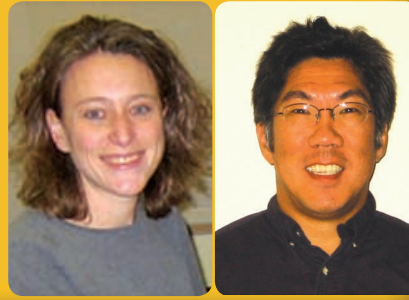


NKG2D and CD28 Costimulation

with

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What is known about the NKG2D receptor?

Mary A Markiewicz and Andrey S Shaw: All human and mouse NK cells constitutively express NKG2D (1,2), but the rest of the cellular expression profile of this receptor differs between mouse and human. Murine NKG2D is expressed on activated, but not naïve, CD8⁺ cytotoxic T cells (CTL), and on subsets of $\gamma\delta$ T cells and NK T cells (3,4). In the human, NKG2D is expressed on all CD8⁺ T cells, all $\gamma\delta$ T cells, and possibly on some activated CD4⁺ T cells (1,5,6). In mice alternative mRNA splicing generates two functionally distinct NKG2D isoforms, NKG2D long (NKG2D-L) and NKG2D short (NKG2D-S), whereas only the NKG2D-L equivalent is expressed in the human (7). There are a number of NKG2D ligands in both mouse and human (3,10,11,12-16). In general, these ligands are not expressed by normal cells, but are up-regulated in virally infected cells, tumor cells, cells with DNA damage, or otherwise "stressed" cells (8,9).

NKG2D does not have intrinsic signaling capabilities, but rather associates with one of two adaptor signaling proteins. The only adapter protein present in mouse and human CD8⁺ T cells and human NK cells that associates with NKG2D is the YXXM-contain-

ing protein DAP10. The YXXM motif allows DAP10 to bind the p85 subunit of PI3K as well as the adaptor protein Grb2 (2,17). The mNKG2D-S isoform, which is present solely in activated murine NK cells, is capable of pairing with either DAP10 or DAP12. DAP12 contains an ITAM sequence that recruits Syk family kinases (7). In the mouse, both DAP10 and DAP12 signaling contribute to NK cell-mediated cytotoxicity, but only DAP12 signaling can mediate effector cytokine production by NK cells (7,18-20).

Although DAP10 signaling is sufficient for NKG2D-mediated killing in NK cells, NKG2D-DAP10 signaling is generally not enough to induce CTL killing. The reason for this difference is not known, and the function of NKG2D on T cells is controversial. Many studies, including our own, suggest that NKG2D can function as a T cell costimulatory molecule, functioning to augment signaling by the T cell receptor (TCR) (1,3,10,21-23). However, there is evidence to suggest that treatment of CTL with specific cytokine regimens can allow for NKG2D-mediated killing independent of TCR ligation (6,24). Further, another study suggests that in the absence of additional costimulation, NKG2D has no function at all on T cells (25).

What are the similarities and differences between NKG2D- and CD28-mediated costimulation of CTL responses?

Mary A Markiewicz and Andrey S Shaw: In our studies we compared qualitatively the costimulation of a transgenic CTL line by NKG2D and the prototypical costim-

ulatory molecule CD28 (24). In many ways, costimulation by the two molecules was similar. Simulation of either NKG2D or CD28 increased antigen-specific CTL responses and antigen-independent IFN- γ secretion in response to the combination of IL-12 and IL-18. There were notable differences between the costimulatory capacity of these molecules, however.

A significant difference between NKG2D and CD28 costimulation was that only CD28 allowed for recovery of a significant number of transgenic T cells following secondary stimulation. This suggests that CD28, but not NKG2D, is capable of enhancing CTL survival. This finding conflicts data suggesting that one of the human NKG2D ligands, *Letal*, enhances CD8⁺ T cell survival (26). This difference could reflect a difference between mouse and human NKG2D function, or it may be that while the one NKG2D ligand tested could not enhance CTL survival, one of the other murine

NKG2D ligands may be able to do so. Since both CD28 and DAP10 are able to bind to the p85 subunit of PI3K and Grb2, but only CD28 is able to enhance CTL survival, these data suggests that these signals alone are insufficient for enhanced T cell survival via CD28.

Another striking difference between NKG2D and CD28 costimulation is that ligation of NKG2D, but not CD28, induced CTL to form an immune synapse (IS) (27-29) in the absence of TCR stimulation. The IS is thought to be important in regulating T cell responses, and previously ligation of the TCR was thought to be required for IS formation (29-32). How NKG2D engagement stimulates IS formation is not clear. We are currently exploring whether DAP10 signaling is required or whether IS formation is driven solely on the high affinity interaction between NKG2D and its ligands. Despite IS formation, NKG2D stimulation alone does not drive any CTL effector function that has been measured. The purpose of this IS formation in the absence of effector function is not clear. One possibility that we are exploring is that interactions of NKG2D with its ligands may target CTL to distressed cells, such as virally infected cells or tumor cells, *in vivo*.

How important is the research about NKG2D costimulation?

Mary A Markiewicz and Andrey S Shaw: Because CTL are essential mediators of many immune responses, both beneficial (anti-viral and anti-tumor) as well as detrimental (autoimmune), understanding the mechanisms involved in the recognition and destruction of various target cells by CTL is critical. While CD28 stimulation is effective in enhancing CTL effector

responses in laboratory models, the expression of the ligands for CD28 is largely restricted to professional APC, disallowing a role for CD28 costimulation in most target cell killing. On the other hand, NKG2D ligands are often upregulated on CTL targets, suggesting NKG2D costimulation could play an important role in CTL-mediated target cell destruction. In fact, this

receptor has been implicated in CTL-mediated autoimmune disease and in CTL-dependent tumor-specific responses (5,21,33,34). In the human, there may also be a role for NKG2D in the primary stimulation of CD8⁺ T cells since this receptor is expressed on naïve human CD8⁺ T cells. This possibility has only begun to be investigated (25,35), and the relevance to human disease has not yet been explored.

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