Minireview

A View to a Kill: Signals Triggering Cytotoxicity[1]

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Abstract

Tumor immunity requires the participation of lymphocyte effector cells that display powerful processes to destroy malignant cells. Natural killer (NK) cells and CTLs, once activated, use the same lytic processes for mediating target cell death. However, they are triggered through distinctly separate antigen receptors. NK cells are currently known to express at least three families of receptors unrelated to the T cell receptor, *i.e.*, NKG2, KIR, and NCR, to mediate cytotoxicity. This review provides a view to a kill, bringing together a unifying concept for a common signal pathway that dictates lytic function. What emerges is a specific Syk/Zap70 → PI3K → Rac → PAK → MEK → ERK signal cascade triggered by target cell recognition, which is responsible for mobilizing the lytic granules containing perforin and granzyme B toward the contacted target cell.

Introduction

NK[3] cells and T cells are key players in tumor immunity, being ultimately responsible for destruction of the malignant cells (1). NK cells participate early in innate immunity, whereas CTLs provide a long-lasting effect. These two types of killer cells use distinctly separate antigen receptors to recognize target cells, but upon antigen ligation, both effector cells deploy the same lytic processes to mediate tumor cell death. One such process is the release of perforin and granzyme B stored in lytic granules in the cytoplasm of NK cells and CTLs (2). Upon contact with tumor cells, the lytic granules are mobilized toward the tumor cell, and perforin forms pores in the target cell membrane, allowing entry of enzymes such as granzyme B to activate caspases and induce apoptosis in the target cell.

Antigen-specific, MHC-restricted tumor cell recognition is the hallmark of T cells. Naïve, resting T cells require the participation of APCs, and this interaction is controlled by the TCR and CD4/CD8 that specifically recognize the processed antigen and MHC class II/class I, respectively, on the surface of APCs such as dendritic cells. Once the TCR-CD4/CD8 complex is ligated on the T cell, together with other costimulatory molecules, signal pathways are triggered that lead to transcriptional activation of genes that cause T-cell differentiation and proliferation, *e.g.*, interleukin 2 and IFN-γ. These signal pathways have been well documented, and it is clear that a complex array of signal cascades are involved in gene transcription, prevention of apoptosis, and cell cycle control (3–5). Once CTLs are activated, however, they must now interact with the tumor cells rather than the APC expressing the processed antigen. TCR engagement with the antigen on the tumor cell results in the triggering of lytic granule mobilization and tumor cell lysis. To bring about tumor cell death, are the same signals triggered in the earlier encounter by TCR engagement that initiated activation, called into play when the activated CTLs are now confronting the tumor cell itself? This review summarizes the types of receptors that are displayed on cytotoxic lymphocytes, particularly NK cells, and the signal molecules reported to mediate cytotoxicity. Supporting evidence is also provided that merges most of these identified molecules into a cohesive signal cascade that can be used by diverse antigen receptors.

The PI3K → Rac → PAK → MEK → ERK Pathway Dictates Lytic Granule Mobilization.

Recently, a number of laboratories have explored the molecular mechanisms associated with lytic function. Because of the restricted growth of tumor-specific CTLs, most investigators have turned to the use of readily available NK cell lines and cultures, which possess the same lytic machinery as CTLs. The first foray into this area uncovered Syk, Rac, Vav, and ERK as critical signal molecules that become activated during NK cell binding to NK-sensitive tumor targets (6–11). Further evidence of the participation of these signal molecules came from the use of vaccinia viral constructs to introduce mutant genes into NK cells. Mutant Syk, Rac, and mitogen-activated protein kinase (MAPK)/ERK, that act as dominant-negative gene products, were independently found to effectively block lytic function by separate studies. Interestingly, dominant-negative Vav did not block NK lytic function, but wild-type Vav markedly enhanced it, suggesting that downstream products of activated Vav could mediate NK function (7). This phenomenon was also seen with LAT and SLP76, where expression of the wild-type but not the dominant-negative form raised the lytic ability of NK cells (12, 13).

How some of these signal molecules contribute to NK lysis of tumor cells was resolved when the sequence of events triggered by NK ligation of tumor cells was systematically investigated with one NK cell line. By use of biochemical analysis of kinase enzyme activation, gene transfer of dominant-negative and constitutively active signal molecules, accompanied by parallel examination of lytic function against ^{51}Cr-labeled tumor cells, it became clear that a specific signal cascade was triggered

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[3] The abbreviations used are: NK, natural killer; ADCC, antibody-dependent cell-mediated cytotoxicity; APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; ERK, extracellular regulatory kinase; ITAM, immunoregulatory tyrosine-based activatory motif; KIR, killer cell immunoglobulin-like receptor; MEK, mitogen-activated protein/ERK kinase; NCR, natural cytotoxic receptor; PI3K, phosphoinositide 3-kinase; TCR, T-cell receptor.

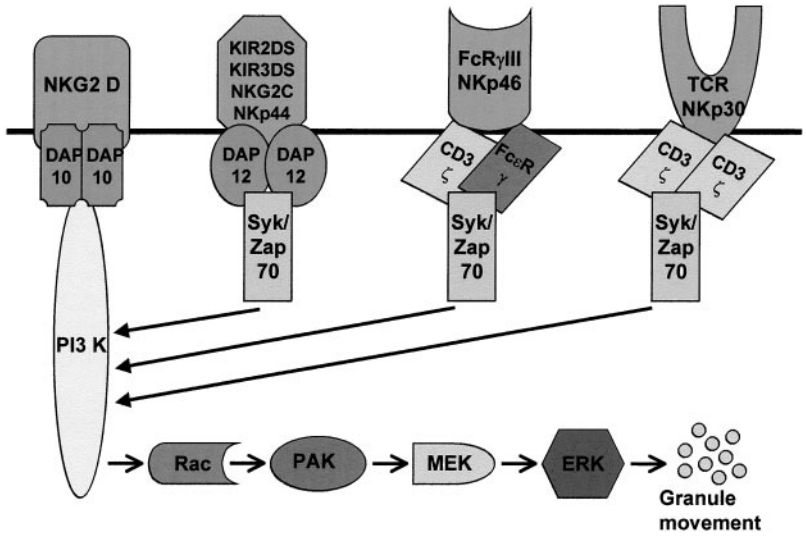

Fig. 1 The signal network that controls cytotoxic function. Different antigen receptors partner with selective homodimeric or heterodimeric adaptor proteins to activate the PI3-Rac-PAK-MEK-ERK signal network to mobilize lytic granules, resulting in target cell death. The homodimeric adaptor, DAP10, directly binds PI3K. The other adaptors, DAP12 homodimer, CD3ζ homodimer, FcεRγ homodimer, and CD3ζ/FcεRγ heterodimer, directly bind Syk/Zap70, which can, in turn, activate PI3K.

in NK cells by exposure to tumor cells. NK cell ligation with tumor cells rapidly caused a transient activation of ERK, which apparently controls lytic granule movement (9). In known systems, ERK is downstream of a series of signal cascades, and of these, it immediately became apparent that the Ras/Raf/MEK/ERK pathway, which is crucial in gene expression and tumor cell growth, is not used in the killing process (14). Instead, the utility of a specific phosphoinositide 3-kinase (PI3K) → Rac → PAK → MEK → ERK pathway was identified in the NK92 cell line, depicted in Fig. 1 (15). It is noteworthy that this same signal cascade for lytic function was also demonstrated in freshly isolated human NK cells, documenting its biological relevance (15).

DAP10 and DAP12 Adaptor Proteins Link NK Receptors to PI3K and Syk to Trigger Lytic Function.

If PI3K is one of the earliest signals to be triggered in NK cells, are there NK receptors that are associated with PI3K? All identified activatory NK receptors have a short cytoplasmic tail with no signaling capacity. Instead they must associate with adaptor proteins that couple these receptors to signal molecules. Recent cloning of the homodimer adaptor protein, DAP10, which couples to one of the NK receptors, NKG2D, has revealed that it has a binding site for PI3K (16). It is therefore likely, through the DAP10/NKG2D complex, that antigen recognition transduces a PI3K signal, resulting in ERK activation, to mobilize lytic granules toward the ligated tumor cell.

NK cells possess other activating antigen receptors with no intrinsic kinase activity, but they contain a transmembrane region containing a positively charged amino acid, aspartic acid or lysine, which allows them to associate with adaptor proteins such as DAP12 (17). DAP12 forms homodimers and contains the ITAM that binds Syk or Zap70. It has many NK receptor partners, including KIR2DS2, KIR3DS, NKG2C/CD94, NKp44 in humans, and LY49D, LY49H in mice (17–20). Does DAP12 then transduce a different signal from DAP10, or do they merge at a later downstream crossroad? Most recently, the latter pathway was demonstrated to be present in NK cells. Using dominant-negative Syk and constitutively active PI3K constructs, NK cells expressing DAP12, upon target ligation, was shown to signal via Syk to reach the PI3K → Rac → PAK → MEK → ERK pathway.[4] This was verified in normal human peripheral blood NK cells. Vav also participates in this pathway because it is known as the Rac1 guanine nucleotide exchange factor (21). Syk, however, may not provide the sole entry point to the PI3K pathway. It is known that DAP12 interacts with Syk or Zap70 (22), and mutant mice that are either Syk −/− or Zap −/− develop normal NK cell development and function, demonstrating the redundancy in Syk/Zap70 *in vivo* (23, 24). Other signal molecules have also been identified in NK lysis, such as Pyk, p38, and 3BP2, but they have not yet been integrated into this pathway (25–29). These molecules may, on the other hand, be part of other signal cascades required for optimal lytic function; and costimulatory receptors, such as integrins, adhesion molecules, and CD2, could provide additional signals that can contribute to cell cytotoxicity. An alternative possibility is that some of these signal molecules participate in cytokine gene induction, which can also be triggered through the NK receptors (30).

It is reasonable, however, to postulate that NK cells will resort to a common signal cascade to mediate granule mobilization for lytic function. With the wealth of NK antigen receptors to trigger one critical function, *i.e.*, target lysis, initial receptor ligation may be different, but the use of common adaptors and compatible ones that all end in PI3K activation would then allow for the specific activation of a unique pathway for lysis, as shown in the model in Fig. 1. This allows for efficiency and signaling economy within NK cells.

[4] K. Jiang, B. Zhong, D. L. Gilvary, B. C. Corliss, E. Vivier, E. Hong-Geller, S. Wei, and J. Y. Djeu. Syk regulation of phosphoinositide 3-kinase for natural killer lysis: possible linkage of DAP10 and DAP12 signaling, J. Immunology, in press, 2002.

Three Families of NK Antigen Receptors: NKG2, KIR, and NCR.

NK cells have a unique propensity to seek out cells undergoing stress or infection, based on several antigen receptors. Abnormal cells undergoing stress or transformation express certain inducible genes. The lectin-like NK receptor, NKG2D, recognizes stress-inducible MICA and MICB, which are distant homologues of HLA class I that play no role in antigen presentation (31). Because MIC A/B are induced along with heat shock protein 70, it is hypothesized that *MIC A/B* genes contain putative heat-shock elements in the promoter region. Although they are restricted in distribution to intestinal epithelia, MICs are also expressed in diverse tumors of epithelial origin (31–33). Thus, NK cells could be involved in innate immunity against gut carcinomas via NKG2D-MIC receptor-ligand interaction. Recently, new data indicate that cytomegalovirus can induce MICs in fibroblasts and endothelial cells, and this phenomenon could explain the high efficiency of NK cells to lyse virally infected cells (34). In addition, NKG2 also appears to recognize the retinoic acid-inducible early gene-1-like proteins (35). Unlike NKG2D, NKG2C dimerizes with CD94 and recognizes the nonclassical MHC class I molecule, HLA-E (36). It is interesting that NKG2D couples to DAP10, whereas NKG2C associates with DAP12 for signaling.

KIRs, on the other hand, are not lectins but contain several extracellular immunoglobulin-like variable domains (37). They recognize classical MHC class I on target cells and exist in two forms (38, 39). The inhibitory KIR isoform expresses a long cytoplasmic tail that contains one or more immunoreceptor tyrosine-based inhibitory motifs that, upon phosphorylation, binds SHP, which is a tyrosine phosphatase that disables the signal molecules required in cytotoxicity. In contrast, the activatory KIR isoform has a truncated cytoplasmic tail but expresses arginic or lysine in the transmembrane domain that allows it to associate with ITAM-containing adaptors such as DAP12 (40). Balance of these activatory and inhibitory KIRs on a given NK cell will determine its final lytic capacity.

In addition to NKG2 lectin and KIR families of receptors that recognize MHC or related molecules, NK cells also express NCRs (41). Because NK cells have long been known to readily lyse MHC-deficient tumor cells, the existence of NCRs that must recognize non-MHC molecules was suspected. Recently, NKp30, NKp44, and NKp46 were cloned and were found to be primarily expressed on resting or interleukin 2-activated NK cells, and not on T cells (19, 42, 43). The ligand for NKp46 is hemagglutinin and hemagglutinin-neuraminidase of influenza and parainfluenza, respectively (44). Expression of NKp46 could thus account for the preferential killing by NK cells of target cells infected with these viruses. Of these receptors, only NKp44 was reported to signal via DAP12 (19). NKp30 couples to CD3ζ (43), whereas NKp46 couples to both CD3ζ and FcεRγ adaptor molecules (42). Like DAP12, CD3ζ and FcεRγ contain ITAMs and can form CD3ζ homodimers or CD3ζ/FcεRγ heterodimers. These adaptors are known to activate Syk or Zap 70 (5). In addition, NKp30 and NKp46 require PI3K for signaling (45). Thus, these NCRs may signal via the common Syk//PI3K pathway described earlier, underscoring the economy of signaling for cytotoxicity.

Other receptors, such as 2B4, and NKp80 have also been identified, and 2B4 appears to associate with LAT, whereas NKp80 ligation can trigger intracellular calcium mobilization (46, 47). Whether these receptors use the pathway in Fig. 1 is not yet known.

Do Tumor-specific CTLs Use the Same Signal Cascade to Lyse Tumor Cells?

In contrast to NK cells, CTLs express specific TCR to recognize unique antigens on target cells. However, this recognition is in the context of self MHC class I. Dual recognition of antigen and class I on the target cell triggers the TCR/CD8 complex on CTLs, resulting in target cell death. Although the antigen receptors are different, it is tempting to speculate that CTLs may signal via the same pathway as NK cells to lyse target cells. Support for this hypothesis comes from the observation that the TCR is coupled to CD3ζ, which associates with Zap70 (48). Of note, CD8 T cells can express NKG2D, which may provide costimulatory action (34), and their interaction with TCR for optimal lytic signaling needs investigation. Activating and inhibitory KIRs have also been identified on CTLs, and the balance of all these receptors could influence the functional outcome (49–53).

CD16-mediated Antibody-dependent Cell Cytotoxicity Couples to CD3ζ and FCεRIγ and Signals via Syk.

In addition to direct lysis of target cells, NK cells also possess the ability to mediate ADCC via expression of FCRγIII (CD16; Ref. 30). Many of the signal molecules identified in direct NK lysis have also been reported to be involved in ADCC. Lck, Syk, PI3K, Vav, Rac, p38, and ERK are separately reported to control ADCC in NK cells (7, 8, 10, 28, 54–57), and it seems likely that the pathway used by NK receptors for direct lysis is also integrated into CD16-mediated tumor lysis. This speculation is supported by the well-known association of CD16 with the adaptors, CD3 ζ and FCεRIγ, both of which contain ITAMs to bind Syk (58).

Conclusions

In summary, NK cells express diverse families of antigen receptors that are distinct from antigen-specific TCRs in CTLs. In addition, NK cells also express CD16 that allows for binding to antibody-ligated target cells. All these receptors on NK cells and T cells, upon recognizing specific ligands, trigger lysis of the ligand-expressing target cells. Despite the diversity of receptors, it appears that the initial signal molecule(s) activated may vary depending on the receptor, but they merge downstream into a common signal cascade that ends in ERK activation that mobilizes lytic granules to cause target cell death. Entry into this cascade can occur at Syk/Zap70, if ITAM-containing adaptor proteins such as DAP12, CD3ζ, or FcεRγ, are used by the NK or T cell receptors. On the other hand, a second entry point can be PI3K, which is coupled to the adaptor protein, DAP10, which associates with the NKG2D lectin-like NK receptor. In addition, antibody-dependent cell cytotoxicity by NK cells can occur via this pathway through FcγRIII (CD16), which couples to CD3ζ, or FcεRγ. Deployment of a common signal pathway (Fig. 1) for all or most activating antigen receptors on

NK and T cells thus provide an effective and economic use of signal transduction to mediate tumor cell destruction.

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