REVIEW

NK cell education via nonclassical MHC and non-MHC ligands

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Natural killer (NK) cell education, a process for achieving functional maturation and self-tolerance, has been previously defined by the interaction between self-major histocompatibility complex class I (MHC-I) molecules and their specific inhibitory receptors. Over the past several years, growing evidence has highlighted the important roles of nonclassical MHC-I and non-MHC-I molecules in NK cell education. Herein, we review the current knowledge of NK cell education, with a particular focus on nonclassical MHC-I- and non-MHC-I-dependent education, and compare them with the classical MHC-I-dependent education theory. In addition, we update and extend this theory by presenting the 'Confining Model', discussing *cis* and *trans* characteristics, reassessing quantity and quality control, and elucidating the redundancy of NK cell education in tumor and virus infection.

INTRODUCTION

In addition to T cells and B cells, natural killer (NK) cells comprise a third major type of lymphocyte. NK cells do not express rearranged, antigen-specific receptors, and thus, NK cells are classified as an important component of the innate immune system and participate in early anti-viral and anti-tumor immune responses. Similar to T cells and B cells, NK cells possess a fine-tuning mechanism known as NK cell education, in which they acquire functional competence and adapt to the host where they develop.[1–3] It is well established that the recognition of self-major histocompatibility complex class I (MHC-I) molecules via their specific inhibitory receptors is critical for the acquisition of functional effector NK cells.[4–8] However, recent studies have identified new educational signals mediated through engagements of nonclassical MHC molecules or even non-MHC molecules (Figure 1a; Table 1). In light of these observations, we review the current data about the nonclassical MHC-dependent and MHC-independent education of NK cells; propose the 'Confining Model', which suggests an update to this signaling process; reassess the controversial 'Cis-interaction Model'; discuss the

plasticity of education; and finally, demonstrate relevant therapeutic implications.

CLASSICAL NK CELL EDUCATION (NK CELL LICENSING)

NK cell activation is largely regulated by self MHC-I molecules. The most widely studied MHC-I-specific receptors are inhibitory Ly49 receptors in mice and KIRs (killer cell immunoglobulin-like receptors) in humans. It is worth noting that a subset of NK cells lacking the expression of inhibitory MHC-I-specific receptors are not autoreactive but have acquired a state of 'hypo-responsiveness' to MHC-I-deficient targets or cross-linked activating signals.[3,9,10] Moreover, NK cells from either MHC-I-deficient mice (for example, $\beta 2m^{-/-}$ mice, $TAP^{-/-}$ mice and H2-K^bD^bKO mice) or Ly49 receptor-deficient mice (for example, NKC^{KD} mice) fail to reject MHC-I-deficient targets and respond poorly to many stimuli.[1,11] These findings indicate that only NK cells that have engaged their inhibitory receptors with self MHC-I molecules during development are functionally competent. This interaction between the self MHC-I molecule and its specific inhibitory receptor that allows NK cells to become functionally mature is termed NK cell licensing or classical NK cell

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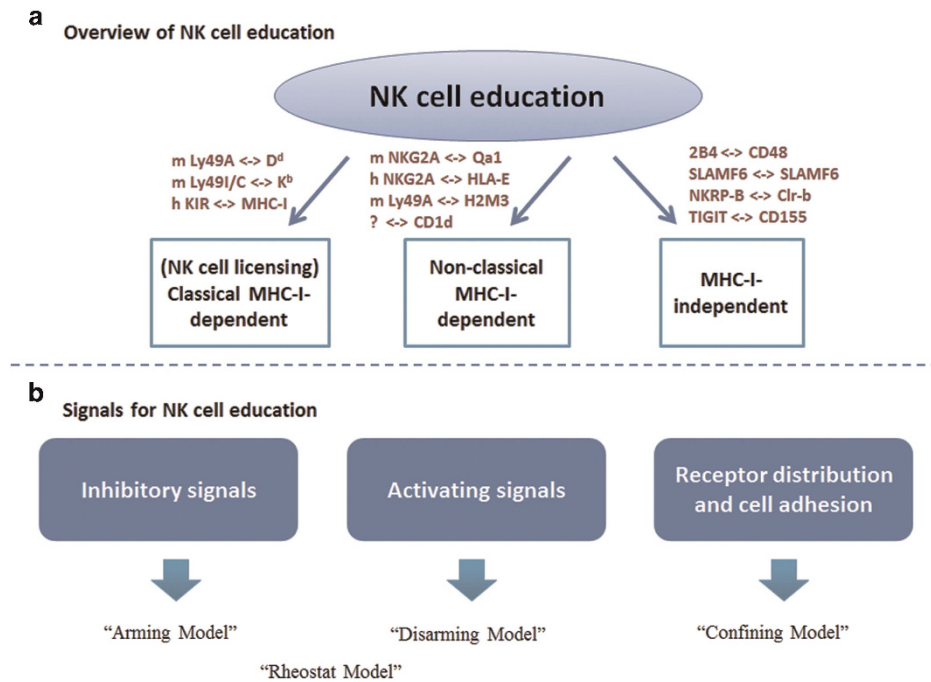E-mail: tzg@ustc.edu.cn

Figure 1 Outline of NK cell education. (**a**) Overview of NK cell education. NK cell education, a broad concept regarding the acquisition of NK cell function and self-tolerance, can be defined as classical MHC-I-dependent, nonclassical MHC-I-independent and MHC-I-independent. (**b**) Signals for NK cell education. Inhibitory signals, activating signals, and adhesion signals are involved in NK cell education. In the 'Arming Model', inhibitory receptors provide critical signals for education through their ITIM motifs. The 'Disarming Model' highlights the important role of activating receptors. NK cells are disarmed (hypo-responsive) if they are without inhibitory signals to balance the chronic activating stimulations. The 'Rheostat Model' proposes a quantitative perspective of NK cell education, suggesting that the increase or decrease in NK cell responsiveness is quantitatively controlled by the strength of the inhibitory input. The 'Confining Model' demonstrates that the confinement of receptor distribution is indispensable for NK cell education. ITIM, immunoreceptor tyrosine-based inhibitory motif; MHC, major histocompatibility complex; NK cells, natural killer cells.

education. The further evidence of licensing is provided by experiments utilizing MHC-I-transgenic mice. The induced expression of an MHC class I single-chain trimer consisting of ovalbumin peptide (SIINFEKL), β2m, and H2K^b heavy chain led to the licensing of Ly49C+ NK cells. Similarly, the transgenic expression of MHC ligand H2-D^d or human leukocyte antigen (HLA) rendered Ly49A+ NK cells and KIR + NK cells, respectively, licensed and responsive.[1,12–14]

Consequently, NK cell licensing results in two types of self-tolerant NK cells. The licensed cells are effective sensors of a missing MHC-I target, but are unable to attack the MHC-I sufficient hosts at the particular locations the inhibitory receptors would be ligated; in contrast, the unlicensed NK cells are hypo-responsive and therefore have a low potential to attack normal cells.

NON-CLASSICAL MHC-I-DEPENDENT NK CELL EDUCATION

Because NK cells possess a wide range of inhibitory receptors in addition to Ly49 receptors and KIRs (Figure 2a), it is critical to determine whether non-classical MHC-I-specific inhibitory receptors can regulate NK cell education and activation.

The C-type lectin-like receptor CD94/NKG2A, another commonly studied inhibitory receptor, recognizes non-classical MHC-Ib molecules (that is, Qa-1 in mice and HLA-E in humans) and is important in educating NK cell tolerance to self and contributes to the inhibition of NK cell-mediated immunity to infections and tumors.[15] NK cells expressing NKG2A are efficient killers of certain targets, especially the ligand deficient ones (specifically Qa1 or HLA-E), which might suggest an educational role via NKG2A signaling.[16–18] It has been shown that the MHC-specific KIRs are not able to educate NK cells in the human fetus and instead induce hypo-responsiveness. However, NKG2A educates fetal NK cells as well as adult peripheral blood NK cells.[17] The mechanism underlying the differential education via NKG2A and KIRs remains to be elucidated.

The inhibitory Ly49A receptor recognizes both the MHC-I molecule D^d and the nonclassical MHC-Ib molecule H2-M3. The Ly49A-H2M3 axis resembles the classical Ly49-H2-D^d system, which triggers the functional maturation of Ly49A+ NK cells and enables them to respond to numerous stimuli.[19] Due to an impairment in NK cell education, H2-M3-deficient mice show an increased risk of tumor invasion in a Ly49A-dependent manner.[20,21] These observations extend our knowledge of the effects of H2-M3 on NK cells and provide new evidence for nonclassical MHC-I-dependent education effects.

Table 1 Animal models for NK cell education

Point	Animal model	Result	References
Classical-MHC-I-dependent education	B6 (H2^b) mice	Ly49C+ NK cells displayed more augmented responsiveness in response to MHC-I-deficient targets and activation receptor cross-linking than Ly49C- NK cells.	1,9
	B10.D2 (K^d D^d) mice	Ly49A+ NK cells showed a higher level of cytokine production and cytotoxicity than Ly49A- NK cells.	1
	MHC-I-deficient mice (β2m−/− mice, TAP−/− mice, H2-K^{b}D^{b}KO mice)	Normal NK cell development and maturation; impaired NK response to MHC-I-deficient targets and activation receptor cross-linking.	1
	NKC^{KD} mice (Ly49-deficient mice)	Normal NK cell development and maturation; defective NK cell response to MHC-I-deficient targets; strong activating signals masked the deficiency of NK cell education.	11
	H2D^d Tg mice	Ly49A+ NK cells, but not CD8αα iIELs, became responsive following the induction of H-2D^d.	12,14
	K^{b}D^{b}KO-TgKIR/HLA mice	NK cells regained functional competence following KIR and HLA induction.	13
	SCT Tg β2m$^{−/−}$ K$^{b−/−}$D$^{b−/−}$ mice	Unlicensed Ly49C+ β2m$^{−/−}$ K$^{b−/−}$D$^{b−/−}$ NK cells became responsive after induced SCT-K^b expression.	1
Nonclassical MHC-I-dependent education	B6 (H2^b) mice	NKG2A+Ly49C/I+ double-positive (DP) NK cells exhibited more augmented responsiveness than the single-positive (SP) or double-negative (DN) cells.	17,18
	H2-M3-deficient mice	Normal NK cell development and MHC-I expression; attenuated NK cell ability to control B16-F10 metastases and MCA-induced fibrosarcoma; impaired NK cell response to activation receptor cross-linking.	19,20
	CD1d1-deficient mice	Normal NK cell maturation; attenuated NK cell 'missing-self' recognition and NKG2D-dependent cytotoxicity.	23
MHC-I-independent education	2B4-deficient mice	Normal NK cell development; dampened NK cell lysis of CD48- targets.	24,27
	CD48-deficient mice	Impaired NK cell lysis of CD48-deficient targets.	24,27
	SLAMF6-deficient mice	Normal NK cell development; defective NK cell responses toward nonhematopoietic cells.	30
	NKR-P1B-deficient mice	Normal NK cell development and maturation; defective rejection of Clr-b-deficient tumors and allogenic cells.	32,35
	Clr-b-deficient mice	Attenuated NK responsiveness of cytokine production in response to PK136 or IL-12 plus IL-18 stimulation.	32,35
	TIGIT-deficient mice	Normal NK cell maturation but impaired NK cell functional degranulation ability; defective NK cell-mediated rejection of CD155-deficient targets.	(He, 2016, unpublished data)
	CD155-deficient mice	Impaired degranulation ability of TIGIT+ NK cells.	
Arming model	Gene transfer of Ly49A mutant	Ly49A lacking ITIM failed to confer licensing.	1
	SHP1-deficient mice (me^v mice, dn SHP1 mice)	Attenuated NK cell cytotoxicity and cytokine production.	43
	SHP1-cKO mice (Ptpn6$^{fl/fl}$-Ncr1^{icre} mice)	Defective NK cell-mediated rejection of MHC-I-deficient transplants and tumors.	42
	SHIP-deficient mice	Diminished NK cell killing of tumor cells.	44,45
	SAP-deficient mice	Compromised NK killing of hematopoietic cells; enhanced NK cell responses to a wide range of nonhematopoietic targets.	28,30
Disarming model	Rae1 Tg mice	Decreased NKG2D expression on NK cells; defective NK cell resistance of Rae1+ targets.	46,47
	m157 Tg mice	Decreased Ly49H expression on NK cells; attenuated NK cell response through Ly49H signaling.	50
Quantity and quality control	B6 (H2^b) mice	NK cell responses increased with the number of types of inhibitory receptors.	32,52,53
	1 (D^b), 2 (K^{b}D^b), or 3 (K^{b}D^{b}D^d) MHC-I-expressing mice	NK cell responsiveness increased with the number of MHC-I alleles; K^b and D^d were strong educational ligands; L^d is relatively weak.	54

Table 1 (Continued)

Point	Animal model	Result	References
Confining model	Wild-type, KbDbKO, KbDbKO-TgKIR/HLA, and KbDbKO-TgKIR mice	Activating receptors of educated NK cells were organically confined in membrane nanodomains; receptors of uneducated NK were dispersed into the actin meshwork.	59
	LFA-1 deficient mice, talin-deficient mice, and Wiskott-Aldrich Syndrome protein (WASP)-deficient mice	Dampened LFA-1 signaling was correlated with impaired reorganization of the actin cytoskeleton and polarization.	61,62
Cis or trans?	Ly49-72A Tg mice	Inhibitory Ly49A receptors interacted with D^d in trans but not in cis; defective NK cell response to H-2D^d -deficient targets.	74
	Ly49A Δa1 Tg mice	Inhibitory Ly49A receptors interacted with D^d in cis but not in trans; defective NK cell response to activation receptor cross-linking.	77
	Ncr-cre D^d mice and CD4-Cre D^d mice (NK cell and T cell-specific H-2D^d deficient mice)	Impaired NK cell response to H-2D^d -deficient targets.	77
	Transfer of NK cells into a different SLAMF6-deficient environment	The absence of trans effects did not alter the SLAMF6-mediated NK cell education.	30

Abbreviations: HLA, human leukocyte antigen; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; NK cells, natural killer cells; SCT, single-chain trimer.

CD1d1, a nonclassical MHC-I molecule crucial for NK T-cell maturation and activation, also binds to NK cells and inhibits NK cell cytotoxicity.[19,22] Interestingly, CD1d1-deficient mice exhibit impaired NK cell activity (due to defective 'missing-self' recognition and NKG2D-dependent cytotoxicity), despite normal NK cell number and development.[23] It is possible that a trans interaction between CD1d1 and an unknown inhibitory receptor enhances NK responsiveness to provide an unknown mechanism of education.

MHC-I-INDEPENDENT NK CELL EDUCATION

Several members of non-MHC-I families, notably signaling lymphocytic activation molecule (SLAM)-related ligand CD48 (2B4 ligand), SLAM family member 6 (SLAM6, self ligand), C-type lectin-related ligand Clr-b (NKRP1-B ligand) and poliovirus receptor CD155 (TIGIT ligand), are known to inhibit the responsiveness of NK cells. Thus, it is critical to determine whether interactions between these non-MHC molecules and their specific inhibitory receptors might have a role in MHC-I-independent education.

2B4 (CD244), a member of the SLAM family of CD2-related receptors, specifically recognizes the cognate SLAM molecule CD48. Murine 2B4 is primarily considered to be a non-MHC-I-dependent inhibitor of NK cells, especially in the absence of its adapter SAP (SLAM-associated protein), as it inhibits NK cells from killing CD48-sufficient tumor cells or allogeneic cells.[24–26] Given that NK cells from either 2B4 knockout mice or CD48 knockout mice fail to reject CD48-deficient tumor cells,[24,27] this 2B4-CD48 engagement is necessary for NK cell-mediated recognition of missing self-CD48 targets and thus represents a MHC-I-independent education pathway.

SLAMF6 (CD352, also known as NTB-A and Ly108), a homotypic receptor of the SLAM family, is highly expressed on hematopoietic cells such as NK, T and B lymphocytes. Similar to 2B4, in the absence of the adapter protein SAP SLAMF6 inhibits NK cell activation when properly engaged with SLFMF6-sufficient hematopoietic target cells.[28,29] On the other hand, SLAMF6-SLAMF6 homotypic interactions enhance NK cell responses to a wide range of non-hematopoietic targets that lack the expression of SLAMF6.[30] This observation suggests that the SLAMF6 receptor confers an educational effect and potentiates NK cell-mediated missing self-recognition of SLAFM6-deficient nonhematopoietic cells.

NKRP1-B, a member of the NKRP1 family, differs from the extensively studied activating receptor NKRP1-C (also called NK1.1) and inhibition of NK cells when properly ligated with its cognate C-type lectin-related ligand Clr-b.[31,32] Although NKP1-B+ NK cells are unable to kill Clr-b-sufficient targets, they can readily kill Clr-b-deficient targets and show higher responsiveness to various stimuli than NKP1-B- NK cells.[33] Researchers have also observed that an NK cell subset co-expressing Ly49I/C and NKRP1-B is more responsive to stimuli, an interaction which mediates a more efficient MHC-I-independent missing self-recognition of Clr-b in both tumor environment and under CMV infections compared with those NK cell subsets expressing only one or neither of the two receptors.[34–36] Furthermore, the absence of either NKRP1-B or Clr-b results in the hypo-responsiveness of NK cells, for example, the dampened rejection of Clr-b missing targets and diminished secretion of interferon-γ.[32,35] Therefore, similar to the MHC-I-specific inhibitory receptors, NKRP1-B is involved in NK cell self-tolerance and the missing self-recognition of Clr-b-deficient targets and hence contributes to NK cell education.

TIGIT (T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain), a recently discovered inhibitory receptor predominantly expressed on T cells and NK cells, inhibits NK cell and T-cell responsiveness upon ligation with its immunoglobulin superfamily ligand CD155.[37–40] Our unpublished data suggest that TIGIT+ NK
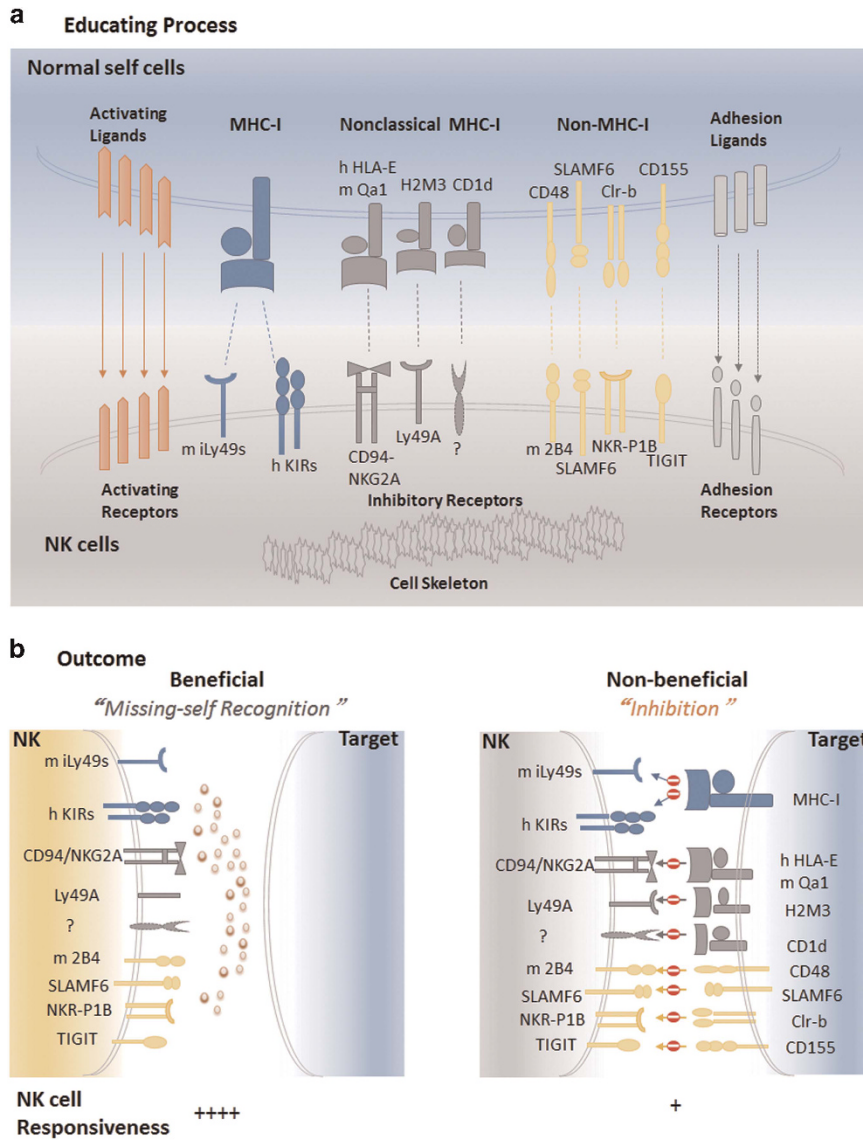
Figure 2 Schematic representation of the role of education on target recognition. (**a**) Educating process. During development, NK cells acquire functional maturation through an adaptation to the host. In this process, inhibitory receptors are directly involved by engaging self-ligands (either MHC-I-dependent or not) to educate NK cells to acquire effector responses. (**b**) Outcome. Differential roles of the education process are shown with respect to the presence of inhibitory ligands on target cells. Education is beneficial to allow NK cells with the expression of inhibitory receptors to sense missing self. However, when inhibitory ligands are sufficient on target cells, the inhibition by ligation of inhibitory receptors with their cognate ligands impedes the activation of educated NK cells. NK cells, natural killer cells.

cells display an augmented responsiveness compared with TIGIT− NK cells to certain stimuli. In addition, the absence of TIGIT or self CD155 in gene knockout mice results in functional defects in NK cells, as demonstrated by impaired recognition and killing of CD155-deficient targets (He, 2016, unpublished data). This finding addresses how the functional maturation of NK cells is impacted by the TIGIT-CD155 system and reveals a certain role for non-MHC-specific receptors in regulating NK cell education (Figure 3).

SIGNALS OF NK CELL EDUCATION
Coordinated integration of inhibitory, activating and adhesion signals regulates the process of NK cell education (Figure 1b).

This next section will address several mechanisms that have been proposed to explain how NK cells become educated.

Inhibitory signals allow NK cell education
The 'Arming Model' was first proposed to address how NK cells can become educated via inhibitory receptors by suggesting that inhibitory signaling directly potentiates NK cells with functional potential.[1] However, this theory is not compatible with the traditional understanding that inhibitory receptors negatively regulate cell functional ability upon ligation.[41] To potentially explain this contradiction, it is important to know that a common feature of inhibitory receptors, MHC dependent or not, is the presence of an essential ITIM in the
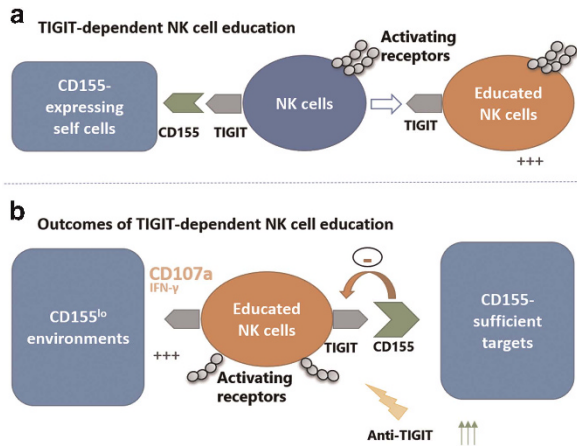
Figure 3 TIGIT-CD155 axis regulates NK cell education (He, 2016, unpublished data). (**a**) During NK cell development, CD155 molecules on self-cells is involved in educating TIGIT+ NK cells to obtain final functional maturation. (**b**) The TIGIT-CD155 system is important for NK cells in sensing missing CD155 targets. However, CD155-sufficient targets inhibit the responsiveness of functional TIGIT+ NK cells. NK cells, natural killer cells.

cytoplasmic tail. ITIM-mutated NK cells cannot be educated and respond poorly to stimuli,[1] which reveals an indispensable role for intact ITIMs and provides strong evidence to support the Arming Model. Initial signaling by ITIM motifs involves the recruitment of several phosphatases, as follows: SH2-domain-containing protein tyrosine phosphatase 1 (SHP1), SHP2 and SH2-domain-containing inositol-5-phosphatase (SHIP). Among these proteins, SHP1 is the most abundant and best understood. NK cell-specific deletion of SHP1 alters the expression of inhibitory Ly49 receptors and impairs NK cell cytotoxicity and cytokine production,[42,43] which suggests that signaling via SHP1 is crucial for the process of NK cell education. Inhibitory signaling through non-MHC inhibitory receptors such as 2B4, TIGIT and KLRG1 is often associated with the recruitment of SHIP and not SHP1, and the absence of SHIP renders NK cells hypo-responsive and uneducated.[44,45] The adapter protein SAP, which disrupts inhibitory signaling mediated by SHP1 or SHIP, impedes NK cell education.[28,30] This phenomenon would suggest that inhibitory receptor-mediated NK cell education is an ITIM-dependent process and is mediated by either SHP1 or SHIP downstream signaling.

Sustained activation induces NK cell hypo-responsiveness

NK cell activation is coordinately regulated by both inhibitory and activating receptors. In general, activating receptors allow NK cells to detect and diminish infected, transformed host cells or tumor cells. However, the sustained engagement of activating receptors with their plate-bound ligands *in vitro* or with tumor ligands *in vivo* induces hypo-responsiveness in NK cells.[46–48] Some studies performed in transgenic mice clearly demonstrate the negative effects of sustained engagement of activating receptors for NK cells. The transgenic expression of Rae-1 results in the reduced expression of the NKG2D receptor and impairment of NKG2D-mediated NK cell function.[46,49] In addition, the transduction of the *m157* gene decreases the expression of activating Ly49H receptor and leads to impaired NK cell activation through Ly49H signaling.[50,51] In agreement with studies conducted in mice, the continuous engagement of the human-activating receptor KIR2DS1 impairs the responsiveness of NK cells.[49] Thus, in the absence of inhibitory signals, NK cells experience sustained activation and become hypo-responsive, or 'disarmed', which is known as the Disarming Model.[2]

Quantity and quality control of NK cell education

The research on MHC-dependent education has demonstrated that NK cells co-expressing NKG2A and inhibitory Ly49 receptors are more responsive to stimuli than those with just one or no MHC-dependent inhibitory receptors.[52,53] Furthermore, studies utilizing MHC-deficient or transgenic mice show that the responsiveness of NK cells increases with the number of MHC-I alleles.[20,53,54] In line with studies of mice, human NK cells with more types of *KIR* gene copies display augmented functional competence,[55] which suggests that NK cell responsiveness varies continuously with inhibitory input.[56] Recent studies have also demonstrated that NK cells expressing both Ly49I/C and NKRP1-B display augmented responsiveness,[32,35] extending the notion of inhibitory input in a MHC-I-independent manner. We have also observed that an NK cell subset co-expressing TIGIT, NKG2A and Ly49C/I exhibit notably higher responsiveness to stimuli (He, 2016, unpublished data), further broadening the concept of quantity control of NK cell education.

In addition to the number of inhibitory receptors or ligands, the education impact increases with the strength of the receptor-ligand interaction, that is, strong ligands (for example, K^b and D^d) show effective education impacts, while the role of weak ligands (for example, L^d) are compromised.[54] Degranulation, as assessed by CD107a expression, and cytokine production, particularly interferon-γ secretion, are two hallmarks of NK cell responsiveness. The achievement of effector interferon-γ function requires stronger inhibitory input than achievement of degranulation ability.[56] Education via non-classical MHC or non-MHC ligands is sufficient to potentiate the ability to degranulate but is too weak to stimulate cytokine secretion. Different inhibitory receptors vary in the strength of educational input, suggesting that NK cell education is not only quantitatively but also qualitatively tuned. This notion is referred to as the Rheostat Model, in which NK cell education is not an 'on or off' procedure but is a continuously ranging process.[56]

Receptor distribution and cell adhesion—the 'Confining Model'

Although the proposed Arming Model and Disarming Model explains what occurs during NK cell education to a certain extent, large gaps still exist between these models and detailed mechanisms. For example, it is important to identify what determines the response capacity of educated NK cells.

Cell–cell contact constitutes the first step in initiating NK cell cytotoxicity. In this type of contact, the activating and inhibitory receptors of NK cells cluster at the synapse, and then inside-out signaling through adhesion molecules is induced to form stable conjugates and to trigger the polarization of granules. Activating receptors, particularly NKG2D and NKp46, structurally form microclusters at the immune synapse, and this compartmentalization is correlated with enhanced actin remodeling and a lowered threshold for activation.[57,58] Research using dynamic fluorescence demonstrates that activating receptors of educated NK cell are organically confined in nanodomains at the membrane, whereas the receptors of hypo-responsive NK cells from MHC-I-deficient mice are dispersed into the actin meshwork.[59] This physical confinement of activating receptors provides an explanation for education-mediated functional competence. Recent data have also demonstrated that NKG2A+ NK cells are more dynamic and form stronger contacts with targets.[60] Our group has also found that TIGIT + NK cells possess an enhanced ability to conjugate with targets (He, 2016, unpublished data), forming a complement in a MHC-independent manner.

Adhesion molecules, notably LFA-1, are critical for NK cells to control the compartmentalization of receptors and enable stable conjugate formation.[61,62] It is worth noting that the weak NK cell contacts of uneducated NK cells can be partially explained by a lack of inside-out signaling mediated by LFA-1.[63] Another well-studied costimulatory adhesion molecule, CD226 (DNAM-1), which is involved in synapse formation and NK-mediated cytotoxicity of certain tumors, co-localizes with LFA-1 and facilitates LFA-1 mediated contacts.[64] The synergy between CD226 and LFA-1 in educated NK cells constitutes an important regulator in cell conjugation formation and promotion of NK cell reactivity.[65] Moreover, the expression pattern of CD226 is correlated with that of MHC-I-specific inhibitory receptors, suggesting that CD226 acts as a sensor of classical NK cell education.[65,66]

The cell receptors associated with adhesion molecules and the cytoskeleton at the plasma membrane form a well-confined distribution to ensure NK cell responsiveness. This idea represents a new Confining Model, in which the education-induced confinement of receptor compartmentalization prompts efficient NK cell contacts and sequential NK cell activation. Overall, NK cell education is a process determined by the coordination of inhibitory, activating and adhesion signals by which adhesion signaling functions as a link through which inhibitory and activating signaling is functionally connected.

UNEDUCATED OR IMMATURE?
Because MHC-I-specific inhibitory receptors, such as Ly49C/I and KIRs, are highly expressed on terminally mature CD11b +CD27 − NK cells,[67,68] a debate exists regarding whether the hypo-responsive, uneducated NK cells are actually at an immature stage of development. Recent studies demonstrate that either MHC-I- or Ly49-deficient mice contain a normal number of terminally mature NK cells.[1,11] In addition, hypo-responsive NK cells from mice deficient in the expression of H2M3, CD1d1, 2B4, CD48, SLAMF6, NKRP1-B, Clr-b or even TIGIT,[26,32,35,69–71] which are either nonclassical MHC-dependent or non-MHC-dependent receptors, likewise undergo normal development and exhibit regular expression of activating receptors, for example, NKG2D and activating Ly49 receptors. Thus, although functionally impaired, uneducated NK cells develop normally and exhibit the characteristics of a mature NK cell.

CIS, TRANS OR BOTH?
Inhibitory Ly49 receptor family members recognize MHC-I in both a cis (receptor and ligand expressed on the same cell) and trans (receptor and ligand expressed on different cells) manner.[72,73] To address which manner of recognition is relevant to NK cell education, two animal models of Ly49A variants were generated. The Ly49-72A variant, which recognizes D^d in trans but not in cis, inhibits the reactivity of NK cells upon ligation but fails to educate NK cells.[74] Although this outcome highlights the importance of the cis interaction, it is contrary to results obtained from adoptive transfer experiments in which uneducated NK cells gain functional competence after transfer into MHC-I-sufficient hosts[13,75] and educated NK cells become functionally defective after transfer into an MHC-I-deficient environment.[13,76] More work is required to clarify this somewhat undefined cis model. The studies of another Ly49A variant, Ly49A Δa1, which interacts with D^d in cis but not in trans, suggests a favorable effect of trans interaction in NK cell education, as NK cells are rendered hypo-responsive in this variant.[77] The recent finding that NK cells from both NK cell-specific and T cell-specific H-2D^d-deficient mice show defective functional competence[77] suggests that both cis and trans interactions might contribute to the process of NK cell education. Cis interactions might provide a relatively short sequestration of unengaged inhibitory receptors to lower the activation threshold,[78] while trans interactions might transmit a relatively durable signal to balance the chronic disarming signals during NK education.

Studies using in vivo adoptive transfer assays and in vitro mixing experiments have shown that trans interactions are insufficient for SLAM receptors to confer an education effect.[30] However, due to the lack of animal models of receptor variants, no additional details have been revealed regarding the impacts of cis or trans interactions on MHC-independent education. Nevertheless, the expression patterns of self-inhibitory ligands provide some clues to as which mode of interaction is important. Because some ligands, for example, CD48 and Clr-b, are expressed on both NK cells and non-NK cells, education via these specific ligands probably involves the cooperation of both cis and trans interactions. Other self-ligands that are hardly expressed on NK cells, for example, H2-M3, Qa1 and CD1d, would only allow interactions in trans to initiate an educational procedure.

REVERSAL OF NK CELL EDUCATION IN TUMOR AND VIRUS INFECTION

Resetting responsiveness of uneducated NK cells

Uneducated NK cells, which are presumed to be hypo-responsive, restore functional competence in inflammatory microenvironments, like viral and bacterial infections and certain tumor invasions.[1,5,10,79] During infection of murine cytomegalovirus (MCMV), the uneducated NK cells, and not the educated ones, dominantly control the infection.[80] One possible explanation for this observation is that cytokine stimulation increases the expression of inhibitory receptors and therefore a large population of NK cells are re-educated.[81] Another explanation is that education deficiency can be masked by a strong activation. To illustrate this phenomenon, a resetting response is triggered by strong signals following NKG2D ligation in which uneducated Ly49-deficient NK cells lyse RMA-S-Rae-1 tumor cells as efficiently as educated wild-type NK cells.[11] Overall, NK cell education is a plastic process that adapts to environmental changes, and this adaptation may explain the existence of responsive NK cell subsets lacking inhibitory receptors specific for self MHC-I molecules.[82]

Ligation of inhibitory receptors offsets education benefits

To further elucidate the effects of education on NK cell reactivity, it is necessary to determine whether target cells express ligands specific for inhibitory receptors. Inhibitory receptors that drive the education of NK cells are not always helpful, especially when self-specific ligands are sufficient on target cells. Licensed NK cells expressing Ly49C/I fail to respond to MHC-sufficient RMA tumor cells.[80] Moreover, in certain viral infections such as MCMV and influenza, the educating receptor Ly49C/I does not potentiate strong responses but instead inhibits the proliferation and activation of NK cells.[80,83,84] Similarly, when potential targets, for example, autoreactive T cells or tumor cells, maintain a high expression of nonclassical MHC Qa-1 or HLA-E, NKG2A-mediated education is not beneficial for NK cell effector function and conversely inhibits NK cell responses.[85–87] Further-more, the high expression of ligands specific for non-MHC inhibitory receptors, once engaged, triggers dominant negative signals to inhibit NK cell reactivity. The Clr-b-NKRP1-B axis, as an example, is critical for the immune escape of spontaneous myc-induced B lymphomas.[32] In light of these observations, functional benefits obtained from education are apparent only when potential targets lack the expression of self-educating ligands.[83,88] Thus, two roles for inhibitory receptors are shown. First, inhibitory receptors trigger beneficial educational impacts to enable NK cells to sense a missing self. Second, inhibitory receptors serve as a negative regulator to limit the reactivity of NK cells upon ligation (Figure 2b). Accordingly, NK cell therapies based on the blockade of inhibitory receptors have attracted increasing attention in recent years. The anti-KIR blocking antibody, for example, relieves educated NK cells from MHC-I-mediated inhibition and provides a promising therapeutic approach for tumor treatment.[88–90]

CONCLUDING REMARKS

Increasing evidence has demonstrated that both MHC-I molecules and the nonclassical MHC-I, or even non-MHC-I, molecules are involved in NK cell education. Interestingly, inhibitory receptors mediate positive signals for NK cells to achieve functional competence, whereas continuous activating signals induce NK cell hypo-responsiveness. Moreover, adhesion molecules link inhibitory and activating receptors together to form confined compartmentalization that ensures NK cell reactivity. However, distinct signals to illuminate MHC-I-independent education theory require further investigation. Another significant finding is that both *cis*-interactions and *trans* interactions contribute to NK cell education. Nevertheless, cell type specific education needs to be further clarified. In addition, elucidating how the education of NK cells adapts to its environment, particularly in tumor invasion and virus infection, can provide valuable therapeutic implications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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