Immunity
Previews

Distinct Mechanisms of Tumor Resistance to NK Killing: Of Mice and Men

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Tumors cells can release natural killer (NK) cell ligands for activating receptor NKG2D that are thought to inhibit NK cell function. In a recent issue of *Science*, Deng et al. (2015) show that, unexpectedly, a soluble NKG2D ligand can enhance anti-tumor NK cell activity.

Natural killer (NK) cells of the innate immune system were first named and described as a cell population with a natural ability to kill tumor cells in vitro. Indeed, simply mixing NK cells with tumor cells results in NK cell activation and tumor cell killing without the addition of exogenous factors. As more was learned about the mechanism of NK cell regulation, it became clear that NK cell activation is regulated by a balance of positive and negative signals, largely delivered through constitutively expressed membrane receptors interacting with self-ligands. Thus, it is the balance of expression of the ligands for these receptors that ultimately determines whether an NK cell becomes activated to kill the target cell (Yokoyama, 2005). The first (Ly49) and best-characterized NK receptors, including the killer inhibitory receptor (KIR) group, recognize subsets of MHC class I molecules independently of the bound peptide (Karlhofer et al., 1992). Killer activating receptors (KAR) are also diverse—a common thread among the even more diverse KAR ligands is that they are upregulated by various forms of cellular stress ranging from highly activated Ras-pathway signaling and DNA damage responses, both of which are common in many cancers (Raulet and Guerra, 2009; Gasser et al., 2005). The best characterized KAR is natural-killer group 2, member D (NKG2D), which has many ligands that are MHC class I-related but do not present peptides like conventional MHC class I. NKG2D is associated with one of two adaptors that contain immune tyrosine activating motifs that become phosphorylated upon NKG2D crosslinking and in turn bind kinases that mediate NK cell activation (Lanier, 2009).

These features of NK cell signaling and activation explain much about the physiologic role of NK cells in the response to viral infection. Most viral infections stress infected cells, inducing upregulation of ligands for KAR, thus shifting the balance toward NK cell activation. In addition, many viruses express proteins that down-regulate MHC class I expression as a means of evading recognition by classic CD8 cytotoxic T lymphocytes (CTLs); this mechanism of immune evasion is countered by enhanced NK activation due to decreased engagement of MHC class I-specific NK cell inhibitory receptors. Children with homozygous mutations in various genes that impair NK cell function are susceptible to certain viral infections (Eidenschenk et al., 2006).

Cancer cells generally exist in a constant state of cellular stress due to hypoxia, chronic proliferative signals (i.e., due to constitutively activating Ras mutations), and ongoing genomic instability. Not surprisingly, many therefore upregulate KAR ligands on their surface, making them susceptible to NK cell killing. Similar to viruses, some cancers down-modulate MHC class I, making them further susceptible to NK cell killing. As with CD8 CTL recognition, cancers must develop mechanisms to evade NK cell killing in order to survive. Understanding these mechanisms is critical because it will provide novel targets for cancer immunotherapy that complement the expanding armamentarium of immunotherapies aimed at conventional anti-tumor T cells.

One mechanism proposed for how evasion of NKG2D-dependent tumor killing by NK cells is secretion of soluble NKG2D ligands that bind NKG2D in a fashion that down-modulates NKG2D on NK cells without triggering it. Because NKG2D is believed to require dimerization by membrane-expressed ligands, soluble monomeric ligand is not activating (Figure 1, bottom). Experimental evidence for this hypothesis was first provided by Spies and colleagues, who focused on a major human ligand for NKG2D, termed MICA (as well as its homolog, MICB). They demonstrated that certain human tumors released soluble MICA and/or MICB and that the soluble ligands indeed down-modulated NKG2D on human NK cells and inhibited their activation in vitro. Some correlative evidence in humans (see below) supports this model, and antibodies that bind and clear soluble MICA/B have indeed been proposed for immunotherapy (Groh et al., 2002).

In striking contrast to this model, Deng et al. present a very different picture of tumor-NK cell dynamics in the latest issue of *Science* (Deng et al., 2015) (Figure 1, bottom). Using elegant murine models, they show that engineering tumors to secrete a soluble form of a different NKG2D ligand, MULT-1, actually results in inhibition of tumor growth. They go on to use a combination of in vitro binding and functional experiments, as well as in vivo gene deficiency experiments (using mice deficient in NKG2D [*Klrk1*] and mice deficient in genes encoding two major murine NKG2D ligands, *Rae1d* and *Rae1e*) to support a model in which chronic interaction of membrane-bound RAE-1 with NKG2D actually leads to down-modulation of NKG2D and also functional "desensitization" or "anergy" of NK cells (analogous to the exhaustion model of CD8 T cells chronically exposed to cognate antigen). Soluble MULT1 competes with the membrane-bound

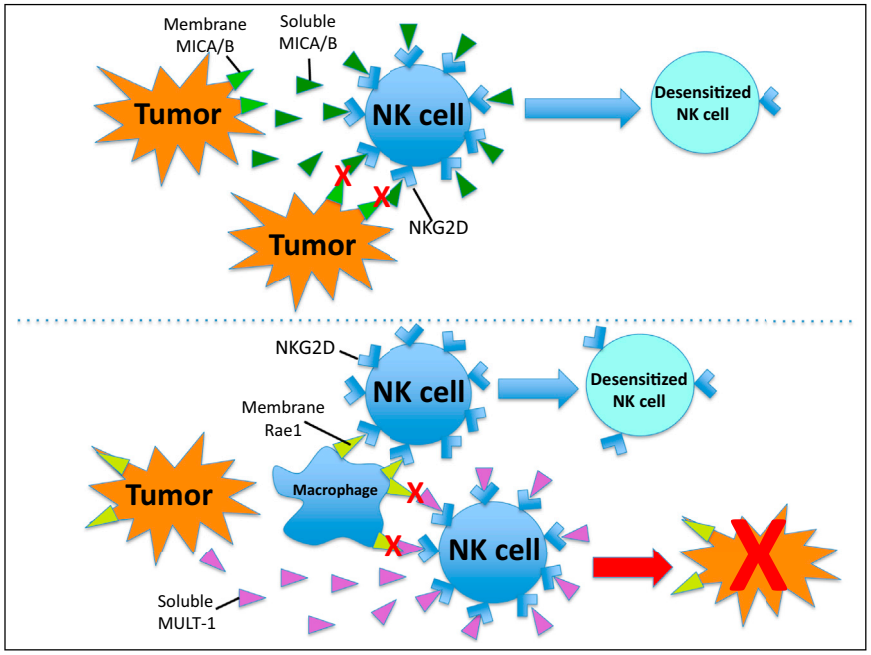

Figure 1. Two Different Models for Tumor Resistance to NK Cell Killing

(Top) Spies and colleagues, based on human data, hypothesize that release of soluble MICA and MICB down-modulates NKG2D on NK cells, thereby rendering them insensitive to triggering by membrane-bound MICA and MICB on tumor cells.

(Bottom) Raulet and colleagues, based on murine data, hypothesize that chronic engagement of NKG2D by Rae1 highly expressed on tumor-associated macrophages desensitizes (along with NKG2D down-modulation) NK cells. Soluble MULT-1 blocks this desensitization and leads to tumor killing by activated NK cells.

RAE-1-NKG2D interaction and thus mitigates NKG2D down-modulation and NK cell desensitization. Not only is this desensitization effect diminished (i.e., NK cell activity is increased) in *Rae1d*$^{-/-}$ *Rae1e*$^{-/-}$ mice, but also in *Klrk1*$^{-/-}$ mice. This finding suggests that chronic RAE-1-NKG2D interactions inhibit NK cell responses mediated by other (non-NKG2D) KARs as well. In tumors, they find that myeloid cells express high levels of RAE-1 molecules on their surface and are thus proposed to be the major source of NK cell-desensitizing chronic NKG2D engagement. In contrast to the Spies model, the implication of the Deng-Raulet model is that treatment of cancer patients with soluble NKG2D ligands could enhance anti-tumor NK responses.

How can we reconcile these two quite opposing models? One resolution, which emphasizes the complexity of this system and its therapeutic modulation, stems from the differences between murine and human NKG2D ligands. Whereas both mice and humans use the RAE-1 family members (the human versions commonly termed ULBP), which seem to represent orthologs between mouse and human, MULT-1 has no human ortholog and MICA and MICB have no known murine orthologs. Furthermore, the affinity of MULT-1 for NKG2D is roughly 100-fold higher than the affinity of MICA or MICB. Thus, although the biology of NK regulation at the 30,000-foot level is likely similar in mouse and human, the effect of specific ligands in mouse versus human might be very different. Supportive evidence for the Spies model in human cancer comes from interesting findings of Dranoff and colleagues (Jinushi et al., 2006) that certain patients treated with cancer vaccines or anti-CTLA-4 develop natural anti-MICA antibodies associated with a decrease in serum levels of soluble MICA and an increase in NKG2D levels on circulating NK cells relative to levels prior to development of the antibody response. This is associated with increased NK cell function in vitro. These findings, while correlative, have been used to argue for the generation of monoclonal antibodies to MICA that clear soluble MICA while not blocking the MICA-NKG2D interaction.

More experimentation will be necessary to determine whether the findings of Deng et al. in mice can be translated to human cancers because, of course, human cancer is the ultimate model for human cancer. However, the study reveals that the role of NKG2D ligands in cancer might be more complicated than was previously appreciated and opens up a new avenue of investigation for immunotherapy.

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