Expression and function of NK cell receptors in CD8+ T cells

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A wide variety of inhibitory and stimulatory NK cell receptors are expressed by some CD8+ cytotoxic T lymphocytes in mice and humans. Recent data address the induction of these receptors on activated or memory CD8+ T cells and have led to hypotheses addressing their function in the CD8+ T cell response.

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Abbreviations

CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
KIR	killer-cell immunoglobulin-like receptor
LCMV	lymphocytic choriomeningitis virus

Introduction

The term 'NK cell receptors' is used to describe the ever-growing collection of stimulatory and inhibitory receptors known to be expressed by NK cells, but in some cases also expressed by other cell types. In NK cells, an integration of positive and negative receptor signaling dependent on ligands expressed by target cells is thought to determine whether an NK cell will attack the target cell.

Many of the NK cell inhibitory receptors have been shown to recognize MHC class I molecules. These inhibitory receptors actively prevent NK cells from attacking cells that express normal levels of class I and thus are a primary determinant of self-tolerance of NK cells [1]; conversely, a lack of inhibitory signals allows them to attack potential targets that have downregulated class I expression, such as transformed or infected cells [2,3]. The mechanisms for NK cell activation are less well understood, although it is likely that the ligands recognized by some stimulatory receptors are constitutively expressed by some normal cells, whereas the ligands for others are only induced in instances of cellular distress [4].

NK cell receptors are not restricted to NK cells and have been detected to varying degrees on most types of hematopoietic cells. This review focuses on the various NK cell receptors expressed by CD8+ cytotoxic T lymphocytes (CTLs) in mice and humans. Unlike the multiple receptor types utilized by NK cells, CD8+ T cells bear a clonally distributed TCR that is the primary determinant of specificity. Therefore, the functional role of NK cell receptors in CD8+ T cells remains uncertain. However, there are several interesting possibilities that will be discussed in this review, with emphasis placed on reports published in the past year.

Expression of NK cell receptors by CTLs

KIR and Ly49 family members: receptors specific for classical MHC class I

The killer-cell immunoglobulin-like receptor (KIR) gene family in humans encodes numerous NK cell receptors specific for different allelic variants of classical MHC class I molecules. In mice, the lectin-like Ly49 family of receptors serve a parallel function, despite their structural dissimilarities to KIRs [3]. Individual NK cells generally co-express several of these receptor-family members. In the case of most NK cells, at least one of the expressed receptors is usually specific for self MHC class I molecules, allowing the cell to directly survey class I levels on potential target cells [1]. Most KIR and Ly49 family members contain cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs) and are therefore inhibitory. However, several members of each family lack ITIMs, and are stimulatory because of noncovalent association with the adapter signaling molecule, DAP12/KARAP.

On average, individual KIR family members are expressed by up to 5% of peripheral blood CD8+ T cells in humans [5,6,7•,8]. A corresponding population has been identified in mice, where approximately 10% of CD8+ T cells express at least one Ly49 family member [9,10•]. In mice, evidence suggests that only the inhibitory Ly49 isoforms are expressed by T cells [9,10•,11]; in humans, both inhibitory and stimulatory KIRs are expressed [12–14], but there are conflicting reports on whether the stimulatory isoforms are functionally active [13,15,16]. Like NK cells, CD8+ T cells express KIR and Ly49 family members in a variegated, overlapping fashion, so that individual cells typically express a distinct, small set of receptors [1,6,7•,8,10•].

Abundant evidence indicates that the inhibitory KIR isoforms can inhibit antigen-mediated T cell effector functions, such as cytokine release and target cell cytolysis [17,18]. In mice, strong functional inhibition of CD8+ T cells by endogenously expressed Ly49 molecules has been difficult to demonstrate to date [10•,19•]. However, when the Ly49A molecule is expressed as a transgene by all T cells, Ly49–ligand interactions potently inhibit T cell proliferation [20–22] and effector responses [23•,24•]. Furthermore, in some cases Ly49 transgenes, when expressed in mice with the corresponding class I ligand, cause the development of a fatal wasting syndrome that is thought to represent autoimmunity due to impaired thymic negative selection [25•,26•,27]. The discordant results obtained with endogenously expressed Ly49 molecules (versus Ly49 expressed by a transgene) may be due to the fact that the cell surface levels of endogenous Ly49 molecules on T cells are relatively low. Alternatively, the receptors may signal differently in the subset of CD8+ T cells that naturally express the receptors as compared with other CD8+ T cells.

KIR and Ly49 molecules are not detectably expressed by naïve CD8+ T cells or by the major thymic populations (with the exception of CD1-restricted NKT cells in mice, discussed separately by Gumperz and Brenner, this issue, pp 471–478). Expression is restricted to CD8+ T cells bearing surface markers associated with a memory phenotype [8,10•]. This expression pattern suggests that KIR and Ly49 expression is induced only in mature T cells and, furthermore, that these cells have previously encountered (or are currently responding to) cognate antigen.

Like conventional CD8+ T cells, the development of normal numbers of murine Ly49+CD8+ T cells requires classical class I molecules. In addition, these cells exhibit a diverse TCR Vβ repertoire analogous to that of conventional CD8+ T cells [10•,28•] and unlike the restricted TCR repertoire characteristic of CD1-restricted NKT cells (see the review by Gumperz and Brenner, this issue, pp 471–478). Unlike other CD8+ T cells, Ly49+CD8+ T cells have additional requirements for normal development: the expression of class II in the host and the expression of class I by host hematopoietic cells [10•]. One interpretation of these observations is that Ly49 induction in CTLs may be dependent upon class I mediated stimulation by (bone marrow derived) professional antigen-presenting cells and undefined signals from (class II restricted) CD4+ T cells.

Nonetheless, it is not yet clear under what circumstances KIR and Ly49 receptors are induced *in vivo* and whether there is something unique about the nature of the T cell antigens recognized. KIR+ or Ly49+ CD8+ T cells specific for tumor (e.g. melanocyte) or non-self (e.g. viral) antigens can be isolated from humans and mice [7•,16,19•,29,30,31•,32]. Interestingly, however, the majority of CTLs specific for a given antigen do not express these receptors, implying either that antigen specificity alone does not determine KIR or Ly49 expression, or that the KIR+ and Ly49+ subpopulations of cells are cross-reactive with other, undefined antigens. Finally, it still remains possible that these cells represent a separate T cell subset with unique developmental requirements and functions, despite the evidence to the contrary discussed above.

CD94–NKG2 heterodimers: receptors specific for nonclassical MHC class I

NKG2A, NKG2C and NKG2E are highly homologous lectin-like NK cell receptors that form heterodimers with the CD94 molecule [33]; NKG2A is inhibitory, whereas the others are stimulatory. These receptors recognize peptides derived from the leader sequence of many classical MHC class I molecules, embedded in the groove of a specialized nonclassical class I molecule called HLA-E (in humans) or Qa-1 (in mice) [34]. Thus, expression of classical class I is surveyed indirectly by CD94–NKG2 receptors.

In healthy humans and mice, CD94–NKG2 receptors are generally expressed by less than 5% of CD8+ T cells ([7•,8,14]; RE Vance, CW McMahon, DH Raulet, unpublished data). Human CTL clones and murine allospecific CTLs have been used to demonstrate that the CD94–NKG2A receptor can inhibit antigen-specific effector functions [30,35•,36]. Whether stimulatory NKG2 isoforms can modulate T cell function is unknown.

The extensive upregulation of CD94–NKG2A in activated CD8+ T cells has been demonstrated directly. Essentially all human CD8+ T cells responding to TCR-mediated stimulation *in vitro* initiate *de novo* expression of CD94–NKG2A when the culture contains either TGF-β or IL-15 [37•,38]. Similarly in mice, CD94–NKG2A is expressed by the vast majority of lymphocytic choriomeningitis virus (LCMV)-specific CTLs during *in vivo* infection and is also induced on naïve cells after antigenic stimulation *in vitro* (CW McMahon, AJ Zajac, R Ahmed, DH Raulet, unpublished data). Conversely, KIR and Ly49 molecules are not demonstrably induced under the conditions listed above. These data indicate that the expression of KIR and Ly49 molecules is restricted to unique activating conditions or to T cells with unique antigen specificities or properties, whereas CD94–NKG2A induction appears to be a more general feature of many CD8+ T cell responses.

NKG2D

The recent description of the stimulatory receptor, NKG2D, and its ligands in humans and mice is an important advance in our understanding of NK cell activation [39,40]. Because some NKG2D ligands are induced in cells that are stressed or transformed, there is the strong suggestion that NK cell activation, like inhibition, is an actively regulated component of target cell specificity. The expression of NKG2D is not restricted to NK cells and it also extends to the majority of γδ T cells and CD8+ T cells found in the peripheral blood of humans [41]. The murine NKG2D ortholog is also constitutively expressed by all NK cells and is induced on the surface of macrophages and CD8+ T cells following cellular activation [42]. Functional evidence that NKG2D can play a role in the stimulation of CD8+ T cells is discussed below.

Despite its name, NKG2D shares little sequence homology with the NKG2 receptor family members described above and does not appear to pair with CD94 [33]. Unlike the activating members of the Ly49 and NKG2 families, NKG2D uses DAP10, rather than DAP12, as an adapter signaling molecule [43]. Interestingly, the ligands for NKG2D include multiple members of at least two gene families, all of which are only distantly related to MHC class I molecules. The ligands described in mice — Rae1 family members and the minor histocompatibility antigen, H60 — are rarely expressed by normal cells but are expressed by many tumor cell lines [42,44]. The syntenic chromosomal region in humans contains the ULBP genes and, although they are only weakly homologous to H60 and the Rae1 family members, the ULBP gene products have a similar domain structure and bind human NKG2D [45]. In addition, the MHC-encoded MICA and MICB are

ligands for human NKG2D [41]. The MIC proteins appear to be stress-inducible and are considerably upregulated in epithelial tumors and in the context of cytomegalovirus (CMV) infection [46••]. Taken together, the observed expression patterns of the MIC and Rae1 NKG2D ligands would suggest that they are predominantly induced as a consequence of oncogenic transformation, viral infection or other forms of cellular distress. There is presently little information concerning the regulation of ULBPs and H60.

The function of NKG2D in CD8+ T cells has been investigated in a recent report using human T cell clones specific for CMV-derived peptides [46••]. It was found that CD8+ T cell effector functions, such as cytotoxicity and cytokine release, were enhanced when peptide-presenting cells were transfected with the NKG2D ligand, MICA. Importantly, MICA+ cells were not able to stimulate T cells in the absence of cognate antigen, suggesting that NKG2D is most likely to deliver a co-stimulatory signal that complements (rather than replaces) TCR-mediated antigen recognition. This signal may be particularly important for CTL IL-2 production, which was difficult to detect *in vitro* in the absence of NKG2D–ligand interactions. There are also indications that at least a fraction of CD28– CTLs in the peripheral blood, previously thought to be refractory to co-stimulation, produce IL-2 in response to NKG2D-mediated co-stimulation [46••]. A question of great interest is whether NKG2D plays an important role in the recognition of transformed and infected cells by CD8+ T cells *in vivo* and how these cells work in concert with NK cells and macrophages, which also recognize and respond to NKG2D ligand expression.

NK cell receptors with unknown ligands

The expression of several additional NK cell receptors by CD8+ T cells has been recently reported. The pan-NK marker NK1.1 (NKR-P1 family members) is expressed by all CD1-restricted NKT cells (see the review by Gumperz and Brenner, this issue, pp 471–478), is found on a fraction of memory phenotype CD8+ T cells in normal mice and is also upregulated to low levels on many conventional murine CTLs responding to viral infection [10•,32,47]. Similarly, IL-2Rβ+CD8+ T cells in lymphokine-activated killer (LAK) cultures can be induced to express NK1.1 in the absence of antigen when exposed to IL-2, IL-4 or IL-15 [48,49]. It has been reported that viral infection in mice also strongly induces expression of the putatively inhibitory NK cell receptor, KLRG1 (also known as MAFA) by effector CD8+ T cells [50]. The significance of NK1.1 and KLRG1 expression by CD8+ T cells is currently hard to gauge because the ligands for these receptors are not yet known.

Why do CD8+ T cells express NK cell receptors?

There are several nonmutually exclusive theories to explain why CD8+ T cells initiate expression of NK cell receptors, four of which are detailed below. Most assume the central tenet that these receptors serve to fine-tune the T cell response by raising or lowering the threshold of TCR triggering.

Do NK cell receptors prevent autoaggression by CTLs specific for self-peptides?

Recent reports have demonstrated that mice expressing a Ly49 transgene in all T cells and thymocytes often develop a fatal inflammatory disease suggestive of autoimmunity [25•,26•,27]. Although thymocytes in normal mice do not express Ly49 molecules, the possible defect in thymic negative selection of autoreactive T cells in Ly49-transgenic mice underscores the general concept that inhibitory receptor expression on T cells can tip the balance of self/non-self discrimination. In this framework, it can be hypothesized that inhibitory KIR and Ly49 molecules are expressed by mature CTLs that encounter and react to self antigens in the periphery, so as to ward off autoimmunity. In light of this hypothesis, it is perhaps significant that KIR+ CTL clones specific for self antigens have been isolated from melanoma patients and healthy individuals [7•,16,29,30,31•]. Expression of inhibitory receptors by self-specific T cells would be useful under at least two circumstances. First, during an antitumor response, class-I-specific inhibitory receptors may prevent CTL attack of normal cells, while permitting lysis of tumor cells that have lost a class I allele (a common attribute of escape variants) [29]. Second, the inhibitory receptor(s) may spare self-reactive CTLs from peripheral deletion, allowing the cells to survive and respond later in situations where tumor cells present increased amounts of a self antigen (or a modified, higher affinity tumor antigen) [16,31•]. It thus seems plausible that inhibitory receptors are induced in CTLs responding to self antigens as a reversible mechanism of peripheral tolerance.

Does NK cell receptor expression result from prolonged CTL stimulation due to chronic infection?

There are some indirect reasons to think that KIR+ and Ly49+ CTLs may arise as a result of chronic, rather than acute, stimulation. First, these receptors are not expressed by a large fraction of activated CTLs during acute primary viral infections with Epstein–Barr virus or hepatitis B in humans [8], or LCMV in mice [19•]. Second, the reported oligoclonality of human KIR+ CTLs [8] is reminiscent of the limited CTL specificities that emerge as a result of chronic stimulation *in vivo*, either during autoimmune disease or during chronic infections with viruses such as CMV, HTLV-I or HIV.

It is perhaps more difficult to ascertain the advantage of using inhibitory receptors to dampen responses to chronic foreign antigens and in fact there are some suggestions that such CTL inhibition could contribute to uncontrolled viral pathogenesis [23•,51]. Possibly, inhibitory-receptor expression represents a mechanism to avoid over-stimulation that would otherwise lead to exhaustion and activation-induced cell death. Although it would seem that inhibitory-receptor expression by CTLs would render them ill-equipped to respond to antigen encountered at a later date, this is not

necessarily the case. There is recent evidence that, in the absence of antigen, KIR+ CTLs downmodulate KIR surface expression to nonfunctional levels; these cells therefore could potentially respond potently to re-encountered antigen (such as a re-activated latent virus), at which point KIR surface levels would again be upmodulated [31•].

Do NK cell receptors control antigen-independent 'NK-like' killing?

Some CD94+, KIR+ and Ly49+ T cells that have been stimulated and cultured in high concentrations of IL-2 display 'NK-like' activity [52]. Although these cells can still react to TCR-mediated stimulation, they are also able to spontaneously lyse several NK-sensitive targets and this lysis can be reduced by triggering inhibitory-receptor–ligand interactions [9,53]. It has been hypothesized that, under unspecified conditions, some CD8+ T cells may bypass the requirement for TCR stimulation, perhaps via the expression of stimulatory NK receptors [54]. Parallel expression of inhibitory NK cell receptors could thus prevent autoaggression against normal cells and allow these CTLs to function essentially as NK cells. However, freshly isolated CD8+ T cells do not spontaneously lyse targets and the majority of KIR+ CTL clones and Ly49+ T cells only respond when their TCRs are engaged, even when inhibitory receptor signaling is blocked [10•,16,19•]. Therefore, it is unknown if this antigen-independent CTL activity exists *in vivo*.

Do NK cell receptors optimally shape CTL responses to infection?

In mice, it is apparent that under some circumstances a variety of NK cell receptors (such as KLRG1, NKR-P1, NKG2D and CD94–NKG2A) are upregulated on the majority of CD8+ T cells following TCR-mediated activation ([32,42,47,50]; AJ Zajac, CW McMahon, R Ahmed, DH Raulet, unpublished data). It is not yet clear whether this phenomenon occurs in humans, although there does seem to be a correlation between CD94–NKG2A expression and an activated CTL phenotype *in vivo* [7•,35•,55,56], and CD94–NKG2A can clearly be induced in human CTLs *in vitro* in the presence of certain cytokines [37•,38]. The expression of these receptors by a large fraction of CD8+ T cells at the peak of the response suggests that they play a role in CD8+ T cell responses to most antigens.

How this class of receptors influences the CTL response presumably varies for the different receptors. In the case of the stimulatory receptor, NKG2D, recent evidence suggests that NKG2D ligands are induced on infected or transformed cells [42,44,46••,57] and serve to costimulate CD8+ T cells [46••]. In the case of inhibitory receptors, negative signaling may restrain CTL proliferation or function during a normal immune response once antigen levels are low. When high levels of antigen are present, activating TCR signals can presumably eclipse inhibitory signals, as has been demonstrated directly *in vitro* [22,30,31•]. Alternatively, inhibitory-receptor engagement may selectively impair the activation of T cells with low affinity TCRs, thus promoting 'affinity maturation' of the CD8+ T cell response. Possibly consistent with the latter hypothesis is the finding that CTLs specific for a subdominant epitope from LCMV-infected Ly49A-transgenic mice were inhibited more strongly by Ly49A engagement than were CTLs specific for dominant epitopes [23•]. Finally, it is possible that inhibitory NK cell receptors prevent CTLs from destroying useful cells such as antigen-presenting cells, as they express higher levels of the class I MHC molecules that are ligands for NK cell receptors.

Following an immune response, the majority of effector CTLs are destined for apoptosis; thus, it is alternatively possible that expression of inhibitory receptors helps to drive CTLs toward this fate. There are few data addressing this possibility, although a recent study suggests that the inhibitory receptor, KLRG1, is a marker for CD8+ T cell replicative senescence following viral infection (D Voehringer, C Blaser, P Brawand, DH Raulet, T Hanke, H Pircher, unpublished data). Conversely, it has been recently proposed that inhibitory receptors may actually encourage the formation or maintenance of long-term memory CTLs, perhaps by reducing the extent of activation-induced cell death that normally follows a T cell response [58••]. This hypothesis is based upon the observation that unusually large numbers of memory CD8+ T cells accumulated in mice expressing transgenes for both a human inhibitory KIR and its cognate HLA molecule, correlating with reduced cell death as the cells divided.

Conclusions

NK cell receptors expressed by CD8+ T cells can be categorized into those that are induced on all or most CD8+ T cells responding to antigen and those that are expressed by a subset of CD8+ T cells with a memory phenotype. The nature of the stimuli that induce expression of the latter class of receptors is unclear. There is accumulating evidence that receptors of both classes can modulate antigen-driven T cell responses, either by inhibiting or by co-stimulating T cell proliferation and effector functions. In future studies, it will be important to define the factors necessary for induction of KIR and Ly49 molecules *in vivo* and to determine whether the receptors help to prevent autoaggression and/or over-stimulation. In addition, future studies will aim to unravel the role of various NK cell receptors in the induction, dampening and memory of the CD8+ T cell response. The use of disease models in mice should prove useful for determining how NK cell receptors help or hinder T cell responses to pathogens and/or cancer cells.

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This report examines the small percentage of Ly49G2-bearing CD8+ T cells observed during an antiviral response and evaluates the antigens recognized by these cells. The Ly49G2+CD8+ T cells were shown to be specific for viral epitopes. The authors argue that this cell population expanded from pre-existing Ly49G2+CD8+ T cells, although it has not been excluded that they instead initiated Ly49 expression during infection. Interestingly, these cells responded poorly to allogeneic stimulator cells and some viral epitopes, suggesting that Ly49+CD8+ T cells have limited TCR specificities compared with CD8+ T cells as a whole.

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This paper is the first to demonstrate that expression of a Ly49 receptor by CD8+ T cells can inhibit the CTL response to an infectious agent. Mice expressing a Ly49A transgene in all T cells were infected with LCMV and it was shown that CTL activity *in vivo* and *in vitro* was greatly reduced in the presence of a strong Ly49A ligand (H-2^d). In addition, the Ly49A transgenic mice had difficulty in controlling infection with a virulent strain of LCMV.

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