

Innate immune recognition by stimulatory immunoreceptors

Andreas Diefenbach* and David H Raulet†

The specificity of cells of the innate immune system is determined in part by various stimulatory receptors that function in different forms of immune recognition. NKG2D, a stimulatory receptor expressed by natural killer (NK) cells, macrophages and certain T cell subsets, recognizes various families of 'induced-self' ligands. The ligands are distantly related to class I MHC molecules and are induced in 'distressed' cells as markers of abnormal self. Another form of innate immune recognition is exemplified by the Ly49H receptor, which is expressed by a subset of NK cells. The Ly49H receptor directly recognizes a virus-encoded protein expressed by cells infected with mouse cytomegalovirus (MCMV) and the *Ly49h* gene is identical to the *Cmv1^{FL}* gene, which confers resistance to MCMV infections. Yet another group of receptors (the triggering receptors expressed by myeloid cells, or TREMs), which are exclusively expressed by myeloid cells, have been shown to amplify cytokine responses to bacterial products and have also been implicated in the pathogenesis of septic shock.

Addresses

Department of Molecular and Cell Biology and Cancer Research Laboratory, 485 Life Sciences Addition, University of California, Berkeley, CA 94720, USA

*e-mail: diefenb_@uclink4.berkeley.edu

†e-mail: raulet@uclink4.berkeley.edu

Current Opinion in Immunology 2003, **15**:37–44

This review comes from a themed issue on
Innate immunity
Edited by Ruslan Medzhitov and Christine A Biron

0952-7915/03/\$ – see front matter
© 2003 Elsevier Science Ltd. All rights reserved.

DOI 10.1016/S0952-7915(02)00007-9

Abbreviations

BCR	B cell receptor
GM-CSF	granulocyte-monocyte colony-stimulating factor
IFN	interferon
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibition motif
KARAP	killer cell-activating receptor-associated protein
MCMV	mouse cytomegalovirus
MHC	major histocompatibility complex
MICA/B	MHC class I chain-related proteins A and B
Rae1	retinoic acid early inducible 1 protein
TCR	T cell receptor
TNF	tumor necrosis factor
TREM	triggering receptor expressed by myeloid cells
ULBP	UL16-binding protein

Introduction

Stimulatory immunoreceptors occupy center stage in the recognition of foreign antigens or pathogens by the

immune system. Archetypal examples of stimulatory immunoreceptors are the B cell receptor (BCR) and T cell receptor (TCR), which are encoded by rearranging genes and are the major structures employed by B and T cells to discriminate between self and nonself molecules. Stimulatory immunoreceptors are multisubunit structures composed of ligand-binding subunits (e.g. the TCR α and β chains and the BCR heavy and light chains) and associated transmembrane signaling adaptor protein(s). The cytoplasmic domains of adaptor proteins contain an immunoreceptor tyrosine-based activation motif (ITAM) with the consensus sequence YxxL/Ix₆₋₈YxxL/I (with x representing any amino acid, the other residues are represented by amino acid one-letter code). The TCR associates with several adaptor proteins including the TCR ζ chain (also called CD3 ζ), and the BCR associates with the Ig α and Ig β adaptor proteins. Crosslinking of stimulatory immunoreceptors leads to activation of the corresponding cells and initiates immune responses against pathogens and tumors.

The cells of the innate immune system do not express highly diversified receptors encoded by rearranging genes. Instead, they express various other stimulatory immunoreceptors that also associate with ITAM-containing signaling adaptor proteins, including CD3 ζ , Fc γ R and killer cell-activating receptor-associated protein (KARAP, also called DAP12). The biological roles of many of these receptors are not well understood, primarily because many of the ligands have not been identified and genetic loss-of-function studies have not yet been carried out in most cases.

This review focuses on three stimulatory receptor systems that fulfill very distinct functions in the innate immune response: NKG2D, Ly49H and the triggering receptors expressed by myeloid cells (TREMs). These three receptor systems illustrate distinct paradigms of how innate immune recognition via stimulatory receptors is achieved.

NKG2D is a stimulatory receptor expressed by lymphoid and myeloid cells that recognizes various cell surface ligands that are distantly related to class I MHC molecules and upregulated in infected, transformed or stressed cells [1]. Ly49H is expressed by a subset of NK cells and directly recognizes a viral gene product from mouse cytomegalovirus (MCMV), suggesting that this receptor may be involved in the immune response to viruses [2^{••}–6^{••}]. The TREMs are a small family of stimulatory receptors exclusively expressed by myeloid cells. The ligands for these receptors are unknown. However, the

blockade of TREM-1 in mice prevents various types of sepsis syndromes, suggesting that this receptor is involved in enhancing innate immune responses to several types of pathogens [7**]. All of these immunoreceptors use the KARAP/DAP12 molecule as a signaling adaptor protein ([6**,7**], see also Update).

Stimulatory receptors expressed by NK cells

The activation of NK cells is regulated by a balance of signals received from inhibitory and stimulatory receptors. NK cells are known to be activated by many tumor cells and virus-infected cells. How NK cells selectively recognize these cells is largely unknown. NK cells express various families of inhibitory receptors, all of which interact with class I MHC molecules. These receptors prevent NK cells from attacking normal self cells, while allowing them to attack cells that downregulate class I MHC molecules, as often occurs in tumor cells and virus-infected cells [8]. This form of interaction is called 'missing self' recognition (Figure 1b; [9]).

Similar to other lymphocytes, NK cells must be triggered to become activated. A variety of stimulatory receptors expressed by NK cells have been identified over the years [1,10], but the specificity and function of most of these receptors has been difficult to determine. Recently, ligands for some of the stimulatory receptors (e.g. NKG2D, Ly49H) have been identified, which has aided in developing a nascent understanding of their functions.

The NKG2D receptor recognizes 'induced self' ligands as markers of abnormal self

NKG2D receptor and its expression

NKG2D is expressed by various lymphoid and myeloid cells. In mice, NKG2D is expressed by all NK cells, by all epidermal $\gamma\delta$ T cells, and by subsets of NKT cells and splenic $\gamma\delta$ T cells (but not by intestinal epithelial $\gamma\delta$ T cells) [11,12**]. Additionally, NKG2D is expressed by essentially all CD8⁺ T cells after T cell receptor triggering and by macrophages after stimulation with LPS, IFN- γ or IFN- α/β [11,12**]. In humans a slightly different expression pattern is observed; in addition to NK cells, all CD8⁺ T cells and all intraepithelial lymphocyte (IEL) $\gamma\delta$ T cells constitutively express NKG2D [13–15].

Ligands for the NKG2D receptor

NKG2D recognizes several families of cellular ligands, all of which are distantly related to MHC class I molecules in sequence and are upregulated on tumor cells, virally infected cells and 'stressed' cells (Figure 1c; [16]). The first ligands to be identified were a family of non-classical class I MHC molecules, the MHC class I chain-related proteins A and B (MICA/B; [13]). These molecules were initially identified as ligands for a subset of human $\gamma\delta$ T cells [17,18*]. Interestingly, MICA/B are not expressed by most normal tissues but are upregulated in

many epithelial tumor cells [19], in cells infected with human cytomegalovirus [20**], in bacterially infected cells [21] and in 'stressed' cells [22]. A low level of MICA/B expression is maintained on the epithelial cells lining the gastrointestinal surfaces, which may be due to interactions of these cells with various environmental 'stressors' [22,23]. No MICA/B homologs have been identified in mice.

We, and others, recently cloned two novel families of ligands for the mouse NKG2D receptor, Rae1 and H60 [11,24]. The retinoic acid early inducible 1 proteins (Rae1s) are encoded by a family of five very closely related genes (*Rae1a–e*) [25,26,27*,28]. H60 was initially identified as a dominant minor histocompatibility antigen in the response of C57BL/6 mice against BALB.B cells [29]. H60 and Rae1 proteins are distantly related to class I MHC molecules in sequence, although they lack an α_3 -like domain. Furthermore, unlike the MHC-encoded *MICA/B* genes, the *H60* and *Rae1* genes are all colocalized away from the MHC on mouse chromosome 10 [25,29]. Most interestingly, the Rae1 proteins are not expressed by most normal cells, but are upregulated by many tumor cells of diverse origin [11,27*,30**]. H60 is expressed by some tumor cells from BALB/c mice but is also expressed at low levels by activated lymphoblasts and at high levels by thymocytes from BALB/c mice [1,11,29].

Interestingly, a region of human chromosome 6 (q24.2–q25.3), which is syntenic to the murine Rae1/H60 locus contains a related gene family that encodes NKG2D ligands. This family of proteins, variously called UL16 binding proteins (ULBPs; [31**]), ALCAN [32] or human RAE1 proteins [33], is encoded by 10 related genes, six of which encode potentially functional glycoproteins and four of which are pseudogenes [33]. The ULBPs were initially identified based on the ability of some members (ULBP1 and 2) to interact with UL16, a protein encoded by human cytomegalovirus (HCMV). It has been proposed that UL16 helps the virus evade the NK cell response by binding to and inactivating ULBPs in infected cells [31**].

NKG2D function

The expression of NKG2D ligands on target cells potently induces NK cell cytotoxicity (Figure 1c; [11,12**,13,30**]). Depending on the levels of NKG2D ligands, the stimulatory signal can override coexisting inhibitory signals provided by the same target cell [11,13,24,30**]. However, the stimulatory signal provided by NKG2D is not entirely refractory to inhibitory signals [14].

Crosslinking of the NKG2D receptor on NK cells triggers several effector mechanisms from NK cells (e.g. mobilization of intracellular Ca²⁺ [12**], production of cytokines

including IFN- γ [11,12^{••},30^{••},34,35], GM-CSF [31^{••},35], TNF- α [34] and TNF- β (LT- α) [31^{••}], and production of chemokines such as macrophage inflammatory protein (MIP-1 β ; [34,35]) and I-309 [31^{••}). Interestingly, triggering of the NKG2D receptor alone is sufficient to stimulate NK cell activation, contradicting the notion that NKG2D is a co-stimulatory receptor in NK cells [12^{••}]. The cross-linking of NKG2D expressed by activated macrophages also led to the induction of effector mechanisms (e.g. production of nitric oxide and TNF- α [11,12^{••}]). By contrast, activated CD8⁺ T cells that express NKG2D cannot be directly stimulated via NKG2D [12^{••},20^{••}]. In CD8⁺ T cells, including CD28⁻CD8⁺ T cells, however, NKG2D provides a co-stimulatory signal that synergizes with T cell receptor signals [12^{••},15,20^{••},30^{••}]. Hence, interactions of NKG2D with induced ligands provide functions appropriate to the cells in which it is expressed: direct activation of innate immune cells and enhancement of the response of antigen-specific CD8⁺ T cells.

NKG2D in anti-tumor immune responses

Many human tumors of epithelial origin express MICA/B [19], and most mouse tumor cell lines of diverse origin also express ligands for NKG2D [11,30^{••}]. Blockade of the NKG2D receptor–ligand interaction resulted in reduced NK killing of all NKG2D ligand-positive tumor cell lines tested, suggesting that NKG2D plays a significant role in natural cytotoxicity to tumor cells [12^{••}].

Ectopic expression of the NKG2D ligands Rae1 or H60 in rare tumor cell lines that do not naturally express ligands (the RMA lymphoma and its MHC class I low variant RMA-S, the EL4 thymoma and the B16-BL6 melanoma) resulted in uniform rejection of the tumor cells by syngeneic mice [30^{••},36^{••},37[•]]. The rejection was mediated by NK cells in the case of the B16-BL6 melanoma, EL4 thymoma and MHC class I⁻ RMA-S lymphoma [30^{••},37[•]], or cooperatively by CD8⁺ T cells and NK cells in the case of the MHC class I⁺ RMA lymphoma [30^{••},37[•]]. Interestingly, rejection of NKG2D ligand-expressing RMA or RMA-S tumor cells required functional perforin but not IFN- γ , suggesting that pore-forming cytotoxic granules, but not IFN- γ from NK cells and CD8⁺ T cells, are the main effector mechanism for tumor rejection [37[•]]. Strikingly, mice that had rejected tumor cells expressing ligands for NKG2D were immune when subsequently rechallenged with the parental tumor cell lines that lacked NKG2D ligands. This efficient vaccination effect was dependent on tumor-specific CD8⁺ T cells [30^{••}]. Also, vaccination of mice with irradiated ligand-positive tumor cells, but not with the ligand-negative parental cells, led to potent priming of tumor-specific CTLs in an NK cell-independent manner [30^{••}]. These data suggest that tumor cell expression of NKG2D ligands can enhance adaptive immune responses specific for tumor cells.

In summary, the data suggest that the NKG2D immunoreceptor is an important ‘sentinel’ used by various lymphoid and myeloid cells to detect cells that have upregulated induced-self ligands as a result of various cellular insults (Figure 1c).

Direct recognition of viral proteins by NK cells

Ly49H is another stimulatory receptor expressed by NK cells. Ly49H is a C-type lectin-like receptor that is a member of the Ly49 family of MHC class I-specific receptors [38]. In contrast to the majority of the Ly49 molecules, Ly49H does not contain an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. Instead, Ly49H associates with the signaling adaptor protein KARAP/DAP12, which contains an ITAM and couples the Ly49H receptor to the syk/ZAP70 signaling pathway [39]. Crosslinking of Ly49H induces cytotoxicity against target cells and the production of cytokines (GM-CSF, IFN- γ ; [40]). Similar to other Ly49 molecules, Ly49H is expressed in a variegated fashion by approximately 50% of NK cells [40,41]. Unlike most other Ly49 family members, however, Ly49H does not bind to any known class I MHC molecule.

Recently, two groups reported evidence that a gene previously shown to confer resistance to MCMV infection, *Cmv1^R*, is identical to the *Ly49h* gene on mouse chromosome 6 [2^{••},3^{••}]. Furthermore, the blockade of Ly49H in C57BL/6 mice, which express *Cmv1^R*, rendered these mice susceptible to infection with MCMV [2^{••},4^{••}]. More recently, two groups reported that Ly49H directly binds to the product of the MCMV *m157* gene, a protein that is expressed on the surface of infected cells [5^{••},6^{••}]. Although the sequence of *m157* is not notably homologous to MHC proteins, structural prediction programs suggest that the protein is similar to class I MHC molecules in structure, with similarities to the H-2M3, MICA and CD1d nonclassical MHC class I molecules [5^{••},6^{••}]. Target cells transfected with *m157* triggered cytotoxicity and the production of IFN- γ and activation-induced T cell derived and chemokine-related cytokine (ATAC)/lymphotoxin by NK cells from C57BL/6 mice [5^{••},6^{••}]. A possible beneficial function of *m157* expression for MCMV was suggested by the finding that, in addition to binding Ly49H in C57BL/6 mice, *m157* binds the inhibitory MHC-specific Ly49I receptor in another mouse strain, 129/J [5^{••}]. Therefore, *m157* may help the virus inhibit and therefore evade NK cells in some mouse strains (Figure 1b). The reactivity of Ly49H with *m157* may have evolved in the host as an immune countermeasure (Figure 1a). These notions would be supported if it could be shown that recombinant viruses lacking the *m157* gene are more pathogenic in resistant strains such as C57BL/6 and less pathogenic in sensitive strains such as 129/J.

Stimulatory receptors expressed by myeloid cells

Several stimulatory receptors have been identified over the years that are expressed by myeloid cells and signal through ITAM-containing adaptor proteins, usually KARAP/DAP12 [42]. As discussed above, NKG2D is one receptor of this type [11,12^{••}; see also Update]. The specificities and biological functions of most of the other receptors in this category are unknown. Recently, the TREM family of receptors has attracted considerable attention based on evidence that one family member (TREM-1) participates in the pathogenesis of septic shock in mice infected with bacteria [7^{••}].

The TREM family

Five *Trem* genes have been identified to date, four of which (*Trem 1–4*) encode potentially functional type I transmembrane glycoproteins [43]. The *Trem* genes are clustered on mouse chromosome 17/human chromosome 6. TREMs display a single extracellular Ig-like domain. For signaling, all known TREMs associate with the KARAP/DAP12 signaling adaptor protein [43–46]. Interestingly, the TREMs are distantly related to a stimulatory receptor expressed by human NK cells, NKp44 [47,48]. NKp44 also associates with KARAP/DAP12 and maps to the same region on human chromosome 6 [44].

The TREMs are expressed by different subsets of myeloid cells. Whereas TREM-2 is exclusively expressed by immature monocyte-derived dendritic cells [45], TREM-1 is expressed by neutrophils and a subset of CD14^{high} monocytes. Significantly, the stimulation of TREM-1-expressing cells with bacteria (both Gram positive and Gram negative) and fungi, or their cell-wall products, results in marked upregulation of TREM-1 on monocytes and neutrophils [7^{••},44]. In addition, TREM-1 is also specifically upregulated *in situ* on neutrophils and monocytes from peritoneal lavages of patients with bacterial peritonitis [7^{••}]. Triggering of TREM-1 on neutrophils leads to the release of IL-8 and myeloperoxidase [44]. Monocytes produce IL-1 β , TNF- α and monocyte chemoattractant protein (MCP)-1 after stimulation of TREM-1 [7^{••},44]. TREM-1 stimulation also upregulates a variety of adhesion molecules (ICAM-1, CD11b, CD49e, CD29) and co-stimulatory receptors (CD40, CD86/B7.2) expressed by monocytes and/or neutrophils [7^{••}].

Importantly, TREM-1 synergizes with LPS in the induction of cytokine and chemokine responses. Notably, TREM-1 engagement enhances the release of TNF- α and IL-1 β , pro-inflammatory cytokines that mediate septic shock. Strikingly, blocking experiments performed with TREM-1–Ig fusion proteins prevented septic shock and inflammatory responses in three different experimental models of septic shock. These results indicate that TREM-1 recognition participates in the pathogenesis of septic shock [7^{••}]. The results also suggest that LPS and

TREM-1 act cooperatively, but how the respective signaling pathways interact is not yet well understood. Furthermore, because TREM ligands have not yet been identified, it is not known whether TREMs recognize components of the pathogen or ‘induced-self ligands’.

Perspectives

Stimulatory receptors on lymphoid and myeloid cells are multisubunit receptor complexes composed of extracellular ligand-binding subunits that associate with various ITAM-containing signaling molecules. Some of these receptors are primary immune recognition receptors (e.g. BCR, TCR, Ly49H), whereas others may act as amplifiers of a response (TREMs may fall into this category). NKG2D does both, functioning as a primary stimulatory receptor on NK cells and macrophages and as a co-stimulatory receptor on CD8⁺ T cells. Despite their similarity in subunit structure, stimulatory receptors play different roles in the immune system, which in some cases illustrates distinct strategies of immunity.

The first and most familiar strategy of immune recognition is the direct recognition of foreign molecular structures (Figure 1a). The adaptive immune system, by virtue of its great diversity of specificities, can respond to virtually any foreign molecular structure. Components of the innate immune system, by contrast, are less diverse and appear to have evolved to recognize a much smaller universe of foreign structures, albeit ones that are characteristic of specific classes of potential pathogens. This concept is well illustrated by the Toll-like receptors (TLRs), which participate in the recognition of various common components of microbes and viruses. The recognition of viral components by Ly49H, and possibly other NK receptors, such as NKp46 and NKp44, is apparently yet another example of this type of recognition (Figure 1a; [5^{••},6^{••},49]).

A second general strategy of immunity is the concept of ‘missing-self’ recognition, in which immune cells express inhibitory receptors specific for markers of normal self cells (Figure 1b; [9]). When expression of the normal self markers is prevented in a cell, inhibition is disrupted and the cell is attacked. This type of recognition operates in the complement system, but perhaps the best characterized example is the recognition of MHC class I molecules by inhibitory NK receptors such as Ly49s, KIRs and CD94/NKG2A. MHC downregulation, as occurs frequently during viral infection and transformation, can lead to susceptibility of an infected cell to NK cell attack. This type of system is vulnerable to evasion by viruses, which may produce proteins that engage inhibitory receptors and therefore prevent NK responses. It appears likely that this has occurred in the case of MCMV m157, which binds to at least one of the inhibitory receptors expressed by mouse NK cells (Figure 1b). As a possible countermeasure, mice have evolved stimulatory Ly49

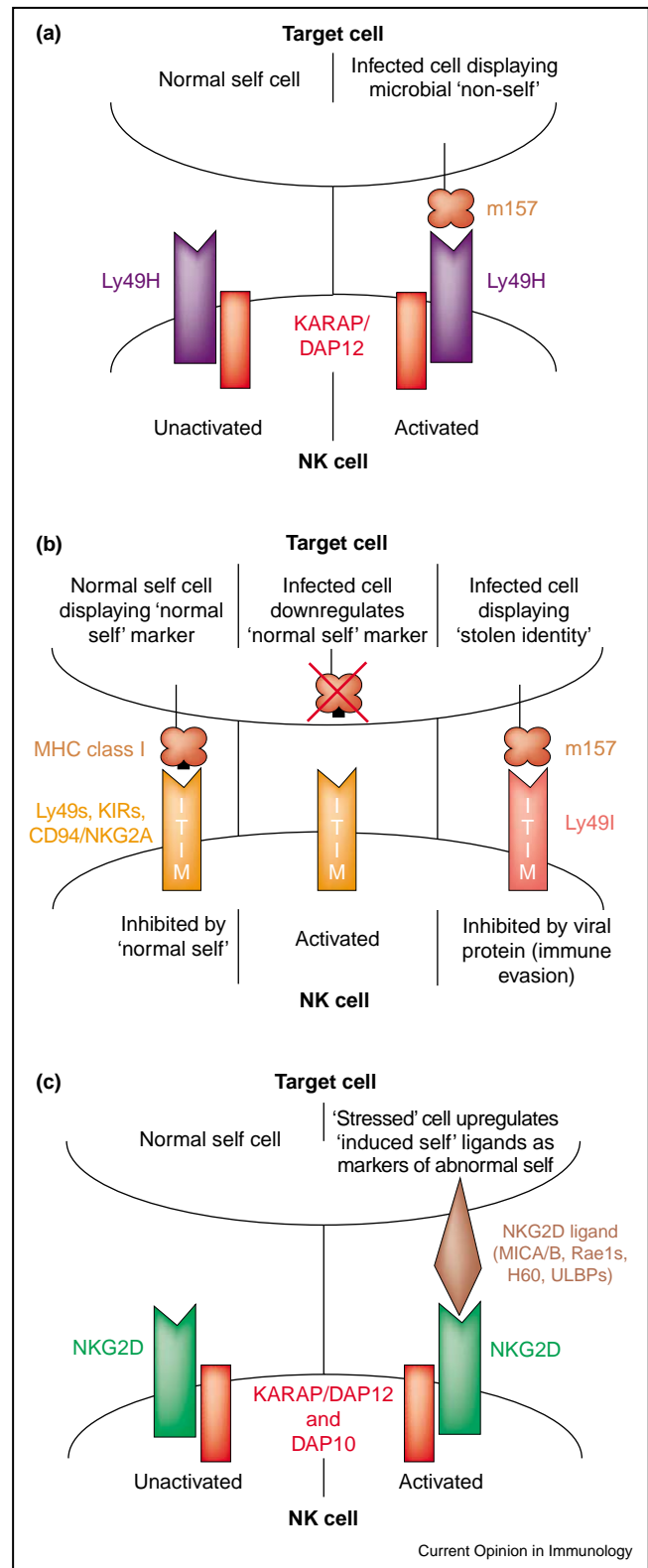
isoforms (e.g. Ly49H), which bind the same viral ligand (Figure 1a).

The recognition of 'induced-self ligands' by the NKG2D receptor is a paradigm for a third strategy of immune recognition (Figure 1c; [1]). NKG2D ligands are all distantly related to class I MHC molecules and are not expressed by most normal cells [1]. Upregulation of these ligands in response to various cellular insults (transformation [11], viral infection [20**], bacterial infection [21] and several other forms of cellular abuse [22]) results in recognition of these cells by the immune system (Figure 1c; [1,50]). Interestingly, this type of recognition system shifts the burden of discriminating 'normal' and 'abnormal' from the immune system itself to the potential target cell, which must 'decide' whether to upregulate the ligands. Strikingly, as has also been revealed in studies of foreign pattern recognition by TLRs, induced self recognition (via NKG2D) activates not only the innate immune system, but also provides key signals in the activation of adaptive immune responses [30**,51**].

Update

Recent studies have shed light on how the NKG2D receptor provides different signals in different cell types. Two NKG2D splice variants associate with different signaling adaptor proteins, resulting in qualitatively different signals [52**]. In CD8⁺ T cells, NKG2D associates solely with the signaling molecule DAP10, which provides a primarily co-stimulatory signal. In NK cells and macrophages, by contrast, NKG2D associates with both DAP10 and the ITAM-containing KARAP/DAP12 signaling molecule, which together provide both co-stimulatory and directly stimulatory signals in these cells. A recent analysis of mice genetically deficient for DAP10 arrived at a similar conclusion [53**]. In these mice, CD8⁺ T cells do not express cell surface NKG2D, and NKG2D function is abolished in these cells. In contrast, activated NK cells show normal surface expression of NKG2D and the function of NKG2D is only moderately impaired in these cells. Most strikingly, tumor cells expressing

Figure 1



Innate immune recognition by NK cells. The figure illustrates three different modes of innate immune recognition, as mediated by NK cells in this example. **(a)** Direct recognition of microbial 'nonself'. MCMV-infected target cell expresses the viral protein m157. Ly49H binds to m157, resulting in activation of the NK cell by KARAP/DAP12-mediated signaling events. **(b)** 'Missing-self' recognition. Inhibitory receptors (Ly49s, KIRs, CD94/NKG2A) expressed by NK cells interact with self class I MHC molecules on normal self cells. The ITIM motif in the cytoplasmic domain of these inhibitory receptors relays an inhibitory signal to the NK cell. Infection or transformation is often accompanied by downregulation of self class I MHC, unleashing the NK cell. Some viruses evade NK immunity by expressing molecules that mimic surface MHC class I, which can inhibit NK cells despite the downregulation of endogenous class I MHC molecules. **(c)** Recognition of 'induced self' ligands as markers of abnormal self. Stressed cells (e.g. infected, transformed) upregulate 'induced self' ligands for the NKG2D receptor, leading to NK cell activation via KARAP/DAP12 and/ or DAP10 signaling events.

NKG2D ligands were rejected to the same extent in DAP10 deficient mice as in wild-type controls. Taken together, these data provide strong evidence that the NKG2D receptor fulfills distinct functions in the innate and adaptive immune system. In NK cells and macrophages NKG2D acts as a direct stimulatory receptor by associating with both ITAM-containing stimulatory adaptor proteins (KARAP/DAP12) and a co-stimulatory adaptor protein (DAP10). In the adaptive immune system NKG2D has a co-stimulatory function, amplifying antigen-specific signals provided by the TCR, by virtue of its sole association with a co-stimulatory adaptor protein (DAP10).

Another recently reported finding is that tumor cells expressing the MICA/B ligands for the human NKG2D receptor can release a soluble form of these proteins that are detectable in the serum of patients with MICA/B-positive tumors [54**]. Binding of soluble MICA/B to NKG2D induces downregulation of cell surface NKG2D on human CD8⁺ T cells as a result of ligand-induced endocytosis and probably lysosomal degradation. Melanoma antigen-specific CD8⁺ T cell clones that have down-regulated NKG2D after incubation with soluble MICA/B proteins can no longer be co-stimulated by NKG2D ligand-positive tumor cell lines. This paper provides the first evidence for an immune evasion mechanism employed by tumor cells to avoid recognition via NKG2D.

Acknowledgements

AD is a Physician Postdoctoral Fellow of the Howard Hughes Medical Institute. Work from this laboratory is supported by grants from the National Institutes of Health to DHR.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Diefenbach A, Raulet DH: **Strategies for target cell recognition by natural killer cells.** *Immunol Rev* 2001, **181**:170-184.
2. Brown MG, Dokun AO, Heusel JW, Smith HR, Beckman DL, Blattenberger EA, Dubbelde CE, Stone LR, Scalzo AA, Yokoyama WM: **Vital involvement of a natural killer cell activation receptor in resistance to viral infection.** *Science* 2001, **292**:934-937.
- This paper demonstrates (together with [3**,4**]) that the *Cmv1^R* locus is identical to the *Ly49h* gene. Blockade of Ly49H in MCMV resistant mice rendered them susceptible to infection.
3. Lee SH, Girard S, Macina D, Busa M, Zafer A, Belouchi A, Gros P, Vidal SM: **Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily.** *Nat Genet* 2001, **28**:42-45.
- See annotation to [2**].
4. Daniels KA, Devora G, Lai WC, O'Donnell CL, Bennett M, Welsh RM: **Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H.** *J Exp Med* 2001, **194**:29-44.
- See annotation to [2**].
5. Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL: **Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors.** *Science* 2002, **296**:1323-1326.
- Together with [6**], this paper demonstrates that Ly49H binds to the MCMV encoded protein m157. The authors provide evidence that m157 activates NK cells via Ly49H and that m157 is structurally related to class I MHC molecules.
6. Smith HR, Heusel JW, Mehta IK, Kim S, Dörner BG, Naidenko OV, Iizuka K, Furukawa H, Beckman DL, Pingel JT *et al.*: **Recognition of a virus-encoded ligand by a natural killer cell activation receptor.** *Proc Natl Acad Sci USA* 2002, **99**:8826-8831.
- See annotation to [5**]
7. Bouchon A, Facchetti F, Weigand MA, Colonna M: **TREM-1 amplifies inflammation and is a crucial mediator of septic shock.** *Nature* 2001, **410**:1103-1107.
- The authors demonstrate that TREM-1 is upregulated on myeloid cells after stimulation with bacteria and fungi, and synergizes with LPS in the production of pro-inflammatory cytokines (TNF- α and IL-1 β). Blocking of TREM-1 prevents sepsis in three experimental sepsis models.
8. Raulet DH, Vance RE, McMahon CW: **Regulation of the natural killer cell receptor repertoire.** *Annu Rev Immunol* 2001, **19**:291-330.
9. Ljunggren HG, Karre K: **In search of the 'missing self': MHC molecules and NK cell recognition.** *Immunol Today* 1990, **11**:237-244.
10. Moretta L, Bottino C, Vitale M, Pende D, Cantoni C, Mingari M, Biassoni R, Moretta A: **Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity.** *Annu Rev Immunol* 2001, **19**:197-223.
11. Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH: **Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages.** *Nat Immunol* 2000, **1**:119-126.
12. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH: **The role of the NKG2D immunoreceptor in immune cell activation and natural killing.** *Immunity* 2002, **17**:19-29.
- This paper provides evidence that NKG2D is a directly stimulating receptor in innate immune cells (NK cells, macrophages) and a co-stimulatory receptor in CD8⁺ T cells. The authors also demonstrate a role for NKG2D in NK killing of tumor target cells.
13. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T: **Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA.** *Science* 1999, **285**:727-729.
14. Pende D, Cantoni C, Rivera P, Vitale M, Castriconi R, Marcenaro S, Nanni M, Biassoni R, Bottino C, Moretta A, Moretta L: **Role of NKG2D in tumor cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin.** *Eur J Immunol* 2001, **31**:1076-1086.
15. Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC, Jabri B: **NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment.** *J Immunol* 2001, **167**:5527-5530.
16. Diefenbach A, Raulet DH: **Natural killer cells: stress out, turn on, tune in.** *Curr Biol* 1999, **9**:R851-R853.
17. Groh V, Steinle A, Bauer S, Spies T: **Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells.** *Science* 1998, **279**:1737-1740.
18. Wu J, Groh V, Spies T: **T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial $\gamma\delta$ T cells.** *J Immunol* 2002, **169**:1236-1240.
- Using a transfection system, the authors demonstrate that MICA binds to both the $\gamma\delta$ receptor and NKG2D.
19. Groh V, Rhinehart R, Secrist H, Grabstein KH, Spies T: **Broad tumor-associated expression and recognition by tumor-derived $\gamma\delta$ T cells of MICA and MICB.** *Proc Natl Acad Sci USA* 1999, **96**:6879-6884.
20. Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T: **Costimulation of CD8 $\alpha\beta$ T cells by NKG2D via engagement by MIC induced on virus-infected cells.** *Nat Immunol* 2001, **2**:255-260.
- Using T cell clones specific for HCMV, the authors provide evidence for the co-stimulatory function of NKG2D expressed by human CD8⁺ T cells.
21. Tieng V, Le Bouguenec C, du Merle L, Bertheau P, Desreumaux P, Janin A, Charron D, Toubert A: **Binding of *Escherichia coli***

- adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA.** *Proc Natl Acad Sci USA* 2002, **99**:2977-2982.
22. Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T: **Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium.** *Proc Natl Acad Sci USA* 1996, **93**:12445-12450.
 23. Bahram S, Bresnahan M, Geraghty DE, Spies T: **A second lineage of mammalian major histocompatibility complex class I genes.** *Proc Natl Acad Sci USA* 1994, **91**:6259-6263.
 24. Cerwenka A, Bakker ABH, McClanahan T, Wagner J, Wu J, Phillips JH, Lanier LL: **Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice.** *Immunity* 2000, **12**:721-727.
 25. Nomura M, Zou Z, Joh T, Takihara Y, Matsuda Y, Shimada K: **Genomic structures and characterization of Rae1 family members encoding GPI-anchored cell surface proteins and expressed predominantly in embryonic mouse brain.** *J Biochem (Tokyo)* 1996, **120**:987-995.
 26. Zou Z, Nomura M, Takihara Y, Yasunaga T, Shimada K: **Isolation and characterization of retinoic acid-inducible cDNA clones in F9 cells: a novel cDNA family encodes cell surface proteins sharing partial homology with MHC class I molecules.** *J Biochem (Tokyo)* 1996, **119**:319-328.
 27. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, Hobby P, Sutton B, Tigelaar RE, Hayday AC: **Regulation of cutaneous malignancy by $\gamma\delta$ T cells.** *Science* 2001, **294**:605-609.
This paper provides evidence that carcinogen-induced skin tumors upregulate ligands for NKG2D.
 28. Carayannopoulos LN, Naidenko OV, Kinder J, Ho EL, Fremont DH, Yokoyama WM: **Ligands for murine NKG2D display heterogeneous binding behavior.** *Eur J Immunol* 2002, **32**:597-605.
 29. Malarkannan S, Shih PP, Eden PA, Horng T, Zuberi AR, Christianson G, Roopenian D, Shastri N: **The molecular and functional characterization of a dominant minor H antigen, H60.** *J Immunol* 1998, **161**:3501-3509.
 30. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH: **Rae1 and H60 ligands of the NKG2D receptor stimulate tumor immunity.** *Nature* 2001, **413**:165-171.
The authors demonstrate that tumor cells ectopically expressing ligands for NKG2D are efficiently rejected by NK cells and/or CD8⁺ T cells. Mice that had rejected ligand expressing tumor cells were immune to the rechallenge with the wild-type tumor, suggesting that this approach may be applicable as an anti-tumor vaccine.
 31. Cosman D, Müllberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ: **ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor.** *Immunity* 2001, **14**:123-133.
The authors describe the identification of a second family of human ligands for the NKG2D receptor, which are related to mouse Rae1 and H60. Interestingly, these proteins also interact with UL16 encoded by HCMV, suggesting that this virus may have developed an immune evasion strategy to avoid recognition via NKG2D.
 32. Onda H, Ohkubo S, Shintani Y, Ogi K, Kikuchi K, Tanaka H, Yamamoto K, Tsuji I, Ishibashi Y, Yamada T *et al.*: **A novel secreted tumor antigen with a glycosylphosphatidylinositol-anchored structure ubiquitously expressed in human cancers.** *Biochem Biophys Res Commun* 2001, **285**:235-243.
 33. Radosavljevic M, Cuillierier B, Wilson MJ, Clement O, Wicker S, Gilfillan S, Beck S, Trowsdale J, Bahram S: **A cluster of ten novel MHC class I related genes on human chromosome 6q24.2-q25.3.** *Genomics* 2002, **79**:114-123.
 34. Kubin M, Cassiano L, Chalupny J, Chin W, Cosman D, Fanslow W, Müllberg J, Rousseau AM, Ulrich D, Armitage R: **ULBP1, 2, 3: novel MHC class I-related molecules that bind to human cytomegalovirus glycoprotein UL16, activate NK cells.** *Eur J Immunol* 2001, **31**:1428-1437.
 35. Sutherland CL, Chalupny NJ, Schooley K, VandenBos T, Kubin M, Cosman D: **UL16-binding proteins, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells.** *J Immunol* 2002, **168**:671-679.
 36. Cerwenka A, Baron JL, Lanier LL: **Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*.** *Proc Natl Acad Sci USA* 2001, **98**:11521-11526.
See annotation to [30**].
 37. Hayakawa Y, Kelly JM, Westwood J, Darcy PK, Diefenbach A, Raulet DH, Smyth MJ: **Cutting edge: tumor rejection mediated by NKG2D receptor-ligand interaction is strictly dependent on perforin.** *J Immunol* 2002, **169**:5377-5381.
This paper presents evidence that rejection of NKG2D ligand expressing RMA and RMA-S tumor cells by NK cells and/or CD8⁺ T cells strictly depends on perforin but not IFN- γ .
 38. Brennan J, Mager D, Jefferies W, Takei F: **Expression of different members of the Ly-49 gene family defines distinct natural killer cell subsets and cell adhesion properties.** *J Exp Med* 1994, **180**:2287-2295.
 39. Smith KM, Wu J, Bakker AB, Phillips JH, Lanier LL: **Ly-49D and Ly-49H associate with mouse DAP12 and form activating receptors.** *J Immunol* 1998, **161**:7-10.
 40. Smith HRC, Chuang HH, Wang LL, Salcedo M, Heusel JW, Yokoyama WM: **Nonstochastic coexpression of activation receptors on murine natural killer cells.** *J Exp Med* 2000, **191**:1341-1354.
 41. Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM: **Specific and nonspecific NK cell activation during virus infection.** *Nat Immunol* 2001, **2**:951-956.
 42. Lanier LL, Bakker AB: **The ITAM-bearing transmembrane adaptor DAP12 in lymphoid and myeloid cell function.** *Immunol Today* 2000, **21**:611-614.
 43. Chung DH, Seaman WE, Daws MR: **Characterization of TREM-3, an activating receptor on mouse macrophages: definition of a family of single Ig domain receptors on mouse chromosome 17.** *Eur J Immunol* 2002, **32**:59-66.
 44. Bouchon A, Dietrich J, Colonna M: **Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes.** *J Immunol* 2000, **164**:4991-4995.
 45. Bouchon A, Hernandez-Munain C, Cella M, Colonna M: **A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells.** *J Exp Med* 2001, **194**:1111-1122.
 46. Daws MR, Lanier LL, Seaman WE, Ryan JC: **Cloning and characterization of a novel mouse myeloid DAP12-associated receptor family.** *Eur J Immunol* 2001, **31**:783-791.
 47. Vitale M, Bottino C, Sivori S, Sanseverino L, Castraconi R, Marcanaro E, Augugliaro R, Moretta L, Moretta A: **NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis.** *J Exp Med* 1998, **187**:2065-2072.
 48. Cantoni C, Bottino C, Vitale M, Pessino A, Augugliaro R, Malaspina A, Parolini S, Moretta L, Moretta A, Biassoni R: **NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily.** *J Exp Med* 1999, **189**:787-796.
 49. Mandelboim O, Lieberman N, Lev M, Lada P, Arnon TI, Bushkin Y, Davis DM, Strominger JL, Yewdell JW, Porgador A: **Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells.** *Nature* 2001, **409**:1055-1060.
 50. Medzhitov R, Janeway CA Jr.: **Decoding the patterns of self and nonself by the innate immune system.** *Science* 2002, **296**:298-300.
 51. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R: **Toll-like receptors control activation of adaptive immune responses.** *Nat Immunol* 2001, **2**:947-950.
Using MyD88-deficient mice, the authors provide evidence that TLRs are involved in the priming of potent adaptive immune responses.
 52. Diefenbach A, Tomasello E, Lucas M, Jamieson AM, Hsia JK, Vivier E, Raulet DH: **Selective associations with signaling molecules determines stimulatory versus costimulatory activity of NKG2D.** *Nat Immunology* 2002, **3**: in press.

This paper provides biochemical, molecular and genetic evidence that NKG2D associates with both KARAP/DAP12 and DAP10 in NK cells and macrophages and solely with DAP10 in CD8⁺ T cells. Accordingly, NK cells and macrophages from KARAP/DAP12 loss-of-function mice were severely impaired in NKG2D-mediated functions, whereas NKG2D co-stimulatory function was unimpaired in CD8⁺ T cells. Ectopic expression of KARAP/DAP12 in CD8⁺ T cells conveyed direct stimulatory functions to the NKG2D receptor in these cells.

53. Gilfillan S, Ho EL, Cella M, Yokoyama WM, Colona M: **NKG2D recruits two distinct adapters to trigger natural killer cell activation and costimulation.** *Nat Immunology* 2002, **3**: in press. This paper describes the generation and analysis of DAP10 gene-targeted mice. Surprisingly, the NKG2D receptor was functional in activated NK cells. Strikingly, DAP10 deficient mice rejected tumor cells

expressing NKG2D ligands. In contrast, NKG2D function in CD8⁺ T cells was abolished. These data support the notion that NKG2D function depends on DAP10 signaling in T cells, whereas in the innate immune system NKG2D uses KARAP/DAP12 to provide directly stimulatory signals.

54. Groh V, Wu J, Yee C, Spies T: **Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation.** *Nature* 2002, **419**:734-738.

This paper provides *ex vivo* evidence that tumor cells release soluble MICA/B proteins that lead to significant downregulation of NKG2D on the surface of CD8⁺ T cells. CD8⁺ T cells that have downregulated NKG2D are no longer co-stimulated by tumor cells expressing NKG2D ligands. These data provide the first evidence for a mechanism employed by tumor cells to evade immune recognition by the NKG2D receptor.