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Critical role of Toll-like receptor signaling in NK cell activation

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Toll-like receptors (TLRs) and NK cell receptors are the most important receptor superfamilies in innate immunity. TLRs act as the sensor of external pathogens, while NK cells detect alterations in endogenous protein expression on target cells through activating and inhibitory receptors. Accumulating data has demonstrated that TLRs and NK cell receptors can coordinate and regulate each other during immune responses, which contributes to the initiation of innate response and the priming of adaptive responses. TLRs can activate NK cell function directly or with the help of accessory cells in a cytokine or cell-to-cell contact dependent manner. More understanding of the recognition of innate receptors and interactions between them may provide important insights into the design of effective strategies to combat tumor and microbial infections. In this review, we summarize how TLRs and NK cells discriminate the self or non-self components respectively. And importantly, we pay more attention to the role of TLR signaling in induction of NK cell activation, responses and the crosstalk between them.

Toll-like receptor, natural killer (NK) cells, NK cell receptor, dendritic cells, crosstalk

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The innate immune system in mammalian has evolved a universal and conservative defense line against the invasion from exotic micro-organisms and autogenous transformed objects. The early concept about innate immunity believed that it can nonspecifically eliminate microbes because innate immune cells exert their function without pre-sensitization; however, the discovery of Toll-like receptors (TLRs) in the mid-1990s shows that pathogen recognization by the innate immune system actually relies on germline-encoded pattern-receptors (PRRs) that have evolved to detect components of foreign pathogens, also known as pathogen-associated molecular patterns (PAMPs) [1–3]. So far four classes of PRRs have been identified: TLRs, RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin receptors (CLRs) [4]. Among them, TLRs have the most greatly advanced understanding of how they recognize conserved structures in pathogens and trigger innate immune response to further prime antigen-specific adaptive immunity.

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Natural killer (NK) cells are innate effector cells that play a critical role in immunosurveillance by eliminating virally infected and transformed cells via generating and secreting cytolytic granules or cytokines, as well as expressing several activating and inhibitory receptors on the surface [5]. Equally as the essential components of the innate immune system, NK cells and TLRs bear unique mechanism to sense dangerous signals and exert immune effect. On the other hand, they coordinate with each other to control the invasion of acataleptic challenges [6,7]. In this review, we survey how TLRs and NK cells discriminate the self and non-self components respectively. And importantly, we pay more attention to the present knowledge of how TLRs affect the function of NK cells and the crosstalk between them.

1 TLRs—The sensors of external stimulator

TLRs, acting as the sensors of external pathogens, are one of the most important PRRs. They are expressed on almost

all immune cells, such as DCs, monocytes, macrophages, T cell, B cell, NK cells, and even on non-immune cells such as fibroblasts, epithelial cells and some tumor cells [8]. Although TLRs are evolutionarily conserved from the worm *Caenorhabditis elegans* to mammals, the expression of TLRs is not stable but constantly variable in response to environmental stress such as virus or bacteria infection [9]. TLRs generally detect microbes-related components which are usually called PAMPs to activate transcriptional programs that initiate innate immune responses and orchestrate acquired antigen-specific resistance to exogenetic insults [10]. Stimulation of TLRs by these microbial products, such as lipids, lipoproteins, lipopolysaccharide (LPS) and nucleic acids, leads to the activation of signaling pathways that result in the up-regulation of antimicrobial genes and the secretion of some inflammatory cytokines or IFNs to start up the defense responses [11,12].

So far, over ten kinds of functional TLRs have been elucidated in mammals, including 10 in humans and 12 in mice respectively, with TLR1-TLR9 being conserved in both species [13]. These TLRs are roughly divided into two subfamilies depending on their cellular localization and respective PAMP ligands. One group is composed of TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11, which are expressed on cell surface and recognize mainly microbial lipids components such as LPS and lipoproteins; the other group mainly includes TLR3, TLR7, TLR8 and TLR9, which are expressed exclusively in intracellular vesicles such as endoplasmic reticulum (ER), endosomes, lysosomes and endolysosomes, where they recognize microbial nucleic acids components such as double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), unmethylated-CpG DNA motifs respectively [14,15]. So it is essential for these "nucleic acid-sensing" TLRs to internalize to the endosome before signaling is possible. Study of mice deficient with each TLR has demonstrated that each TLR has a distinct deflection in terms of PAMP recognition to perform their duty to counteract the extraneous invasion.

For example, TLR4 stimulation with LPS is the main force to resist the Gram-negative bacterial infection. For recognition, TLR4 needs the presence of complex from CD14 and MD-2 in the assist of LPS-binding protein (LBP) [16]. For infection of Gram-positive bacteria, TLR2, rather than TLR4, plays a major role to clear away the pathogenic bacteria due to the deficiency of LPS. TLR2 forms heterodimers with TLR1 or TLR6 to recognize its ligands, the microbial components from Gram-positive bacterial, fungi, parasites and virus, including LTA, lipoproteins, and PG [17,18]. Moreover, some co-receptors on the cell surface can assist PAMP recognition of TLR2. These include CD36, which acts together with the TLR2-TLR6 heterodimer to mediate the sensing of some TLR2 agonists [19]; and dectin-1, a C-type lectin that binds to fungus β-glucan and induces its internalization [20]. In addition to recognizing compounds derived from Gram-positive bacteria, TLR2 also binds LPS in the presence of LBP and CD14 and induces nuclear factor (NF)-κB activation. LPS treatment has been demonstrated to enhance the oligomerization of TLR2. Concomitant with receptor oligomerization, the IL-1R-associated kinase is recruited to the TLR2 complex [21]. Viral structural components, including viral DNA, dsRNA, ssRNA, can activate "nucleic acid-sensing TLRs", including TLR3, TLR7, TLR8 and TLR9 to eliminate the viral-invasion cells via inducing type I IFN and antiviral factors production [22].

Structurally, TLRs are type I trans-membrane proteins characterized by the extracellular domains containing leucine-rich repeats that mediate the recognition of PAMPs, transmembrane domains, and the intracellular Toll-interleukin 1 (IL-1) receptor (TIR) domains [23]. After binding with corresponding ligands, TLRs dimerize and undergo conformational changes required for the recruitment of TIR-domain-containing adaptor molecules to the TIR domain of the TLRs. Although with structural similarity, accumulating evidence suggests that the signaling pathways associated with each TLR are not identical, and therefore, result in different biological responses. For example, activation of TLR3 and TLR4 signaling pathway generates both type I IFN and inflammatory cytokine responses whereas TLR1/TLR2, TLR2/TLR6 and TLR5 activation induces mainly inflammatory cytokines such as IL-6, TNF-α and IL-8 [24].

2 Recognition of NK cell receptors: Discrimination of "self" and "non-self"

NK cells participate in immunosurveillance by directly eliminating virally infected or transformed cells through killing target cells, and priming adaptive lymphocytes through secretion of cytokines, such as IL-12 and IFN-γ [25]. NK cells, identical to other lymphocytes, have the potential to attack autologous cells. However, there are unique mechanisms to ensure that NK cells can keep the "self-tolerance" to normal self cells but are standby to danger signals. The NK cell function relies upon signals from activation and inhibitory receptors whose ligands are largely endogenous, that is, encoded by host genes. During effector responses, NK cells may detect alterations in endogenous protein expression on target cells by integrating signals from the balance between inhibitory and activating receptors [26,27].

2.1 Missing-self recognition by NK cells or NK cell self-tolerance

NK cells are lymphocytes that derived from bone-marrow precursors. During the development of NK cells, NK-cell precursors (NKp) generated from multipotent haematopoietic precursors develop into immature NK cells, which express NK-cell-specific markers partially, including similar levels of NKp46, NKG2D, CD11b and CD16, and express perforin and granzyme B both in mice and humans [28,29].

With the further differentiation of immature NK cells, MHC-class-I-binding receptors, also known as the inhibitory receptors, are expressed in a multiplex and omnipresent manner. This process is also referred to as "NK-cell education" [28,30]. So far the MHC class I-specific inhibitory receptors include the killer cell immunoglobulin-like receptors (KIRs) in humans, the lectin-like Ly49 dimers in mouse, and the lectin-like CD94-NKG2A heterodimers in both species [30,31]. The education process generates NK cells that are tolerant to self and obtain the capacity to response to stimulation with regard to their activating receptors. So the recognition and lysis of MHC-class-I^{low} or MHC-class-I defection target cells by NK cells is aptly referred to as "missing-self" recognition [32,33]. Self MHC recognition can activate an immunoreceptor tyrosine-based inhibitory motif (ITIM)-dependent phosphatase SHP-1 or -2 and thus initiates an inhibitory signaling cascade [34]. The suitable expression of MHC class I molecules in normal cells and engagement of inhibitory receptors can avoid the autoimmune activity of NK cells.

2.2 Induced-self recognition by NK cells

NK cells can be incompetent or in-activated via the recognition of inhibitory receptor while encountering "healthy-self" cells. On the other hand, NK cells are regarded as the major force to oppose the virus infection and tumor development. So the integration of activation signal from the stimulatory receptors should conquer the inhibition of NK cells to exert their lytic and cytokine producing function. Transformation or infection might induce expression of stimulatory ligands so that constitutive inhibition delivered by inhibitory receptors is overcome. The magnification of activation response due to the up-regulation of stimulatory ligands is known as "induced-self" recognition [35].

NKG2D is an important activating receptor of NK cells, and is the main force to initiate "induced-self" recognition by NK cells. NKG2D is expressed on all NK cells and a set of γδ T cells and NKT cells in mouse, as well as on all activating CD8+ T cells and intraepithelial γδ T cells in human [36]. NKG2D identifies MHC class I chain-related A chain (MICA) or B chain (MICB) in humans [37] and a diverse family of ligands shared by human and mice called the retinoic acid early transcripts (RAET1) family, which includes RAE-1, H-60 and murine UL16-binding protein-like transcript 1 (MULT1) in mice and the UL16-binding protein (ULBP) in humans [38,39]. Interestingly, MICA and MICB are nearly not expressed on normal cells or tissues but can be induced in many epithelial tumor cells, bacterial or viral infected cells and in "stressed" cells [40]. Similarly, Rae-1 and H-60 are also barely located in tumor cells of diverse origin but not expressed on "non-stressed" cells. So NKG2D recognizes endogenic ligands that are upregulated on the surface of most tumor or infected cells. And all the ligands are "induced-self" relatives of MHC class I molecules and adopt a MHC class I-like structure. The activation of NK cells mediated by "induced-self" recognition is usually transmitted through ITAM-containing adaptor proteins, as known as KARAP/DAP12 [41]. But in $CD8^{+}$T cells, NKG2D associates solely with the signaling molecule DAP10. Collectively, the crosslink of NKG2D and the self ligands which are induced in stress triggers responsive mechanisms including the production of cytokines and the enhancing natural cytotoxicity, which are vital for the elimination of infected or transformed cells.

3 TLR signaling on NK cell activation

It is well-known that NK cells act as first line in defense against tumor, viral and microbial infections. During microbial infections, NK cells can be activated to kill infected or transformed cells and secrete large amount of cytokines to prime innate and adaptive immune responses. However, for a long time, it is not known whether NK cells can directly recognize pathogens. Until recently, it has been pointed that NK cells can be activated directly via TLRs. In other words, TLRs on NK cells can induce NK cell activation via integrating with their respective ligands.

It has been reported that almost all TLRs can be differentially expressed on human NK cells (Table 1). TLR2, TLR3, TLR5 and TLR6 have the primary expression but relatively low expression of TLR4, TLR7, TLR8 or TLR9 on human NK cells [42]. The expression profile might differ in different

Table 1 Expression of TLRs in NK and their exogenous and endogenous ligands

TLR	Expression	Exogenous ligands	Endogenous ligands	Adaptor
TLR2	High	Peptidoglycans, glycolipids	Hsp60, HMGB1	TIRAP/MyD88
TLR3	High	dsRNA, polyI:C, siRNA	dsRNA	MDA5, TICAM-1
TLR5	High	Flagellin	Gp96, fibrinogen, α-defensin2	MyD88
TLR6	High	Di-acetylated lipopeptides		TIRAP/MyD88
TLR4	Low	LPS, lipid A		TIRAP/MyD88
TLR7	Low	ssRNA40	ssRNA	MyD88
TLR8	Low	ssRNA40	ssRNA	MyD88
TLR9	Low	CpG-DNA	DNA-containing immunocomplex	MyD88

NK-cell populations. For example, $CD56^{bright}$ NK cells were found to preferentially express TLR2, while $CD56^{dim}$ NK cells were found to preferentially express TLR3 [43].

Stimulation with various TLR3 ligands can directly promote NK function, leading to the secretion of cytokines, the enhancement of cell lysis against virally infected or tumor cells. Total NK cells and $CD56^{dim}$ subset have been sorted from donors and were stimulated by poly I:C, an agonist of TLR3. As expected, poly I:C directly induced the secretion of IFN-γ, as well as pro-inflammatory cytokine IL-6 and IL-8 by NK cells in a dose-dependent manner without the help of other cytokines [44]. And NK cells showed a up-regulation of CD69 expression and enhanced cytotoxicity targeting K562 tumor cells [44,45]. Similarly, poly I:C also directly activated human NK cell lines, NK92, YTCl2 and YTS, which all had a notable TLR3 expression [43]. Poly I:C stimulation up-regulated the cytotoxic activity of NK cells targeting K562 and 721.221 cell lines, promoted the secretion of IFN-γ and induced the CXCL10 mRNA without additional cytokines or cell assist [44,45]. Further study proved that the TLR3 stimulation can directly induce the activation of TRIF/TICAM-1-dependent transcription factor IRF-3 and p38 MAPK in these human NK cell lines, and activation of p38 MAPK was essential for the activation of NK cells [43]. The activation of NK cells mediated by poly I:C has been applied to the research of tumor treatment or anti-virus responses, looking forward to finding an effective method for tumor or infection control in clinic. In head and neck squamous cell carcinoma (HNSCC) patients, the HNSCC microenvironment made TLR3 on surface of NK cells internalized rapidly, but afresh stimulation of NK cells with poly I:C can impair the TLR3 internalization and lead to the activation of local NK cells, as shown as high levels of IFN-γ secretion and up-regulation of cytotoxicity targeting the tumor cells [46]. Similarly, TLR3 expressed on NK cells also plays a major role on other circumstances such as virus infection, especially for MCMV infection. Moreover, similar activation effect of NK cells stimulated by TLR9 and possibly TLR7 and TLR8 signaling also occurs during MCMV infection [47].

There are two disparate situations on the expression of TLR7/8 on NK cells. TLR7/8 proteins are relative low expressed in $CD56^{dim}$ and $CD56^{bright}$ NK cells from normal healthy donors but are distinctly expressed in NK cell lines [48]. TLR7 is solely located in the NKL cell line, while TLR8 is highly expressed in all NK cell lines (NKL, NK92, and YT) [49]. Nevertheless, accumulating data show that TLR7/8 stimulation promotes NK cell activation and exerts powerful anti-tumor and anti-viral responses. NK cell activation through TLR7/8 agonists mainly requires the help of other cytokines, such as IL-12, IL-18, and IFN-α/β, which are secreted by DCs or monocytes [49,50]. ssRNA40 which contains a uridine-rich ssRNA sequence derived from the LTR region of HIV-1 can serve as a TLR7/8 ligand and activate DCs and even NK cells. The critical role of ssRNA40 on NK cell activation has been shown in patients with acute viremic HIV-1 infection, in which the $CD14^{+}$ monocytes help the activation of NK cells by secretion of IFN-α and IL-12, and also with a cell-to-cell contact involvement [51]. During the past years, with the elicitation of ssRNA40, several other single-stranded immunostimulatory RNAs (ssisRNAs), which are characterized as uridine-rich oligonucleotide, have been identified to be able to stimulate NK cells via TLR7 recognition [52]. And more important is that unlike other TLR7/8 agonists, these ssRNAs can mediate the activation of NK cells directly, represented as up-regulation of cytotoxicity against HNSCC cell lines BHY, PCI-1 and PCI-13, induction production of IFN-γ, granzyme B and perforin by NK cells [53]. VTX-2337, a novel small-molecular TLR8 agonist, is recently found to be able to activate NK cells directly and augment ADCC by rituximab in PBMCs [54]. Stimulation with imiquimod, also known as a TLR7 ligand, enhances the cytotoxic activity of expanded NK and γδ T cells from cancer patients *in vitro* [55]. Our recent results showed that Imiquimod and another TLR7 agonist, Gardiquimod, promoted the proliferation and activation of splenic T, NK and NKT cells, and increased the cytolytic ability of NK cells against melanoma B16. When combined with DC vaccine, both Imiquimod and Gardiquimod exert more powerful inhibition of the growth of subcutaneous B16 melanoma and suppression of the pulmonary metastasis, demonstrating that TLR7-stimulated anti-tumor effect by NK cells needs the help of DCs [56]. Taken together, the activation of NK cells mediated by TLR7/8 agonists should be accompanied by the stimulation of other cytokines.

TLR9 regarded as another "nucleic acid-sensing" TLR also has a functional expression in NK cells. The classical ligand of TLR9 is oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides (CpG ODNs) which is mimics of bacterial DNA. So far three CpG ODN classes, also known as ODN A, ODN B and ODN C, have been identified and proved to bear differential capability to stimulate human NK cells [57]. First of all, stimulation of freshly isolated NK cells with ODN C shows similar or higher levels of CD69 and CD25 expression as compared with ODN A, whereas treatment with ODN B is consistently less efficient [57,58]. However, in line with the TLR7/8, TLR9 ligands induce NK activation in virtue of suboptimal doses of pro-inflammatory cytokines, such as IL-12 or IL-18, due to low expression of TLR9 in human NK cells [59]. In addition, ODN C administration can up-regulate both cytolytic activity against tumor cell lines and cytokine production more significantly than ODN A and ODN B [60]. These results suggest that ODN C might represent a good volunteer to support anti-tumor therapy.

CpG ODN has gained much attention to the tumor treatment in mice model, because CpG ODN has been proved to be able to activate monocytes directly to secrete the Th1-like cytokine IL-12 and type I IFNs, promote NK cells re-

sponse with increased lytic activity and IFN-γ secretion, and even drive dendritic cell and B cell maturation and thus enhance their Ag presentation capability [61]. However, the spontaneous antitumor immunity primed by CpG ODN can be tampered when ODN is applied systemically *in vivo*, therefore developing some vector-based agents for selective delivery of CpG-ODN is important for the activation maintaining. In RIP1-Tag5-transgenic mouse model of pancreatic islet tumorigenesis, developing insulinomas are deeply embedded in the exocrine pancreas and thus out of reach for intra-tumoral injection. CpG-ODN has been packaged into the vascular targeting peptide (RGR peptide) which specifically gets to the tumor blood vessels. RGR peptide-coated stealth liposomes showed a long time stimulation of spontaneous antitumor immunity and anti-vascular effects. RGR-liposomes plus CpG-treated RIP1-Tag5 mice display long-term survival, and $CD8^+$T and NK cells are major force for this effect, because $CD8^+$T and NK cells but not the $CD4^+$T cells depletion impaired the survival [62]. And more important is that sorted NK cells from tumor sites exhibit more vigorous ability of producing IL-12 and IFN-γ and natural cytotoxicity, even accompanied by distinct inhibition of angiogenesis target tumor cells [62,63].

In addition to controlling tumor growth, TLR9-dependent signaling in plasmacytoid dendritic cells (pDCs) is a key contributor to innate immune defense against virus or bacteria infection. For instance, TLR2 and TLR9 have been proved to act in concert to mount the first line of defense against HSV infection, in which the production of various proinflammatory cytokines, such as type I IFNs, IL-6, IL-12, RANTES, and TNF-α, plays the major role [64]. On the other hand, although expression of the early activation marker CD69 was comparable between splenic $NK1.1^+$ cells from WT and TLR2/9 DKO, reduced NK cell recruitment to the spleen has been observed, resulting in more virus load [64]. Further study suggested that the secretion of chemokines such as CXCL9 and CXCL3 induced by TLR2 or TLR9 stimulation is important for the NK activation [64,65]. This is also the case for MCMV infection. Overall, during the virus infection, TLR2/9 stimulation can mediate NK recruitment directly via the production of chemokines, as well as enhance the CD69 expression and cytokine secretion. Just during the cutaneous leishmaniasis challenge, NK cells represent an important source for early IFN-γ, and TLR9 is essential for the induction of NK cell-related IFN-γ secretion and cytotoxicity during the innate phase of *Leishmania (L.) major* infection, because lymph node (LN) NK cells from $TLR9^{-/-}$ mice acquired no cytolytic activity after stimulation with *L. major* [66]. Further study showed that activation of DCs via TLR9 was essential for the induction of hypertrophy in leishmaniasis, due to the crosstalk between NK cells and DCs [67].

Besides the TLRs mentioned above, TLR2 and TLR5 expressing on NK cells at a middle degree also can stimulate NK cells directly via combining with their ligands. NK activation mediated by TLR2 and TLR5 mainly participates in the defense of bacteria and fungi challenge. Klebsiella pneumoniae (KpOmpA) and flagellin, which represent the ligand of TLR2 and TLR5 respectively, can activate $CD56^+CD3^-$NK cells directly, demonstrated as induction of IFN-γ production or increment of proinflammatory cytokines such as TNF-α in the presence of IL-2 [68]. More importantly, KpOmpA and flagellin rapidly promote NK cells to constitutively produce α-defensins which disrupted the bacterial membrane, leading to pathogen death [68]. Similarly, TLR2 is involved to assist NK cells to recognize *H. pylori* to promote the production of IFN-γ which is the major inflammation factor in the human gastric mucosa [69]. In vaccinia viral infection, TLR2 was able to directly activate NK cell in a TLR2/MyD88-dependent manner and was critical for the control of vaccinia viral infection *in vivo* [70]. TLR2 agonist polysaccharide krestin (PSK) was also reported to activate human NK cells directly and indirectly with the help of IL-12. Notably, it can enhance the antitumor effect of HER2-targeted monoclonal antibody therapy [71]. In addition, a bacterial fimbrial protein FimH, a novel TLR4 ligand, was recently reported to directly activate human and murine natural killer cells via TLR4-MyD88 pathways [72].

Ligation of TLRs with respective agonists at the site of infection or cellular stress activates NK cells followed by the release of inflammatory mediators, including cytokines and chemokines, and initiation of target-cell lysis. However, the activation of NK cells mediated by TLRs is also involved in some pathologic process of autologous diseases. For example, poly I∶C treatment can induce the accumulation and activation of NK cells in liver, leading to autoimmune hepatitis [73,74]. Pre-activation of T cells aggravates poly I∶C-induced liver injury by up-regulating NK cell function [75]. HBs-transgenic mice are over-sensitive to poly I∶C-induced liver injury, which is dependent on the presence of NK cells and IFN-γ produced by intrahepatic NK cells [76,77]. In primary biliary cirrhosis (PBC), stimulation with ligands for TLR3 and TLR4 enhanced NK cell-mediated cytotoxicity against biliary epithelial cells (BECs), during which IFN-α produced by TLR3 ligand-stimulated monocytes enhances the cytotoxicity of TLR4 ligand-activated NK cells [78]. In addition, the stimulation of TLR3 can also trigger murine uterus NK cell activation followed by amounts of TNF-α and IFN-γ secretion. These two factors can induce the reconstruction of artery and promote the abortion [79].

4 The crosstalk between TLR and NK cell receptors

Accumulating evidence has shown that NK cells can recognize pathogen-associated structures directly or indirectly.

However, it is not clear how TLR signaling results in NK cell activation. Increasing data has indicated that there exists crosstalk between TLR and NK cell receptors, and therefore regulates NK cell function.

KIRs are human NK receptors that recognize allotypic determinants of human leukocyte antigen (HLA) class I. The major function of inhibitory KIRs is discriminating normal cells from tumor or virus-infected cells via recognizing the HLA class I molecules (HLA-A, -B or -C allotypes). As one of the members of KIRs, KIR3DL2 has been reported to be capable of binding CpG-ODNs (such as ODN-C) and transiting themselves into the endosomal compartments of NK cells and therefore down-modulating surface KIR3DL2 expression [80]. The disappearance of KIR3DL2 mediated by TLR9 stimulation is beneficial for the activation of NK cells to counteract the pathogen challenge. The stimulation of CpG-ODNs did not impair the expression of other surface receptors, including NKG2D, NKG2A, CD16, CD56, DNAM-1, CD2, LFA-1, 2B4 and NTBA [81,82].

The expression of activating receptors and their ligands is also regulated by TLR pathway to influence NK cell function when suffering the microbe infection or cell stress. The NKG2D receptor which is the primary activating receptor can be upregulated when "stressed-induced" signals emerge. Accumulating data demonstrates that TLR signaling might tempt the NKG2D expression on NK cells or other NKG2D-expressing lymphocytes, and/or simultaneously induce expression of NKG2D ligands on NK-targeting cells to strengthen NK activation. For instance, during the infection of intracellular *Mycobacterium tuberculosis (M. tb)*, lysis of infected mononuclear phagocytes mediated by NK cells is the main force to clear away microbial pathogens, in which natural cytotoxicity receptors on NK cells such as NKp30, NKp44 and NKp46 play a pivotal role [83,84]. The expression of NKp46, NKp30 and NKG2D has been identified to be markedly increased on NK cells upon exposure to *M. tb*-infected cells, and NKG2D and NKp46 are vital for NK cell lysis of *M.tb*-infected monocytes, which was confirmed by neutralization antibodies. More importantly, *M. tb* infection can up-regulate ULBP1 expression on monocytes through stimulating TLR2 signaling [84]. This induction of both receptor and ligand can enhance cytotoxicity of NK cells. So these results indicate that interactions between the TLR2 pathway and NK cells can contribute to the local immune response to *M. tb* infection.

Large amount of data has shown that TLR signaling enhances NK cell activation through strengthening the interactions between NKG2D and its ligands via up-regulating NKG2D ligands on macrophages, monocytes or tumor cells. Previously reports showed that LPS preferentially upregulated MICA but not MICB, while CL097 stimulation resulted in upregulation of both MICA and MICB. Multiple mechanisms downstream of TLR4 stimulation, such as ATM/ATR kinases and miRNAs, regulate expression of NKG2D ligands, which were involved in the macrophage/NK cell crosstalk [85]. Our recent published data showed that poly I:C-stimulated murine macrophages can enhance NK cell-mediated anti-tumor effects, which is dependent on the interaction of NKG2D and its ligands RAE-1, H60, and MULT-1 [86]. Similar effects also occur in crosstalk between NK and monocytes. The up-regulation of MICA expression and human monocytes activation upon LPS stimulation in turn enhances the NK cells activity manifested by the induction of IFN-γ secretion via NKG2D-ligands interaction and augments NK cytolysis against tumor cells. More important was that activated NK cells did not display cytotoxicity against MICA-expressing monocytes to avoid autologous immune disorder [87].

In addition to inducing the activation of NK cells via up-regulating the NKG2D ligands on macrophages or monocytes to complete the mission of resisting microbe infection or tumor challenge, TLR signaling-induced NK cell activation may also mediate autologous disorder via affecting the reciprocity of NKG2D-ligands. In a polyI:C/D-GalN-induced fulminant hepatitis, in which TLR3 stimulation with polyI:C triggered the up-regulation expression of Rae-1 on the surface of Kupffer cells, the crosstalk between NK cells and Kupffer cells via NKG2D-Rae1 recognition resulted in IFN-γ and TNF-α secretion which were major factors involved in liver injury [88]. Poly I:C was also reported to activate hepatic NK cells and ameliorate liver fibrosis through enhancing NKG2D expression on NK cells and Rae-1 expression on hepatic stellate cells and then killing stellate cells in NKG2D- and TRAIL-dependent manner [89].

Besides mediating the liver injury via intensifying the interaction of NKG2D and respective ligand, TLR stimulation can induce other immune disorders through promoting NK activation. In human skeletal muscle cell lines such as myoblasts and human rhabdomyosarcoma cells (TE671), there are low mRNA expression of TLR1-7 and TLR9 but constitutively expression of TLR3 which can be up-regulated by poly(I:C) [90]. Moreover, TLR3 engagement with poly(I:C) or other dsRNA is capable of initiating the increase of NKG2D on NK cells, and also the up-regulation of respective ligands such as MICA, MICB, ULBP-2 and ULBP-3 in human muscle cells [91]. The interaction between NKG2D and ligands results in autologous cytotoxicity and the secretion of IL-8 and tissue inhibitor of metalloproteinase-2 (TIMP-2), which can aggravate the development of sarcolysis [90]. The activation of TLR signaling also breaks down epithelial homeostasis of small intestine in mice. Poly I:C stimulation could urge intestinal epithelial cells (IECs) to express RAE-1 and promote NKG2D expression on $CD8^+$ intestinal intraepithelial lymphocytes in the presence of IL-15 derived from TLR3-activated IECs. The blockade of interaction between NKG2D and RAE-1 inhibited the cytotoxicity of intraepithelial lymphocytes

against IECs consequently to alleviate epithelial destruction and acute mucosal injury of small intestine mediated by poly I:C [92]. This is also the case for the full development of renal ischemia-reperfusion injury (IRI). Published results have proved that ischemic injury resulted in the generation or release of various endogenous ligands of TLRs, including HMGB1, biglycan, and heat shock proteins. The coalescent of TLR4 and its ligand HMGB1 has been found to be involved in the progress of IRI [93]. Recent finding showed that in ischemic kidney of BABL/c mouse, RAE-1 and MULT-1 expression on renal tubular epithelial cells and NKG2D expression on renal NK cells both could be up-regulated and TLR2/MyD88 signaling was responsible for the increase of NKG2D and respective ligands directly because further induction was restricted in both $TLR2^{-/-}$ and $MyD88^{-/-}$ mice [94].

Collectively, the crosstalk between TLR and NK cell receptors not only plays major roles in control of NK cell function and participates in the defense of NK cells against infection and transformation, but also participates in pathogenesis of some diseases.

5 TLRs—The hinge connecting DCs and NK cells

Customarily, DCs are known as the professional antigen presentation cells which present antigens to acquired immune cells such as T and B lymphocytes, followed by the robust activation of entire immune system. But more and more data have shown that DCs also play a very important role in control of the innate immune responses. It is undoubted that DCs can trigger NK cells directly via the secretion of cytokines, such as IL-12 and IL-15, which promote NK-cell survival and differentiation, NK cell-mediated cytotoxicity and IFN-γ secretion [95]. Herein we demonstrate that TLRs highly expressed on DCs act as a bridge between DCs and NK cells, that is, TLR ligands can stimulate DCs, and consequently activate NK cells through cytokine secretion and/or cell-to-cell contact.

pDCs are bone marrow-derived leukocytes that secrete large amounts of type I IFN in response to a variety of viruses. And two endosomal sensors, TLR7 and TLR9 both have a remarkable expression in pDCs. Moreover, pDCs are responsible for the early peak of IFN production in response to most viruses or TLR7 and TLR9 agonists [96]. In line with previous reports, the activation of NK cells mediated by TLR9 recognition depends on the presence of cytokines probably from other immune cells, but the exact role of pDCs played in the activation of NK cells induced by TLR has not been validated. It has been proved that TLR9-engagement with CpG-A in human pDCs induces NK cell activation, represented as the up-regulation of CD69 expression, as well as proliferation of $CD56^{bright}CD16^{-}$ NK cells. Moreover, extensive research showed that the activation and proliferation of NK cells promoted by pDCs after TLR9 stimulation could be augmented by the addition of $CD4^{+}$ $CD25^{-}$ Th cells in a IL-2-dependent manner, but was completely abrogated by the $CD4^{+}CD25^{hi}$ Treg cells [97]. It has been demonstrated that during MCMV infection, the production of type I IFN by pDCs is vital for NK cell activation and function. Recent findings show that during persistent MCMV infection, IFN-γ production and cytotoxic capacity of NK cells is inhibited dramatically due to the defect of type I IFN response from pDCs [98]. When wild-type (WT) BALB/c mice encounter the challenge of some Gram-negative bacteria, such as *Klebsiella pneumoniae*, pDCs expressing TLR9 accumulate rapidly, followed by the increase of total number of $DX5^{+}$cell. If CpG-stimulated $CD11c^{dulll}$ $B220^{+}$ pDCs are injected into the infected mice, the recruitment of NK cells to the peritoneum can be induced drastically followed by the high expression of CXCR3 and CD62L, and moderate expression of CCR5 [99]. And these NK cells isolated from the peritoneum inoculated with TLR7/8 or TLR9-stimulated pDCs exhibit highlighted cytotoxicity against YAC-1 tumor cells. These findings suggest that although the TLRs stimulation on the NK cells can directly enhance their ability to produce cytokines and lyse infected or transformed cells, the response of DCs to TLR agonists is pivotal for the recruitment and proliferation of NK cells. About the recruitment of NK cells mediated by DCs, there are many related details. By using a mouse model for the ablation of DCs, the priming of NK cell responses to viral and bacterial pathogens was proved to require the help of $CD11c^{high}$ DCs [98]. After peripheral TLR stimulation, NK cells were recruited to local lymph nodes, and their interaction with DCs resulted in the emergence of effector NK cells in the periphery. NK cell priming was dependent on the recognition of type I IFN signals by DCs and the subsequent production and trans-presentation of IL-15 by DCs to resting NK cells. $CD11c^{high}$ DC-derived IL-15 was vital and sufficient for the priming of NK cells.

Conventional DCs (cDCs) in fact are veiled cells of myeloid origin which are capable of efficiently processing and presenting antigens to prime naive T cells [100]. Although pDCs are characterized as the type I IFN-production in response to virus or bacterium components, they cannot excrete IFN-α by poly I:C stimulation. In contrast, $CD11c^{+}$-$B220^{-}$ cDCs produce type I IFNs in response to poly I:C. Moreover, $CD8\alpha^{+}$ cDCs in response to poly I:C stimulation can enhance cytotoxicity of NK cells targeting YAC-1 tumor cells, and up-regulate the expression of granzyme B and IFN-γ due to the activation of IPS-1 and TRIF signaling pathway and consequently up-regulate the expression of type I IFNs, IL-6, and IL-12p40 in DCs [101]. Similar to pDCs, cDCs also promote the recruitment of NK cells after TLRs-related ligands stimulation via up-regulating the secretion of chemokines. Some investigators showed that in mice with depletion of $CD11c^{+}$ MHC class II^{+} cDCs, the recruitment of $CXCR5^{+}DX5^{+}$ NK cells to draining lymph nodes is abated after treatment with TLR4 and TLR5 ago-

nists, although these TLR ligands also can activate NK cells in the draining lymph nodes [102]. DCs are now accepted as crucial regulators of innate and adaptive immunity, in particular NK cells function. It is undoubtful that the IFN-γ secretion, cytotoxicity, CD69 expression and proliferation of resting NK cells can be induced by mouse DC cell lines, mouse bone-marrow-derived DCs (BMDCs), human monocyte-derived DCs and human cord-blood-derived DCs [103]. So far the mechanisms involved in which DCs activate NK cells include the direct cell-cell contact and soluble factors, including IL-12, IL-18 and type I IFN. Moreover, some researchers have begun to reveal intrinsic relations between NK cells and DCs when stimulated by diversified TLR agonists or other stimulus. Myeloid DCs (mDCs), which comprise many subsets, including BMDCs and CD8+ DCs, possess subset-specific pattern-recognition systems. After stimulation with TLR agonists, adaptor MyD88 or the Toll/IL-1 receptor homology domain-containing adaptor molecule (TICAM)-1 (TRIF) is recruited and transduces signaling to engaged in type I IFN induction and NK activation via partly overlapped but distinct pathways in a cell type-specific manner [104]. MyD88 in pDC can be stimulated after administration of TLR7 or TLR9 ligand to promote IFN-γ secretion, and consequently, NK cells demonstrate further activation [95,96]. This is also the case for TLR2 agonist, because cell contact-mediated NK activation is hampered when the cells are stimulated with Pam2 lipopeptide after coincubation with MyD88$^{-/-}$ mDC [70]. In contrast, poly I:C-induced NK activation is tampered in double KO (IPS-1$^{-/-}$ and TICAM-1$^{-/-}$) mice, suggesting that these two pathways are both required for poly I:C-mediated NK activation [105]. Moreover, regulatory molecule which is responsible for the crosstalk between NK and mDC has been reported. INAM (IRF-3-dependent NK cell activating molecule), a poly I:C-inducible membrane protein, has been identified to participate in mDC-mediated NK cell activation and contribute to mDC-NK reciprocal activation [104,106]. Notably, INAM does not act as an NK-activating ligand; it works only on mDC/macrophage for NK activation. In addition, MyD88 in NK cells also participates in direct NK activation.

6 Future prospect

TLRs and NK cell receptors are the most important receptor superfamilies in innate immunity. TLRs, acting as the sensor of external pathogens, lie in the first defense line to resist the invasion from xenogenic organisms by directly recognizing PMAPs. NK cells can eliminate pathogenic infection or transformed-cells via detecting alterations in endogenous protein expression on target cells through activating

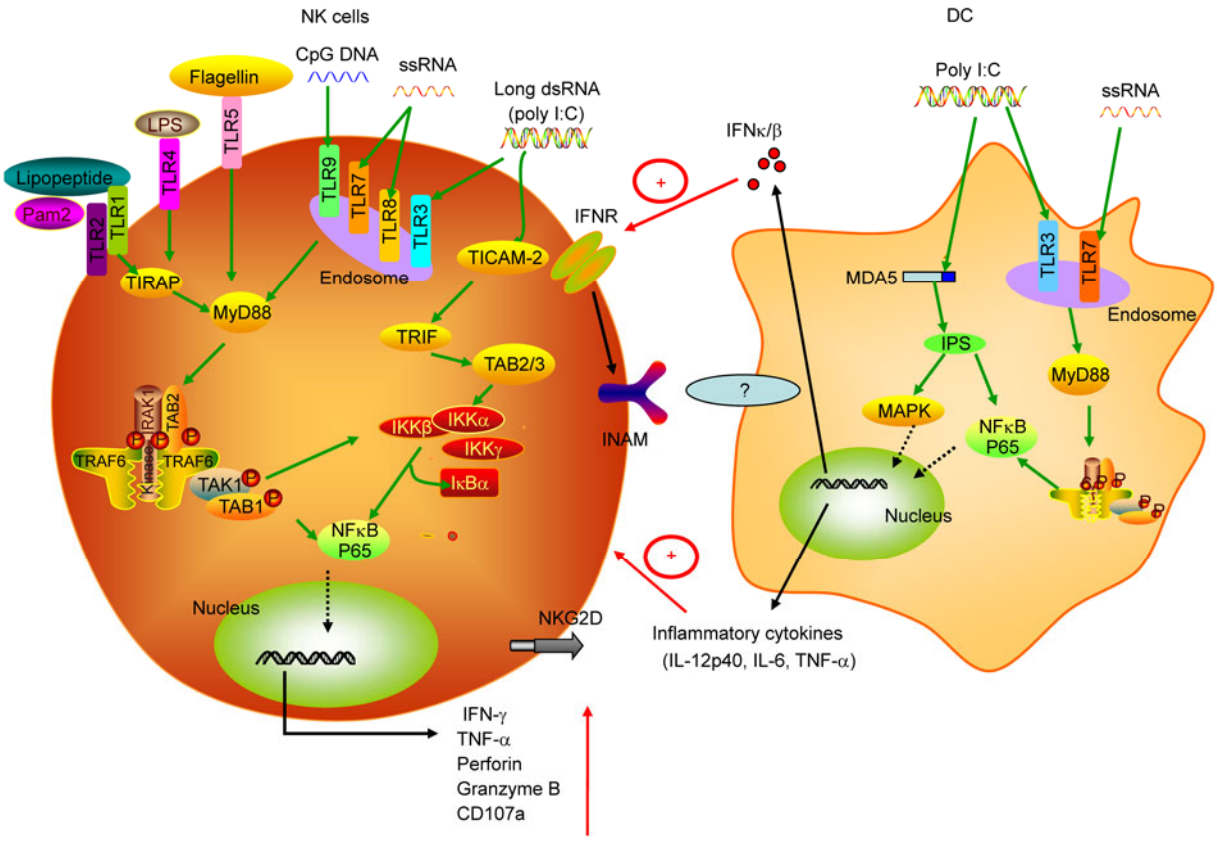

Figure 1 The TLR signaling in NK cell activation.

and inhibitory receptors. Accumulating data has demonstrated that TLRs and NK cell receptors can coordinate and regulate each other during immune responses, which contributes to the initiation of innate response and the priming of adaptive responses. TLRs can activate NK cell function directly or with the help of accessory cells in a cytokine or cell-to-cell contact dependent manner. In addition, TLR signaling can also induce the expression of NKG2D ligand and thus indirectly activate NK cells. As shown in Figure 1, NK cells express diversified TLRs, and they can be activated directly by TLR ligands, followed by the activation of TLR signaling pathway via the recruitment of TIR domain-containing adaptor molecules, including MyD88, TIRAP (Mal), TRIF and TRAM. In most cases, TLR-induced NK cell activation needs the help of accessory cells, such as DCs, macrophages and monocytes. For example, TLR agonists stimulate TLRs on the surface or in the endosome of DCs and finally promote the production of inflammatory cytokines such as IL-12, IL-15, and type I IFN, which are vital for NK activation. Moreover, cell-to-cell contact between DC and NK cells is crucial for NK cell activation. DC activation can induce the expression of INAM to enhance the function of NK, although the exact role of INAM players in the mDC-NK contact is still unidentified. Taken together, TLR signaling can directly or indirectly induce the activation of NK cells, thereby promoting IFN-γ secretion and enhancing cytotoxicity against tumor or infected cells.

In conclusion, the TLR signaling is critical for NK cell activation and function in the control of viral or bacterial infection and cancer. Other innate immune receptors, such as RLRs, NLRs, and CLRs, may also interact with NK cell receptors or intracellular signaling, and contribute to NK cell activity. Although the progress has been gained in recent years, the precise crosstalk between different immune cells and different receptors should be investigated more intensively. More understanding of the recognition of innate receptors and interactions between them may provide important insights into the design of effective strategies to combat tumor and microbial infections.

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1 Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. Nat Immunol, 2010, 11: 373–384
2 Janeway C A Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol, 2002, 20: 197–216
3 Pisegna S, Pirozzi G, Piccoli M, et al. p38 MAPK activation controls the TLR3-mediated up-regulation of cytotoxicity and cytokine production in human NK cells. Blood, 2004, 104: 4157–4164
4 Yoneyama M, Fujita T. RNA recognition and signal transduction by RIG-I-like receptors. Immunol Rev, 2009, 227: 54–65
5 Raulet D H. Interplay of natural killer cells and their receptors with the adaptive immune response. Nat Immunol, 2004, 5: 996–1002
6 Colonna M, Jonjic S, Watzl C. Natural killer cells: Fighting viruses and much more. Nat Immunol, 2011, 12: 107–110
7 Tu Z K, Bozorgzadeh A, Pierce R H, et al. TLR-dependent cross talk between human Kupfer cells and NK cells. J Exp Med, 2008, 205: 233–244
8 Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell, 2006, 124: 783–801
9 Pukkila-Worley R, Ausubel F M. Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium. Curr Opin Immunol, 2012, 24: 3–9
10 Pietrocola G, Arciola C R, Rindi S, et al. Toll-like receptors (TLRs) in innate immune defense against *Staphylococcus aureus*. Int J Artif Organs, 2011, 34: 799–810
11 Jin M S, Lee J O. Structures of the Toll-like receptor family and its ligand complexes. Immunity, 2008, 29: 182–191
12 Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity, 2011, 34: 637–650
13 Thompson M R, Kaminski J J, Kurt-Jones E A, et al. Pattern recognition receptors and the innate immune response to viral infection. Viruses, 2011, 3: 920–940
14 Blasius A L, Beutler B. Intracellular Toll-like receptors. Immunity, 2010, 32: 305–315
15 Barbalat R, Ewald S E, Mouchess M L, et al. Nucleic acid recognition by the innate immune system. Annu Rev Immunol, 2011, 29: 185–214
16 Akashi-Takamura S, Miyake K. TLR accessory molecules. Curr Opin Immunol, 2008, 20: 420–425
17 Oeckinghaus A, Hayden M S, Ghosh S. Crosstalk in NF-kappaB signaling pathways. Nat Immunol, 2011, 12: 695–708
18 Anand P K, Tait S W, Lamkanfi M, et al. TLR2 and RIP2 pathways mediate autophagy of Listeria monocytogenes via extracellular signal-regulated kinase (ERK) activation. J Biol Chem, 2011, 286: 42981–42991
19 Hoebe K, Georgel P, Rutschmann S, et al. CD36 is a sensor of diacylglycerides. Nature, 2005, 433: 523–527
20 Goodridge H S, Underhill D M. Fungal recognition by TLR2 and Dectin-1. Handb Exp Pharmacol, 2008, 183: 87–109
21 Yang R B, Mark M R, Gurney A L. Signaling events induced by lipopolysaccharide-activated Toll-like receptor 2. J Immunol, 1999, 163: 639–643
22 Yan N, Chen Z J. Intrinsic antiviral immunity. Nat Immunol, 2012, 13: 214–222
23 O'Neill L A, Bowie A G. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol, 2007, 7: 353–364
24 Yamamoto M, Takeda K. Current views of Toll-like receptor signaling pathways. Gastroenterol Res Pract, 2010, 2010: 240365
25 Sun J C, Lanier L L. NK cell development, homeostasis and function: Parallels with CD8 T cells. Nat Rev Immunol, 2011, 11: 645–657
26 Raulet D H, Vance R E. Self-tolerance of natural killer cells. Nat Rev Immunol, 2006, 6: 520–531
27 Raulet D H. Missing self recognition and self tolerance of natural killer (NK) cells. Semin Immunol, 2006, 18: 145–150
28 Huntington N D, Vosshenrich C A, Di Santo J P. Developmental pathways that generate natural-killer-cell diversity in mice and humans. Nat Rev Immunol, 2007, 7: 703–714
29 Leavy O. Maturation and function of NK cells. Nat Rev Immunol, 2012, 12: 150–151
30 Thielens A, Vivier E, Romagne F. NK cell MHC class I specific receptors (KIR): From biology to clinical intervention. Curr Opin Immunol, 2012, 24: 239–245
31 Orr M T, Lanier L L. Inhibitory Ly49 receptors on mouse natural killer cells. Curr Top Microbiol Immunol, 2011, 350: 67–87
32 Vivier E, Romagne F. Good news, bad news for missing-self recognition by NK cells: Autoimmune control but viral evasion. Immunity, 2007, 26: 549–551
33 Lanier L L. NK cell recognition. Annu Rev Immunol, 2005, 23: 225–274
34 Sullivan L C, Clements C S, Rossjohn J, et al. The major

histocompatibility complex class Ib molecule HLA-E at the interface between innate and adaptive immunity. Tissue Antigens, 2008, 72: 415–424
35 Vivier E, Tomasello E, Baratin M, et al. Functions of natural killer cells. Nat Immunol, 2008, 9: 503–510
36 Zloza A, Kohlhapp F J, Lyons G E, et al. NKG2D signaling on CD8(+) T cells represses T-bet and rescues CD4-unhelped CD8(+) T cell memory recall but not effector responses. Nat Med, 2012, 18: 422–428
37 Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science, 1999, 285: 727–729
38 Raulet D H. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol, 2003, 3: 781–790
39 Cosman D, Mullberg J, Sutherland C L, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity, 2001, 14: 123–133
40 Huang B, Sikorski R, Sampath P, et al. Modulation of NKG2D-ligand cell surface expression enhances immune cell therapy of cancer. J Immunother, 2011, 34: 289–296
41 Turnbull I R, Colonna M. Activating and inhibitory functions of DAP12. Nat Rev Immunol, 2007, 7: 155–161
42 Lauzon N M, Mian F, MacKenzie R, et al. The direct effects of Toll-like receptor ligands on human NK cell cytokine production and cytotoxicity. Cell Immunol, 2006, 241: 102–112
43 Pisegna S, Pirozzi G, Piccoli M, et al. p38 MAPK activation controls the TLR3-mediated up-regulation of cytotoxicity and cytokine production in human NK cells. Blood, 2004, 104: 4157–4164
44 Schmidt K N, Leung B, Kwong M, et al. APC-independent activation of NK cells by the Toll-like receptor 3 agonist double-stranded RNA. J Immunol, 2004, 172: 138–143
45 Duluc D, Tan F, Scotet M, et al. PolyI:C plus IL-2 or IL-12 induce IFN-gamma production by human NK cells via autocrine IFN-beta. Eur J Immunol, 2009, 39: 2877–2884
46 Xie L, Pries R, Kesselring R, et al. Head and neck cancer triggers the internalization of TLR3 in natural killer cells. Int J Mol Med, 2007, 20: 493–499
47 Tabeta K, Georgel P, Janssen E, et al. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc Natl Acad Sci USA, 2004, 101: 3516–3521
48 Gorski K S, Waller E L, Bjornton-Severson J, et al. Distinct indirect pathways govern human NK-cell activation by TLR-7 and TLR-8 agonists. Int Immunol, 2006, 18: 1115–1126
49 Hart O M, Athie-Morales V, O'Connor G M, et al. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. J Immunol, 2005, 175: 1636–1642
50 Girart M V, Fuertes M B, Domaica C I, et al. Engagement of TLR3, TLR7, and NKG2D regulate IFN-gamma secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. J Immunol, 2007, 179: 3472–3479
51 Lester R T, Yao X D, Ball T B, et al. Toll-like receptor expression and responsiveness are increased in viraemic HIV-1 infection. AIDS, 2008, 22: 685–694
52 Alter G, Suscovich T J, Teigen N, et al. Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. J Immunol, 2007, 178: 7658–7666
53 Pries R, Wulff S, Kesselring R, et al. Up-regulation of NK cell function against head and neck cancer in response to ss-isRNA requires TLR7. Int J Oncol, 2008, 33: 993–1000
54 Lu H, Dietsch G N, Matthews M A, et al. VTX-2337 is a novel TLR8 agonist that activates NK cells and augments ADCC. Clin Cancer Res, 2012, 18: 499–509
55 Shojaei H, Oberg H H, Juricke M, et al. Toll-like receptors 3 and 7 agonists enhance tumor cell lysis by human gammadelta T cells. Cancer Res, 2009, 69: 8710–8717
56 Ma F, Zhang J, Zhang J, et al. The TLR-7 agonists, imiquimod and gardiquimod, improve DC-based immunotherapy for melanoma in mice. Cell Mol Immunol, 2010, 7: 381–388
57 Vollmer J, Weeratna R, Payette P, et al. Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur J Immunol, 2004, 34: 251–262
58 Sivori S, Carlomagno S, Moretta L, et al. Comparison of different CpG oligodeoxynucleotide classes for their capability to stimulate human NK cells. Eur J Immunol, 2006, 36: 961–967
59 Schleicher U, Liese J, Knippertz I, et al. NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs. J Exp Med, 2007, 204: 893–906
60 Takeshita F, Leifer C A, Gursel I, et al. Cutting edge: Role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. J Immunol, 2001, 167: 3555–3558
61 Krieg A M. Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. Oncogene, 2008, 27: 161–167
62 Hamzah J, Altin J G, Herringson T, et al. Targeted liposomal delivery of TLR9 ligands activates spontaneous antitumor immunity in an autochthonous cancer model. J Immunol, 2009, 183: 1091–1098
63 Geary S M, Lemke C D, Lubaro V D M, et al. Tumor immunotherapy using adenovirus vaccines in combination with intratumoral doses of CpG ODN. Cancer Immunol Immunother, 2011, 60: 1309–1317
64 Lima G K, Zolini G P, Mansur D S, et al. Toll-like receptor (TLR) 2 and TLR9 expressed in trigeminal ganglia are critical to viral control during herpes simplex virus 1 infection. Am J Pathol, 2010, 177: 2433–2445
65 Liese J, Schleicher U, Bogdan C. TLR9 signaling is essential for the innate NK cell response in murine cutaneous leishmaniasis. Eur J Immunol, 2007, 37: 3424–3434
66 Carvalho L P, Petritus P M, Trochtenberg A L, et al. Lymph node hypertrophy following Leishmania major infection is dependent on TLR9. J Immunol, 2012, 34: 1394–1401
67 Rozy A, Chorostowska-Wynimko J. Bacterial immunostimulants—Mechanism of action and clinical application in respiratory diseases. Pneumonol Alergol Pol, 2008, 76: 353–359
68 Chalifour A, Jeannin P, Gauchat J F, et al. Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers α-defensin production. Blood, 2004, 104: 1778–1783
69 Lindgren A, Pavlovic V, Flach C F, et al. Interferon-gamma secretion is induced in IL-12 stimulated human NK cells by recognition of *Helicobacter pylori* or TLR2 ligands. Innate Immun, 2011, 17: 191–203
70 Martinez J, Huang X, Yang Y. Direct TLR2 signaling is critical for NK cell activation and function in response to vaccinia viral infection. PLoS Pathog, 2010, 6: e1000811
71 Lu H, Yang Y, Gad E, et al. TLR2 agonist PSK activates human NK cells and enhances the antitumor effect of HER2-targeted monoclonal antibody therapy. Clin Cancer Res, 2011, 17: 6742–6753
72 Mian M F, Lauzon N M, Andrews D W, et al. FimH can directly activate human and murine natural killer cells via TLR4. Mol Ther, 2010, 18: 1379–1388
73 Dong Z, Wei H, Sun R, et al. Involvement of natural killer cells in PolyI:C-induced liver injury. J Hepatol, 2004, 41: 966–973
74 Wang J, Xu J, Zhang W, et al. TLR3 ligand-induced accumulation of activated splenic natural killer cells into liver. Cell Mol Immunol, 2005, 2: 449–453
75 Wang J, Sun R, Wei H, et al. Pre-activation of T lymphocytes by low dose of concanavalin A aggravates Toll-like receptor-3 ligand-induced NK cell-mediated liver injury. Int Immunopharmacol, 2006, 6: 800–807
76 Chen Y, Wei H, Sun R, et al. Increased susceptibility to liver injury in hepatitis B virus transgenic mice involves NKG2D-ligand interaction and natural killer cells. Hepatology, 2007, 46: 706–715
77 Chen Y, Sun R, Jiang W, et al. 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
78 Shimoda S, Harada K, Niiro H, et al. Interaction between Toll-like receptors and natural killer cells in the destruction of bile ducts in primary biliary cirrhosis. Hepatology, 2011, 53: 1270–1281
79 Zhang J, Sun R, Wei H, et al. Toll-like receptor 3 agonist enhances

IFN-gamma and TNF-alpha production by murine uterine NK cells. Int Immunopharmacol, 2007, 7: 588–596
80 Sivori S, Falco M, Carlomagno S, et al. A novel KIR-associated function: Evidence that CpG DNA uptake and shuttling to early endosomes is mediated by KIR3DL2. Blood, 2010, 116: 1637–1647
81 Sivori S, Falco M, Moretta L, et al. Extending killer Ig-like receptor function: From HLA class I recognition to sensors of microbial products. Trends Immunol, 2010, 31: 289–294
82 Lodoen M B, Lanier L L. Natural killer cells as an initial defense against pathogens. Curr Opin Immunol, 2006, 18: 391–398
83 Guerra C, Johal K, Morris D, et al. Control of *Mycobacterium tuberculosis* growth by activated natural killer cells. Clin Exp Immunol, 2012, 168: 142–152
84 Vankayalapati R, Garg A, Porgador A, et al. Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. J Immunol, 2005, 175: 4611–4617
85 Eissmann P, Evans J H, Mehrabi M, et al. Multiple mechanisms downstream of TLR-4 stimulation allow expression of NKG2D ligands to facilitate macrophage/NK cell crosstalk. J Immunol, 2010, 184: 6901–6909
86 Zhou Z, Zhang C, Zhang J, et al. Macrophages help NK cells to attack tumor cells by stimulatory NKG2D ligand but protect themselves from NK killing by inhibitory ligand Qa-1. PLoS ONE, 2012, 7: e36928
87 Kloss M, Decker P, Baltz K M, et al. Interaction of monocytes with NK cells upon Toll-like receptor-induced expression of NKG2D ligand MICA. J Immunol, 2008, 181: 6711–6719
88 Hou X, Zhou R, Wei H, et al. NKG2D-retinoic acid early inducible-1 recognition between natural killer cells and Kupffer cells in a novel murine natural killer cell-dependent fulminant hepatitis. Hepatology, 2009, 49: 940–949
89 Radaeva S, Sun R, Jaruga B. Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. Gastroenterology, 2006, 130: 435–452
90 Schreiner B, Voss J, Wischhusen J, et al. Expression of Toll-like receptors by human muscle cells *in vitro* and *in vivo*: TLR3 is highly expressed in inflammatory and HIV myopathies, mediates IL-8 release and up-regulation of NKG2D-ligands. FASEB J, 2006, 20: 118–120
91 Champsaur M, Lanier L L. Effect of NKG2D ligand expression on host immune responses. Immunol Rev, 2010, 235: 267–285
92 Zhou R, Wei H, Sun R, et al. NKG2D recognition mediates Toll-like receptor 3 signaling-induced breakdown of epithelial homeostasis in the small intestines of mice. Proc Natl Acad Sci USA, 2007, 104: 7512–7515
93 Wu H, Chen G, Wyburn K R, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. J Clin Invest, 2007, 117: 2847–2859
94 Shigeoka A A, Holscher T D, King A J, et al. TLR2 is constitutively expressed within the kidney and participates in ischemic renal injury through both MyD88-dependent and -independent pathways. J Immunol, 2007, 178: 6252–6258
95 Cooper M A, Fehniger T A, Fuchs A, et al. NK cell and DC interactions. Trends Immunol, 2004, 25: 47–52
96 Reizis B, Colonna M, Trinchieri G, et al. Plasmacytoid dendritic cells: One-trick ponies or workhorses of the immune system? Nat Rev Immunol, 2011, 11: 558–565
97 Romagnani C, Della C M, Kohler S, et al. Activation of human NK cells by plasmacytoid dendritic cells and its modulation by CD4+ T helper cells and CD4+ CD25hi T regulatory cells. Eur J Immunol, 2005, 35: 2452–2458
98 Swiecki M, Gilfillan S, Vermi W, et al. Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8(+) T cell accrual. Immunity, 2010, 33: 955–966
99 Persson C M, Chambers B J. Plasmacytoid dendritic cell-induced migration and activation of NK cells *in vivo*. Eur J Immunol, 2010, 40: 2155–2164
100 Zanoni I, Granucci F. Regulation of antigen uptake, migration, and lifespan of dendritic cell by Toll-like receptors. J Mol Med (Berl), 2010, 88: 873–880
101 Miyake T, Kumagai Y, Kato H, et al. Poly I:C-induced activation of NK cells by CD8 alpha+ dendritic cells via the IPS-1 and TRIF-dependent pathways. J Immunol, 2009, 183: 2522–2528
102 Vulink A, Radford K J, Melief C, et al. Dendritic cells in cancer immunotherapy. Adv Cancer Res, 2008, 99: 363–407
103 Romani N, Gruner S, Brang D. Proliferating dendritic cell progenitors in human blood. J Exp Med, 1994, 180: 83–93
104 Seya T, Kasamatsu J, Azuma M, et al. Natural killer cell activation secondary to innate pattern sensing. J Innate Immun, 2011, 3: 264–273
105 McCartney S, Vermi W, Gilfillan S, et al. Distinct and complementary functions of MDA5 and TLR3 in poly(I:C)-mediated activation of mouse NK cells. J Exp Med, 2009, 206: 2967–2976
106 Ebihara T, Azuma M, Oshiumi H, et al. Identification of a polyI:C-inducible membrane protein that participates in dendritic cell-mediated natural killer cell activation. J Exp Med, 2010, 207: 2675–2687