Natural killer cells: **Stress out, turn on, tune in**

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Natural killer cells attack tumor cells, infected cells and some normal cells, but the basis of their specificity is not completely understood. Recent studies indicate that epithelial tumor cells upregulate a stress-induced MHC class-I-like protein termed MICA, triggering NK cells via a recently described receptor called NKG2D.

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At one time, natural killer (NK) cells were considered boring. Little was understood about their specificity or their biological role. They were known to be lymphocytes that secrete cytokines and mediate direct cytotoxicity, and were thought to play an important role in tumor rejection and immunity to viruses and other infections, but that was the extent of our knowledge. In recent years, many receptors expressed on the surface of NK cells have been identified and several interesting concepts have been advanced concerning their mode of recognition. NK cells are now anything but boring, although great holes still persist in our understanding of them.

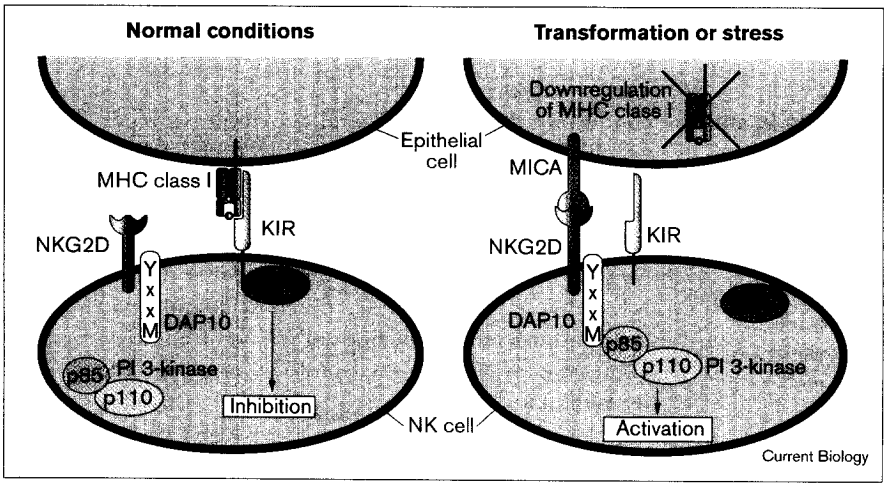
Although there is now an embarrassment of NK receptors, the functions of many of them are currently conjectural. Moreover, none of these receptors fits the bill of 'the NK receptor', analogous to the B- or T-cell receptors that are the major source of specificity for activation on those lymphocytes. As will be discussed below, this is likely to reflect a real difference between the recognition strategies used by T cells, which are relatively single-minded, and NK cells, which use many different types of stimulatory receptors. Nevertheless, some stimulatory NK cell receptors may be more interesting than others. As described in two recent reports [1,2], a recently cloned NK receptor, NKG2D, may have a critical role in the recognition of tumor cells by NK cells via binding to stress-induced ligands that are related to major histocompatibility complex (MHC) class I molecules.

Activation of NK cells is an early event during an immune response and does not involve the expansion of rare, antigen-specific cells as is characteristic of the adaptive immune response. NK cells are therefore part of the innate, immediate-acting arm of the immune system. NK cells can attack normal (non-self) cells under certain conditions, but they generally exhibit stronger activity against tumor cells and virus-infected cells. A key principle of the specificity of NK cells was revealed by studies showing that NK cells prefer to attack cells that have downregulated classical MHC class I molecules. The 'missing self hypothesis' [3] proposes that this function of NK cells serves to protect the host against transformed cells and infected cells, both of which often downregulate expression of MHC class I proteins to escape recognition by cytotoxic T cells.

Great progress has been made in understanding how NK cells detect loss of MHC class I molecules on target cells. NK cells express inhibitory MHC class-I-specific receptors from at least three families of proteins: two C-type lectin families, the CD94–NKG2 heterodimeric receptors (in humans and mice) and Ly49 (in mice), and the killer cell immunoglobulin-like receptor (KIR) family (in humans) [4]. These inhibitory receptors all contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains that binds to the Src homology 2 (SH2) domain of protein phosphatases, such as SHP-1 and possibly SHP-2. Loss of MHC class I expression on target cells prevents the inhibitory interaction between the NK cell and the target cell, and thereby unleashes the NK cell. But although inhibitory recognition of MHC class I molecules plays an important role in regulating NK cell activity, the sensitivity of target cells to NK cells does not always correlate with MHC class I expression [5]. Thus, NK cell activation may require interactions with stimulatory ligands on target cells. The decision whether to attack a target cell would then be determined by the balance of stimulatory and inhibitory interactions.

So what stimulates NK cells to attack target cells? A new and exciting candidate that triggers NK cells to attack tumor cells is the non-classical MHC class I molecule termed MICA (for MHC I chain-related-A). The 43 kDa MICA glycoprotein and a close relative, MICB, are encoded by genes located near the locus for the human MHC class I molecule HLA-B [6]. Unlike most MHC class I molecules, MICA is not associated with β_2 microglobulin and probably does not bind peptide. Interestingly, the MIC gene transcriptional regulatory sequences contain heat-shock elements similar to those in the human Hsp70 promoter [7]. Indeed, MICA expression is substantially upregulated following heat-shock treatment of various intestinal cell lines. As also observed for some other stress-induced proteins, MIC expression is also upregulated on many epithelial tumors [8].

Figure 1



MICA, NKG2D and NK cell recognition. Normal epithelial cells inhibit NK cells via inhibitory MHC class-I-specific receptors (KIR), which recruit and activate the protein tyrosine phosphatase SHP-1. Dephosphorylation of activating signal molecules by SHP-1 prevents NK cell activation. Cellular transformation leads to induction of stress proteins including MICA ('stress out'). Recognition of MICA by NKG2D–DAP10 'turns on' the NK cell. Engaging the NKG2D receptor complex induces binding of the p85 subunit of PI 3-kinase to a YxxM motif in the DAP10 cytoplasmic domain, leading to NK cell activation. Inhibitory recognition of MHC class I molecules could moderate or 'tune' the activating signal. Maximal NK cell activation would occur when the target cell downregulates MHC class I molecules as shown on the right.

These interesting findings suggested that MIC expression could be a flag raised by tumor cells to recruit anti-tumor lymphocytes such as T cells or NK cells. Indeed, target cells transfected with MICA were susceptible to attack by intestinal epithelial T-cell lines expressing $V_\delta1$ γδ T-cell receptors [9]: γδ T cells represent a small proportion of the total T-cell population and relatively little is known about their function. MICA-positive tumors were predominantly infiltrated by $V_\delta1$ γδ T cells. These results suggested that MIC molecules are stress-induced antigens recognized by γδ T cells, which could play an important role in the surveillance of transformed, infected and damaged cells.

As it is now well established that other MHC class I molecules can be targets of both T-cell receptors and NK receptors, it should perhaps be no surprise that MIC molecules are no exception. Bauer *et al.* [1] provide striking evidence that soluble multimeric MICA binds to essentially all NK cells and cytotoxic $CD8^+$ T cells. They show that the receptor for MICA on these cells is NKG2D, a receptor expressed by NK cells in both humans [10] and mice [11]. NKG2D is a C-type lectin encoded in the NK receptor gene complex. Despite its name, NKG2D is no more related to NKG2A, B, C and E receptors than to other receptors encoded in the NK receptor gene complex. It does not associate with CD94 and does not exhibit the same specificity as CD94–NKG2A receptors.

Bauer *et al.* [1] report that binding of MICA on target cells to NKG2D on NK cells strongly induces cytotoxic activity in the NK cell, even overriding inhibitory signals provided by the same target cell. The cytotoxicity of a human NK cell clone against MIC-expressing tumor cell lines was at least partially dependent on the recognition of MICA by NKG2D. The finding that a stress-inducible MHC class-I-like ligand is targeted by NK cells represents the best elucidated instance of how the innate immune system can target transformed cells and raises the question of whether MIC molecules are induced in infected cells as well. Given the binding of MICA to $CD8^+$ T cells, the possible role of NKG2D in these cells is also intriguing.

NKG2D contains a positive charge in its transmembrane domain, a hallmark of all activating NK receptors. The positive charge promotes interactions with acidic residues in the transmembrane domains of adaptor proteins, most of which contain immunoreceptor tyrosine-based activation motifs (ITAMs) for the recruitment of protein tyrosine kinases. NK cells express several adaptor proteins, including the CD3ζ signalling chain of the T-cell receptor, Fcγ receptor and a recently identified protein called DAP12 (originally named KARAP) [12]. DAP12 associates with several stimulatory NK receptors and initially seemed a good candidate for the adaptor protein of NKG2D. Instead, Wu *et al.* [2] found that NKG2D associates with a newly discovered adaptor protein, DAP10, which is expressed in a variety of hematopoietic cells. DAP10 and DAP12 are extremely closely linked on human chromosome 19q13.1, but differ considerably in their amino acid sequences. Surprisingly, the DAP10 cytoplasmic domain may not contain an ITAM, but instead contains an SH2-binding YxxM motif (in single-letter amino acid code with x representing any amino acid) that appears to recruit the p85 catalytic subunit of phosphatidylinositol (PI) 3-kinase. Interestingly, the T-cell costimulatory molecule CD28 contains a similar motif. Precisely how NKG2D activates NK cells and whether it also requires reinforcing stimulatory signals — as is the case with CD28 — remains to be determined.

Taken together, these recent studies provide a plausible model for at least one mode of recognition of malignant cells by NK cells (Figure 1): patrolling NK cells receive only weak activating signals from normal cells, and these signals are nullified by the inhibitory recognition of self-MHC class I molecules. Stress signals that accompany cellular transformation upregulate MIC, which is recognized by the stimulatory NKG2D–DAP10 receptor. If these stimulatory signals are sufficiently strong, they may overcome inhibition mediated by inhibitory recognition of classical MHC class I molecules. If the offending cell has also downregulated classical MHC class I molecules, the balance would swing even further in favor of activation of the NK cell. This model can account for the fact that some tumor cells are susceptible to NK cells despite expressing normal levels of classical MHC class I molecules, whereas other cells are resistant to NK cells despite expressing low levels of classical MHC class I molecules.

Interestingly, the human proteins NKG2D and DAP10 are quite conserved in mice [2,11], but there is no obvious murine MIC ortholog. An interesting parallel concerns the CD94–NKG2 receptors. The human and mouse ligands for these receptors, the nonclassical MHC class I proteins HLA-E and Qa-1^b, respectively, are clearly functional homologs. Their overall sequences are no more similar, however, than any pair of unrelated MHC class I proteins in mouse and human. Perhaps the functional MIC homolog in mice is also quite divergent in sequence from its human counterpart.

The centrality of NKG2D to NK cell activation remains to be established. For one thing, most hematopoietic tumor cell lines do not express MIC molecules [6], yet many are highly sensitive to NK cells. Indeed, NK cells express a plethora of other stimulatory receptors. Among these are close relatives of the inhibitory, MHC class-I-specific receptors. Several of these stimulatory isoforms are specific for the same MHC class I molecules that are recognized by their inhibitory relatives. Other activating molecules on NK cells are the NKR-P1A and C molecules, whose natural ligands have not been identified. NK cell lines lacking NKR-P1 molecules exhibited reduced capacity to attack one tumor cell line that was tested, but not several others [13]. Recent studies in the human system have identified two new immunoglobulin-like NK cell receptors — NKp44 and NKp46 — that may be involved in recognition of some tumor cells [14]. These receptors probably recognize non-MHC ligands on target cells, but the nature of these ligands is not established.

A key difference between the stimulatory receptors expressed by NK cells and those of B and T cells is that many of the stimulatory NK receptors are co-expressed by individual NK cells. It appears that NK cells use several optional triggering receptors, which may differ in specificity but target overlapping sets of susceptible cells. It is as if an NK cell, given access to receptors of relatively limited diversity, equips itself with a large variety of them, whereas individual T and B cells make do with a single highly sophisticated and more specific model. This state of affairs befits the differing strategies inherent in the innate versus adaptive immune response. Much effort is being exerted to identify new stimulatory receptors on NK cells, and to determine their biological function. We can expect lots of new surprises to come as research on the enigmatic NK cell progresses.

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