

The NK cell receptor repertoire: formation, adaptation and exploitation

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The identification of NK cell receptors specific for MHC class I molecules has greatly improved our knowledge of NK cell reactivity and specificity. Inhibitory receptors prevent NK cell activation directed against cells expressing self-MHC class I molecules. Consequently, diseased cells that do not express self-MHC class I molecules become susceptible to NK cell-mediated attack. Because of the specificity and distribution of inhibitory NK cell receptors, cells that express non-self (allogeneic) MHC class I molecules are also susceptible to NK cell reactions. This feature has been exploited in a clinical setting to treat leukemia patients.

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Abbreviations

GvHD	graft versus host disease
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibition motif
KIR	killer cell immunoglobulin-like receptor
MCMV	mouse cytomegalovirus
NK	natural killer
NKG	NK group
NKR	NK cell receptor specific for MHC class I molecules
SH2	src homology 2
SHIP	SH2-containing inositol phosphatase
TCR	T-cell receptor

Introduction

Natural killer (NK) cell function is controlled by opposing activating and inhibitory signals generated during the interaction with a target cell. Target cells are killed when NK cells receive an excess of activation signals, which can occur in two (non-exclusive) ways. When target cells lack self-MHC class I molecules, NK cells no longer receive inhibitory signals via MHC class I-specific inhibitory receptors ('missing self' recognition; [1]). As the interaction with a target cell is often sufficient to result in significant NK cell activation, lysis is induced. Alternatively, MHC class I-dependent inhibition may be overridden by *de novo* expression of ligands on target cells,

which are recognized by constitutively expressed NK cell activation receptors ('induced self' recognition; [2]).

In this review, we will consider NK cell receptors specific for MHC class I molecules (NKRs). Their hallmark is differential, subset-restricted expression. This type of expression pattern is not unique to MHC class I receptors and may mark different NK cell subtypes or lineages (as proposed for CD56; [3]). Other cell-surface receptors may delineate developmental stages (for example, CD11b; [4]) or are currently of unknown importance (for example, killer cell lectin-like receptor G1 [KLRG1]; [5]). Even though some of these latter receptors probably influence the functional repertoire of NK cells significantly, they are not within the scope of this review and will not be discussed further. In this review, we will summarize recent advances in our understanding of the formation of a repertoire of NK cell receptors specific for MHC class I molecules, alterations in this repertoire imposed by self-MHC class I molecules and other factors, and emerging clinical applications of this knowledge.

MHC class I-specific NK cell receptors

NK cells express either killer cell immunoglobulin-like receptors (KIRs, human) or C-type lectin-like Ly49 receptors (mouse) [6,7]. MHC class I receptor heterodimers of CD94 and NK group 2A (NKG2A), NKG2C or NKG2E are expressed by both species [8,9]. Each type of receptor family (KIR, Ly49 and CD94–NKG2 heterodimers) includes activating and inhibitory forms of receptors. Inhibitory receptors are characterized by the presence of one or more immunoreceptor tyrosine-based inhibition motifs (ITIMs; V/IxYxxL in amino acid one-letter codes, where x represents any amino acid), whereas activating receptors lack ITIMs and instead associate with the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor molecule, DAP-12 (also called KARAP; [10]). Therefore, MHC class I expression on target cells can also activate NK cells. This may play a role when target cells express increased amounts of MHC class I.

Specificity of NK cell receptors

Certain KIRs and Ly49 receptors are specific for allelic determinants on classical MHC class I molecules. As the MHC and the KIR or Ly49 clusters are inherited independently, humans and mice may in fact express NKRs for which they have no MHC ligand. At the other end of the spectrum, human immunoglobulin-like transcript-2 (ILT2, an additional Ig-like receptor) displays rather broad MHC class I reactivity, and CD94–NKG2 receptors

recognize non-polymorphic HLA-E in humans or Qa1^b in mice (for recent reviews see [11,12]).

Recent work has identified ligands for NKR in addition to the MHC class I molecules of the respective species. The activating murine Ly49D receptor, which was initially shown to be H-2D^d-specific, also recognizes xenogeneic MHC-encoded ligands on rat lymphoblasts [13] and Chinese hamster ovary cells [13,14,15^{*}]. Thus, activating NKRs may play a role in xenotransplant rejection. Furthermore, the activating Ly49H receptor, for which a class I ligand has not yet been described, was recently shown to bind to the mouse cytomegalovirus (MCMV)-encoded MHC class I-like protein, m157 [16^{**},17^{**}]. In addition, the protein m157 was also found to bind to an allelic variant of the inhibitory Ly49I receptor [16^{**}]. As viruses subvert host genes rather than 'invent' them, these latter findings raise the possibility that functional m157 homologs exist in the murine and human genomes and that the specificity of KIRs or Ly49 receptors extends to MHC class I-independent self-molecules.

The NK cell receptor repertoire

The number of KIR and Ly49 genes varies considerably between different individuals or mouse strains, respectively [18,19]; approximately ten receptors are actually expressed in each. Single NK cells can express more than one NKR, and the usual figure is between one and five [20,21]. Indeed, individual NK cells express different combinations of KIR or Ly49 and CD94–NKG2 receptors (for a detailed recent review, see [11]). The term 'NKR repertoire' thus refers to the NKRs used by an individual or a mouse strain, as well as the combinations of NKRs expressed by single NK cells.

The signals that induce NKR expression during NK cell development, and the molecular basis for the combinatorial NKR distribution, are not well understood. Cytokines may play a role in the induction of CD94–NKG2 expression [22,23]. By contrast, bone marrow stromal cell-derived factors seem to be required for inducing KIR or Ly49 expression [24–26,27^{*}]. *In vitro* differentiation experiments indicate that Ly49 receptors or KIRs are acquired in a non-random order during NK cell development [25,26,27^{*}]; for example, in murine NK cells, Ly49A appears before Ly49G, which appears before Ly49I [26]. Staged Ly49 acquisition may be comparable to the successive generation and expression of T cell receptor (TCR) α chains during T-cell development [28,29]. The difference between NKR expression and TCR expression is that in NK cells the primary receptor choice is not extinguished by subsequent receptor choices. Consequently, NK cells can accumulate several NKRs.

An additional process seems to act as the actual driving force for diverse receptor expression. Only a fraction of NK cells acquire an NKR at the permissive stage of NK

cell development. The restricted acquisition of Ly49 receptors, which is evident at the level of the NK cell population, may find its correlate at the single cell level as a predominant mono-allelic expression of Ly49 receptors [30,31]. Thus, receptor distribution may be based on an inefficient activation of individual *Ly49* genes. Compatible with this idea, the transcription factor T-cell factor (TCF)-1 is limiting for the acquisition of the Ly49A receptor [32,33]. Maintenance of the expression patterns of KIRs is ensured epigenetically through DNA methylation [34^{**}].

It may be asked why NK cells need such a fine receptor specificity and an elaborate receptor distribution system, if all they have to recognize is self-MHC class I molecules? Consider what would happen if each NK cell expressed all the available inhibitory NKRs; complete loss of MHC class I from target cells would be required for NK cells to react. Receptor selectivity and clonal distribution can generate NK cell clones that express a single inhibitory NKR specific for a single self-MHC class I allele. For such a clone the loss of a single class I allele from a target cell will be sufficient for an NK cell reaction to occur. Loss or downregulation of a single class I allele is indeed a relatively frequent event during transformation [35] or infection with certain viruses [36,37].

Adaptation of the NK cell receptor repertoire I: MHC class I

Even though it is clear that NK cells functionally adapt to their MHC class I environment [38], the expression of MHC-specific receptors, as we can currently assess it, is only relatively subtly modified by MHC class I expression. Changes in expression include reduced cell-surface levels of inhibitory Ly49 receptors (but not of KIRs) in the presence of the MHC ligand. This has previously been attributed to ligand-induced receptor downmodulation, although recent results have raised the possibility that it may result from reduced receptor accessibility, due to an interaction of the receptor with its MHC class I ligand in the plane of the NK cell's membrane [39,40].

Contrary to what may be expected, the presence of MHC ligand reduces rather than increases the number of Ly49 receptors expressed per cell. This finding is compatible with the model in which NKRs are acquired sequentially, and suggests that engagement with MHC class I limits further receptor acquisition. This scenario is supported by the analysis of mice expressing a self-MHC-specific NKR transgene. Limited acquisition of endogenous Ly49 receptors in transgenic mice is observed when the transgenic receptor is engaged, irrespective of whether the endogenous receptor is self-MHC specific or not [41^{*},42^{*}].

The findings described above provide important insights into the adaptation of the NKR repertoire to the MHC environment. However, the questions of whether potentially auto-aggressive NK cells (i.e. NK cells that fail

to acquire a self-MHC-specific inhibitory receptor) arise, and how auto-aggression is prevented (NK cell tolerance induction), are not yet resolved. In cloned human NK cells, a characteristic of the repertoire is, in fact, that each NK cell expresses at least one inhibitory NKR with specificity for self-MHC class I [20]. This suggests that auto-aggressive clones either do not arise, or they arise but cannot be cloned. In fact, the failure to engage NKRs during NK cell development does not represent a death sentence for NK cells. NK cells develop in normal numbers in the absence of MHC class I molecules in various mouse strains and human transporter associated with antigen processing (TAP)-deficient patients, yet such NK cells display reduced functional capacities [43,44]. NK cell non-reactivity (tolerance) towards non-infected, non-transformed self cells can thus also be ensured independently from the engagement of NKRs with MHC class I ligands.

Adaptation of the NK cell receptor repertoire II: NK cell activation

In conjunction with MHC class I molecules, NK cell activation seems to play a role in the adaptation of the Ly49 receptor repertoire. The lysis of 'normal missing-self' targets (i.e. non-infected, non-transformed lymphoblasts, which lack MHC class I molecules) is dependent in part on the src family kinase Fyn, suggesting a role for Fyn in NK cell activation. Fyn-deficient NK cells express fewer inhibitory Ly49 receptors than wild-type cells [45]. In contrast, mice lacking syk/ZAP-70 (which are unable to induce NK cell activation via ITAM-associated NK cell receptors) kill normal missing-self target cells. These NK cells display a normal Ly49 receptor repertoire [46]. Collectively, these data raise the possibility that NKRs are acquired until the inhibitory signals generated by them are able to match the activation levels provided by their interaction with normal self cells.

Consistent with this scenario, activating NK cell receptors appear before (or in the absence of) KIRs or Ly49 receptors on developing NK cells [24,47]. A possible mechanism for keeping NK cells in check while they try to match their activation levels with MHC-dependent inhibition has been recently proposed [47]. In the presence of the cytoplasmic molecule SAP (for signaling lymphocyte activation molecule [SLAM]-associated protein), CD244 (also called 2B4) acts as a co-receptor for NK cell activation [48]. In the absence of SAP, CD244 associates with src homology 2 (SH2)-containing protein tyrosine phosphatase (SHP)-1 and consequently inhibits NK cells [49]. Intriguingly, during NK cell development SAP is not expressed, suggesting that CD244 inhibits NK cells via the interaction with its ubiquitously expressed ligand CD48 [47].

An additional factor influencing the Ly49 receptor repertoire is SH2-containing inositol phosphatase (SHIP; [50]). Certain Ly49 receptors recruit SHIP to their cytoplasmic tail. In the absence of SHIP, these Ly49-defined NK cell

subsets selectively and gradually expand over time, suggesting that SHIP may normally limit their proliferation or survival [50]. It remains to be shown whether this process is also dependent on MHC class I expression.

Exploitation of the NK cell receptor repertoire

NK cells are able to kill normal missing-self targets, as the relevant inhibitory receptor(s) is no longer engaged by the target cell's MHC molecules. Obviously, NK cells will also react to target cells expressing a foreign MHC class I allele, as long as the relevant inhibitory receptor(s) is not engaged. Thus, one explanation for why NK cells react to normal allogeneic cells is that non-self MHC, exactly like no-self MHC, is unable to mediate NK cell inhibition [51].

This principle has been elegantly exploited to direct donor NK cells to recipient's leukemic cells following bone marrow transplantation in which the recipient MHC class I molecules do not block all donor-derived NK cells [52]. In this case, the hematopoietic graft gave rise to some NK cells that were not inhibited by the host's MHC class I molecules. These NK cells prevented not only the relapse of leukemia in the host but also provided additional benefits, such as enhanced engraftment and absence of graft versus host disease (GvHD).

Although they represent an exciting new opportunity for exploiting NK cell reactivities, the outcome of the Ruggieri studies [52,53] would not have been predicted based on experiments in mice. Radiation bone marrow chimeras, with an MHC mismatch between the radio resistant (non-hematopoietic) and the radio sensitive (hematopoietic) compartments, revealed that NK cell reactivity to missing-self is determined by MHC class I molecules of both hematopoietic and non-hematopoietic cells [54–57]. It is therefore surprising that NK cell reactivity in the human situation seems to be dictated at least transiently by the hematopoietic cells alone. Along these lines, it is known that the use of a large number of MHC class I-deficient stem cells can eventually override bone marrow graft rejection in MHC class I-sufficient mice [58]. Perhaps the use of a large number of stem cells similarly allows for the generation of an early wave of NK cells, which escape tolerization by non-hematopoietic cells. Indeed, donor-type NK cells are detected very rapidly after transplantation, and alloreactive NK clones can be recovered early but are no longer detected 4 months after transplantation [53]. Thus, clinical work may also teach us a new lesson in NK cell biology.

Conclusions

Knowledge of NK cell reactivity and specificity has greatly expanded in recent years. As a first major payoff from the basic research, it may now become possible to exploit the peculiarities of NK cell reactivity in clinical settings. Nevertheless, major issues of NK cell biology,

such as the specificity of NK cell activation, the molecular basis for diverse NKR expression, the acquisition of missing-self reactivity and the basis for NK cell tolerance, remain to be resolved.

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