Journal of Autoimmunity 81 (2017) 1–12

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Contribution of inhibitory receptor TIGIT to NK cell education

Yuke He [a], Hui Peng [a, b], Rui Sun [a, b, **], Haiming Wei [a], Hans-Gustaf Ljunggren [c], Wayne M. Yokoyama [d], Zhigang Tian [a, b, *]

[a] Institute of Immunology, Key Laboratory of Innate Immunity and Chronic Disease of Chinese Academy of Science, School of Life Sciences and Medical Center, University of Science and Technology of China, Hefei, 230027, China
[b] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, 310003, China
[c] Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, 16451, Sweden
[d] Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri, 63123, USA

ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form
1 April 2017
Accepted 3 April 2017
Available online 21 April 2017

Keywords:
NK cells
Education
Licensing
MHC-I
TIGIT
CD155

ABSTRACT

Engagement of inhibitory receptors by cognate host MHC-I molecules triggers NK cell education, resulting in functional maturation and allowing NK cells to sense missing-self. However, NK cells also express inhibitory receptors for non-MHC-I ligands and their role in NK cell education is poorly understood. TIGIT is a recently identified inhibitory receptor that recognizes a non-MHC-I ligand CD155. Here, we demonstrated that TIGIT$^+$ NK cells from wild-type mice exerted augmented responsiveness to various stimuli, including targets that lacked expression of CD155 ligand. TIGIT$^+$ NK cells derived from CD155-deficient hosts, however, exhibited functional impairment, indicating that the engagement of TIGIT receptor by host CD155 promoted NK cell functional maturation. Furthermore, TIGIT deficiency impaired NK cell-mediated missing-self recognition and rejection of CD155$^-$ targets, such as allogenic splenocytes and certain tumor cells, in an MHC-I-independent and CD226-unrelated manner. Thus, TIGIT-CD155 pathway is also involved in the acquisition of optimal NK cell effector function, representing a novel MHC-I-independent education mechanism for NK cell tolerance and activation.

1. Introduction

NK cells are important components of innate immunity that involved in the rejection of both tumor cells and damaged cells. Their functions are accomplished through direct killing and the release of cytokines, such as interferon (IFN)-γ. According to the "missing-self" hypothesis, host MHC-I molecule acts as a molecular marker of "self" and, NK cells mediate effective responses to target cells with aberrantly low MHC-I expression [1,2]. These MHC-I-dependent effects are conferred by inhibitory receptors, including human KIRs and murine Ly49 receptors, which recognize classical MHC-I ligands and, when properly engaged, transduce inhibitory signals through immunoreceptor tyrosine-based inhibitory motifs (ITIM) and prevent NK cell-driven autoimmunity [3,4].

To ensure functional competence, NK cells undergo a process known as "licensing" or "education" during development and maturation [5–8]. It is widely accepted that MHC-I is critical not only for functional inhibition but also for NK cell licensing, because NK cells from MHC-I-deficient mice exhibit intrinsically defective responses to various stimuli [5]. This finding also explains why NK cells from MHC-I-deficient hosts do not cause autoimmunity, when considering "missing-self" effects in normally matured NK cells. Evidence also shows that only NK cells expressing inhibitory receptors for MHC-I are capable of mediating missing-self immunosurveillance, whereas those lacking inhibitory receptors are rendered hypo-responsive [5,9,10]. Thus, engagement of inhibitory receptors by host MHC-I molecules determines whether NK cells become educated (functionally competent) or uneducated (hyporesponsive). Recently, several members of non-classical MHC-I families, notably mouse Qa-1 and H2M3 and human HLA-E, which are cognate ligands for inhibitory receptors, have been shown to be missing-self determinants and to be required for optimal NK cell

* Corresponding author. School of Life Sciences and Medical Center, University of Science and Technology of China, 443 Huangshan Road, Hefei, Anhui, 230027, China.
** Corresponding author. School of Life Sciences and Medical Center, University of Science and Technology of China, 443 Huangshan Road, Hefei, Anhui, 230027, China.
E-mail addresses: sunr@ustc.edu.cn (R. Sun), tzg@ustc.edu.cn (Z. Tian).

http://dx.doi.org/10.1016/j.jaut.2017.04.001
0896-8411/

responsiveness [11,12]. These findings broaden the concept that NK cell education can occur in a non-classical MHC-I-dependent manner.

Under certain conditions, however, NK cell responsiveness cannot be explained by MHC-I-dependent NK cell education. On a C57BL/6 background, CIN (Ly49C/I and NKG2A) negative NK cells or cells from MHC-deficient and Ly49-deficient mice, which are thought to be unlicensed, exhibit robust NK cell responses to mouse cytomegalovirus (MCMV)- or influenza-infected cells and to several types of tumor cells [13–15]. Therefore, the acquisition of NK cell function appears to occur *via* alternative pathways. Some inhibitory receptors of NK cells recognize neither MHC-I nor non-classical MHC-I ligands [16–19]. Subsequent studies on NKR-P1B and SLAMF6 receptors have provided promising clues, because these inhibitory receptors enhance NK cell effector function under certain circumstances [20,21]. Yet, it remains to be confirmed whether interactions between non-MHC-specific inhibitory receptors and their cognate ligands are able to regulate NK cell education.

TIGIT is a novel inhibitory receptor expressed on both T cells and NK cells [22,23]. Ligated TIGIT mediates inhibitory signals by recruitment of SHIP phosphatases to ITIM and inhibits the activation of T cells and NK cells [24,25]. CD155, also known as poliovirus receptor (PVR), belongs to the immunoglobulin superfamily and is a high-affinity ligand for TIGIT [26,27]. Similarly to MHC-I molecules, CD155 is widely expressed on multiple cell types, such as epithelial and endothelial cells in many tissues [27]. Over the past several years, the engagement of TIGIT by CD155 on damaged self-cells or certain tumor cells has been shown to limit NK/T cell-mediated autoimmune, anti-viral or anti-tumor immune responses in both humans and mice [18,23,28–32]. Absence of CD155 in recipients results in aggravated graft-versus-host disease (GVHD), indicating a role for T cell mediated recognition of missing-CD155 in allogeneic responses [33]. Loss of CD155 expression on macrophages also stimulates TIGIT$^+$ NK cells, suggesting that CD155 may act as a non-MHC missing-self determinant [34]. Adding complexity to this network, activating receptor CD226 is another receptor for CD155 ligand with an affinity lower than TIGIT [35,36]. As has been reported, the expression of CD226 on educated NK cells constitutes an important regulator in cell conjugation and enhances NK cell reactivity [37]. CD226 distinguishes between two functionally distinct NK cell subsets and acts as an intrinsic marker for MHC-I-dependent education [37,38]. Recent studies have also demonstrated that CD155 is sufficient to mediate MHC-independent thymic selection of T cells [39,40]. However, the role of TIGIT/CD226-CD155 network in NK cell education is still unclear.

Here, we showed that loss of CD155 expression on splenocytes led to their rejection by NK cells in a TIGIT-dependent manner, thus confirming the role of TIGIT in mediating MHC-I-independent missing-self recognition by NK cells. We also found that TIGIT$^+$ NK cells were rendered hypo-responsive in CD155-deficient hosts, thus indicating that host CD155 was necessary for the acquisition of optimal effector function of TIGIT$^+$ NK cells. Further, NK cells from TIGIT-deficient mice responded poorly to various stimuli, especially to CD155-missing targets. Moreover, the TIGIT-CD155 pathway functioned in an MHC-I-independent and CD226-unrelated manner in regulating NK cell education and effector function. Our findings indicated that TIGIT mediated recognition of host CD155 imparted NK cell education similar to the role of engagement of Ly49 and NKG2A receptors *via* their MHC-I ligands in mice.

2. Material and methods

2.1. Animals

C57BL/6 mice were purchased from the Shanghai Experimental Animal Center (Shanghai, China). CD155-deficient (*Pvr*$^{-/-}$) C57BL/6 mice were kindly provided by Dr. Yoshimi Takai (Kobe University, Japan) and Dr. Stephan Gasser (National University of Singapore). *Tigit*$^{-/-}$ C57BL/6 mice were kindly provided by Bristol-Myers Squibb. *Rag1*$^{-/-}$ C57BL/6 mice were obtained from the Model Animal Research Center (Nanjing, China). *Tigit*$^{-/-}$*Rag1*$^{-/-}$ mice were generated and bred in house. NOD/SCID mice and *B2m*$^{-/-}$ NOD/SCID mice were purchased from the Model Animal Research Center (Nanjing, China), which obtained the mice from the Jackson Laboratory. CD45.1 C57BL/6 mice were purchased from the Jackson Laboratory. *Tigit*$^{fl/fl}$ C57BL/6 mice were generously provided by Dr. Zusen Fan (Institute of Biophysics, Chinese Academy of Sciences, China). *Tigit*$^{fl/fl}$*Ncr1*$^{icre/+}$ mice (TIGIT conditional knockout mice, TIGIT-cKO mice) were generated by crossing *Tigit*$^{fl/fl}$ mice with *Ncr1*$^{icre/+}$ mice (a kind gift form Dr. Eric Vivier, Center d'Immunologie de Marseille-Luminy, France). All mice were maintained in a specific pathogen-free facility. Treatment was randomly allocated to animal subjects, and all animal experiments and protocols were approved by the Committee on the Ethics of Animal Experiments of University of Science and Technology of China.

2.2. Mixed bone marrow chimeras

CD45.2$^+$ congenic mice were lethally irradiated with 11 Gy and used as recipients for bone marrow transfer 8 h later. Bone marrow cells were isolated from the femurs of CD45.1 *Tigit*$^{+/+}$ and CD45.2 *Tigit*$^{-/-}$ mice. A 1:1 mixture of 10^6 CD45.1 *Tigit*$^{+/+}$ and 10^6 CD45.2 *Tigit*$^{-/-}$ bone marrow cells was injected intravenously into recipient mice. The resulting chimeras were analyzed 8 weeks after bone marrow transplantation.

2.3. CD155 overexpression

CD155 was cloned into the pLVTHM vector. Lentiviral supernatant was generated by co-transfecting 293T cells with pLVTHM-CD155 plasmid, the envelope pMD2.G plasmid and the packing psPAX2 plasmid. Virus supernatant was harvested 2 or 3 times every 12 h. RMA-S cells [41] were incubated with virus supernatants for 48 h. The efficiency of CD155 overexpression was determined by flow cytometry, and single-cell clones of the lentivirus-infected RMA-S cells were generated by using serial dilution. All the cell lines have been authenticated by FACS and verified free of mycoplasma contamination.

2.4. Antibody staining and flow cytometry

Monoclonal antibodies (mAbs) against CD3e (cat#557655), CD19 (cat#557655), NK1.1 (cat#552878), CD11c (cat#553801), CD69 (cat#553236), CD155 (cat#151036), Ly49C/I (cat#553277), CD27 (cat# 558754) and CD11b (cat#550993) were purchased from BD Biosciences (San Jose, CA). Abs against TIGIT (cat#50-9501), NKG2A (cat#12-5897), perforin (cat#17-9392), Granzyme B (cat#12-8822) and NKp46 (cat#50-3351) were purchased from eBioscience (San Diego, CA). Abs against F4/80 (cat#123128), Gr-1 (cat#108412), CD45 (cat#103114), CD96 (cat#131705), CD107a (cat#121606), IFN-γ (cat#505822) and CD226 (cat#132006) were purchased from BioLegend (San Diego, CA). Prior to staining with antibodies, cells isolated from various organs or cultures were incubated with rat immunoglobulin for 30 min to block Fc receptors. Data were acquired with an LSR II flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).