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REVIEW

Activating and inhibitory receptors of natural killer cells

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Natural killer (NK) cells are potent immune effector cells that can respond to infection and cancer, as well as allowing maternal adaptation to pregnancy. In response to malignant transformation or pathogenic invasion, NK cells can secrete cytokine and may be directly cytolytic, as well as exerting effects indirectly through other cells of the immune system. To recognize and respond to inflamed or infected tissues, NK cells express a variety of activating and inhibitory receptors including NKG2D, Ly49 or KIR, CD94–NKG2 heterodimers and natural cytotoxicity receptors, as well as co-stimulatory receptors. These receptors recognize cellular stress ligands as well as major histocompatibility complex class I and related molecules, which can lead to NK cell responses. Importantly, NK cells must remain tolerant of healthy tissue, and some of these receptors can also prevent activation of NK cells. In this review, we describe the expression of prominent NK cell receptors, as well as expression of their ligands and their role in immune responses. In addition, we describe the main signaling pathways used by NK cell receptors. Although we now appreciate that NK cell biology is more complicated than first thought, there are still facets of their biology that remain unclear. These will be highlighted and discussed in this review.
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Natural killer (NK) cells are lymphocytes that are part of the innate immune system. They are an important part of the first line of defense that protects the body from pathogen invasion and malignant transformation. NK cells comprise 5–10% of peripheral blood lymphocytes, however, this proportion can vary with age.[1,2] NK cells can also be found in the spleen, lungs and liver, as well as in the uterus and in small numbers in the lymph nodes.[3–6] NK cells can respond rapidly to activation signals and, through the activity of perforin and granzymes, they can be directly cytolytic without the requirement for transcription or proliferation. NK cell responses are mediated through cell surface receptors that can either be inhibitory or activating. Although NK cells can also respond to cytokines, the following review focuses on NK cell receptors and provides details of their importance in directing NK cell responses.

RECEPTOR-MEDIATED NK CELL RESPONSES

NK cells do not have the exquisite antigen specificity of T cells and B cells. Rather they express a series of activating and inhibitory receptors. These receptors provide signals, the balance of which forms the decision of whether an NK cell becomes activated or activation is inhibited. This recognition system does have some degree of flexibility, although unlike T and B cells, flexibility is not achieved through the rearrangement of gene clusters. Instead, NK cell recognition receptor families have achieved flexibility through rapid genetic evolution (within a species) and reported promiscuity of ligand binding.[7]

To contribute to the first line of defense, NK cells are poised ready to attack infected or malignant cells. This immediate response capacity of NK cells may present a danger to healthy cells in the event of inappropriate NK cell activation, and consequently the process of NK cell activation is tightly regulated. Part of this regulation is inherent in the type of receptors that NK cells use to recognize and respond to target cells. Two suggested hypotheses of NK cell activation are the 'missing self' and 'induced self' theories.[8,9] The 'missing self' hypothesis suggested that NK cells attack target cells that show reduced or aberrant major histocompatibility complex (MHC) or human leukocyte antigen (HLA) class I (that is, when the cells are missing expression of self-molecules, which are usually expressed on healthy tissue). Thus, when MHC class I are expressed on cells, activation of NK cells is inhibited. However, further studies have indicated that NK cell activation may be determined, not only by lack of MHC class I expression, but also the expression of ligands for NK cell-activating receptors.[10–12] The presence of activating receptors was implicit in the original 'missing self' model, however, Karre,[13] suggested that activating receptors recognized ubiquitous ligands, and that inhibitory signals were determinant of a functional response. The 'induced self' model of NK cell activation is therefore not entirely exclusive from the missing self model and describes the recognition of cellular stress ligands, induced upon malignant transformation or viral invasion. For example, MHC class I chain-related gene *MICA*/*MICB* are ligands for the NKG2D-activating receptor. Expression of these

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molecules is induced under situations of cellular stress, such as viral infection.[14] Hence, NK cells can become activated by the induced expression of stress-related proteins.

NK CELL RECOGNITION AND RESPONSE RECEPTORS

NK cell inhibitory and activating receptors are a complex group of receptors that use opposing signaling motifs to stimulate or inhibit activation. The main signaling pathways used by NK cell receptors will be briefly described in this section followed by a more detailed description of some NK cell recognition receptors.

Inhibitory receptors signal through intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), located in the cytoplasmic tail of these receptors. Commonly, Src homology 2 domain containing phosphatases (SHP1 or 2) are recruited after phosphorylation of a tyrosine residue.[15] How inhibitory signals interfere with activating signals remain unclear, but recent reports suggest that ITIM-mediated signaling result in both dephosphorylation and specific phosphorylation of intracellular components. One recent report implicated β-arrestin 2 in the inhibition of NK cell activation, through the recruitment of SHP1 and 2.[16] Another report suggested that a common point of NK cell activation signaling could be targeted, in which a SHP1 phosphorylation site (Vav1) could be dephosphorylated during inhibitory signaling.[17] This report showed that inhibitory signaling can prevent not only NK cell-mediated cytotoxicity, but also interfere with adhesion of NK cells to target cells. In contrast, Long and Peterson[18] have described the specific phosphorylation of a tyrosine adapter, Crk, as a result of ITIM engagement. Therefore, it is now clear that while ITIM signaling prevents intracellular phosphorylation, it is likely to involve more complex signaling than originally thought. The more we can learn regarding the inhibitory signals use by NK cells, the better placed we will be to manipulate these signals for the optimization of NK cell-based therapies.

In contrast, some activating receptors signal through immunoreceptor tyrosine-based activating motifs (ITAMs), although these are not contained in the receptors' cytoplasmic tails but rather in associated molecules. After phosphorylation of a tyrosine residue in the tail, the Src homology 2 domain containing kinases (Syk or ZAP70) are recruited, leading to a signal cascade, which results in degranulation and transcription of cytokine and chemokine genes.[15] Further investigation has revealed a requirement for PKC-θ in sustained ITAM signaling, which results in NK cell activation, and suggest that ITAM-mediated activation of NK cells does not require co-stimulatory signals.[17,19] However, there are reports that stimulation of only one activating receptor is insufficient to stimulate cytotoxicity and cytokine secretion, and stimulation of more than one receptor is required for function.[20] Therefore, the question of NK cells requiring co-stimulation for activation remains unclear.

Other activating receptors, including NKG2D, use an alternate signaling mechanism, using either DAP-10 or DAP-12, which signal differently to each other. DAP-12 signals through an ITAM as described above, whereas DAP-10 binds either Grb2 or p85 and signals through phosphatidylinositol-3 kinase and other signaling pathways.[21–23] Although the subsequent signaling events are not well characterized, it is clear that the outcomes of DAP-10 and DAP-12 signaling differ, wherein DAP-12 signaling results in cytokine secretion and cytotoxicity and DAP-10 signaling results in cytotoxicity.[24,25]

A third activation/inhibition signaling pathway is possible in NK cells, and results from stimulation of the CD244 (2B4) receptor. This receptors cytoplasmic tail contains an immunoreceptor tyrosine-based switch motif, which recruit Src homology 2 domain containing adapter proteins SAP or ERT.[26] Recruitment of SAP results in activation of NK cell function, wherein recruitment of ERT inhibits NK cell function. The advantage of using several different activating pathways is not entirely clear, however, it has been shown that activating signals can be overridden with signaling of an ITIM-containing receptor.[17,27]

Receptor family more easily defines the recognition receptors on NK cells than the functional categories of 'inhibitory' or 'activating.' This is due to the fact that some receptor families contain both activating and inhibitory receptors, as described above for 2B4. The reasons for these phenomena are unclear, although it is hypothesized to increase the ability of NK cells to discriminate normal, healthy tissue from infected or malignant tissue, thereby preventing inappropriate NK cell activation. We will now discuss each receptor, with respect to structure, proposed function and regulation.

Ly49 FAMILY RECEPTORS

The C-type lectin-like Ly49 receptors are a large receptor family in mice. The majority of these receptors are inhibitory and signal through an ITIM, although activating Ly49 receptors do exist and use the DAP-12 molecule for signaling.[28] The specificities of Ly49 receptors are mainly MHC class I molecules and related proteins (see Table 1), in which Ly49A binds H-2D^{d,} H-2D^{k}[29] and Ly49C binds H-2K^{b} and H2D^{b}.[30] Activation receptor Ly49D has been shown to bind H-2D^{d}, although this remains controversial.[31,32] Other Ly49 receptors include activating Ly49P and Ly49W, which are also reported to interact with H-2D^{d}.[33,34] The 'recognition' of MHC class I by Ly49 receptors require the presence of a peptide bound in the groove of the MHC molecule, although the specificity of this peptide-binding varies between Ly49 receptors.[35–38] Interestingly, it has been reported that Ly49 molecules can bind MHC molecules in *cis* (that is, on the same membrane), which can reduce the capacity for *trans* binding and hence reduce the required signaling threshold for activation.[39,40] Therefore, the nature of Ly49 receptor and MHC binding (*cis* or *trans*) can affect the signaling outcome.

With respect to receptor expression regulation, the initial determination of Ly49 gene expression was found to be controlled by *cis*-acting regulatory elements. However, the level of expression of Ly49 genes is affected by the level of MHC class I expression in the particular mouse.[41,42] That is, the NK cell Ly49 repertoire is influenced by the host, and consequently it is found to be highly polymorphic.[43] In a deviation from the recognition of MHC class I by Ly49 receptors, it has been shown that Ly49H binds m157, a viral glycoprotein expressed on cells infected with MCMV. This protein resembles MHC class I and may have evolved as a viral-encoded immune evasion strategy, as it also binds the inhibitory Ly49I (in the 129/J mouse strain).[44]

KIR FAMILY RECEPTORS

In humans and primates, the Ly49 family of receptors is absent but is replaced with the structurally distinct killer immunoglobulin-like receptors (KIR). The similarities between these receptor families, including the presence of both inhibitory and activating receptors, the common ITIM and DAP-12 ITAM signaling, and their recognition of MHC class I is evidence of their convergent evolution.[45] In further support of this, evidence exists showing that both activating KIR and Ly49 receptors have both evolved from the respective ancestral inhibitory receptors.[46] In spite of these evolutionary similarities, structurally, Ly49 and KIR are very different. KIRs have evolved from the Ig-superfamily and consist of type 1 transmembrane glycoproteins with two or three Ig-like domains[47,48] and possess either a short or long cytoplasmic tail. The decision of which KIRs are

Table 1 NK cell receptors

Receptor family	*Species*	*Ligands*	*Activation/ inhibitory*
Ly49	M	MHC class I	ACT/INHIB
Ly49A		H-2D^{d,k,p}	Inhib
Ly49C		H-2K^{b,d}, H-2D^{b,d,k}	Inhib
Ly49D		H-2D^{d}	Act
Ly49H		m157	Act
Ly49I		H-2K/D^{b,d,s,q,v}	Inhib
Ly49P		H-2D^{d}	Inhib
KIR	H	HLA-A/-B/-C	ACT/INHIB
KIR2DL1		HLA-C2	Inhib
KIR2DL2/3		HLA-C1	Inhib
KIR2DL4		HLA-G	Act
KIR2DL5		?	Inhib
KIR3DL1		HLA-Bw4	Inhib
KIR3DL2		HLA-A3, -A11	Inhib
KIR2DS1		HLA-C2	Act
KIR2DS2		HLA-C1	Act
KIR2DS3		?	Act
KIR2DS4		?	Act
KIR2DS5		?	Act
KIR3DS1		HLA-Bw4	Act
CD94-NKG2	H/M	H: HLA-E M: Qa1b	ACT/INHIB
NKG2A			Inhib
NKG2C			Act
NKG2E			Act
NKG2D	H/M	H: MIC-A/-B, ULBP1/2/3/4 M: RAE-1, MULT-1, H60	ACT
NCRs	H/M	Viral HA	ACT
NKp30		BAT-3, HSPG, B7-H6	Act
NKp44		Viral HA	Act
NKp46		Viral HA, HSPG	Act
NKp80		AICL	Act
LILR	H/M	MHC class I, UL18	INHIB
2B4	H/M	CD48	ACT/INHIB
KLRG1	H/M	Cadherins	INHIB
NKR-P1	M	Ocil/Clr-b	ACT/INHIB
DNAM-1	H/M	PVR, CD122	ACT
PILR	M	CD99	ACT

Abbreviations: ACT, activation; BAT-3, HLA-B-associated transcript 3; H, human; HA, hemagglutinin; HLA, human leukocyte antigen; INHIB, inhibitory; KIR, killer immunoglobulin-like receptor; KLRG1, killer cell lectin-like receptor G1; LILR, leukocyte immunoglobulin-like receptor; M, mouse; MHC, major histocompatibility complex; MULT-1, mouse UL16-binding-like transcript-1; NCR, natural cytotoxicity receptor; NK, natural killer; PVR, polio virus receptor; RAE-1, retinoic acid early transcript-1.

expressed on each NK cell seems to be random and is regulated by the methylation of *KIR* gene loci.[49] The overall KIR repertoire is determined by KIR genotype, however, there is evidence of some modulation of KIR expression by HLA class I.[50,51] The repertoire of *KIR* genes expressed within one individual forms a KIR haplotype, and expression of each *KIR* gene varies between haplotypes, but three *KIR* genes are common to all haplotypes (KIR3DL3, KIR2DL4 and KIR3DL2). Inhibitory KIRs signal through an ITIM,[52,53] while activating KIRs associate with the DAP-12 molecule to signal activation.[54,55] KIR receptors specifically bind HLA-A, -B and -C molecules and recognize polymorphisms in these class I molecules (see Table 1). Structurally, it has been shown that KIRs bind the peptide-binding region of HLA molecules when a peptide is bound.[56–60] For the prototypical Ly49 family member, Ly49A, this binding event is dependent on site 2, with no peptide selectivity.[35,36,39] However, KIR bind HLA in a similar manner to TCRs whereas Ly49 binding of MHC class I was shown to be concentrated in two specific areas.[29,61,62] With respect to KIR repertoire, the expression of KIRs on different NK cells within one individual can vary with respect to allelic variants and levels of expression, and therefore each individual has different populations of NK cells that express an assortment of KIRs.[63]

KIR, as well as inhibitory Ly49 family members described above, are thought to have a role in the induction of NK cell tolerance of self-tissue. One suggested method of tolerance induction involves a process termed 'licensing'.[64] In this process, NK cells must express at least one inhibitory receptor specific for a self MHC molecule to be permitted to become responsive to later encounters with target cells. Evidence for this process exists in both mice and humans.[65–67] This prevents activation of NK cells against normal tissue and is known as the 'at least one' hypothesis.[68,69] However, there is mounting evidence to suggest that this is not the only means of inducing NK tolerance toward normal tissue. This evidence includes the observation that HLA and KIR segregate independently and the expression of KIRs is not driven by HLA.[7,70–72] Further to this, it has been shown that a significant number of individuals do not express a KIR that specifically recognize their own HLA ligands.[73] An alternate model for the induction of NK cell self-tolerance has been suggested that involves unlicensed NK cells. This model suggests that NK cells can lack expression of a self MHC-specific receptor yet remain tolerant to self-tissue, which lacks MHC class I, as these NK cells are hypo-responsive.[74] Thus, the assumption that KIR expression is required to maintain tolerance to self-tissue may not be correct, and this may become important when predicting the alloreactivity of these cells.

CD94–NKG2 HETERODIMER RECEPTORS

Another C-type lectin family of receptors is the CD94–NKG2A/C/E heterodimers. These receptors react to the level of non-classical MHC class I on the surface of potential target cells, and are thought to be important in the prevention of inappropriate NK cell activation.[75] The expression of these receptors is not stable like Ly49 receptors, and in contrast, expression levels can be affected by cytokines present in the surrounding environment.[76–78] In humans, the expression of these receptors may be related to *KIR* gene expression, as NK cell clones lacking expression of an inhibitory KIR were shown to express an inhibitory CD94–NKG2 heterodimer.[79]

Human CD94–NKG2A/C/E heterodimers recognize the non-classical MHC molecule, HLA-E,[80] whereas the corresponding mouse heterodimers recognize Qa1^{b}.[81] The ability of NK cells to monitor expression of HLA-E (and Qa1^{b} in mice) is thought to be a mechanism by which NK cells can monitor the expression of classical MHC class I molecules on target cells. Both the human and mouse ligands (HLA-E and Qa1^{b}) bind peptides derived from the leader sequence of classical MHC class I molecules.[75,82] Therefore, the peptides presented by HLA-E or Qa1^{b} directly reflect expression of other MHC class I molecules expressed on the cell. This allows indirect monitoring of expression of MHC class I molecules on a target cell.

Heterodimers, CD94–NKG2C and CD94–NKG2E, have been shown to associate with DAP-12 and are thought to be activating receptors.[75,81,83] In humans, the inhibitory, ITIM-containing

CD94–NKG2A receptor and activating, DAP-12-associating CD94–NKG2C receptor both bind HLA-E, a non-classical HLA class I molecule.[80,84,85] The reason for having one activating and one inhibitory receptor specific for the same molecule remains unclear. This phenomenon may allow more specific discrimination between normal and distressed or infected tissue, as expression of this ligand may not necessarily lead to NK cell activation.[86]

NKG2D RECEPTOR

NK cells recognize 'stressed' cells through the activating receptor NKG2D, which is expressed on almost all mouse NK cells.[87] This receptor has been shown to be important in the NK cell-mediated control of some cancers.[88] Only distantly related to the NKG2 family, NKG2D does not form a heterodimer with CD94, but is expressed as a homodimer and signals by recruiting DAP-10 or DAP-12 molecules.[22,89] Structural analysis has revealed that one NKG2D homodimer actually associates with four DAP-10 chains.[90] In the mouse there are two isoforms of the NKG2D molecule, a longer isoform (NKG2D-L), which can only recruit DAP-10, and a shorter isoform (NKG2D-S), which recruits either DAP-10 or DAP-12.[91,92] After NKG2D stimulation of mouse NK cells, it is thought that signaling through DAP-12 results in both cytokine secretion and cytotoxicity, and that DAP-10 stimulates a strong cytotoxic response, although in certain circumstances DAP-10 signaling can result in cytokine secretion.[93] Human NK cells only express the long isoform of NKG2D and it associates with DAP-10 to induce both a cytotoxic and cytokine-mediated response.[22,25]

The NKG2D molecule recognizes several different ligands, this ability is thought to be due to a single binding site in the receptor, with side chains that show a limited flexibility resulting in a rigid body interaction model of ligand binding.[94] NKG2D ligands include MHC class I-related proteins whose expression is regulated by both the DNA damage and heat shock response pathways, which are often activated in tumors.[95,96] The expression of NKG2D ligands is tightly regulated, however, they can be expressed on healthy tissues, generally at baseline levels, below a functionally relevant threshold.[97] The ligands of human NKG2D include the stress-inducible MHC class I chain-related gene (MIC)-A and MIC-B, and ULBP1, ULBP2, ULBP3 and ULBP4.[89,98,99] Expression of cellular stress ligands MIC-A and MIC-B has been reported to be induced upon malignant transformation, reportedly as a result of the DNA damage response pathway.[100,101] There is also evidence to suggest that expression of MIC genes is related to the heat shock response pathway.[96] In the mouse, NKG2D binds to retinoic acid early transcript-1 molecules (α, β, γ, δ and ε), as well as mouse UL16-binding-like transcript-1 and histocompatibility 60 (H60) molecules.[11,102–104] These molecules may compete for NKG2D binding, however, H60 has been shown to have a greater affinity for the NKG2D molecule, in spite of only being expressed in the BALB/c mouse strain.[11,105] Mouse retinoic acid early transcript-1 transcription has been shown to be induced after Toll-like receptor stimulation or viral infection, showing a direct link between cellular stress and NK cell activation.[106,107] Further to this, retinoic acid early transcript-1 molecules are reportedly upregulated on various tumor cell lines and carcinogen-induced tumors.[11,102,108] Mouse UL16-binding-like transcript-1 expression in mice is not directly driven by the DNA damage or heat shock response pathways, but can be upregulated through these pathways through posttranscriptional regulation.[109,110] Thus, NKG2D-mediated recognition of these stress ligands enables NK cell-mediated monitoring of stressed or malignant cells.

NKG2D has been shown to have a role in the immune response to certain immunogenic tumors, as well as the induction of CTL, Th1 and Th2 responses.[11,111] Furthermore, the importance of this receptor in immunosurveillance has been illustrated by a report describing Eμ-myc mice that also lack NKG2D. These double-mutant mice showed a more rapid onset of lymphoma compared with single-mutant Eμ-myc mice.[88] Indeed, NKG2D has such an important role in the immune response to tumor it has become the target of immune evasion strategies. Several tumors have been reported to secrete NKG2D ligands, such as MIC-A, which can serve as a decoy to NK cells.[112,113] Another mechanism of tumor-mediated NKG2D evasion is the secretion of transforming growth factor-β1 from tumor cells, which can lead to downregulation of expression of NKG2D on NK cells.[114] In addition, in mice, transforming growth factor-β has been shown to downregulate expression of NKG2D-ligands on malignant glioma cells.[115] Given the immune-stimulatory nature of NK cells, NKG2D-mediated recognition of tumor cells is integral for an optimal immune response to some tumors.

NATURAL CYTOTOXICITY RECEPTORS

An additional group of activating receptors, referred to as natural cytotoxicity receptors (NCRs), also belong to the Ig-superfamily.[7] The appearance of these receptors (more specifically NKp44) in recently evolved species, illustrated the rapid nature of the evolution of NK cell receptors.[116] In humans, NCRs NKp46, NKp80 and NKp30 are expressed on activated and resting NK cells, but NKp44 is upregulated upon interleukin-2 stimulation of some NK cells.[117,118] Reported ligands for NKp46 and NKp44 include viral hemagglutinins. Cellular ligands probably exist, given that anti-NCR antibodies abrogate NK cell-mediated lysis of many tumor cell types.[119–123] Other ligands of NCRs include nuclear factor HLA-B-associated transcript 3, which can be released from tumor cells and binds NKp30.[124] NKp46 and NKp30 have also been shown to bind heparin sulfate proteoglycans and NKp80 binds activation-induced C-type lectin.[125,126] More recently, NKp30 has also been shown to bind B7-H6.[127] The NCRs have been suggested to be one of the main mechanisms by which NK cells kill tumor targets.[119,122] This is supported by studies showing that deletion of a single NCR reduces the ability of NK cells to lyse tumor targets *in vivo*.[122,128,129] NKp30 has also been implicated in NK cell–dendritic cell interactions, resulting in NK cell-mediated apoptosis and maturation of dendritic cells.[119,130] These receptors will surely become more important in the use of NK cells in cancer therapy as we learn their true roles in the anti-tumor immune response.

Leukocyte immunoglobulin-like receptor

The leukocyte immunoglobulin-like receptors (also known as LIR, ILT or CD85) are inhibitory receptors that are expressed on NK cells and bind MHC class I molecules.[131,132] The nature of the interactions between leukocyte immunoglobulin-like receptor and their ligand differ with each different receptor's structure, however, it has been shown to involve the α3 and β2 microglobulin domains of the MHC class I molecule.[133] The function of LIR in the regulation of NK cell activation is unclear, as leukocyte immunoglobulin-like receptor receptors are able to inhibit NK cell activation, although inhibitory KIR and CD94–NKG2 receptors are thought to be more dominant.[134] One specific LIR receptor has been shown to bind UL18, a human cytomegalovirus-encoded protein, with far greater affinity than for HLA class I.[135] The relevance of UL18–LIR interactions remains unclear, as expression of UL18 was found to increase target cell lysis by NK cells.[136]

2B4 RECEPTORS

The 2B4 receptor (CD244) is present on all human and mouse NK cells, and binds CD48, which is expressed on all hematopoietic

cells.[137–140] In both humans and mice, there are conflicting reports regarding the outcome of stimulation through this receptor. Activation or inhibition results from the signaling induced by the recruited adapter proteins.[139,141,142] Therefore, it has been postulated that 2B4 could be a multi-functional receptor, wherein the outcome of triggering may be dependent on the stage of NK cell maturation.[143] In addition, the presence of isoforms may affect the adapters recruited and hence receptor-stimulating outcome. Human 2B4 is expressed as one of two isoforms, of which only one has been shown to activate NK cell cytotoxicity.[144] The two mouse isoforms have different cytoplasmic domains, signaling either activation or inhibition.[145,146] In humans, this receptor has been reported to be important in the rejection of melanoma cells expressing CD48, although its involvement in the immune response to other tumor types is unknown.[147]

KLRG1 RECEPTOR

Killer cell lectin-like receptor G1 (KLRG1) is an inhibitory receptor that signals through an ITIM to inhibit NK cell function.[148] The ligands for this receptor were shown to be classical cadherins, (E-, N- and R-cadherins).[149] These cadherin ligands are expressed in healthy, solid tissues and therefore may have a role in the prevention of lysis of healthy tissues. Ito *et al.*,[149] showed that expression of KLRG1 on immune-experienced NK cells resulted in a higher threshold for activation against E-, N- and R-cadherin positive targets. This could prevent damage to healthy tissues by restraining the activation of 'experienced' NK cells. Further to this, this receptor is thought to have a role in the 'missing self'-mediated activation of NK cells. The KLRG1 ligand, E-cadherin has been shown be downregulated on malignant epithelial tumors and may allow these tumors to metastasize.[150,151] Consequently, it has been suggested that the KLRG1 receptor not only sets a threshold for NK cell activation, but may also serve as a strategy for NK cells to detect malignant epithelial tissue with abnormal E-cadherin expression.[152]

CO-STIMULATORY RECEPTORS

There are several other NK cell receptors, which are viewed as co-stimulatory. These receptors provide further stimulation to the cell, although alone are not sufficient to trigger NK cell activation. Hence, not only do they provide an alternate mechanism of activation, but also ensure that the NK cells are not activated to respond to normal or healthy tissue. These receptors include DNAM-1, the NKR-P1 receptors and the PILR receptor, which are discussed below.

NKR-P1 RECEPTORS

In the mouse, NKR-P1 receptors are either activating or inhibitory co-stimulatory receptors, however, only one non-polymorphic NKR-P1 gene exists in human.[153] NKR-P1 receptors are encoded by the gene family of the same name, and are type II membrane glycoprotein receptors that belong to the C-type lectin family. NK1.1, which is the prototypical NK cell marker (in C57BL/6 mice), belongs to this family.[154] Five receptors NKR-P1A, -B, -C, -D and -F have been identified, in which NKR-P1D/B both contain an ITIM suggesting inhibitory function.[155,156] However, it was found that NKR-P1C (NK1.1) associates with the ITAM-containing FcεRI to induce NK cell activation, although the biological relevance of this remains unclear.[44,157] NKR-P1A receptor signaling has not been fully characterized, although it may activate acid sphingomyelinase, which was suggested to result in NK cell resistance to apoptosis.[153,158] Ligands for NKR-P1B and -P1D have been identified as Ocil/Clr-b, a glycoprotein expressed on hematopoietic cells[159] and Clr-g, a C-type lectin expressed on activated NK cells.[160] These receptors may also be involved in the NK cell-mediated anti-tumor responses as expression of Ocil/Clr-b can be downregulated on tumor cells, in some form of 'missing self' recognition of target cells.[159] Therefore, in spite of the presence of both activation and inhibitory NKR-P1 receptors, this family of unique receptors in believed to represent a novel, MHC-independent mechanism for self/non-self discrimination across species.[161]

DNAM-1 RECEPTOR

DNAM-1 receptor (also known as CD226) is a member of the Ig-superfamily, and is constitutively expressed upon approximately 50% of NK cells.[162] The ligands for this co-stimulatory activating receptor are CD155 (also referred to as Polio virus receptor, PVR or Necl-5) and CD112 (Nectin-2), and these ligands can be upregulated on some tumor cells, implicating DNAM-1 in some NK cell-mediated anti-tumor responses.[163–165] Indeed, upregulation of CD155 on multiple myeloma cells has been reported, and resulted in increased sensitivity to NK cell-mediated lysis.[166] In addition, DNAM-1 has been shown to be involved in the lysis of tumor cells that do not express ligands for NK cell-activating receptors, this therefore broadens the scope of tumors susceptible to NK cell-mediated responses, and suggests this receptor is more than just co-stimulatory.[167] This receptor is also reportedly involved in the NK cell-mediated immunosurveillance of methylcholanthrene-induced sarcoma cells and tumor cells expressing CD70 or CD80.[168,169] It remains a possibility that this receptor has a role in the migration of NK cells, as DNAM-1 has been shown to allow movement of monocytes between endothelial cell junctions.[170] In addition, this receptor has been associated with lymphocyte function-associated antigen-1, an adhesion molecule important in the lysis of target cells, and has the capacity to bind intracellular adhesion molecule-1.[162,171,172] This resulted in actin polymerization and activation of other surface receptors, thus DNAM-1 may permit stable interactions between NK and target cells.[173] Furthermore, DNAM-1 has been shown to be involved in co-stimulation of T cells.[174,175] The involvement of this receptor in the NK cell-mediated responses to tumor is beginning to be elucidated and results to date suggest that DNAM-1 has a role, an important role in the recognition of tumor cells and migration of NK cells.

PIL RECEPTORS

The paired Ig-like 2 receptor (PILβ) is a type 1 glycoprotein receptor that can associate with DAP-12, and is regarded as an activating receptor.[176] An additional isoform, PILRα, has an ITIM in its cytoplasmic domain and is reported to be an inhibitory receptor.[177] The ligand for these receptors is PILR-L (CD99) and mouse NK cells have been shown to lyse PILR-L$^+$ target cells.[176] Recognition of PILR-L by these receptors has been shown to be dependent on the pattern of sialylated O-linked sugar chains.[178] These findings suggest a role for PILR in the NK cell-mediated recognition of carbohydrate chains, as opposed to proteins, on target cells, thereby broadening the range of target cells that NK cells can recognize.

CONCLUSIONS

NK cells are a diverse population of potent effector cells that can be divided into different subsets. Further investigation into the expression of various activating and inhibitory receptors will reveal more regarding the heterogeneity of these cells and indicate the differential roles of these subsets in an immune response. Furthermore, research that identifies new ligands and their role in trafficking and regulation will contribute to the understanding of NK cell diversity. A more in

depth knowledge of these cells and the complex signaling involved in their responses will allow further development of effective NK cell-based immunotherapies.

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