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Review

# NK cell self tolerance, responsiveness and missing self recognition

Nataliya Shifrin, David H. Raulet*, Michele Ardolino

*Department of Molecular and Cell Biology and Cancer Research Laboratory, Division of Immunology, University of California at Berkeley, Berkeley, CA 94720, USA*

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ABSTRACT

Natural killer (NK) cells represent a first line of defense against pathogens and tumor cells. The activation of NK cells is regulated by the integration of signals deriving from activating and inhibitory receptors expressed on their surface. However, different NK cells respond differently to the same stimulus, be it target cells or agents that crosslink activating receptors. The processes that determine the level of NK cell responsiveness have been referred to collectively as NK cell education. NK cell education plays an important role in steady state conditions, where potentially auto-reactive NK cells are rendered tolerant to the surrounding environment. According to the "tuning" concept, the responsiveness of each NK cell is quantitatively adjusted to ensure self tolerance while at the same time ensuring useful reactivity against potential threats. MHC-specific inhibitory receptors displayed by NK cells play a major role in tuning NK cell responsiveness, but recent studies indicate that signaling from activating receptors is also important, suggesting that the critical determinant is an integrated signal from both types of receptors. An important and still unresolved question is whether NK cell education involves interactions with a specific cell population in the environment. Whether hematopoietic and/or non-hematopoietic cells play a role is still under debate. Recent results demonstrated that NK cell tuning exhibits plasticity in steady state conditions, meaning that it can be re-set if the MHC environment changes. Other evidence suggests, however, that inflammatory conditions accompanying infections may favor high responsiveness, indicating that inflammatory agents can over-ride the natural tendency of NK cells to adjust to the steady state environment. These findings raise many questions such as whether viruses and tumor cells manipulate NK cell responsiveness to evade immune-recognition. As knowledge of the underlying processes grows, the possibility of modulating NK cell responsiveness for therapeutic purposes is becoming increasingly attractive, and is now under serious investigation in clinical studies.



## 1. Introduction

Natural Killer (NK) cells are an innate lymphoid population that develop mainly in the bone marrow from the common lymphoid progenitor. Unlike T- and B-lymphocytes, the receptors expressed on their surface are not derived from somatic recombination, but rather belong to different families of innate receptors. Functionally, NK cells were initially defined by their ability to kill target cells without requiring prior immunization [1–3], but subsequent studies indicate that they exhibit low killing activity unless pre-activated briefly with cytokines or other inflammatory stimuli [4–6]. NK cells kill by the same killing mechanisms as CD8 T cells do: they release onto target cells cytotoxic granules containing proteins that permeabilize the membrane, allowing entry of apoptosis-inducing effector proteins [7,8]. In addition, they express death receptor ligands such as TRAIL or CD95-L, which can engage death receptors on the surface of target cells and initiate target cell apoptosis [9,10].

*Abbreviations:* adcc, antibody dependent cellular cytotoxicity; β2m, β2-microglobulin; CIN-, Ly49C$^-$Ly49I$^-$CD94/NKG2A$^-$; CRACC, CD2-like receptor-activating cytotoxic cells; DNAM-1, DNAX accessory molecule 1; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer-cell immunoglobulin-like receptor; LFA-1, lymphocyte function-associated antigen 1; LMCV, lymphocytic choriomeningitis virus; MCMV, mouse cytomegalovirus; MICA/B, MHC class I polypeptide-related chain A/B; MHC, major histocompatibility complex; MHV, mouse hepatitis virus; MULT1, murine UL-16 binding protein like transcript 1; NK, natural killer; NKG2, natural killer cell group 2; NKp46, natural killer protein 46; RAE-1, retinoic acid early-inducible 1; SLAM, signaling lymphocytic activating molecule; TRAIL, TNF-related apoptosis-inducing ligand; Tregs, regulatory T cells; ULBP, UL-16 binding protein.

* Corresponding author. Tel.: +1 510 642 9521; fax: +1 510 642 1443.
*E-mail address:* raulet@berkeley.edu (D.H. Raulet).

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NK cell activation is governed by the integration of activating and inhibitory signals they receive subsequent to encountering a target cell [11–13]. In the next two sections we will briefly describe some of the activating and inhibitory receptors expressed by NK cells.

### 1.1. Activating receptors

A number of activating receptors expressed by NK cells have been identified in the last 20 years. The best-characterized activating NK cell receptor is NKG2D. NKG2D recognizes "induced-self" ligands – molecules that are not expressed or are expressed at low levels on most normal cells, but which are upregulated on unhealthy cells due to the activation of pathways associated with malignancy, infection or stress [14–16]. NKG2D ligands include MICA/MICB and ULBP1-6 in humans, and MULT1, RAE-1α-ε, and H60a-c in mice [16]. The NKG2D/NKG2D-ligand axis is the defining example of "induced-self" recognition, wherein immune cells recognize self ligands that are upregulated on unhealthy cells. NKG2D recognition of target cells is a major mode of natural killing of tumor cells [17], and has been implicated in some autoimmune diseases as well [18–20]. Other receptors on NK cells, such as DNAM-1, recognize self molecules that are widely expressed by healthy cells but can be further up-regulated under conditions of stress. DNAM-1 interacts with the adhesion receptor LFA-1 on the surface of NK cells and may therefore play a role in regulating adhesion [21].

A distinct and so far unique form of NK cell recognition is represented by the receptor Ly49H in mice, whose ligand is a cytomegalovirus-encoded protein, m157, expressed on the surface of infected cells [22,23]. Ly49H/m157 interactions are crucial for NK cell mediated control of MCMV infection, as shown using Ly49H-deficient mice, in which the virus cannot be efficiently cleared [24–26].

Another important NK cell stimulatory receptor is the Fc receptor FcγR, which triggers antibody-dependent cell cytotoxicity (ADCC) when it binds IgG bound to antigens on the surfaces of other cells [27,28]. Numerous other activating receptors exist and have been implicated in host defense, but the corresponding ligands remain to be clearly defined. Among these are NKp46 and NKR-P1C.

Recently the SLAM family of activating receptors has been shown to be important in the recognition of hematopoietic cells by NK cells [29]. This family of proteins includes SLAM, CD48, Ly9, 2B4, CD84, Ly108, and CRACC. SLAM receptors are broadly and selectively expressed on various hematopoietic cell types. All SLAM family receptors, with the exception of SLAM, are expressed by NK cells. Most SLAM receptors interact homotypically with the same protein on another cell. The exception is 2B4, which binds CD48 on target cells [28]. NK cells with defective SLAM receptor signaling capacity fail to kill diverse hematopoietic target cells, including MHC-deficient spleen cells and bone marrow cells, but retain the capacity to kill non-hematopoietic target cells.

### 1.2. Inhibitory receptors

NK cells are also regulated by inhibitory receptors, most of which engage MHC I molecules expressed by target cells [30–32]. In mice, the Ly49 receptors directly recognize MHC class Ia molecules. Surprisingly, humans have only a single, nonfunctional, Ly49 gene; instead MHC class Ia recognition is performed by a distinct set of receptors called killer cell immunoglobulin-like receptors (KIRs), which are not present in mice. The KIR and Ly49 families include about 10 members each, which vary in their specificity for different polymorphic MHC I molecules. Both mice and humans also express a CD94/NKG2A heterodimeric receptor that recognizes a complex of a peptide derived from the class Ia leader sequence presented by a specific nonclassical MHC I molecule (HLA-E in humans and Qa-1 in mice) [33–35]. Inhibitory receptors signal through immunoreceptor-tyrosine based inhibitory motifs (ITIMs) in their cytoplasmic tails. Upon receptor engagement, ITIMs are tyrosine-phosphorylated and recruit protein tyrosine phosphatases. The phosphatases are believed to inhibit activation by dephosphorylating one or more critical signaling molecules, including Vav1 [36–39].

### 1.3. NK cell education

Evidence for NK cell education came from several approaches, including studies of the NK cell repertoire in normal mice [40]. In the C57BL/6 (B6) mouse strain only 2 of the Ly49 receptors, Ly49I and Ly49C, appreciably recognize self MHC (both recognize H-2K$^b$) [41]. The CD94/NKG2A receptor in these mice recognizes the leader peptide of H-2D$^b$ presented on the non-classical MHC I molecule Qa1 [34]. Hence, only three self MHC-specific NK receptors are available in these mice. The acquisition of the inhibitory receptors is stochastic, such that many different combinations of the available inhibitory receptors are expressed on distinct NK cells. In B6 mice, each NK cell can express between 0 and 3 of the three self MHC-specific inhibitory receptors [42], with approximately 85% of splenic NK cells expressing at least one of these receptors [40]. Consequently, ~15% of NK cells in these mice lack self MHC-specific receptors. These NK cells display a mature phenotype and express normal amounts of activating receptors, yet are demonstrably self-tolerant – they fail to kill self cells. Furthermore, such NK cells fail to kill MHC I-deficient normal lymphocytes in vivo and in vitro, and kill tumor cells less efficiently than other NK cells. Indeed, they exhibit a generalized hyporesponsiveness to stimulation via engagement of activating receptors [40]. A separate study employed MHC transgenic mice to arrive at a similar conclusion [43]. The initial finding that appreciable fractions of NK cells are devoid of self MHC-specific inhibitory receptors has been generalized by other groups to human NK cells as well [44]. These findings refuted a widely held hypothesis that all NK cells express at least one inhibitory receptor specific for self MHC as a means to prevent NK cell autoreactivity [45].

The findings just outlined interfaced with earlier results concerning the impact of MHC-deficiency on NK cell functions. In mice lacking all MHC Ia molecules due to various targeted mutations (such as a mutation in β2-microglobulin, abbreviated β2m), NK cells were present in normal numbers but exhibited self tolerance to the surrounding MHC I-deficient cells. Indeed, these NK cells were similarly hyporesponsive and shared other similarities with the subset of NK cells in normal mice that lack inhibitory receptors for self MHC. Hence, the absence of NK receptors for self MHC, and the absence of MHC altogether, result in similar outcomes: NK cell hyporesponsiveness and tolerance to normal MHC-deficient cells [45]. This review addresses the mechanisms that confer NK cell self tolerance in light of the fact that many NK cells in normal mice and humans lack self-MHC-specific inhibitory receptors. The findings are likely important for evaluating the role of NK cells in inflammatory diseases and the failure of NK cells to control certain tumors or infections.

### 1.4. The rheostat concept

The rheostat model considers NK responsiveness from a quantitative point of view. The discussion until now has implied that NK responsiveness reflects two states (responsive vs. hyporesponsive). The rheostat concept, in contrast, is based on the observation that NK responsiveness varies quantitatively, rather than reflecting only two states [45,46]. As stated previously, due to the stochastic nature of inhibitory receptor expression by NK cells, each cell can

express between 0 and 3 self MHC I specific inhibitory receptors (in B6 mice) [42,46]. This fact, along with the evidence that the affinity of the various inhibitory receptors for MHC ligands is known to vary, leads to the conclusion that NK cells encountering cells in the normal environment are exposed to varying degrees of inhibition, depending on their inhibitory receptor repertoire and the available MHC molecules that are expressed. Studies examining the responsiveness of NK cell as a function of the number of self-specific inhibitory receptors they express showed that responsiveness is directly proportional to the number of these receptors. NK cells expressing multiple inhibitory receptors that can be engaged by neighboring cells in the steady state comprise the most inherently responsive subset and responsiveness diminishes as the number of inhibitory receptors decreases [42,46]. Similar results were generated in the analysis of human NK cells [47–49]. Furthermore, progressively decreasing the number of MHC molecules expressed by mice, using gene knockouts and transgenic animals, resulted in progressively lower responsiveness of the NK cells in the animals [50]. Recently, a role for the non-classical MHC class I molecule H2-M3 in NK cell education has been highlighted. In B6 mice, Ly49A does not bind canonical MHC molecules but still can tune NK cell responsiveness by binding H2-M3, adding therefore another level of complexity to the process [51]. Collectively, these findings suggest that responsiveness of NK cells varies quantitatively depending on the amount of inhibitory signaling the cell encounters. This variation has been likened to a "rheostat" that governs NK cell activity quantitatively.

## 2. NK cell education

### 2.1. Models for NK cell education

While there is no doubt that MHC class I molecules play a critical role in NK cell education, there is considerable debate regarding the types of cell interactions (or lack thereof) that influence this process. Initially, two hypotheses were proposed based on the hyporesponsiveness of NK cells devoid of self-specific inhibitory receptors, and all NK cells in MHC I-deficient mice. Since NK cells with receptors that bind MHC I are responsive and kill MHC-deficient hematopoieitic cells and those that lack such receptors are hyporesponsive and fail to kill nonhematopoietic cells, one possibility is that encounters of NK cells with MHC I-expressing cells endow NK cells with high responsiveness to MHC I-deficient cells. The converse possibility is that the sustained interactions of NK cells with target cells in which MHC I is not engaged leads to hyporesponsiveness and self tolerance. These possibilities have been described in different ways in the literature and conferred with different names. In one treatment [52], the two extremes were described as "disarming" and "arming" as detailed here:

*Disarming*: According to this model, high responsiveness is the default state for NK cells, but encounters with normal cells that lack ligands for inhibitory receptors drives the cells into a hyporesponsive state ("disarms" them). The simplest version of this proposal is that during such encounters the NK cells receive persistent stimulation because the encountered target cells display ligands that activate the NK cells without opposing inhibitory ligands. Persistent stimulation has been shown to induce anergy of self-reactive B and T cells in some instances, suggesting the plausibility of this model. The cells may initially respond but later be rendered hyporesponsive.

*Arming*: The Arming model postulates that when inhibitory receptors on NK cells engage MHC I molecules on neighboring normal cells a higher state of responsiveness is induced. When this interaction is impossible, NK cells persist in a hyporesponsive state, which is often characterized as an immature state. We consider the arming concept to be essentially equivalent to the "licensing" concept of NK cell education [53], which suggests that encounters with MHC I license the maturation of NK cells. It should be noted that the notion that hyporesponsive NK cells are immature is not evident from their cell surface phenotype or other properties [40], and conflicts with evidence that responsive NK cells can be converted into hyporesponsive NK cells after exposure to an MHC-deficient environment [54] (discussed in detail below).

*Tuning model*: The arming/disarming concepts are not mutually exclusive, and many researchers in the field now favor the tuning model, which incorporates the arming and disarming concepts as well as the rheostat concept. It proposes that each NK cell, depending on the inhibitory (and stimulatory) receptors expressed, and the MHC genotype of the animal, receives a varying amount of stimulation and inhibition when encountering neighboring cells. Stimulatory and inhibitory signals are thought to be integrated by the NK cell. The model proposes further that depending on the net stimulation received, the NK cell assumes a quantitatively appropriate responsiveness state. Strong net stimulation (e.g. steady state stimulation without opposing inhibition) drives the NK cell to its lowest responsive state, whereas weak (or no) net stimulation drives the cell to its highest responsive state. Intermediate net stimulation results in intermediate responsiveness.

### 2.2. The roles of cis and trans interactions between MHC I and inhibitory receptors in NK cell education

Members of the Ly49 receptor family have the capacity to bind MHC I on neighboring cells (in *trans*) and on the NK cells themselves (in *cis*) [55,56]. *Trans* binding but not *cis* binding mediates NK cell inhibition [55]. However, it has been suggested that *cis* binding may play a role in NK cell education. It has been proposed that binding of inhibitory receptors to MHC I in *cis* leads to sequestration of these receptors and makes them unavailable for *trans* binding resulting in enhanced activation of NK cells [57]. A study of NK cells that express an engineered variant of Ly49A which retains *trans* but not *cis* binding to its ligand H-2D$^d$ showed that this receptor inhibited killing of H-2D$^d$ expressing cells but did not contribute to the education of NK cells, suggesting a possible role for *cis* interactions between MHC I and Ly49 receptors in this process [57]. A reciprocal study involving NK cells that express a Ly49A variant that binds its ligand in *cis* but not in *trans* showed that *cis* interactions lead to alterations in the Ly49 repertoire, further supporting the possible role of such interactions in NK cell education [58].

### 2.3. Role of activating receptors in NK cell education

There is still incomplete information concerning the role of activating receptors in NK cell education. Presumably, normal hematopoietic cells (and perhaps certain subsets of nonhematopoietic cells) display activating ligands for NK cells, such that when these cells lack inhibitory MHC I molecules they are subject to lysis by wild type NK cells. The relevant activating ligands appear likely to play a role in NK cell education, by providing the activation signals necessary to tune NK cell responsiveness. As noted earlier in this review, NK cells from mice lacking SLAM receptor function fail to kill MHC-deficient hematopoietic cells, or certain MHC-deficient tumors [29], suggesting that SLAM receptors may play a role in NK cell tuning. Consistent with this proposal, NK cells from SLAM-deficient mice showed an *increased* capacity to lyse non-hematopoietic tumor cell lines [29]. An interpretation of this finding is that the decreased amount of steady state stimulation experienced by NK cells in these mice because the absence of SLAM receptor function tunes the NK cells to a higher basal level of responsiveness. The activating receptor NKG2D may also play a role in tuning NK cells, since mice carrying a deletion of the gene

encoding this receptor were slightly more responsive than WT NK cells when exposed to certain stimuli [59,60].

In another study, mice with a mutation in the gene encoding a distinct activating receptor, NKp46 [61], also displayed increased NK activity against several target cells, suggesting the possibility that steady state NKp46 signaling may also play a role in NK cell tuning [62]. This proposal implies that ligand(s) for NKp46 must be expressed on normal cells, but this prediction has not been tested because the relevant ligand has not yet been discovered. Surprisingly, however, a recent study showed that NK cells from mice with a targeted disruption of the *Ncr1* gene (which encodes the NKp46 receptor) were not hyper-responsive [60]. It remains to be determined whether these differing results depend on the specific nature of the mutations studied, a knock-out in one case vs. a missense mutation in the other.

The potential for activating NK receptors to influence NK cell education has also been investigated by over-expressing the ligands on normal cells in transgenic mice. Transgenic expression of NKG2D ligands resulted in some studies in reduced capacity of NK cells to attack MHC-deficient cells, but this was not recapitulated in another transgenic line [63,64]. It is possible that the differing results depend on the extent of over-expression of the NKG2D ligands. In the case of the Ly49H activating receptor, transgenic over-expression in mice of the viral m157 ligand for Ly49H caused reduced responses to m157-bearing target cells but did not cause global inactivation of NK cells [65]. Despite these variations, the results as a whole support the conclusion that steady state activating signals influence NK cell tuning. We propose that the variations in outcomes observed in different studies depend on the intensity of activating signals that the NK cells encounter, which may vary in these different experimental settings. It is also possible that variations in the quality of the activating signal – that is, which signaling molecules participate – also play an important role.

### 2.4. Cell types that are responsible for NK cell education

The MHC-expressing cell types that interact with NK cells and set the tolerance and responsiveness of NK cells remain largely unknown. Both hematopoietic and non-hematopoietic cells may play a role. Because mice deficient in adaptive lymphocytes ($Rag^{-/-}$) have grossly normal NK cells and are tolerant of MHC-deficient cells [43,52], it is unlikely that $T_{regs}$ or other T cell or B cell subtypes are necessary for this process. Myeloid cells are a candidate population, especially in light of multiple studies showing the importance of dendritic cells for NK cell functions [66,67], evidence that both macrophages and dendritic cells appear to be important for the final stages of NK cell maturation [68], and the findings that neutrophils influence NK cell activity [69]. Experimental evidence for a role for hematopoietic cells in NK cell education has been recently brought forward by using mice with inducible expression of MHC class I molecules [70]. In this model, NK cells are hyporesponsive until MHC I is induced. Using bone marrow chimeras, the authors conclude that hematopoietic cells play a major role in educating NK cells. However, we should note that these findings conflict with our own unpublished data (N.S., Nathalie Joncker, and D.H.R., in preparation).

Cells of non-hematopoietic origin are also likely players in this process. Little is known in detail about the requirement for non-hematopoietic cells in NK cell development, but several findings suggest that such interactions are important in the process. For example, the cytokine IL-15 and its high affinity receptor IL-15Rα are critical for NK cell development and maintenance [71,72], and it was demonstrated that IL-15Rα expression on non-hematopoietic cells alone is sufficient to support differentiation and survival of NK cells [72]. In light of evidence that IL-15 is trans-presented on the cell surface by IL-15Rα [73,74] these findings suggest that non-hematopoietic cells may play a critical role in presenting IL-15 to NK cells [75]. Both IL-15 and IL-15Rα are broadly expressed to multiple tissue and cell types [76], therefore precluding a specific prediction as to the relevant cell type. Another clue as to the origin of the critical non-hematopoietic cell type is the finding that a set of receptor tyrosine kinases called the TAM family, consisting of three related kinases (TYRO3, AXL and MER), plays a critical intrinsic role in the development of NK cell functional activity [77,78]. The three TAM family kinases bind to two cell surface ligands, GAS6 and protein S, which are expressed by non-hematopoietic stromal cells and not by hematopoietic cells [79]. The results suggest that interactions of NK cells with non-hematopoietic cells expressing GAS6 and protein S play a critical role in NK cell development and it is attractive to hypothesize that MHC expression by these critical stromal cells may also play a role in educating NK cells by determining NK cell responsiveness.

## 3. Functional plasticity of NK cells

### 3.1. Resetting of NK cell responsiveness in steady-state conditions

Recent work from our lab and others addressing the plasticity of NK cell education demonstrated that responsiveness can be re-set following adoptive transfer of NK cells into a different MHC I environment. Mature, responsive NK cells from WT mice undergo a decrease in responsiveness a few days after transfer into MHC I-deficient animals [54]. This decrease, which has been called "downward resetting", is also accompanied by acquisition of tolerance toward MHC I-deficient target cells. Conversely, upon transfer to WT animals, hyporesponsive NK cells from MHC I-deficient mice acquire a responsive phenotype [54,80], which has been called "upward resetting". According to the tuning model, NK cells displaced to a new MHC environment are subject to a new round of tuning that results in appropriately altered responsiveness of the cells.

These data show that the responsiveness of NK cells remains plastic even after the cells reach maturity. The capacity of NK cells to reset their responsiveness might represent a mechanism to set the triggering threshold of NK cells to an appropriate level under steady state conditions, in order to prevent autoreactivity, while preserving optimal reactivity to MHC I-deficient or other stimulatory target cells.

### 3.2. Resetting of NK cell responsiveness in inflammatory conditions

The plasticity of NK cell responsiveness in steady state conditions, as revealed by cell transfer experiments, has the capacity to tune NK cell activity in the face of changing steady state conditions. However, such plasticity could be maladaptive under conditions of infection, were it to lead to desensitization of NK cells that would otherwise serve a protective role. These considerations suggest that infections may create conditions that modify NK cell responsiveness, preventing or reversing hyporesponsiveness. We first reported that hyporesponsive NK cells in B6 mice (i.e. those that lack inhibitory receptors Ly49C, Ly49I and NKG2A, also called CIN-cells) respond as powerfully as other NK cells in terms of IFN-γ production when the mice are infected with *Listeria monocytogenes* [40]. Listeria is known to cause major inflammation in infected mice, and it is reasonable to hypothesize that the potent responses of otherwise hyporesponsive NK cells are due at least in part to the cytokines produced in the course of the infection. Earlier studies had shown that hyporesponsive NK cells from MHC I-deficient mice killed efficiently after culturing the cells in IL-2 for several days [81].

Other reports show that viral infections can strongly influence the responsiveness of NK cells. In one study, hematopoietic chimeras were employed to show that a state of tolerance to MHC-deficient cells established in steady state conditions could be reversed when the mice were infected with mouse cytomegalovirus [82]. In another report, the same group showed that during MCMV infection in wildtype B6 mice, the Ly49C$^-$Ly49I$^-$ NK cells, which exhibit low responsiveness in steady state conditions, expand preferentially and provide better protection than Ly49C$^+$Ly49I$^+$ NK cells [83]. Similar preferential expansions of Ly49C$^-$Ly49I$^-$ NK cells were observed in LCMV, vaccinia and MHV infections [25]. One interpretation of these data is that the initially low responsive NK cells are re-activated by the cytokines produced during the infection and are enabled to expand preferentially because the cells lack inhibitory receptors for self MHC, which inhibit expansion of Ly49C$^+$Ly49I$^+$ NK cells.

Taken as a whole, the available data demonstrate that responsiveness is both plastic and contextual. Changes in steady state MHC expression can lead to gain or loss of responsiveness, but the inflammatory conditions associated with infections act to sustain a higher state of responsiveness, enabling initially hyporesponsive NK cells to provide protective activity, and to do so without being subject to inhibition by self MHC I molecules.

### 3.3. Role of inhibitory MHC I-specific receptors on NK cells

If NK cells lacking self MHC I-specific inhibitory receptors play the most important protective roles in certain infections, what is the role of the major population of NK cells, which express such receptors and are inhibited by self MHC I molecules? Evidence clearly indicated that such receptors inhibit rejection of tumor cells expressing self MHC molecules [84], and previous studies showed that NK cells that express inhibitory receptors for self MHC mediate the rejection of MHC I-deficient bone marrow grafts [40]. Therefore, it is reasonable to propose that these NK cells function to eliminate MHC-deficient cells that arise by mutations in normal cells [85], mutations in tumor cells, or as a result of MHC I-downregulation that occurs in certain viral infections. Such cells could also play a role (along with T cells and antibodies) in eliminating allogeneic (MHC-different) cells transferred sexually, and therefore serve to prevent tumors from becoming sexually transmissible.

Mouse cytomegalovirus (MCMV) is an example of a viral infection that causes MHC I downregulation, and it may therefore be somewhat surprising that NK cells expressing self MHC-specific inhibitory receptors fail to play a predominant role in protection from the virus. Virus-induced MHC I down-regulation occurs only at certain stages of MCMV infection, and is incomplete, possibly explaining this discrepancy. Indeed, in unpublished experiments, we observed that an MCMV mutant lacking the evasins responsible for MHC I downregulation was no less sensitive to in vivo control by NK cells than wild type MCMV (D. Serna and D.H.R., unpublished data).

It remains likely that a substantive role of NK cells expressing inhibitory self MHC I-specific receptors is in the elimination of newly arisen MHC I-deficient tumor cells. Various studies suggest that MHC-deficient tumor cells occur frequently in vivo in naturally arising tumors, possibly because of a high burden of mutations and selection for MHC-low tumor cells by tumor antigen-specific T cells [86]. How does this role for NK cells square with the finding that NK cells exposed to MHC-deficient cells in vivo under steady state conditions are rendered hyporesponsive? This is a question that is currently under investigation. Our unpublished data show that when NK cells infiltrate implanted MHC-deficient tumors, they are rendered anergic within a few days. These results show that when tumor cells with low expression of MHC molecules survive the initial NK cell attack, they can escape NK cell-mediated surveillance by inducing a state of functional hyporesponsiveness. Notably, treatment of MHC-deficient tumor bearing mice with pro-inflammatory cytokines substantially improved the survival of the animals in an NK cell-dependent fashion (M.A. and D.H.R., unpublished data). These findings are consistent with the findings that NK cells undergo downward resetting when exposed to MHC I-deficient cells in non-inflammatory conditions, but can be restored to high functional activity when exposed to inflammatory conditions.

### 3.4. Educated, uneducated, licensed or unlicensed?

The changes in NK cell responsiveness induced by the environment, especially as a result of variations in the expression of MHC I molecules or activating ligands, have been described by many descriptive terms and abbreviations. Given that the underlying molecular mechanisms are likely to be complex, and that many of the consequences remain to be understood, we consider the term “education” a suitably generic and unbiased phrase to describe these processes in toto. However, much confusion arises when researchers apply this and other terms inappropriately. Recently, for example, numerous publications refer to “educated” vs. “uneducated” NK cells, to describe responsive vs. hyporesponsive NK cells, respectively. The data show, however, that fully mature, responsive NK cells convert to hyporesponsive NK cells after exposure to an MHC I-deficient environment [54]. In this case, hyporesponsive NK cells are therefore as educated as responsive NK cells, just with a different outcome. The use of the terms “educated” vs. “uneducated” has the additional effect of implying that hyporesponsive NK cells are immature, but a great deal of evidence argues against this proposition. As we have previously argued, the use of the terms “licensed” vs. “unlicensed” NK cells similarly imposes the concept of maturity vs. immaturity for responsive vs. hyporesponsive NK cells, which appears to be incorrect given the available evidence. Both of these terminologies can lead to confusing statements such as “(unlicensed or uneducated) NK cells are protective in viral infections”. Using these terms in this manner fails to account for the plasticity of NK cell responsiveness or the critical role of the physiological context in shaping it. Furthermore, the terms are binary, and therefore fail to convey the quantitative component of education, wherein NK cells acquire different amounts of responsiveness depending on the intensity of receptor signaling encountered in steady state conditions.

## 4. Concluding remarks

The role of NK cells as effectors of the innate immune response has been extensively studied through the years, and yet we are still far from a complete understanding of how NK cell responsiveness is regulated and the impact of these processes in determining the roles that NK cells play in infections and tumor surveillance. Future research will need to identify the molecular mechanisms underlying the induction of NK cell tolerance as well as possible clinical applications focused on the modulation of NK cell responsiveness. A promising clinical approach to enhancing tumor elimination by NK cells is to block inhibitory receptors in vivo with anti-KIR antibodies [87–89]. In mouse models, antibody-treated mice cleared tumors more efficiently than control mice. Anti-KIR antibodies are now in clinical trials for various tumor indications. A better understanding of how NK cell responsiveness is modulated when inhibitory signaling is prevented will be key to optimizing these therapeutic approaches.

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