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Review

NKG2D: A versatile player in the immune system

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ABSTRACT

NKG2D is known as a potent activating receptor of the immune system. It is expressed on a multitude of immune cells, including NK cells and different subsets of T cells. NKG2D recognizes various MHC I-like ligands that are induced on target cells exposed to stressors such as viral infection, DNA damage and oncological transformation. NKG2D drives or facilitates cytotoxic and cytokine responses towards cells expressing its ligands to eliminate the threat. Therefore, NKG2D is usually classified as a sensor that translates cellular stress into activation signals for immune cells. However, more recently it has become evident that NKG2D plays a role beyond direct killing of target cells. Lack of NKG2D affects development of NK cells in the bone marrow, resulting in hyperreactive NK cells. NKG2D deficiency on CD8 T cells affects the ability of effector cells to produce cytokines in response to T cell receptor engagement and reduces their capacity to establish immunological memory. Although NKG2D is not expressed on B cells subsets, lack of this receptor in hematopoietic precursors affects B cell development. Homing of mature B2 cells is altered in NKG2D-deficient mice and they have a strong reduction in peripheral B1a cell numbers, resulting in increased susceptibility to bacterial infections. The exact molecular mechanisms *via* which NKG2D mediates these versatile functions is still being explored, but appears to depend on the control of activation thresholds, either in hematopoietic precursors or mature immune cell subsets. In this review, we will elaborate on the underappreciated developmental and regulatory roles of NKG2D.

Properties of the NKG2D receptor

NKG2D is one of the best studied activating immune receptors within the past two decades [1–3]. It is expressed on all NK cells and several T cell subsets. On NK cells, expression of NKG2D is not restricted to mature cells, but is already induced on the earliest NK-committed progenitors in the bone marrow [4]. The role of NKG2D in mediating effector functions of the immune system have been well documented [5]. Important roles of NKG2D have been shown in immune-surveillance of viral infections and tumor growth as well as in rejection of organ transplants and in autoimmunity [6–10]. In contrast, developmental and regulatory functions of NKG2D have been given relatively little attention. Only in recent years several aspects of its regulatory roles emerged [11–13].

NKG2D is a type II transmembrane protein classified as killer cell lectin-like receptor of the subfamily K member 1 (KLRK1) [14,15]. It is encoded by the *Klrk1* gene located on chromosome 6 and chromosome 12 of mice and humans respectively. NKG2D is known as member D of the NK group 2 (NKG2) family of receptors. In contrast to the other members (A/B, C, E) of the family, which form heterodimers with CD94 and recognize MHC Ib ligands, NKG2D is a homodimer that recognizes stress-induced MHC I-like proteins [16,17]. The molecular structure of NKG2D allows promiscuous binding of variety of ligands with different affinities. In humans, the ligands are MICA (MHC class I chain–related protein A), MICB and 6 members of the ULBP (UL16-binding proteins) family. In mice, glycoprotein MULT1 (murine UL16-binding protein-like transcript 1), three related proteins (a, b, c) of the histocompatibility antigen 60 (H60) group and five GPI-anchored proteins (α, β, γ, δ, ε) of the RAE-1 (retinoic acid early inducible-1) family [3,17–19] have been shown to activated NKG2D. In healthy tissues, ligands for NKG2D are usually not or weakly expressed [20–23]. They are strongly induced whenever cells are exposed to various types of cellular-stress like in viral infections or oncogenic transformation [19].

NKG2D has a very short intra-cytoplasmic domain which does not have any signaling properties [15]. Through charged amino acid residues in its transmembrane domain, NKG2D binds to two adaptor proteins which transduce NKG2D signaling, *i.e.* DAP10 (DNAX-activating protein of 10 kDa) and DAP12 (DNAX-activating protein of 12 kDa) [24–26]. DAP10 possesses a YxxM motif and recruits PI3K, Grb2 and Vav-1. This signaling cascade is presumed to be mainly responsible for cytotoxicity and survival of cells [2,26,27]. DAP12 has ITAM (immunoreceptor tyrosine-based activation motif) and recruits Syk and Zap70

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kinases [28] and was thought to be the main inducer of cytokine release and cytotoxicity [28,29]. In mice, NKG2D is expressed in two isoforms: a long (NKG2D-L) and a short (NKG2D-S) variant, with differ thirteen amino acids in length and is the result of alternative splicing of the *Klrk1* transcript [24,25]. Human cells express only NKG2D-L. The isoforms differ in their capability to bind adaptor molecules. While NKG2D-S can make complexes with DAP10 and DAP12, NKG2D-L only makes a complex with DAP10 [30,31].

The ability of NKG2D to induce immune cell activation appears to depend on signal input and activation status. In human NK cells, NKG2D signals only through Dap10, which is able to induce direct cytotoxicity in activated, but not resting cells upon ligand engagement [32,33]. Murine NKG2D signals both through Dap10 and Dap12, which is sufficient to induce direct cytotoxicity upon target binding [24].

NKG2D in regulation of NK cell-development and function

NK cells are the third largest lymphoid population after B and T cells and the main lymphoid effectors of innate immunity. Whereas NK cells have been identified decades ago, only recently they were recognized as Type 1 group members of innate lymphoid cells (ILCs) [34–37]. NK cells play important role the in early defense against tumors and different intracellular pathogens. They are armed with a range of activating and inhibitory receptors [38]. The balance between their signals determines whether NK cells are activated. Under homeostatic conditions, inhibitory receptors recognize normal levels of MHC I (Ia and Ib) molecules on target cells and their signals prevail and keep NK cells in a quiescent state. However, in the case of reduced levels of these molecules ("missing self"), NK cells get activated. Many intracellular pathogens as well as tumors actively downregulate MHC I molecules to protect themselves from CD8 T cell-mediated surveillance, and thus become sensitive to NK cell mediated control. In addition to this layer of regulation, NK cells can also recognize stress-induced ligands ("induced self"). These ligands belong to MHC I-like and other unknown molecules that are recognized by NKG2D (see above) and NCR1(NKp46) [39,40] respectively. Both activating mechanisms ("missing self" and "induced self") usually operate synchronously. When activated, NK cells exert effector functions through cytokine production and/or cytotoxicity.

NKG2D is constitutively expressed on all cells from the NK cell lineage, from the earliest stages of development onwards [4]. In early NK precursors NKG2D levels are relatively low, but its expression increases with time and stays high in mature cells [41]. NKG2D-deficient mice revealed an unexpected role of NKG2D in NK cell development [5,11,42]. NK cell precursors showed enhanced proliferation in the bone marrow, faster maturation and augmented sensitivity to apoptosis [5,11]. NKG2D deficiency also resulted in differences in their receptor repertoire, with altered levels of c-kit (CD117), inhibitory Ly49 receptors and the adhesion molecule DNAM-1 [11,42]. As expected, *Klrk1*$^{-/-}$ NK cells were considerably less responsive to target cells expressing NKG2D ligands [6,11]. Surprisingly, following stimulation with non-NKG2D targets, they showed a hyper-reactive phenotype in terms of IFNγ production [11,42] and better control of mouse cytomegalovirus (mCMV) infection [11]. These data suggest that NKG2D negatively regulates activation thresholds of other activating receptors early in NK cell-development (Fig. 1a). This would be a novel concept to the conventional model of NK cell education, where engagement of inhibitory receptors at the immature stage is setting thresholds for activating receptors [43,44]. Our recent data indicates that NKG2D regulation is specifically directed to some activating receptors and that its deficiency does not induce a general state of hyper-responsiveness (unpublished data).

NKG2D plays an important role in surveillance of viral infections and tumors. One of the best viral examples are herpesviruses, which are susceptible to NK cell mediated control. To reduce this susceptibility, herpesviruses express several viral proteins which interfere with the surface expression of NKG2D ligands [45,46]. Many tumors, at least in the early stages of their development, induce NKG2D ligands and become sensitive to NKG2D-mediated immune-surveillance [6,47]. Transfection of resistant tumors with NKG2D ligands increases their susceptibility to NK cell mediated control *in vivo* and *in vitro* [48,49]. However, sustained engagement of NKG2D can cross-tolerize NK cell activation [8,50–52]. It causes reduced activity of the CD16 and NK1.1 receptors, which do not signal through Dap10/12 adaptors [52]. This study suggests that prolonged NKG2D stimulation modulates cytoplasmic signaling downstream of FcRγ. Deng et al. added an additional layer of complexity to NKG2D mediated regulation and introduced the "desensitization" model [10]. Sustained binding of low-affinity ligands to NKG2D leads to its downregulation, inhibition of NK cell activity and their capacity to eliminate tumors. Addition of high-affinity soluble ligand (MULT-1) restored activation of NK cells through NKG2D and improved tumor control [10]. Thus, competitive binding of high-affinity NKG2D ligands can "desensitize" NKG2D on mature cells and drive effector functions (Fig. 1b). This finding puts previous observations that tumor-shaded NKG2D ligands dampen NKG2D-mediated surveillance in a different view [53,54] and opens novel perspective for tumor therapies. However, the "desensitization" model does not explain the developmental phenomenon observed in NKG2D-deficient mice and hyperreactivity of NK cells driven by other activating receptors [11]. Thus, NKG2D-deficiency uncovered another layer of NKG2D activity which is associated with the development of NK cells.

In conclusion, it seems NKG2D plays a bifurcate role in the biology of NK cells. On one side, it shapes development of NK cells and sets general activation thresholds for specific activating receptors. On the other, it drives effector responses that are tightly regulated by variety of stress-induced ligands.

NKG2D in regulation of T cell functions and memory development

On T cells, the role of NKG2D is subject to several layers of regulation which differ between subsets and activation status of cells. On innate-like T cells, such as NKT cells and γδ T cells, NKG2D is constitutively expressed [5]. Naïve murine CD8 T cells do not express NKG2D, whereas their human equivalents do [1,55]. Activation of CD8 T cells results in induction of receptor expression for both humans and mice [56]. CD4 T cells typically do not have NKG2D on their cell surface, but its expression can be observed under conditions of chronic inflammation, such as in patients with multiple sclerosis, in colitis models or in cytomegalovirus infection on cytotoxic CD4 T cells [57–59].

Even though NKG2D expression is mostly associated with cytotoxic immune cell subsets, in T cells this receptor is thought to primarily function as a co-stimulatory molecule rather than a direct mediator of cytotoxicity. In mice, CD8 T cells express low levels of Dap12 [24], whereas humans only contain the short isoform of NKG2D which does not associate with Dap12 [60]. NKG2D signaling in T cells is therefore thought to be primarily mediated *via* Dap10 [24]. Indeed, mice deficient for Dap10 show a strong reduction in cell surface expression of NKG2D on activated CD8 and γδ T cells, whereas its expression is reduced but still present on NK cells [25]. Moreover, NK cells from mice deficient for Dap10 are still able to mediate cytotoxic responses to cells overexpressing NKG2D ligands [25]. These observations led to formulation of a model where, in response to NKG2D engagement, Dap12 mediates cytotoxicity and Dap10 enhances signaling through other activating receptors [61]. This concept was supported by the observation that in many systems, NKG2D engagement on CD8 T cells does not result in direct cytotoxicity, but rather enhances effector function after T cell receptor engagement (TCR) alone [62,63], or even only after combined TCR and CD28 triggering [64].

However, studies using mice deficient for Dap12 or for Syk and Zap70, the signaling modalities downstream of Dap12, showed that NK cells are still capable of cytotoxic responses after engagement of the