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Commentary

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Setting traps for NKG2A gives NK cell immunotherapy a fighting chance

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The equilibrium of signaling through activating and inhibitory receptors dictates whether a given NK cell will execute cellular cytotoxicity. In this issue of the *JCI*, Kamiya et al. describe a novel approach to efficiently inhibiting surface expression of the inhibitory receptor CD94/NK group 2 member A (NKG2A) through retention of the protein in the endoplasmic reticulum. In adoptive transfer experiments into tumor-bearing immunodeficient mice, NKG2A^{null} NK cells were significantly more effective at eliminating HLA-E–expressing tumor cells than NKG2A^{+} NK cells. This study provides proof of concept for a new immunotherapeutic approach using NKG2A^{null} NK cells.

A central role for NKG2A in NK cell inhibition

CD94/NK group 2 member A (NKG2A) and NKG2C were identified in the mid-1990s as cell surface glycoproteins that form disulfide-bonded heterodimers with CD94 and bind the nonclassical MHC class Ib molecule HLA-E. NKG2C engagement in CMV-seropositive individuals imparts NK cell activation (with activated cells termed adaptive NK cells), while engagement of CD94/NKG2A transduces an inhibitory signal, consistent with the presence of two I/VxYxxL immunoreceptor tyrosine-based inhibitory motifs (ITIMs) within the cytoplasmic domain of NKG2A (1–4). While NKG2A expression and NKG2C expression are usually mutually exclusive, the frequency of NKG2A^{+} NK cells is considerably higher than that of NKG2C^{+} NK cells, especially in CMV-seronegative donors (5). Therefore, alongside killer immunoglobulin-like receptors (KIR), NKG2A represents a dominant inhibitory receptor on NK cells. The NKG2A phospho-ITIMs interact directly with the SH2 domains of the tyrosine phosphatases SHP-1 and SHP-2 (6). One of the major targets of SHP-1–mediated dephosphorylation in NK cells is the guanine exchange factor and adaptor protein Vav1. Dephosphorylation of Vav1 prevents Rac1-dependent rearrangement of the actin cytoskeleton and amplification of activating signals (7). Engagement of CD94/NKG2A by HLA-E within inhibitory signaling clusters can also lead to the phosphorylation of the signaling adaptor protein Crk and disruption of actin-dependent signaling upstream of Vav1 (8). NKG2A is uniformly high on immunoregulatory CD56^{bright} NK cells, whereas cytotoxic CD56^{dim} NK cells exhibit more heterogeneous expression, with a general decrease associated with terminal differentiation (9).

HLA-E molecules are expressed at low levels in most tissues and primarily present peptides derived from the leader sequences of classical class Ia HLA molecules (10). However, HLA-E is commonly expressed at high levels on the surface of a variety of different cancers (11–13). The mechanistic basis of HLA-E overexpression is not entirely clear. However, it has been shown that IFN-γ treatment can induce high levels of HLA-E in ovarian carcinoma cell lines (14). Intriguingly, the human cytomegalovirus protein gpUL40 can also upregulate HLA-E (15). Thus, it is possible that HLA-E overexpression in some types of cancer could be driven by viral infection. While CMV-induced NKG2C^{+} NKG2A$^{lo/-}$ NK cells may be highly effective in fighting cancer (16), high levels of HLA-E on tumor cells from cancer patients are negatively correlated with survival and are assumed to restrain the cytotoxic effector function of both NK cells and subsets of CD8^{+} T cells that upregulate NKG2A in response to inflammatory cytokines (13, 17). Given these correlative clinical findings, there is a strong rationale for targeting the NKG2A/HLA-E axis as a means of enhancing immunotherapy.

Trapping NKG2A to unleash NK cell antitumor function

The current study by Kamiya et al. employs a clever approach to eliminating NKG2A surface expression on primary peripheral blood NK cells (18). The authors designed a novel construct termed NKG2A protein expression blocker (PEBL). The construct consists of a single-chain variable fragment (scFv) derived from the sequence of the Z199 anti-NKG2A antibody clone linked to different endoplasmic reticulum (ER) retention domains. The idea behind the use of a NKG2A PEBL is that, once endogenous NKG2A proteins are translated by ribosomes in the ER, they will be immediately "captured" by the anti-NKG2A antibody fragment and retained within the ER instead of trafficking to the cell surface. Indeed, NK cells retrovirally transduced with NKG2A PEBLs exhibited marked reduction in NKG2A surface expression. In particular, constructs containing KKMP (lysine, lysine, methionine, proline) domains together with a CD8α

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Conflict of interest: JSM serves on the scientific advisory board of, and consults for, GT BioPharma Inc. and Fate Therapeutics. He has received research funds from these relationships. He also serves on the scientific advisory boards for CytoSen Therapeutics and Onkimmune. FC consults for Fate Therapeutics and has received research funds from this relationship.

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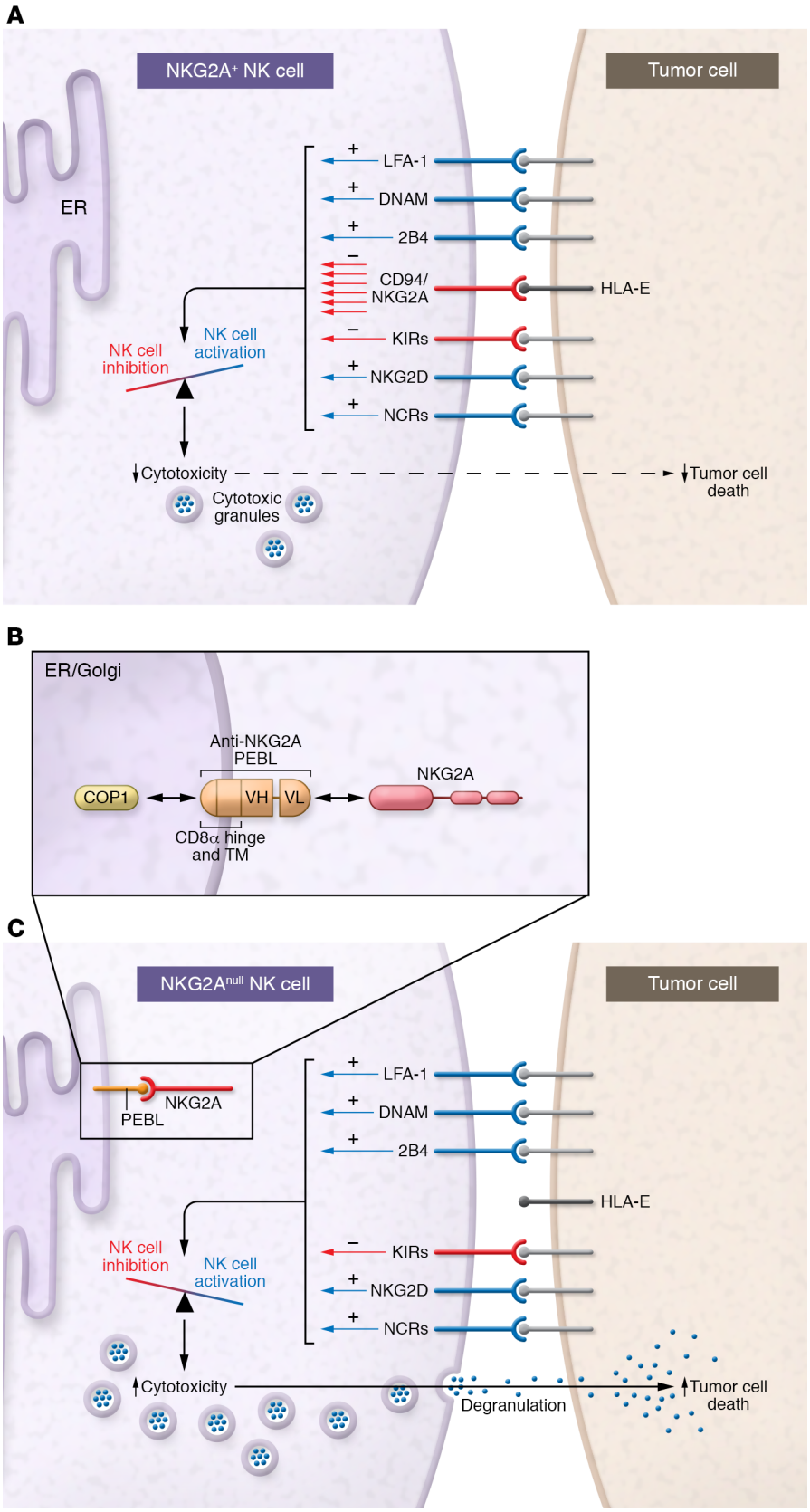

Figure 1. Trapping NKG2A in the ER enhances NK cell cytotoxicity against HLA-E–expressing tumors. (**A**) High HLA-E$^+$ tumors deliver a potent inhibitory signal that leads to inhibition of NK cell function (five relative activating signal units vs. seven inhibitory). (**B**) Kamiya et al. developed a series of NKG2A PEBLs consisting of an scFv derived from an anti-NKG2A antibody linked to ER-retention domains. Transduction of human peripheral blood NK cells with retrovirus containing PEBL cassettes leads to efficient intracellular retention of NKG2A. (**C**) After NKG2A knockdown by PEBLs, the resulting NKG2A^{null} NK cells exhibit greater cytotoxicity against HLA-E–expressing tumor cells due to a net lack of inhibitory signaling (five relative activating signal units vs. one inhibitory).

hinge and transmembrane domain, allowing for direct binding to the ER protein COP1, almost completely blocked NKG2A surface expression (Figure 1B).

After confirming that no other NK cell receptors were altered in response to NKG2A PEBL transduction, the authors generated multiple tumor cell lines with strong NKG2A-binding potential by transducing them with HLA-E plus HLA-G signal peptide. Relative to control NKG2A$^+$ NK cells (expressing only GFP), NKG2A^{null} NK cells (NKG2A PEBL-transduced) exhibited markedly higher cytotoxicity against these HLA-E–overexpressing lines. Therefore, the balance in signaling inputs was tipped; NKG2A$^+$ NK cells that received a negative signal in response to HLA-E ligation were restrained in their killing (Figure 1A), while activating signals prevailed in NKG2A^{null} cells to trigger cytotoxicity (Figure 1C). The authors also tested the function of control and NKG2A^{null} NK cells against tumor cell lines with endogenous HLA-E expression that could be further elevated after exposure to IFN-γ. Again, NKG2A^{null} NK cells exerted significantly higher cytotoxicity with or without the addition of IFN-γ in three out of the four lines tested. Importantly, more robust cytotoxicity was also observed by NKG2A^{null} NK cells in response to acute myeloid leukemia (AML) specimens that were collected from patient bone marrow and treated with IFN-γ.

Kamiya et al. wrap up this study with xenogeneic adoptive transfer experiments designed to assess the antitumor capabilities of NKG2A^{null} NK cells in vivo. They engrafted the Ewing sarcoma cell line

ES8 or the osteosarcoma line U2OS (both transduced with HLA-E plus HLA-G signal peptide) into immunodeficient mice. Mice were then given two infusions of either control NKG2A^{+} NK cells or NKG2A^{null} NK cells at days 1 and 5 after tumor injection along with IL-2 (3 times per week). In both tumor models, control NKG2A^{+} NK cells were effective only at delaying tumor development, while the administration of NKG2A^{null} NK cells resulted in long-term survival for most mice. These impressive in vivo results provide proof of concept for the use of NKG2A^{null} NK cells as a novel immunotherapy for treating patients with cancer.

Future directions

There are three ways to block or diminish NKG2A inhibition: Trapping NKG2A as described here, CMV exposure to bias the repertoire towards NKG2C^{+}/NKG2A$^{low/-}$ adaptive NK cells, and with therapeutic antibody blockade. Two recent publications in *Cell* (19, 20) demonstrate the beneficial impact of targeting NKG2A with blocking antibodies for enhanced cytotoxic immune response against cancer. André et al. recently reported results from a phase 2 trial using monalizumab, a humanized anti-NKG2A antibody, in combination with the anti-EGFR antibody cetuximab in previously treated squamous cell carcinoma patients. They observed a 31% objective response rate in this patient population (20). van Montfoort et al. showed that antibody blockade of NKG2A potentiated CD8^{+} T cell responses induced by a peptide-based cancer vaccine in a mouse model for HPV16-induced carcinoma (19). As a treatment option, administration of a monoclonal anti-NKG2A antibody to cancer patients would be more cost effective and logistically straightforward relative to cell therapy in which NK cells would need to be expanded and transduced under GMP conditions. However, NK numbers and function are frequently suppressed in patients with advanced cancer (21). Thus, the administration of blocking antibodies may not be effective for patients with very low cytotoxic lymphocyte numbers and/or defective cytotoxicity. Adoptive transfer of NKG2A^{null} NK cells, as suggested by Kamiya and colleagues, may be a promising therapy for cancers that are refractory to checkpoint inhibitory receptor blockade. It should be noted that, in order to efficiently transduce NK cells and generate large numbers of cells, the authors of the current study cocultured NK cells with the genetically modified K562-mbIL-15-41BBL cell line. What effects this potent stimulation might have on NK cell persistence and homing after adoptive transfer into humans are still largely unknown. Going forward, it is clear that NKG2A is an important inhibitory receptor on cytotoxic lymphocytes and that cancers can highly overexpress its HLA-E ligand. Future studies to compare the clinical benefit of utilizing NKG2A^{null} cells generated by intracellular protein retention with strategies aimed at increasing CMV-induced NKG2C^{+}NKG2A$^{lo/-}$ adaptive NK cells or therapeutic antibody-mediated NKG2A blockade are needed.

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