[Cancer Biology & Therapy 2:6, 610-616; November/December 2003]; ©2003 Landes Bioscience

Review

Regulation of Natural Killer Cell Function

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Received 10/28/03; Accepted 10/28/03

Previously published online as a CB&T E-publication:
http://www.landesbioscience.com/journals/cbt/toc.php?volume=2&issue=6

KEY WORDS

NK Cells, MHC, tumor, Ly49

ABBREVIATIONS

B6	C57BL/6
NK	natural killer
MCMV	mouse cytomegalovirus
HCMV	human cytomegalovirus
KIR	killer cell Ig-like receptor
NOD	non-obese diabetic
MHC	major histocompatibility complex
LRC	leukocyte receptor complex
NKC	natural killer gene complex
ITIM	immunoreceptor tyrosine-based inhibitory motif
ITAM	immunoreceptor tyrosine-based acti -vation motif
BAC	bacterial artificial chromosome
NCR	natural cytotoxicity receptors
LAK	lymphokine activated killer
GVHD	graft-versus-host-disease
APC	antigen presenting cell

ACKNOWLEDGEMENTS

This work was supported in part by an operating grant from the Canadian Institutes for Health Research. This project has also been funded in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400. A.P.M is a scholar of the Canadian Institutes for Health Research (New Investigator Award).

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

ABSTRACT

Individuals lacking natural killer (NK) cells have persistent viral infections and as a consequence die prematurely. In addition, mice with decreased NK cell function are more susceptible to carcinogen-induced cancers. Current evidence strongly suggests that downregulation of MHC by certain tumors and virally-infected cells results in NK cell attack due to the inability to trigger inhibitory Ly49, KIR, and NKG2A/CD94 class Ia and Ib MHC receptors. Extreme haplotype diversity is present in both mouse and human chromosomal segments coding for NK cell class Ia MHC receptors resulting in different numbers and types of receptors being expressed in individuals and different inbred mouse strains. Whether the absence or presence of a particular NK cell receptor gene is advantageous or deleterious for an individual with respect to immunity to pathogens and cancer is a question of paramount importance. Recent advances in our understanding of NK cell function are due to the identification of activating NK cell receptors, such as Ly49H and NKG2D, for specific viral and tumor ligands (m157 and Rae1, respectively). In a clinical setting, such MHC class I receptor diversity is advantageous with respect to preventing leukemic relapse in individuals treated for leukemia and receiving bone marrow transplants. Further delineation of NK cell receptors and tumor ligands will help researchers to exploit the innate immune system to better treat such diseases.

INTRODUCTION—WHY ARE NK CELLS IMPORTANT?

NK are bone marrow derived lymphocytes originally identified by their large granular morphology and their ability to spontaneously lyse certain tumor targets in vivo and in vitro without prior sensitization.[1] NK cells have the ability to 'home-in' on tumor metastases by extravasation from the circulation.[2] The potency of uncontrolled or inappropriate NK cell responses is evident in disease conditions such as allograft rejection, graft vs. host disease, diabetes, various autoimmune and neurological diseases, and aplastic anaemia/neutropenia. The importance of normal NK cell function in the immune system is best demonstrated in reports of individuals lacking NK cells and/or NK cell-like activity. One such patient was constantly afflicted with viral infections including severe chicken pox and varicella pneumonia, and eventually developed critical human cytomegalovirus (HCMV) and HSV infections.[3] Amazingly, all these infections took place in the presence of an apparently normal adaptive immune system as measured by in vitro B and T cell assays. In addition, papilloma viral infections and the resulting recurrent cervical carcinoma have also been linked with the absence of NK cells in another patient.[4] Finally, HIV^+ individuals show NK cell defects late after infection.[5] Collectively, these observations show that NK cells are a vital component of the immune response that constantly protects an individual from life-threatening infections.

NK cells are also important mediators of anti-tumor immunity. This is clearly displayed in transgenic mice with severely decreased levels of $NK1.1^+CD3^-$ (classical mouse NK cell phenotype) cells. Such mice have decreased resistance to B16 experimental lung metastases and RMA tumor outgrowth.[6] Furthermore, protection from the development of carcinogen-induced tumors is dependent on NK cells.[7] NK cell-mediated tumor rejection also functions to evoke long-term T cell memory. Tumor cells expressing CD70, a ligand for the co-stimulatory NK cell expressed CD27, were rejected more efficiently than $CD70^-$ tumor cells. Interestingly, secondary challenge of mice that had rejected $CD70^+$ tumors with the $CD70^-$ parental tumor line resulted in a tumor-specific T cell rejection response.[8]

NK cell responses toward tumor cells are mediated in part by expression of receptors for ligands often expressed on tumor cells such as the MIC/Rae1 stress-induced molecules recognized by NKG2D (discussed below). Also, the commonly observed phenomenon of tumor cell loss of class I MHC expression,[9] which is thought to be a defense against T

cells, confers susceptibility to NK cell lysis (Fig. 1). Normal class Ia and Ib MHC expression is thought to result in NK cell tolerance through inhibitory class I MHC receptors such as KIR, Ly49, and NKG2A. Finally, analogous to HIV infection, patients with cancer have decreased NK function as measured by cytotoxicity, production of cytokines and proliferation.[10]

According to current scientific knowledge, NK cells mediate their protective effect in two ways: cytotoxicity and cytokine production. Cytotoxicity in NK cells (and cytotoxic T lymphocytes) is carried out by granule exocytosis of membrane pore-forming molecules (perforin) and proteases (granzymes) or death ligand interactions (FasL and TRAIL). Upon target cell binding, NK cells will also produce large amounts of cytokines such as IFN-γ, TNF, and GM-CSF.[11] Natural and induced mutations of cytokine (IFN-γ and IL-2/IL-15Rβ) and cytotoxicity-associated genes (perforin, granzyme A and B, FasL) that are expressed by NK cells also negatively affect anti-viral responses and the rejection of tumors.[12] For example, mice deficient in perforin, TNF-α, or TRAIL cannot efficiently reject MHC class I-deficient tumors in vivo.[13-15] Interestingly, NK cells attack tumor cells in different organs in different ways. For example, perforin is important in lung and spleen, but not liver.[16] In the liver, TRAIL-mediated killing appears to be important for NK mediated protection against tumorigenesis.[15]

HOW DO NK CELLS RECOGNIZE 'TARGET' CELLS?

Developing NK cells expand in the bone marrow after acquiring receptors to the products of the major histocompatibility complex (MHC) and then take up residence in the spleen, blood and liver.[17] An important clue as to the regulation of NK cells came from the observation that MHC-deficient tumor cells were attacked by NK cells in vivo.[18] Subsequently, it was observed that bone marrow grafts and T cell lymphoblasts from β2-microglobulin$^{-/-}$ mice were attacked by NK cells from syngeneic wild-type mice.[19,20] MHC antigens in the mouse (H-2) and in humans (HLA) are ubiquitously expressed and act as self-recognition molecules for T and NK cells in normal healthy cells. Human and mouse tumor tissues and cell lines frequently have defects in MHC antigen expression. Such defects can range from total loss of class I MHC, loss of one haplotype, loss of a locus, loss of specific alleles, compound phenotypes, or unresponsiveness to MHC upregulation by interferons, and aberrant non-classical MHC expression.[21] It is believed that any such defects allow NK cells to kill affected target cells.

NK cells differ from B and T lymphocytes in not recombining genes for cell surface receptors involved in the differentiation of self and non-self. Instead, NK cells express a diverse array of receptors for both foreign and self molecules expressed by target cells such as killer immunoglobulin-like receptor (KIR), Ly49, 2B4, NKG2/CD94, NKG2D, LIR/ILT, NKp44, NKp46 and others.[22] Many of these proteins are expressed in both mouse and human NK cells and play a role in self/non-self discrimination. The ligands of these receptors are diverse and include MHC, viral MHC mimics, integrins, sialic acid, viral hemaglutinins and MHC-related 'stress' proteins. The importance of these receptors in innate immunity against infection is shown by a recent report of the association of KIR3DS1 (an activating KIR) with HIV control and progression to AIDS in population studies.[23] A potential role for the NK cell receptor 2B4 in resistance to EBV infection is suggested by the observation that patients suffering from X-linked lymphoproliferative syndrome possess a defect in signalling by the NK cell receptor 2B4 and are highly susceptible to EBV infection.[24] Similarly, resistance to

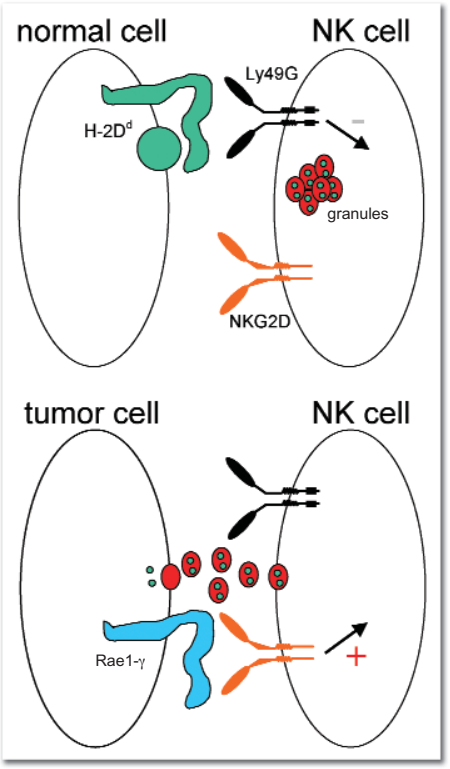

Figure 1. NK cell cytotoxic regulation. Malignant transformation or viral infection can result in the downregulation of class I MHC (H-2D^d) expression and the induction of 'stress' markers such as Rae1-γ. Either of these events will result in NK cell attack by the loss of self-inhibition and/or new activating signals which are mediated by inhibitory and activating receptors, Ly49G and NKG2D, respectively.

MCMV infection is controlled in part by the expression of Ly49H on mouse NK cells.[25-27] NK cells also attack target cells by indirect recognition using antibody dependent cell-mediated cytotoxicity.

Ly49, KIR, NKG2/CD94

In humans, normal cells express self-class I MHC proteins that act as ligands for inhibitory KIR proteins on NK cells and are thus spared from attack. KIRs have either two or three Ig domains along with a short (activating) or long (inhibitory) cytoplasmic tail. Many of the KIR ligand specificities have been elucidated and all have been found to be HLA class I.[28] Depending on haplotype, 4-14 *KIR* genes are found clustered together on human chromosome 19q13.1 in a region termed the leukocyte receptor complex (LRC). In addition to the KIR, the LRC contains other Ig-related receptors (such as the *ILT/LAIR* family) that are expressed on NK and other hematopoeitic cells, some of which have specificity for MHC. Individual KIR haplotypes are highly variable in gene content, such that haplotypes from unrelated individuals are likely to have different numbers and types of these genes.[29] Heterozygosity and the large number of KIR allelic variants in humans further compound the complexity of NK cell receptor expression. Furthermore, a given human NK cell expresses only a subset of *KIR* genes.[30] Thus, NK cell populations within heterozygous individuals are highly heterogeneous with respect to class I MHC receptor expression. Such diversity is thought to be advantageous in NK cell responses to selective MHC downregulation by always ensuring an NK cell sub-population exists that will respond to loss of a single MHC ligand.

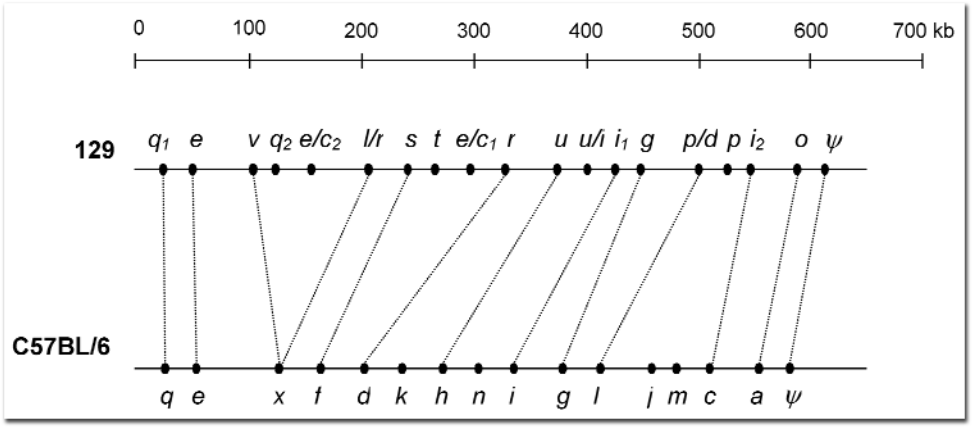

Figure 2. Comparison of 129/J and B6 Ly49 genomic organization. The B6 gene map is reproduced from available GenBank BAC sequence data and previous reports. The most similar genes between the two strains, which may represent alleles, are indicated by a dotted line. All gene markers were based on exon 4 positions. The maps are drawn so that the centromere and telomere are to the left and right, respectively. The *Ly49b* gene is approximately 750kb telomeric of the clusters. α represents a group of Ly49-related gene fragments.

The analogous genes in mice are called *Ly49*. The *Ly49* gene cluster is composed of 15 genes, pseudogenes, and gene fragments in the well characterized C57BL/6 (B6) mouse (*Ly49a-n*, *q*, and *x*), arranged in tandem with identical transcriptional orientation and without interruption by non-*Ly49* genes (Fig. 2).[31] The *Ly49* cluster is located in a region termed the NK complex (NKC). The NKC is found on mouse chromosome 6 and is composed of gene families that code for C-type lectin-related type II transmembrane proteins expressed on NK cells such as *Ly49*, *CD69*, *NKG2/CD94*, and *Nkrp1*.[32] Although they represent structurally distinct protein families, the lectin-related Ly49 proteins and the Ig-related KIR proteins associate with identical signalling molecules to achieve either activation or inhibition of NK cells in response to specific MHC class I ligands,[33] indicating convergent evolution of function. Furthermore, like *KIR*, *Ly49* gene expression in individual NK cells appears to be generally stochastic.[34]

Unlike KIR and Ly49, humans and mice both express activating and inhibitory versions of the C-type lectin-related and NKC-encoded NKG2/CD94 heterodimers. The human ligand is HLA-E, while the mouse analog is Qa-1^b, and both proteins present the leader sequence of class I MHC. Inhibitory function of NKG2A/CD94 is shown in the relative inability of NK cells to clear RMA-S expressing $Qa1^b$ versus normal RMA-S tumor cells.[35] The function of activating NKG2/CD94 isoforms is unknown. But, it is of interest that both HLA-E and Qa-1^b can present peptides derived from bacteria.[36]

Ly49, KIR, and NKG2 families have both activating and inhibitory members. The presence or absence of immuno-receptor tyrosine-based inhibitory motif (ITIM) domains in the cytoplasmic tail can distinguish inhibitory from activating receptors. ITIM domains are essential for the recruitment of phosphatases (SHP-1, SHP-2, and SHIP) to inhibit signal transduction pathways required for cellular activation. Inhibitory Ly49 and KIR receptors are generally agreed to be important for the prevention of autoimmunity, by suppressing NK cell reactivity to normal cells through recognition of self-MHC. Activating Ly49, KIR, and NKG2 do not have ITIMs, but instead have a charged residue in the transmembrane region for the association with the immuno-receptor tyrosine-based activation motif (ITAM)-containing DAP12 molecules.[37] Binding of activating receptors such as Ly49D on NK cells leads to cytotoxicity and cytokine production.[38] Activating receptors Ly49D, P, and W have been shown to bind and in some cases be activated by class I MHC,[39-41] however the physiological role for such an interaction has not been determined. A clue may be present in the observation that the activating KIR3DS1 provides protection against HIV only when its ligand, HLA-B Bw4-80Ile, is present in an infected individual.[23] This suggests that an HIV-derived peptide may be presented for KIR3DS1$^+$ NK cell recognition. The binding of inhibitory Ly49 to class I MHC has been shown, in some cases, to be dependent on the type of peptide being presented.[42]

While the role of inhibitory Ly49 receptors is generally accepted to be for protection against autoimmunity, the purpose of activating Ly49 receptors has been a mystery until recently. The role of at least one activating Ly49 appears to be viral recognition. The ability of a resistant mouse strain (B6) to control splenic murine cytomegalovirus (MCMV) proliferation has been genetically mapped to the NKC and this locus has been termed *Cmv1*.[43] MCMV resistance (*Cmv1^r*) has been found to correlate with the presence of the *Ly49h* gene, and depletion of Ly49H$^+$ NK cells leads to higher viral titres in infected organs.[25-27] NK cells are activated by Ly49H recognition of the viral protein m157, a class I homologue encoded by the MCMV genome, expressed on the surface of infected cells.[44] Formal proof of *Ly49h* as *Cmv1^r* was provided by transgenic-rescue of the MCMV-susceptible mouse strain FVB/N.[45] Resistance to other viruses (ectromelia and HSV) has also been mapped to the *Ly49* gene-containing NKC.[43,46] Using congenic and intra-NKC recombinant mice, the development of other diseases such as diabetes and malarial infection have been shown to be controlled by genes mapped near the NKC.[47,48] Thus, the NKC, in addition to possessing genes regulating the inhibition of NK cells, also contains genes coding for activating receptors involved in the recognition of target cells through class I MHC-related ligands. As the *Ly49* gene cluster contains up to 20 different *Ly49* genes (depending on strain background), it is highly likely that other disease resistance markers will map to individual *Ly49* genes.

Ly49 MULTIGENE FAMILY—POLYMORPHIC ALLELES AND EXTREME HAPLOTYPE DIVERSITY

The *Ly49* gene cluster in C57BL/6 mice is a large family of highly related genes spread over approximately 600 kb that code for class I MHC receptors on NK cells (Fig. 2). The *Ly49* gene cluster is found at the telomeric end of the ~4.7 Mb NKC on mouse chromosome 6.[49] Like the *Ly49* sub-region, the rest of the NKC was found to also be polymorphic among various inbred mouse strains based on PCR-RFLP markers, indicating that this region is rapidly evolving.[50,51] Each individual *Ly49* gene consists of approximately a 10 kb promoter region followed by 20 kb encoding exons and introns.[31] There appears to be little DNA that does not encode *Ly49* genes, however many pseudogenes have been identified. Interestingly, most pseudogenes are of the activating type suggesting a rapid rate of evo-

lution for these genes that may reflect their role in pathogen recognition.

Our research has centered on the *Ly49* genes possessed by the 129/J and related mouse strains, and has revealed that the Ly49 repertoire is extremely polymorphic among inbred mouse strains. We initially discovered that the activating *Ly49p* and *Ly49l* genes are transcribed in 129/J and CBA/J, respectively, but not in the B6 mouse strain.[52,53] Both of these receptors are functional and can activate NK cells through the associated signaling molecule DAP12. Weak class I MHC binding was been observed with the use of soluble MHC tetramers on Ly49P or Ly49L-transfected cells (ref. 54, unpublished observations). It is a possibility that all activating Ly49 are pathogen receptors like Ly49H, and that MHC cross-reactivity is a result of their evolution from inhibitory Ly49 or a reflection of their viral ligands mimicry of class I MHC.

Further screening of a 129/J NK cell cDNA library led to the discovery of 10 full length *Ly49* sequences (*Ly49e*, *g*, *i*, *o*, *p*, *r-v*). Ly49e was identical to the B6 cDNA, and *Ly49g* and *i* were very similar to B6 alleles. Three apparently functional activating receptor sequences were identified (*Ly49p*, *u* and *r*) compared to the two known for B6 mice (*Ly49d* and *h*). The remaining 129/J Ly49 sequences were divergent to the point where allele relationships to known B6 genes could not be identified. Some sequences, such as *Ly49p*, *t* and *v*, were obviously new genes. The novel 129/J-derived Ly49 receptors displayed class I MHC binding characteristics that were quite distinct from those observed in B6 mice.[54] For example, the pan-MHC receptor in B6 mice is Ly49C, while in 129/J mice Ly49V seems to fulfill this role even though Ly49V is of a different Ly49 subfamily (Ly49A-like). Also, the putative $Ly49D^{B6}$ allele, $Ly49R^{129}$, had readily observable affinity for $H\text{-}2D^{d}$ by MHC tetramer binding, while none was observed for $Ly49D^{B6}$.[42] Compared to B6 mice, 129/J mice possess very different innate immune responses to intracellular infections such as Sendai virus and tumor induction via carcinogenic agents.[55,56] Also, 129/J mice display a reduced ability to reject allo- and xenogeneic bone marrow transplants.[57] Whether or not Ly49 repertoire polymorphism is a direct indicator for any of these phenomena are important questions. Interestingly, non-obese diabetic (NOD) strain mice appear to have allelic variants of 129-like (*Ly49p*) and B6-like (*Ly49a*, *d*, and *m*) genes as well as the unique *Ly49w*.[58] There are likely to be many other distinct Ly49 haplotypes as shown for human KIR repertoires.

We have recently completed a bacterial artificial chromosome (BAC) contig map of the *Ly49* region of the 129/J mouse strain and found that the *Ly49* cluster contains 18 genes (excluding the separate *Ly49b* gene), with a centromeric to telomeric order of: $Ly49q_1$, *e*, *v*, q_2, e/c_2, *l/r*, *s*, *t*, *e/c1*, *r*, *u*, *u/i*, i_1, *g*, *p/d*, *p*, i_2, and *o*.[59] Comparison to the only other available *Ly49* gene map (from the B6 strain) indicates that many of the *Ly49* genes do not have corresponding alleles in the two strains (Fig. 2). Overall, the *Ly49* cluster is of similar size in the two strains, where there are framework genes (i.e., conserved genes present in most or all haplotypes) like *Ly49q*, *e*, *g*, and *b*. Based on gene order, intronic and exonic sequence similarity, some probable alleles can be deduced such as *Ly49r* and *d*, *Ly49s* and *f*, and *Ly49h* and *u*. In addition, there are areas of Ly49 gene blocks present in 129/J mice (for example: $Ly49e/c_2$, q_2, and *v*) not present in B6 mice and vice versa. This finding shows a further similarity of mouse Ly49 to the human KIR. Sequencing of two KIR gene haplotypes has revealed conserved genes with interspersed regions of highly variable gene content.[60] Therefore, like the *KIR*, different *Ly49* haplotypes contain different numbers and combinations of activating and inhibitory receptor genes. Significant haplotype differences in regions coding for NK cell receptors (*KIR* or *Ly49*) may provide a genetic explanation for why some mouse strains or human individuals are resistant or susceptible to various diseases.

Despite extreme chromosomal reorganization among Ly49 haplotypes, some allele relationships are obvious based on sequence conservation and relative position to other genes. For example, all strains studied have an *Ly49g*-like gene; the same is probably true for *Ly49e*, *b*, and *q* based on the high homology between 129 and B6 genomic sequences. However, Ly49 alleles can function differently; $Ly49G^{BALB}$ recognition of $H\text{-}2D^{d}$ and $H\text{-}2L^{d}$ is stronger than that of $Ly49G^{B6}$.[61] Likewise, a study of Ly49A alleles also showed a wide-range of $H\text{-}2D^{d}$ affinities.[62] A most interesting comparison of the effects of Ly49 allele divergence was shown in the lack of binding of Ly49U, the putative 129 allele of Ly49H, to MCMV-m157.[44] This correlated with susceptibility of 129 mice to MCMV infection.[51] Moreover, the inhibitory $Ly49I^{129}$ was able to bind m157, while $Ly49I^{B6}$ showed no reactivity,[44] possibly magnifying the phenotypes of these mice.

NK CELL REGULATION THROUGH OTHER RECEPTORS

NKG2D—An Activating Receptor for Tumor Cells. Like *Ly49*, *Nkg2d* is found within the NKC and codes for a lectin-related protein expressed at the cell surface as a homodimer unlike other NKG2 family members, which associate with CD94. NKG2D is an activating receptor and signals through DAP10 or DAP12 for the induction of cytotoxicity.[63,64] NKG2D is expressed on freshly isolated NK cells, anti-CD3 activated $CD8^{+}$ T cells, γδ T cells, and LPS-activated macrophages.[65,66] In mice, the ligands for NKG2D are H60 and the Rae1 family of genes that are distantly-related to MHC. These ligands are expressed on numerous tumor cells, are inducible by carcinogens on normal cells, and trigger NK cell cytotoxicity and cytokine release.[65,67] Furthermore, NKG2D-ligand (Rae1β or H60) bearing tumors are vigorously rejected after introduction into mice. Challenged mice are then immune to parental tumor cells that do not express NKG2D ligands.[68] In contrast, RMA ectopically expressing Rae1γ or δ were also rejected by mice in an NK cell dependent manner, but provided no long term T cell memory.[69]

Human NKG2D functions in a similar manner in response to ligands MICA, MICB, and UL16-binding proteins, which are class I MHC related molecules that are expressed in tumors and are induced by intracellular infection or transformation.[66,70] Interestingly, many individuals with MIC^{+} tumors have low surface expression of NKG2D on effector cells. This appears to be caused by soluble MICA secretion by tumor cells resulting in surface modulation and degradation of NKG2D.[71] This finding may explain the lack of NK cell function in some cancer patients.[10]

NKp30, NKp44, and NKp46—Natural Cytotoxicity Receptors. Several new Ig-related NK cell receptors, NKp30, NKp44, and NKp46, have been identified in the Moretta laboratory and appear to be activating natural cytotoxicity receptors (NCR) for tumor cells.[72-74] These receptors are expressed on resting or activated NK cells in varying densities. NCR signal through various adaptor proteins, including DAP12, CD3ζ, and FcεRIγ.[72,73] The ligands for these receptors are still being characterized, but at present hemaglutinins from influenza and Sendai viruses appear to be ligands for NKp44 and NKp46.[75,76]

Unknown NK Cell Receptors and Their Ligands. Recent studies have shown that in addition to $Qa\text{-}1^{b}$, other non-classical MHC

proteins can also modulate NK cell activation. Specifically, the Qa-2 family member, Q9, which is expressed in immunologically privileged tissues, can inhibit lymphokine activated killer (LAK) (both $NK1.1^+TCR^+$ and $NK1.1^+TCR^-$) cell killing of melanoma cells.[77] However, the presence of Qa-2 does not always protect targets such as ConA-activated T cell blasts. The NK cell receptor responsible for Qa-2 recognition is not known, but did not appear to be Ly49C, G, or I based on antibody blocking studies, which correlates with the fact that the inhibitory effect was detectable using NK cells from mouse strains with different Ly49 haplotypes.[77] Recently, it has been demonstrated that blastocyst MHC, a potential murine homologue of HLA-G expressed in blastocysts and placenta, can abrogate NK cell attack in vitro and protect from NK cell-mediated rejection in vivo of blastocyst MHC-transfected RMA-S tumor cells.[78] As blastocyst MHC was not expressed on the surface of RMA-S, the protective ability was thought to be at least partially a consequence of $Qa\text{-}1^b$ upregulation and subsequent recognition by NKG2A/$CD94^+$ NK cells. An actual receptor for surface-expressed blastocyst MHC is still unknown.

In contrast, the ligand for NK cell activation receptor Nkrp1c (NK1.1) has not been identified. Cross-linking of Nkrp1c on NK cells leads to IFN-γ production and cytotoxicity in re-directed killing assays.[79] Furthermore, transfection of a receptor-deficient NK cell line with the rat orthologue of Nkrp1c (NKR-P1A) confers the ability to selectively destroy specific tumor cells.[80] Recently, the Yokoyama laboratory reported that Nkrp1d and Nkrp1f NK cell receptors bind Clrb and Clrg, respectively.[81] Clrb-transduced tumor cells were less susceptible to LAK cell killing and this could be reversed by the addition of antibody to Nkrp1d. Interestingly, members of the Clr family are shuffled among the *Nkrp1* gene family within the NKC,[82] and unlike the *Ly49* cluster, the Clr region is conserved between 129 and B6 mice.[81] Therefore, not all NKC-encoded receptors will bind to MHC-related ligands.

CLINICAL APPLICATIONS—THE ROLE OF INHIBITORY NK CELL RECEPTORS IN LEUKEMIC RELAPSE

The effect of NK cells on allogeneic bone marrow transplantation is currently a field of intense research. Patients with leukemia or lymphoma must have their immune system reconstituted by bone marrow transfer after irradiation to destroy malignant cells. However, usually the only available bone marrow donor is an immediate family member that is mismatched at one or more HLA loci. Engraftment and eradication of remaining malignant cells (graft vs. leukemia) is a major role for donor T cells, however, these cells also promote graft vs. host disease (GVHD). GVHD is responsible for most transplant failures resulting in leukemic relapse, infection and eventually death. One way of overcoming T cell-mediated GVHD is to deplete the transplant of such cells. This has led to the discovery that alloreactive NK cells in the transplant are expanded and will destroy host antigen presenting cells (APC) and acute myeloid leukemia cells, thus overcoming both GVHD and leukemia, respectively.[83]

Theoretically, inhibitory KIR on donor NK cells specific for class I MHC expressed by surviving leukemic cells will increase the chance for relapse to occur. Recent studies have supported this; patients with acute myeloid leukemia who received bone marrow transplants from HLA mismatched family members are much less likely to have a leukemic relapse after five years.[83] Researchers believe this phenomenon is due to HLA antigens expressed in the recipient tissues for which no inhibitory KIR receptors are expressed on the allogeneic donor NK cells, and this would result in NK cell destruction of leukemic cells. Surprisingly, allogeneic NK cell pre-conditioning does not lead to NK cell mediated GVHD; in fact it protects against this disease by the ablation of host APCs.[83] In mice, conditioning with donor allogeneic NK cells allows the engraftment of large numbers of allogeneic T cells with no GVHD.[83] For unknown reasons, allogeneic donor NK cells only seem to attack recipient hematopoietic cells (including leukemia and APCs) and not organ tissues.

Studies in mice have shown that pre-treatment of leukemia-containing bone marrow with syngeneic NK cells that were pre-blocked with antibodies to inhibitory Ly49 receptors for the leukemic cells also] resulted in leukemia-free bone marrow reconstitution.[84] Furthermore, leukemic cell killing is even more efficient when the inhibitory receptor-blocked NK cells are H2-allogeneic to the recipient.[85] NK cell receptor incompatibility in the graft vs. host direction was recently confirmed to be a significant advantage with respect to overall and disease free survival in a five year study of 130 patients with hematologic malignancies who were given hematopoietic stem cell transplants.[86]

CONCLUSION

NK cells display diverse mechanisms through a large number of receptors to ascertain the appropriateness of killing potential target cells. Some receptors appear to be well conserved, while others are evolving very rapidly. The KIR and Ly49 repertoire heterogeneity resulting from allelic divergence and total haplotype reorganization may be a population specific defense mechanism, always ensuring that certain individuals are resistant to any one pathogen by the expression of activating forms of these receptors. On the other hand, conserved receptors such as NKG2D and NCR may be more important in individual defense against tumorigenesis. However, the different types of NK cell receptors are likely to be important for cross-defense. Variegated expression of *Ly49* and *KIR* ensures that some NK cells will always be able to respond to myriad forms of MHC dis-regulation by tumor cells. Also, there is evidence that NCR, like NKp44, may recognize viral structures. As we begin to understand how NK cells perform their role, we can more effectively apply this knowledge to the treatment of disease.

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