gastrointestinal tract, is continuously challenged by potential antigens, and thus, a strong regulatory mechanism is required to minimize deleterious effects. There are two lungs, placed on either side of the body: the left lung is divided into two lobes, including an upper lobe and a lower lobe, while the right lung is divided into three lobes, including an upper lobe, a middle lobe and a lower lobe. 90% of immune cells in the lower lobes are alveolar macrophages, 10% of which are lymphocytes. The alveolar macrophages originate from bone marrow and proliferate locally in the lung. In a normal human body, alveolar macrophages constitute approximately 95% of all immune cells, lymphocytes constitute approximately 1%–4% and neutrophils constitute approximately 1%. In normal mice, alveolar macrophages constitute almost 100% of all immune cells. Pulmonary lymphocytes are primarily composed of T lymphocytes, B lymphocytes and NK cells, which are major effectors against external pathogens.[114] NK cells constitute approximately 1%–20% of all lymphocytes, and NKT cells constitute less than 1%. Recently, it was noted that NKT cells, M2 alveolar macrophages, regulatory T cells and regulatory NK cells play critical roles in the maintenance of lung immunotolerance.

Phenotypes and functions of pulmonary NK cells

In healthy mice, a higher frequency of NK cells is observed in the lungs than in other tissues, and a more mature phenotype is maintained, with higher expression of inhibitory receptors and lower expression of activating receptors, co-stimulatory molecules, and migration/adhesion-associated molecules.[9] Lung-derived NK cells differ from bone marrow-derived NK cell progenitors due to the expression of the NK cell receptor Ly49 family. More specifically, pulmonary NK cells express a variety of Ly49 receptors, while the majority of NK cells derived from bone marrow progenitors express $CD94^{-}NKG2$ heterodimers instead of Ly49 receptors, suggesting the possible influence of the lung microenvironment on the development of NK cells.[28] During a respiratory infection, pulmonary NK cells are activated and respond to infections swiftly by expressing functional molecules such as CD107a and IFN-γ.[9] The production of tumor-necrosis factor is enhanced by pulmonary NK cells after infection with *S. aureus*. In the lung, alveolar macrophage-derived IL-15 supports NK cell proliferation and concurrently, the engulfment of *S. aureus* by lung macrophages is enhanced by the presence of NK cells.[115] It has been suggested that the unique pulmonary environment modifies NK cells with lung-specific phenotypes.

NK cells in lung immunotolerance

The major function of the lung is to exchange gas between an organism and its external environment under normal conditions. The lung, being an organ that is open to the air, encounters constant stimulations by microorganisms and foreign substances from the external environment. Therefore, a fast response of innate immune cells is required, and concurrently, this local immune response must be regulated by specialized immune regulatory mechanisms to protect the host from self-attacks and ensure normal physical functions. Only a few hours after the occurence of inflammation in the lungs, large numbers of NK cells are recruited to the lungs and become activated as cytokine-secreting cells, primarily secreting IFN-γ.[116,117] When inflammation occurs, alveolar macrophages rapidly produce inhibitory cytokines such as IL-10 and TGF-β to inhibit inflammation,[118] thus inhibiting the cytotoxic functions of pulmonary NK cells, even though the NK cells remain able to bind to the target cells.[119,120] The functional abilities of pulmonary NK cells are restored upon stimulation with type I interferon.[121,122] Soluble factors, such as alveolar macrophage-derived prostaglandin,[123] surface active substances produced by type II alveolar epithelial cells[124] and TGF-β,[125] all contribute to the regulation of pulmonary NK cells. The activation of pulmonary NK cells is thus dependent on the balance between pro-inflammatory cytokines and regulatory cytokines.

Pulmonary NK cells in lung diseases

NK cells in the lung provide defense against viruses and endogenous pathogens. These cells are activated in response to infections and are recruited to the lung to aid in pathogen clearance. In both humans and mice, NK cells are recruited to the lung after the first few days of influenza virus infection,[126] and the depletion of NK cells during viral infection increases the chances of both morbidity and mortality.[127,128] Recruited pulmonary NK cells interact with epithelial cells, monocytes, dendritic cells and T cells on the virus and participate in protection against influenza virus, limiting early viral replication and promoting effective cytotoxic T lymphocyte responses. Respiratory syncytial virus is a major cause of bronchiolitis and pneumonia in children. Respiratory syncytial virus infection promotes the accumulation and activation of pulmonary NK cells highly expressing the activating receptors NKG2D and CD27 at the early stage of infection in BALB/c mice. Activated NK cells produce a large amount of IFN-γ, leading to severe acute immune injury in the lung, which is significantly attenuated by NK cell depletion.[129]

Currently, one-third of the global population is infected with *Mycobacterium tuberculosis*, and approximately two million people die each year due to tuberculosis. In most humans infected with tuberculosis, the infection remains latent, and a weak immune response inhibits the spread of the bacteria without inducing serious immunopathology. *M. tuberculosis* survives in macrophages, although the complete activation of macrophages kills the bacteria. The induction of a Th1 response and the production of IFN-γ are critical for protection against tuberculosis.[130,131] The production of IFN-γ by cells is greater when stimulated with *M. tuberculosis*, and thus, IFN-γ level is a potential indicator for the severity of the disease.[132] NK cells isolated from the peripheral blood of tuberculosis patients show reduced expression of NKp46 and cytotoxicity due to the inhibitory effect of IL-10 and monocytes.[132–134] Glutathione, a tripeptide protecting cells against oxidizing agents, free radicals and reactive oxygen intermediates in their reduced forms, is significantly decreased in red blood cells and peripheral blood mononuclear cells isolated from individuals with active tuberculosis.[135] When used in combination with IL-2 and IL-12, glutathione enhances NK cell functions in controlling *M. tuberculosis* infection,[133] possibly by increasing the expression of NK cytotoxic ligands (such as FasL and CD40L).[136] Dhiman *et al.*[137] found in their research that $NK1.1^+$ cells and IL-22 contribute to the efficacy of vaccine-induced protective immunity against *M. tuberculosis*.

Three hundred million people are infected with asthma worldwide, and most of these cases are related to allergies to environmental antigens. The accumulation of extracellular matrix and the enlargement of muscle and goblet cells lead to thickening of the airways, contributing to the abnormal respiratory functions observed in asthma patients.[138] The inflammation and pathology of asthma are induced by the production of Th2 cytokines (such as IL-4, IL-5, IL-9 and IL-13).[139,140] The activity of peripheral blood mononuclear cell-derived NK cells increases in patients with asthma, and the proportion of $IL\text{-}4^+CD56^+$ NK2 cells in peripheral blood mononuclear cells from asthmatic patients is higher than that from healthy individuals, leading to the hypothesis that the NK2 cell subset contributes to type 2 cytokine-biased status in patients with asthma.[141] Interestingly, murine NK cells activated by asthma and the subsequent activation of antigen-specific $CD8^+$ T cells together eliminate influenza virus-infected cells.[142]

CONCLUSIONS

The study of NK cells in immune tolerance in mice and humans is just beginning. Recent progress indicates that NK cells play an important role in the mechanisms underlying the induction and maintenance of immunotolerance, including but not limited to interactions with different cell types, the secretion of a variety of cytokines, and interactions between inhibitory receptors and their corresponding ligands. Nonetheless, the detailed underlying molecular mechanisms are far more complex than expected. Once we understand the role of NK cells in these immunotolerant organs, the next challenge will be to design future interventions to combat organ-specific diseases by using NK cell manipulations.

1 Martini A, Burgio GR. Tolerance and auto-immunity: 50 years after Burnet. *Eur J Pediatr* 1999; **158**: 769–775.
2 Bach JF. Induction of immunological tolerance using monoclonal antibodies: applications to organ transplantation and autoimmune disease. *C R Biol* 2006; **329**: 260–262.

3 Sakaguchi S, Powrie F, Ransohoff RM. Re-establishing immunological self-tolerance in autoimmune disease. *Nat Med* 2012; **18**: 54–58.
4 Kang TH, Mao CP, La V, Chen A, Hung CF, Wu TC. Innovative DNA vaccine to break immune tolerance against tumor self-antigen. *Hum Gene Ther* 2012; **24**: 181–188.
5 Veenbergen S, Samsom JN. Maintenance of small intestinal and colonic tolerance by IL-10-producing regulatory T cell subsets. *Curr Opin Immunol* 2012; **24**: 269–276.
6 Beland K, Lapierre P, Djilali-Saiah I, Alvarez F. Liver restores immune homeostasis after local inflammation despite the presence of autoreactive T cells. *PLoS ONE* 2012; **7**: e48192.
7 Tiegs G, Lohse AW. Immune tolerance: what is unique about the liver. *J Autoimmun* 2010; **34**: 1–6.
8 Gregoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E *et al*. The trafficking of natural killer cells. *Immunol Rev* 2007; **220**: 169–182.
9 Wang J, Li F, Zheng M, Sun R, Wei H, Tian Z. Lung natural killer cells in mice: phenotype and response to respiratory infection. *Immunology* 2012; **137**: 37–47.
10 Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal–fetal tolerance. *Nat Immunol* 2006; **7**: 241–246.
11 Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med* 2000; **343**: 338–344.
12 Murphy K, Travers P, Walport M, Janeway C. *Janeway's Immunobiology*. 8th ed. New York: Garland Science, 2012.
13 Cerwenka A, Lanier LL. Ligands for natural killer cell receptors: redundancy or specificity. *Immunol Rev* 2001; **181**: 158–169.
14 Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. *Science* 2004; **306**: 1517–1519.
15 Nguyen-Pham TN, Yang DH, Nguyen TA, Lim MS, Hong CY, Kim MH *et al*. Optimal culture conditions for the generation of natural killer cell-induced dendritic cells for cancer immunotherapy. *Cell Mol Immunol* 2012; **9**: 45–53.
16 Luevano M, Madrigal A, Saudemont A. Generation of natural killer cells from hematopoietic stem cells *in vitro* for immunotherapy. *Cell Mol Immunol* 2012; **9**: 310–320.
17 Cheng M, Zhang J, Jiang W, Chen Y, Tian Z. Natural killer cell lines in tumor immunotherapy. *Front Med* 2012; **6**: 56–66.
18 Caligiuri MA. Human natural killer cells. *Blood* 2008; **112**: 461–469.
19 Inngjerdingen M, Kveberg L, Naper C, Vaage JT. Natural killer cell subsets in man and rodents. *Tissue Antigens* 2011; **78**: 81–88.
20 Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001; **22**: 633–640.
21 Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. *Blood* 2010; **115**: 2167–2176.
22 Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol* 2008; **9**: 495–502.
23 Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with $CD8^+$ T cells. *Nat Rev Immunol* 2011; **11**: 645–657.
24 Hayakawa Y, Smyth MJ. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *J Immunol* 2006; **176**: 1517–1524.
25 Ndhlovu LC, Lopez-Verges S, Barbour JD, Jones RB, Jha AR, Long BR *et al*. Tim-3 marks human natural killer cell maturation and suppresses cell-mediated cytotoxicity. *Blood* 2012; **119**: 3734–3743.
26 Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA *et al*. Expression patterns of NKG2A, KIR, and CD57 define a process of $CD56^{dim}$ NK-cell differentiation uncoupled from NK-cell education. *Blood* 2010; **116**: 3853–3864.
27 Chan A, Hong DL, Atzberger A, Kollnberger S, Filer AD, Buckley CD *et al*. $CD56^{bright}$ human NK cells differentiate into $CD56^{dim}$ cells: role of contact with peripheral fibroblasts. *J Immunol* 2007; **179**: 89–94.
28 Shi FD, Ljunggren HG, La Cava A, van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011; **11**: 658–671.
29 Kim S, Iizuka K, Kang HS, Dokun A, French AR, Greco S *et al*. *In vivo* developmental stages in murine natural killer cell maturation. *Nat Immunol* 2002; **3**: 523–528.
30 Chiossone 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**: 5488–5496.
31 Ehlers M, Papewalis C, Stenzel W, Jacobs B, Meyer KL, Deenen R *et al*. Immunoregulatory natural killer cells suppress autoimmunity by down-regulating antigen-specific $CD8^+$ T cells in mice. *Endocrinology* 2012; **153**: 4367–4379.
32 Carrega P, Ferlazzo G. Natural killer cell distribution and trafficking in human tissues. *Front Immunol* 2012; **3**: 347.
33 Tomasello E, Yessaad N, Gregoire E, Hudspeth K, Luci C, Mavilio D *et al*. Mapping of $NKp46^+$ cells in healthy human lymphoid and non-lymphoid tissues. *Front Immunol* 2012; **3**: 344.
34 Zhao E, Xu H, Wang L, Kryczek I, Wu K, Hu Y *et al*. Bone marrow and the control of immunity. *Cell Mol Immunol* 2012; **9**: 11–19.
35 Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. *Annu Rev Immunol* 2004; **22**: 405–429.
36 Thapa M, Kuziel WA, Carr DJ. Susceptibility of CCR5-deficient mice to genital herpes simplex virus type 2 is linked to NK cell mobilization. *J Virol* 2007; **81**: 3704–3713.
37 Hokeness KL, Kuziel WA, Biron CA, Salazar-Mather TP. Monocyte chemoattractant protein-1 and CCR2 interactions are required for IFN-alpha/beta-induced inflammatory responses and antiviral defense in liver. *J Immunol* 2005; **174**: 1549–1556.
38 Salazar-Mather TP, Orange JS, Biron CA. Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1alpha (MIP-1alpha)-dependent pathways. *J Exp Med* 1998; **187**: 1–14.
39 Gregoire C, Cognet C, Chasson L, Coupet CA, Dalod M, Reboldi A *et al*. Intrasplenic trafficking of natural killer cells is redirected by chemokines upon inflammation. *Eur J Immunol* 2008; **38**: 2076–2084.
40 Beider K, Nagler A, Wald O, Franitza S, Dagan-Berger M, Wald H *et al*. Involvement of CXCR4 and IL-2 in the homing and retention of human NK and NK T cells to the bone marrow and spleen of NOD/SCID mice. *Blood* 2003; **102**: 1951–1958.
41 Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L *et al*. Natural killer cell trafficking *in vivo* requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* 2007; **8**: 1337–1344.
42 Eksteen B, Afford SC, Wigmore SJ, Holt AP, Adams DH. Immune-mediated liver injury. *Semin Liver Dis* 2007; **27**: 351–366.
43 Kita H, Mackay IR, van de Water J, Gershwin ME. The lymphoid liver: considerations on pathways to autoimmune injury. *Gastroenterology* 2001; **120**: 1485–1501.
44 Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. *Hepatology* 2008; **47**: 729–736.
45 Yamagiwa S, Kamimura H, Ichida T. Natural killer cell receptors and their ligands in liver diseases. *Med Mol Morphol* 2009; **42**: 1–8.
46 Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA* 2009; **106**: 1915–1919.
47 McIntyre KW, Welsh RM. Accumulation of natural killer and cytotoxic T large granular lymphocytes in the liver during virus infection. *J Exp Med* 1986; **164**: 1667–1681.
48 Liaskou E, Wilson DV, Oo YH. Innate immune cells in liver inflammation. *Mediators Inflamm* 2012; **2012**: 949157.
49 Hata K, Zhang XR, Iwatsuki S, van Thiel DH, Herberman RB, Whiteside TL. Isolation, phenotyping, and functional analysis of lymphocytes from human liver. *Clin Immunol Immunopathol* 1990; **56**: 401–419.
50 Norris S, Collins C, Doherty DG, Smith F, McEntee G, Traynor O *et al*. Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. *J Hepatol* 1998; **28**: 84–90.
51 Gordon SM, Chaix J, Rupp LJ, Wu J, Madera S, Sun JC *et al*. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity* 2012; **36**: 55–67.
52 Jiang X, Chen Y, Peng H, Tian Z. Single line or parallel lines: NK cell differentiation driven by T-bet and Eomes. *Cell Mol Immunol* 2012; **9**: 193–194.

53 Takeda K, Cretney E, Hayakawa Y, Ota T, Akiba H, Ogasawara K *et al*. TRAIL identifies immature natural killer cells in newborn mice and adult mouse liver. *Blood* 2005; **105**: 2082–2089.
54 Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005; **23**: 225–274.
55 Lanier LL. Natural killer cell receptor signaling. *Curr Opin Immunol* 2003; **15**: 308–314.
56 Ishiyama K, Ohdan H, Ohira M, Mitsuta H, Arihiro K, Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. *Hepatology* 2006; **43**: 362–372.
57 Subleski JJ, Hall VL, Back TC, Ortaldo JR, Wiltrout RH. Enhanced antitumor response by divergent modulation of natural killer and natural killer T cells in the liver. *Cancer Res* 2006; **66**: 11005–11012.
58 Krueger PD, Lassen MG, Qiao H, Hahn YS. Regulation of NK cell repertoire and function in the liver. *Crit Rev Immunol* 2011; **31**: 43–52.
59 Jinushi M, Takehara T, Tatsumi T, Yamaguchi S, Sakamori R, Hiramatsu N *et al*. Natural killer cell and hepatic cell interaction *via* NKG2A leads to dendritic cell-mediated induction of CD4 CD25 T cells with PD-1-dependent regulatory activities. *Immunology* 2007; **120**: 73–82.
60 Jinushi M, Takehara T, Tatsumi T, Kanto T, Miyagi T, Suzuki T *et al*. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. *J Immunol* 2004; **173**: 6072–6081.
61 Tu Z, Bozorgzadeh A, Pierce RH, Kurtis J, Crispe IN, Orloff MS. TLR-dependent cross talk between human Kupffer cells and NK cells. *J Exp Med* 2008; **205**: 233–244.
62 Lassen MG, Lukens JR, Dolina JS, Brown MG, Hahn YS. Intrahepatic IL-10 maintains NKG2A$^+$Ly49$^-$ liver NK cells in a functionally hyporesponsive state. *J Immunol* 2010; **184**: 2693–2701.
63 Spaan M, Janssen HL, Boonstra A. Immunology of hepatitis C virus infections. *Best Pract Res Clin Gastroenterol* 2012; **26**: 391–400.
64 Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51–68.
65 Shi CC, Tjwa ET, Biesta PJ, Boonstra A, Xie Q, Janssen HL *et al*. Hepatitis B virus suppresses the functional interaction between natural killer cells and plasmacytoid dendritic cells. *J Viral Hepat* 2012; **19**: e26–e33.
66 Sun C, Fu B, Gao Y, Liao X, Sun R, Tian Z *et al*. TGF-beta1 down-regulation of NKG2D/DAP10 and 2B4/SAP expression on human NK cells contributes to HBV persistence. *PLoS Pathog* 2012; **8**: e1002594.
67 Wu L, Zhang C, Zhang J. HMBOX1 negatively regulates NK cell functions by suppressing the NKG2D/DAP10 signaling pathway. *Cell Mol Immunol* 2011; **8**: 433–440.
68 Dong Z, Wei H, Sun R, Hu Z, Gao B, Tian Z. Involvement of natural killer cells in PolyI:C-induced liver injury. *J Hepatol* 2004; **41**: 966–973.
69 Chen Y, Sun R, Jiang W, Wei H, Tian Z. Liver-specific HBsAg transgenic mice are over-sensitive to Poly(I:C)-induced liver injury in NK cell- and IFN-gamma-dependent manner. *J Hepatol* 2007; **47**: 183–190.
70 Yin W, Xu L, Sun R, Wei H, Tian Z. Interleukin-15 suppresses hepatitis B virus replication *via* IFN-beta production in a C57BL/6 mouse model. *Liver Int* 2012; **32**: 1306–1314.
71 Han Q, Zhang C, Zhang J, Tian Z. Involvement of activation of PKR in HBx-siRNA-mediated innate immune effects on HBV inhibition. *PLoS ONE* 2011; **6**: e27931.
72 Han Q, Zhang C, Zhang J, Tian Z. Reversal of hepatitis B virus-induced immune tolerance by an immunostimulatory 3p-HBx-siRNAs in a retinoic acid inducible gene I-dependent manner. *Hepatology* 2011; **54**: 1179–1189.
73 Huang M, Sun R, Wei H, Tian Z. Simultaneous knockdown of multiple ligands of innate receptor NKG2D high efficiently prevents NK cell-mediated fulminant hepatitis. *Hepatology* 2013; **57**: 277–288.
74 Kramer B, Korner C, Kebschull M, Glassner A, Eisenhardt M, Nischalke HD *et al*. Natural killer p46High expression defines a natural killer cell subset that is potentially involved in control of hepatitis C virus replication and modulation of liver fibrosis. *Hepatology* 2012; **56**: 1201–1213.
75 Golden-Mason L, Cox AL, Randall JA, Cheng L, Rosen HR. Increased natural killer cell cytotoxicity and NKp30 expression protects against hepatitis C virus infection in high-risk individuals and inhibits replication in vitro. *Hepatology* 2010; **52**: 1581–1589.
76 Nattermann J, Feldmann G, Ahlenstiel G, Langhans B, Sauerbruch T, Spengler U. Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C. *Gut* 2006; **55**: 869–877.
77 Wei H, Wang H, Tian Z, Sun R. Activation of natural killer cells inhibits liver regeneration in toxin-induced liver injury model in mice *via* a tumor necrosis factor-alpha-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G275–282.
78 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; **14**: 353–356.
79 Lash GE, Robson SC, Bulmer JN. Review: functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. *Placenta* 2010; **31** Suppl: S87–S92.
80 Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, Aplin JD *et al*. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. *FASEB J* 2012; **26**: 4876–4885.
81 Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S *et al*. Decidual NK cells regulate key developmental processes at the human fetal–maternal interface. *Nat Med* 2006; **12**: 1065–1074.
82 Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy–an inflammatory view. *Trends Immunol* 2006; **27**: 399–404.
83 Deniz G, Christmas SE, Brew R, Johnson PM. Phenotypic and functional cellular differences between human CD3-decidual and peripheral blood leukocytes. *J Immunol* 1994; **152**: 4255–4261.
84 Manaster I, Mandelboim O. The unique properties of uterine NK cells. *Am J Reprod Immunol* 2010; **63**: 434–444.
85 Hanna J, Mandelboim O. When killers become helpers. *Trends Immunol* 2007; **28**: 201–206.
86 Higuma-Myojo S, Sasaki Y, Miyazaki S, Sakai M, Siozaki A, Miwa N *et al*. Cytokine profile of natural killer cells in early human pregnancy. *Am J Reprod Immunol* 2005; **54**: 21–29.
87 Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F *et al*. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003; **198**: 1201–1212.
88 Zhang J, Chen Z, Smith GN, Croy BA. Natural killer cell-triggered vascular transformation: maternal care before birth? *Cell Mol Immunol* 2011; **8**: 1–11.
89 Karimi K, Arck PC. Natural killer cells: keepers of pregnancy in the turnstile of the environment. *Brain Behav Immun* 2010; **24**: 339–347.
90 Mjosberg J, Berg G, Jenmalm MC, Ernerudh J. FOXP3$^+$ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. *Biol Reprod* 2010; **82**: 698–705.
91 Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. *Science* 2000; **287**: 498–501.
92 Makrigiannakis A, Zoumakis E, Kalantaridou S, Coutifaris C, Margioris AN, Coukos G *et al*. Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. *Nat Immunol* 2001; **2**: 1018–1024.
93 Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O, Martal J. TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion reexamining the TH1/TH2 paradigm. *Int Arch Allergy Immunol* 2004; **134**: 93–119.
94 Hiby SE, Walker JJ, O'Shaughnessy K M, Redman CW, Carrington M, Trowsdale J *et al*. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 2004; **200**: 957–965.

95 Moffett A, Loke C. Immunology of placentation in eutherian mammals. *Nat Rev Immunol* 2006; **6**: 584–594.
96 Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, Masters L *et al*. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. *J Immunol* 2008; **181**: 39–46.
97 Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R *et al*. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 2007; **13**: 1450–1457.
98 Fu B, Li X, Sun R, Tong X, Ling B, Tian Z *et al*. Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal–fetal interface. *Proc Natl Acad Sci USA* 2012; in press.
99 Zhu XY, Zhou YH, Wang MY, Jin LP, Yuan MM, Li DJ. Blockade of CD86 signaling facilitates a Th2 bias at the maternal–fetal interface and expands peripheral $CD4^+CD25^+$ regulatory T cells to rescue abortion-prone fetuses. *Biol Reprod* 2005; **72**: 338–345.
100 Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H *et al*. A critical role for the programmed death ligand 1 in fetomaternal tolerance. *J Exp Med* 2005; **202**: 231–237.
101 Poehlmann TG, Busch S, Mussil B, Winzer H, Weinert J, Mebes I *et al*. The possible role of the Jak/STAT pathway in lymphocytes at the fetomaternal interface. *Chem Immunol Allergy* 2005; **89**: 26–35.
102 D'Addio F, Riella LV, Mfarrej BG, Chabtini L, Adams LT, Yeung M *et al*. The link between the PDL1 costimulatory pathway and Th17 in fetomaternal tolerance. *J Immunol* 2011; **187**: 4530–4541.
103 Ayatollahi M, Geramizadeh B, Samsami A. Transforming growth factor beta-1 influence on fetal allografts during pregnancy. *Transplant Proc* 2005; **37**: 4603–4604.
104 Blois SM, Barrientos G, Garcia MG, Orsal AS, Tometten M, Cordo-Russo RI *et al*. Interaction between dendritic cells and natural killer cells during pregnancy in mice. *J Mol Med (Berl)* **86**: 2008; 837–852.
105 Karimi K, Blois SM, Arck PC. The upside of natural killers. *Nat Med* 2008; **14**: 1184–1185.
106 Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; **5**: 266–271.
107 Croy BA, van den Heuvel MJ, Borzychowski AM, Tayade C. Uterine natural killer cells: a specialized differentiation regulated by ovarian hormones. *Immunol Rev* 2006; **214**: 161–185.
108 Ozturk OG, Sahin G, Karacor ED, Kucukgoz U. Evaluation of KIR genes in recurrent miscarriage. *J Assist Reprod Genet* 2012; **29**: 933–938.
109 Bao SH, Shuai W, Tong J, Wang L, Chen P, Sun J. Increased expression of Toll-like receptor 3 in decidual natural killer cells of patients with unexplained recurrent spontaneous miscarriage. *Eur J Obstet Gynecol Reprod Biol* 2012; **165**: 326–330.
110 Karami N, Boroujerdnia MG, Nikbakht R, Khodadadi A. Enhancement of peripheral blood $CD56^{dim}$ cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or *in vitro* fertilization failure. *J Reprod Immunol* 2012; **95**: 87–92.
111 Fukui A, Yokota M, Funamizu A, Nakamua R, Fukuhara R, Yamada K *et al*. Changes of NK cells in preeclampsia. *Am J Reprod Immunol* 2012; **67**: 278–286.
112 Dunk C, Ahmed A. Expression of VEGF-C and activation of its receptors VEGFR-2 and VEGFR-3 in trophoblast. *Histol Histopathol* 2001; **16**: 359–375.
113 Molvarec A, Blois SM, Stenczer B, Toldi G, Tirado-Gonzalez I, Ito M *et al*. Peripheral blood galectin-1-expressing T and natural killer cells in normal pregnancy and preeclampsia. *Clin Immunol* 2011; **139**: 48–56.
114 Zhang J, Dong Z, Zhou R, Luo D, Wei H, Tian Z. Isolation of lymphocytes and their innate immune characterizations from liver, intestine, lung and uterus. *Cell Mol Immunol* 2005; **2**: 271–280.
115 Small CL, McCormick S, Gill N, Kugathasan K, Santosuosso M, Donaldson N *et al*. NK cells play a critical protective role in host defense against acute extracellular *Staphylococcus aureus* bacterial infection in the lung. *J Immunol* 2008; **180**: 5558–5568.
116 Morrison BE, Park SJ, Mooney JM, Mehrad B. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *J Clin Invest* 2003; **112**: 1862–1870.
117 Schuster M, Tschernig T, Krug N, Pabst R. Lymphocytes migrate from the blood into the bronchoalveolar lavage and lung parenchyma in the asthma model of the brown Norway rat. *Am J Respir Crit Care Med* 2000; **161**: 558–566.
118 Wissinger E, Goulding J, Hussell T. Immune homeostasis in the respiratory tract and its impact on heterologous infection. *Semin Immunol* 2009; **21**: 147–155.
119 Robinson BW, Pinkston P, Crystal RG. Natural killer cells are present in the normal human lung but are functionally impotent. *J Clin Invest* 1984; **74**: 942–950.
120 Weissman DN, deShazo RD, Banks DE. Modulation of natural killer cell function by human alveolar macrophages. *J Allergy Clin Immunol* 1986; **78**: 571–577.
121 Bordignon C, Villa F, Allavena P, Introna M, Biondi A, Avallone R *et al*. Inhibition of natural killer activity by human bronchoalveolar macrophages. *J Immunol* 1982; **129**: 587–591.
122 Roth MD, Golub SH. Inhibition of lymphokine-activated killer cell function by human alveolar macrophages. *Cancer Res* 1989; **49**: 4690–4695.
123 Lauzon W, Lemaire I. Alveolar macrophage inhibition of lung-associated NK activity: involvement of prostaglandins and transforming growth factor-beta 1. *Exp Lung Res* 1994; **20**: 331–349.
124 Wilsher ML, Hughes DA, Haslam PL. Immunomodulatory effects of pulmonary surfactant on natural killer cell and antibody-dependent cytotoxicity. *Clin Exp Immunol* 1988; **74**: 465–470.
125 Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005; **6**: 600–607.
126 Ennis FA, Meager A, Beare AS, Qi YH, Riley D, Schwarz G *et al*. Interferon induction and increased natural killer-cell activity in influenza infections in man. *Lancet* 1981; **2**: 891–893.
127 Nogusa S, Ritz BW, Kassim SH, Jennings SR, Gardner EM. Characterization of age-related changes in natural killer cells during primary influenza infection in mice. *Mech Ageing Dev* 2008; **129**: 223–230.
128 Stein-Streilein J, Guffee J. *In vivo* treatment of mice and hamsters with antibodies to asialo GM1 increases morbidity and mortality to pulmonary influenza infection. *J Immunol* 1986; **136**: 1435–1441.
129 Li F, Zhu H, Sun R, Wei H, Tian Z. Natural killer cells are involved in acute lung immune injury caused by respiratory syncytial virus infection. *J Virol* 2012; **86**: 2251–2258.
130 Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. *J Allergy Clin Immunol* 2008; **122**: 1043–1051; quiz 1052–1043.
131 Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* 1993; **178**: 2243–2247.
132 Schierloh P, Yokobori N, Aleman M, Musella RM, Beigier-Bompadre M, Saab MA *et al*. Increased susceptibility to apoptosis of $CD56^{dim}CD16^+$ NK cells induces the enrichment of IFN-gamma-producing CD56bright cells in tuberculous pleurisy. *J Immunol* 2005; **175**: 6852–6860.
133 Millman AC, Salman M, Dayaram YK, Connell ND, Venketaraman V. Natural killer cells, glutathione, cytokines, and innate immunity against Mycobacterium tuberculosis. *J Interferon Cytokine Res* 2008; **28**: 153–165.
134 Vankayalapati R, Wizel B, Weis SE, Safi H, Lakey DL, Mandelboim O *et al*. The NKp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2002; **168**: 3451–3457.
135 Venketaraman V, Millman A, Salman M, Swaminathan S, Goetz M, Lardizabal A *et al*. Glutathione levels and immune responses in tuberculosis patients. *Microb Pathog* 2008; **44**: 255–261.
136 Guerra C, Johal K, Morris D, Moreno S, Alvarado O, Gray D *et al*. Control of *Mycobacterium tuberculosis* growth by activated natural killer cells. *Clin Exp Immunol* 2012; **168**: 142–152.

137 Dhiman R, Periasamy S, Barnes PF, Jaiswal AG, Paidipally P, Barnes AB *et al*. NK1.1$^+$ cells and IL-22 regulate vaccine-induced protective immunity against challenge with *Mycobacterium tuberculosis*. *J Immunol* 2012; **189**: 897–905.
138 Lloyd CM, Robinson DS. Allergen-induced airway remodelling. *Eur Respir J* 2007; **29**: 1020–1032.
139 Jira M, Antosova E, Vondra V, Strejcek J, Mazakova H, Prazakova J. Natural killer and interleukin-2 induced cytotoxicity in asthmatics. I. Effect of acute antigen-specific challenge. *Allergy* 1988; **43**: 294–298.
140 Timonen T, Stenius-Aarniala B. Natural killer cell activity in asthma. *Clin Exp Immunol* 1985; **59**: 85–90.
141 Wei H, Zhang J, Xiao W, Feng J, Sun R, Tian Z. Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. *J Allergy Clin Immunol* 2005; **115**: 841–847.
142 Ishikawa H, Sasaki H, Fukui T, Fujita K, Kutsukake E, Matsumoto T. Mice with asthma are more resistant to influenza virus infection and NK cells activated by the induction of asthma have potentially protective effects. *J Clin Immunol* 2012; **32**: 256–267.