PERGAMON

Molecular Immunology 38 (2001) 1029–1037

Molecular Immunology

www.elsevier.com/locate/molimm

Review

Asymmetric ligand recognition by the activating natural killer cell receptor NKG2D, a symmetric homodimer

Roland K. Strong*

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Received 5 September 2001; accepted 26 November 2001

Abstract

Natural killer (NK) cells function through a diverse array of cell-surface natural killer receptors (NCRs). NCRs specific for classical and non-classical MHC class I proteins, expressed in complex patterns of inhibitory and activating isoforms on overlapping, but distinct, subsets of NK cells, play an important role in immunosurveillance against cells that have reduced MHC class I expression as a result of infection or transformation. Another NCR, NKG2D, is an activating NCR first identified on NK cells, but subsequently found on macrophages and a variety of T cell types. NKG2D ligands in rodents include the MHC class I-like proteins RAE-1 and H60 and, in humans, ULBPs and the cell stress-inducible proteins MICA and MICB. NKG2D–MIC and –RAE-1 recognition events have been implicated in anti-viral and -tumor immune responses. Crystallographic analyses of NKG2D–MICA and –RAE-1 complexes reveal an unusual mode of recognition that apparently tolerates a surprising degree of ligand plasticity while generating affinities that are among the strongest TCR– or NCR–ligand affinities, thus, far described.

Keywords: Natural killer cell receptors; NKG2D; MICA; RAE-1; Receptor–ligand recognition; Structural immunology

1. Immunoreceptors on T cells and NK cells

Cytotoxic responses by the cellular arm of the adaptive immune system are ultimately mediated by recognition events between αβ T cell receptors (TCRs) on the surfaces of T cells and processed peptide fragments of endogenous proteins. These peptides are presented to TCRs as complexes on the cell-surface with major histocompatibility complex (MHC) class I proteins (Germain and Margulies, 1993). MHC class I proteins are integral-membrane, hetero-dimeric proteins with ectodomains consisting of a polymorphic heavy chain, comprising three extracellular domains (α1, α2 and α3), associated with a non-polymorphic light chain, β_2-microglobulin (β_2-m) (Bjorkman and Parham, 1990). Association with both peptide and β_2-m is required for folding and normal cell-surface expression. The α1 and α2 domains together comprise the peptide- and TCR-binding "platform" domain; the α3 and β_2-m domains have C-type immunoglobulin folds. Crystal structures of TCR–MHC complexes show that the TCR variable domains sit diagonally on the MHC platform domain, making contacts to the peptide and the MHC α1 and α2 domains (Fig. 1) (Garcia et al., 1999). T cell activation requires an interaction between TCRs and appropriate MHC–peptide complexes in the context of appropriate co-stimulatory signals from, for example, engagement of the CD28 receptor on T cells with CD80 or CD86 ligands on target cells (Lenschow et al., 1996). Diverse cell-surface molecules also modulate T cell activation, including receptors first identified on natural killer (NK) cells that have since been found expressed on a range of cell types including T cells.

NK cells constitute an important component of the innate immune system, providing surveillance against cells undergoing tumorigenesis or infection by viruses or internal pathogens (Trinchieri, 1989). NK cells act to regulate innate and acquired immune responses through the release of various immune modulators, such as interferon-γ, or by directly destroying compromised cells. They function through a diverse array of cell-surface inhibitory and activating receptors. Many cell-surface NK receptors (NCRs) are specific for classical (HLA-A, -B and -C) and non-classical (HLA-E) MHC class I proteins and occur in paired activating and inhibitory isoforms (Bakker et al., 2000).

Abbreviations: β_2-m, β_2-microglobulin; huNKG2D, human NKG2D; KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; muNKG2D, murine NKG2D; NCR, natural killer receptor; NK, natural killer; NKD, C-type lectin-like NK receptor domain; TCR, T cell receptor; ULBP, hCMV UL16 binding protein

* Tel.: +1-206-667-5587; fax: +1-206-667-7730.

E-mail address: rstrong@fhcrc.org (R.K. Strong).

0161-5890/02/$ – see front matter
PII: S0161-5890(02)00032-9

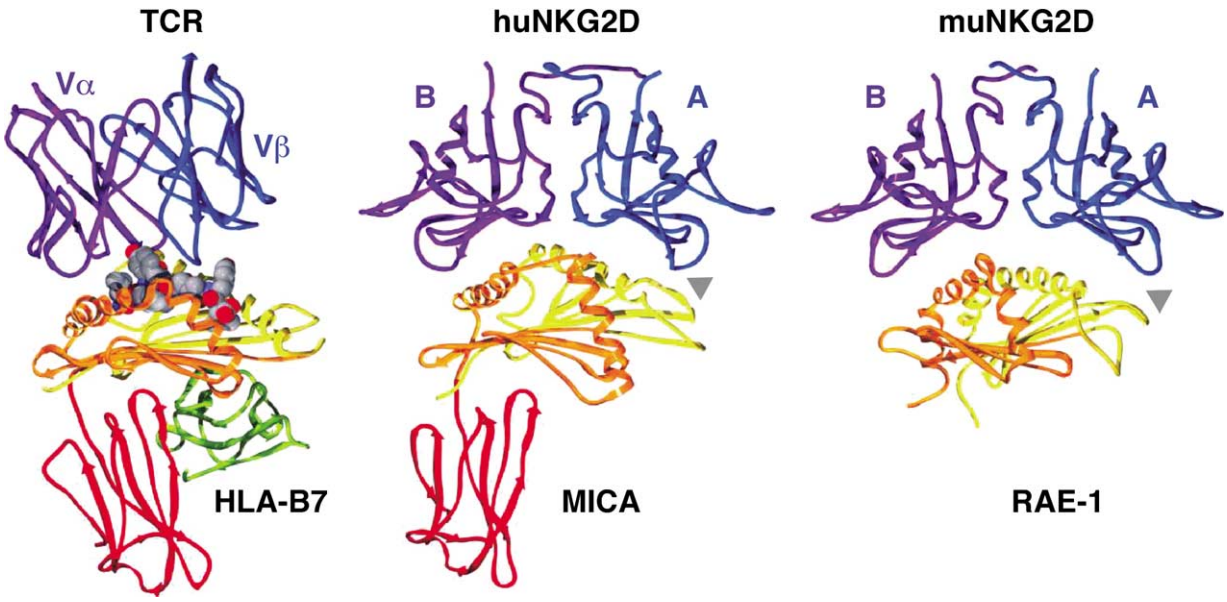

Fig. 1. Immunoreceptor–ligand complexes. Ribbon representations of the crystal structures of three complexes: TCR–MHC class I, exemplified by the B7–Tax–HLA-A2 complex (Ding et al., 1998) (left), huNKG2D–MICA (middle) and muNKG2D–RAE-1 (right). Individual domains are colored as α1: yellow; α2: orange; α3 (when present): red; and β_2-m (when present): green for the ligands and blue and purple for the receptors. Receptor domains are labeled. α-Helices are represented as coils; β-strands as arrows. The peptide in the MHC class I protein is shown in space-filling style and colored by atom-type. Arrows indicate contacts between the $\beta1\beta_2$ loops in the α1 domains of MICA and RAE-1 and the β5′β5 stirrup loops of human and muNKG2D. Views are perpendicular to the homodimer dyad axis of the NKG2Ds; the TCR–MHC class I complex is positioned such that the platform domain of the MHC class I protein is in the same orientation as MICA and RAE-1 in those complexes.

Different NCRs, with different MHC class I specificities, are expressed on overlapping, but distinct, subsets of NK cells, where the strength of the inhibitory signals may be stronger than stimulatory signals. Thus, NK cell effector functions are regulated by integrating signals across the array of stimulatory and inhibitory NCRs engaged upon interaction with target cell-surface NCR ligands, resulting in the elimination of cells with reduced MHC class I expression, a common consequence of infection or transformation (Lanier, 2000).

NCRs can be divided into two families based on structural homologies. The first family, including the killer cell inhibitory receptors (KIR), consists of type I transmembrane glycoproteins containing one to three tandem immunoglobulin-like domains in the ectodomain. The second NCR family comprises homo- and hetero-dimeric type II transmembrane glycoproteins containing C-type lectin-like NK receptor domains (NKDs) (Weis et al., 1998). Crystal structures are available for members of both families: the immunoglobulin-like p58 (Fan et al., 1997), KIR2DL1 (Fan et al., 1997), KIR2DL2 (Snyder et al., 1999), KIR2DL3 (Maenaka et al., 1999), KIR2DL2 in complex with HLA-Cw3 (Boyington et al., 2000) and KIR2DL1 in complex with HLA-Cw4 (Fan et al., 2001); and the NKDs human CD94 (Boyington et al., 1999), murine Ly49A in complex with the murine class I protein H-$2D^d$ (Tormo et al., 1999), human NKG2D (huNKG2D) in complex with MICA (Li et al., 2001) and murine NKG2D (muNKG2D) in complex with RAE-1 (Li et al., 2002).

NKG2D is an activating, NKD-type immunoreceptor whose expression was first recognized on NK cells, but was subsequently found on $CD8^+$ αβ T cells, γδ T cells and macrophages making it one of the most widely distributed NCRs currently described (Bauer et al., 1999; Wu et al., 1999). Other members of the NKG2 family (A, C, E and F) form obligate hetero-dimers with CD94, are highly homologous to each other (approximately 90% identical) and are specific for the non-classical MHC class I protein HLA-E. NKG2D displays only limited sequence similarity to other NKG2 family members and CD94 (20–30% identical), has not been demonstrated to directly interact with MHC class I proteins and forms homodimers (Li et al., 2001; Steinle et al., 2001). NKG2D engagement is signaled by recruitment of phosphatidylinositol 3-kinase through the adapter molecule DAP10 (Wu et al., 1999, 2000), whereas other activating NCRs utilize the DAP12 adaptor molecule (Lanier et al., 1998).

HuNKG2D ligands include the closely related proteins MICA and MICB (MHC class I chain-related) (Bahram et al., 1994; Bahram and Spies, 1996; Groh et al., 1996) and the ULBPs (CMV UL16-binding proteins) (Cosman et al., 2001). All are distant MHC class I homologs that do not function in conventional peptide antigen presentation. HuNKG2D–MIC recognition events stimulate effector responses from NK cells and γδ T cells and may positively modulate $CD8^+$ αβ T cell responses, thus, serving a co-stimulatory function (Bauer et al., 1999; Groh et al.,

1998). On macrophages, stimulation through huNKG2D triggers TNFα production and release of nitric oxide (Diefenbach et al., 2000). On NK cells, the huNKG2D-MIC signal can override any inhibitory signals present (Bauer et al., 1999; Cerwenka et al., 2000).

Unlike the widely- and constitutively-expressed classical and non-classical MHC class I proteins, MICA and MICB are induced in response to cellular stress on intestinal epithelium and epithelially-derived tumors (Groh et al., 1996, 1999). While MICA and MICB are quite similar to each other (84% identical (Bahram et al., 1996; Bahram and Spies, 1996)), they have diverged significantly from the MHC class I family as a whole, with identities of 28–35% domain-by-domain when aligned with the human MHC class I proteins. MICA and MICB are conserved in most mammals except rodents. MIC proteins do not require either peptide or β_2-m for stability or cell-surface expression (Groh et al., 1996).

ULBPs are homologous to the α1α2 peptide-binding platform domains of MHC class I proteins, but lack α3 domains, and are anchored in the membrane by GPI-linkages. ULBP1, 2 and 3 are 23–27% identical in sequence to MICA, MICB or classical MHC class I proteins (Cosman et al., 2001). The functional significance of the huNKG2D–ULBP interaction remains to be determined, though UL16–ULBP/UL16–MIC binding blocks huNKG2D–ULBP/huNKG2D–MIC interactions, thus, potentially representing a viral strategy to mask these antigens, blocking activation through NKG2D and preventing anti-viral innate immune responses (Cosman et al., 2001).

MuNKG2D and huNKG2D ectodomains are 69% identical. Rodents lack any recognizable homologs of MICA and MICB, but muNKG2D ligands include the RAE-1 (retinoic acid early inducible) family of proteins and H60 (Cerwenka et al., 2000; Diefenbach et al., 2000; Zou et al., 1996). Like the ULBPs, RAE-1 and H60 are homologous to the platform domains of MHC class I proteins (RAE-1 is 19–20% identical to a bovine MHC class I protein (Zou et al., 1996)), lack α3 domains, and are also anchored in the membrane by GPI-linkages. RAE-1 and H60 show only weak homology to each other (approximately 24%) or to MICA and MICB (approximately 20%) (Cerwenka et al., 2000). The RAE-1 family comprises four highly-homologous isoforms (≥92% identical), RAE-1α, β, γ and δ, which are highly expressed during embryonic development, but are rare in normal adult tissues (Cerwenka et al., 2000; Diefenbach et al., 2000). It has been recently shown that tumors expressing RAE-1 molecules can be recognized by NK cells and rejected (Cerwenka et al., 2001). Like huNKG2D–MIC stimulation of NK cells, RAE-1 mediated rejection can override inhibitory signals from the expression of self MHC class I proteins on the tumor cells. H60 was originally identified as an immunodominant minor histocompatibility antigen (Malarkannan et al., 1998, 2000). Though differentially expressed in inbred mouse strains, H60 transcripts were found at low levels in embryonic tissue but at higher levels on macrophages and dendritic cells in the spleen (Malarkannan et al., 2000).

2. Solution studies of NKG2D receptor–ligand interactions

Most NCR–ligand and many TCR–ligand interactions with host MHC proteins have dissociation constants in the tens-of-micromolar range (Table 1). However, huNKG2D– and muNKG2D–ligand interactions are one to two orders of magnitude stronger (Table 1). Kinetic analysis of the huNKG2D–MICA interaction yielded a dissociation rate constant (k_{off}) at 37° of $0.04\,s^{-1}$, indicating that the huNKG2D–MICA interaction may also be relatively more stable than TCR–ligand and other NCR–ligand complexes (Li et al., 2001). The association rate constant (k_{on}), $4–7 \times 10^4\,M^{-1}\,s^{-1}$, was slow relative to many other cell-surface interactions, which typically have $k_{on} \geq 1 \times 10^5\,M^{-1}\,s^{-1}$ (Willcox et al., 1999). The analysis also indicated that both association and dissociation rate constants are somewhat temperature dependent, indicating that activation energy barriers impede association and dissociation. This behavior is shared with TCR–MHC interactions, where binding is proposed to be accompanied by ordering flexible loops in the TCR (Willcox et al., 1999). The huNKG2D–MICA kinetic analysis also shows that ionic interactions do not predominate, as is the case for some other NCR–ligand interactions, particularly KIRs.

Binding studies show that RAE-1 and H60 compete directly for occupancy of muNKG2D, demonstrating a shared, or overlapping, binding site (O'Callaghan et al., 2001). The muNKG2D–H60 interaction is more temperature dependent

Table 1
Immunoreceptor affinities

Receptor	Ligand	K_D (μM)	References
TCR	MHC class I	1–90	Davis et al. (1998); Willcox et al. (1999)
CD8	MHC class I	65–200	Kern et al. (1999); Wyer et al. (1999)
NKG2A–CD94	HLA-E	11.23	Vales-Gomez et al. (1999)
KIR	MHC class I	∼10	Vales-Gomez et al. (1998)
LIR-1	MHC class I	15–100	Chapman et al. (1999)
huNKG2D	MICA	0.3	Li et al. (2001)
muNKG2D	H60	0.0189	O'Callaghan et al. (2001)
muNKG2D	RAE-1α, β, γ, δ	0.345–0.726	O'Callaghan et al. (2001)

and makes greater use of electrostatic interactions (estimated as contributing 37% to the free energy of binding) than the muNKG2D–RAE-1 interaction (estimated as contributing 21% to the free energy of binding (O'Callaghan et al., 2001). The binding of muNKG2D to RAE-1 and H60 was characterized by relatively fast association rates compared to TCR–MHC class I interactions (O'Callaghan et al., 2001). Entropic changes during binding suggest that dominant effect in the muNKG2D–H60 interaction is from stabilization of flexible protein loops at the interface, and from displacement of bound water at the muNKG2D–RAE-1 interface (O'Callaghan et al., 2001).

The binding analysis of the muNKG2D–H60 and –RAE-1 interactions, and size-exclusion chromatographic analysis of the huNKG2D–MICA interactions (Steinle et al., 2001), indicate that the stoichiometry of the NKG2D complexes were 2:1, meaning that one NKG2D homodimer binds a single monomeric ligand, either H60, RAE-1 or MICA. This is an unusual, though not an unprecedented, result in that the simplest binding mode for a symmetric homodimer is to interact with two monomeric ligands through two identical binding sites (Fig. 2). 2:1 complexes can result when the symmetric receptor binding sites are positioned such that binding of ligand at one site blocks binding at the second (for example, both Ly49A binding sites on H-2D^d (Tormo et al., 1999)) or when the receptor binding site has evolved the ability to recognize two distinct ligand surfaces (for examples, CD8αα binding to HLA-α2 (Gao et al., 1997) or H-2K^b (Kern et al., 1998) or the human growth hormone receptor–ligand complex (de Vos et al., 1992)). The CD8–MHC class I interaction, however, has one CD8 monomer dominating the binding to ligand, and growth hormone receptor generates two different ligand binding sites, interacting with multiple, distinct surfaces on the hormone ligand, partly through interdomain flexibility in the receptor.

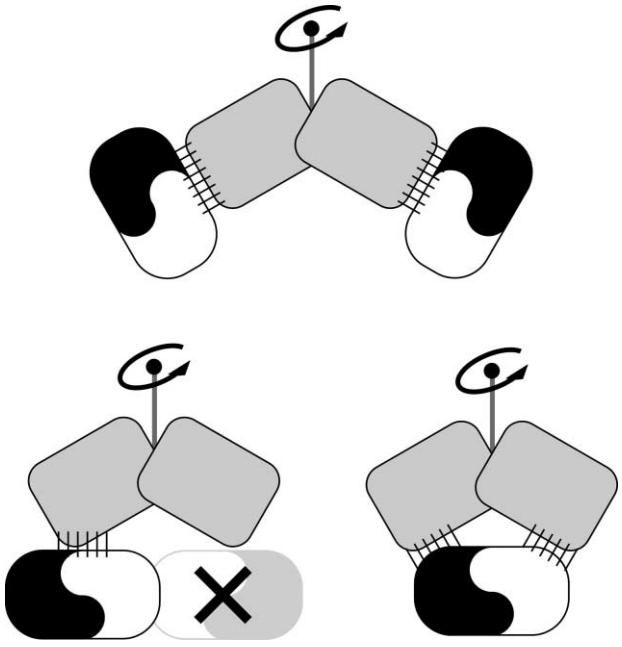

Fig. 2. Possible interactions between symmetric receptors and asymmetric ligands. Using identical binding sites on a symmetric, homodimeric receptor, an asymmetric ligand can bind in three different ways. The simplest interaction (top) is where two ligands bind in an identical manner to two identical, independent receptor binding sites yielding a 2:2 complex. 2:1 receptor:ligand complexes can result either when the two identical receptor binding sites are positioned such that binding of ligand at one site sterically blocks ligand binding at the second (bottom left) or if the two identical receptor binding sites are capable of interacting with distinct surfaces on the strictly asymmetric ligand (bottom right).

3. Structural studies of NKG2D receptor–ligand interactions

Crystal structures of MICA (Li et al., 1999) and RAE-1 (Li et al., 2002) revealed very distorted examples of MHC class I platform domains (Fig. 3). The platform domain of MHC class I proteins comprises two long, roughly parallel, α-helices, interrupted by bends, arranged on an eight-stranded β-sheet. These α-helices define the peptide binding groove in MHC class I homologs that bind peptides. MICA introduced the novel feature that a 10-residue segment, corresponding to the center section of the α2 domain helix of MHC class I proteins, was disordered, presumably forming a flexible loop. Flexible loops have also been observed in H-2T22, but corresponding to different secondary structure elements (Wingren et al., 2000). The platform and α3 domains of MICA are joined through a flexible linker, allowing considerable inter-domain flexibility, a feature unique to MICA among MHC class I homologs. The structure of RAE-1 is distorted from other MHC homologs in the arrangement of helical elements and displayed non-canonical disulfide bonds—one actually between helices in the α1 and α2 domains. The loss of any remnant of a peptide binding groove in RAE-1 is facilitated by the close approach of the groove-defining helices through a hydrophobic, leucine-rich interface, where other non-peptide-binding MHC class I homologs usually close off the groove mostly through salt bridges. MICA retains a small pocket in the center of the platform domain, but due to its size and polar character, is unlikely to bind any peptide or small-molecule ligand.

The recent crystal structures of the MICA–huNKG2D (Li et al., 2001) and the RAE-1β–muNKG2D (Li et al., 2002) complexes revealed that NKG2D homodimers bound to both MICA and RAE-1 monomers in interactions analogous to αβ T cell receptor–MHC class I protein complexes, with the NKG2D homodimer diagonally overlaid on the surface of the ligand platform (Fig. 1). This despite the facts that TCRs are constructed from immunoglobulin-like domains while NKG2D is assembled from NKDs, and that there are significant structural differences between the platform domains of MHC class I proteins and MICA and RAE-1. The stoichiometry was clearly one NKG2D homodimer binding to one ligand monomer. Each NKG2D monomer

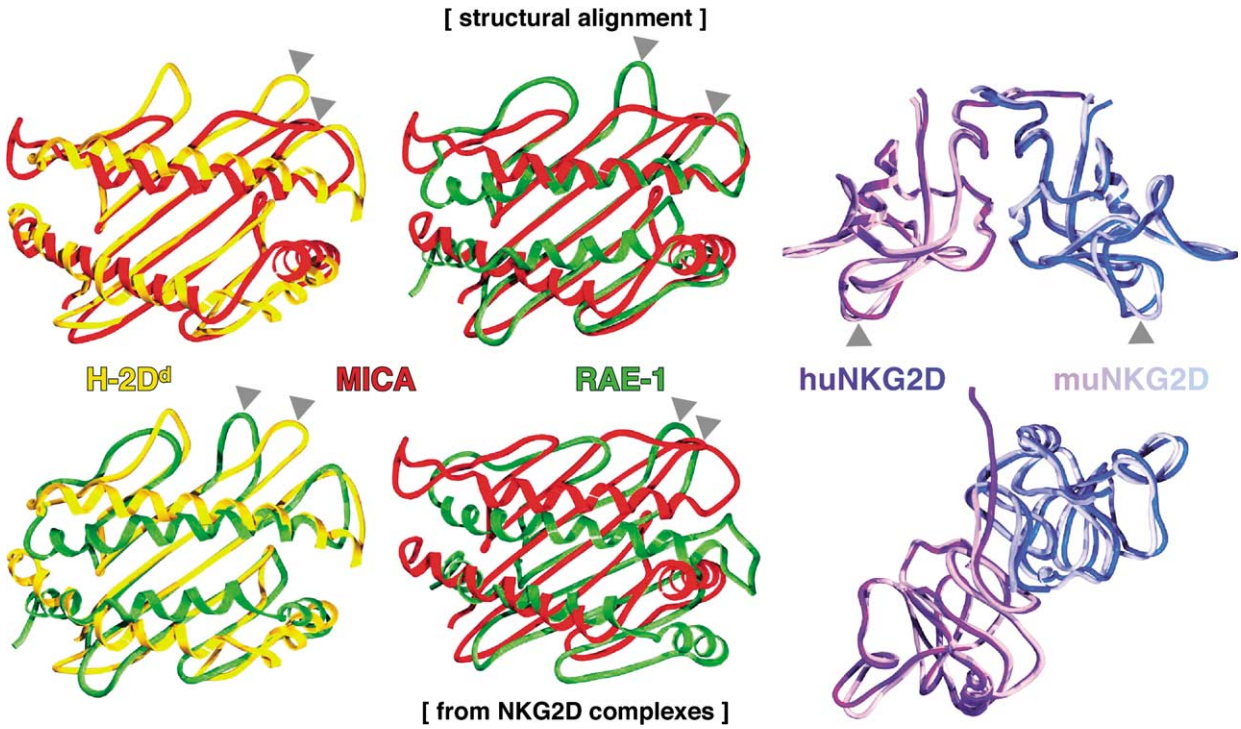

Fig. 3. Structural comparisons between MHC class I platform domains and NKG2Ds. Ribbon representations of superpositions between the platform domains of H-2D^d (yellow), MICA (red) and RAE-1 (green) and between huNKG2D (blue and purple) and muNKG2D (light blue and light purple). The ligand platform domain superpositions are based on structurally conserved Cα atoms in the platform domain β-sheet except for the bottom middle pair (MICA and RAE-1), which are superimposed on the basis of the alignment of muNKG2D and huNKG2D in the two complexes, thus, highlighting the relative repositioning of the ligand between the two complexes. NKG2Ds are superimposed on the basis of all common Cα atoms. Views of the ligand platform domains are down onto the receptor binding surface, as seen from the receptor. Two views of NKG2D are shown: perpendicular to the homodimer dyad axis (top right) and looking down onto the top of the homodimer down the homodimer dyad axis (bottom right). Arrows indicate analogous points in the $\beta1\beta_2$ loops in the α1 domains of the ligand platform domains and the β5′β5 stirrup loops of human and muNKG2D.

(NKG2D-A and -B) predominately contacts either the α1 or α2 domains of MICA or RAE-1, with the two sub-site interactions contributing approximately equally to the overall interaction, unlike the CD8αα–MHC class I interaction. As in TCR–MHC class I complexes, a peak on the surface of the α2 domain, between the α1 and α2a helices, helps lock in the diagonal receptor binding orientation. NKG2D binding sites on its MHC ligands are quite different from the footprints of the murine NKD immunoreceptor Ly49A on its ligand, the murine MHC class I protein H-2D^d (Tormo et al., 1999). The huNKG2D–MICA interaction is more extensive and involves greater shape complementarity than most TCR–MHC and NCR–ligand interactions while the muNKG2D–RAE-1 interface displays values more typical for these types of receptor complexes (Table 2). The shape complementarity in both complexes is sufficient to completely exclude solvent from the interface; no solvent-mediated contacts are seen in either crystal structure. The structures of muNKG2D and RAE-1 undergo little or no conformational changes upon complex formation, and, by extension, neither would huNKG2D. MICA, however, undergoes a dramatic ordering of the disordered loop in the α2 domain when complexed with huNKG2D. The loop forms over two additional turns of α-helix and a segment of random coil, and contributes a series of contacts with huNKG2D-B. Taken together, these structural details account for the quantitative binding results. The crystallographic and binding data show that carbohydrate does not play a role in NKG2D–ligand interactions.

NKG2D homodimers bind to MICA and RAE-1 through a surface comparable to the ligand binding surface of Ly49A (Tormo et al., 1999) and the proposed ligand binding surface of CD94 (Boyington et al., 1999). The increased curvature of the binding surface saddle of NKG2D, relative to both Ly49A and CD94, precisely complemented the decreased inter-helical distances of MICA and RAE-1 relative

Table 2
NKG2D complex parameters

Complex	Buried surface area (Å^2)	Surface complementarity
"Typical" protein–protein	1600	0.64–0.68 (Ab–Ag interactions)
TCR–MHC class I	1700–1900	0.43–0.70
KIR2DL–MHC class I	1485–1540	0.69–0.71
Ly49A–H-2D^d (site #1)	990	0.78
Ly49A–H-2D^d (site #2)	3350	0.54
huNKG2D–MICA	2180	0.72
muNKG2D–RAE-1	1700	0.63

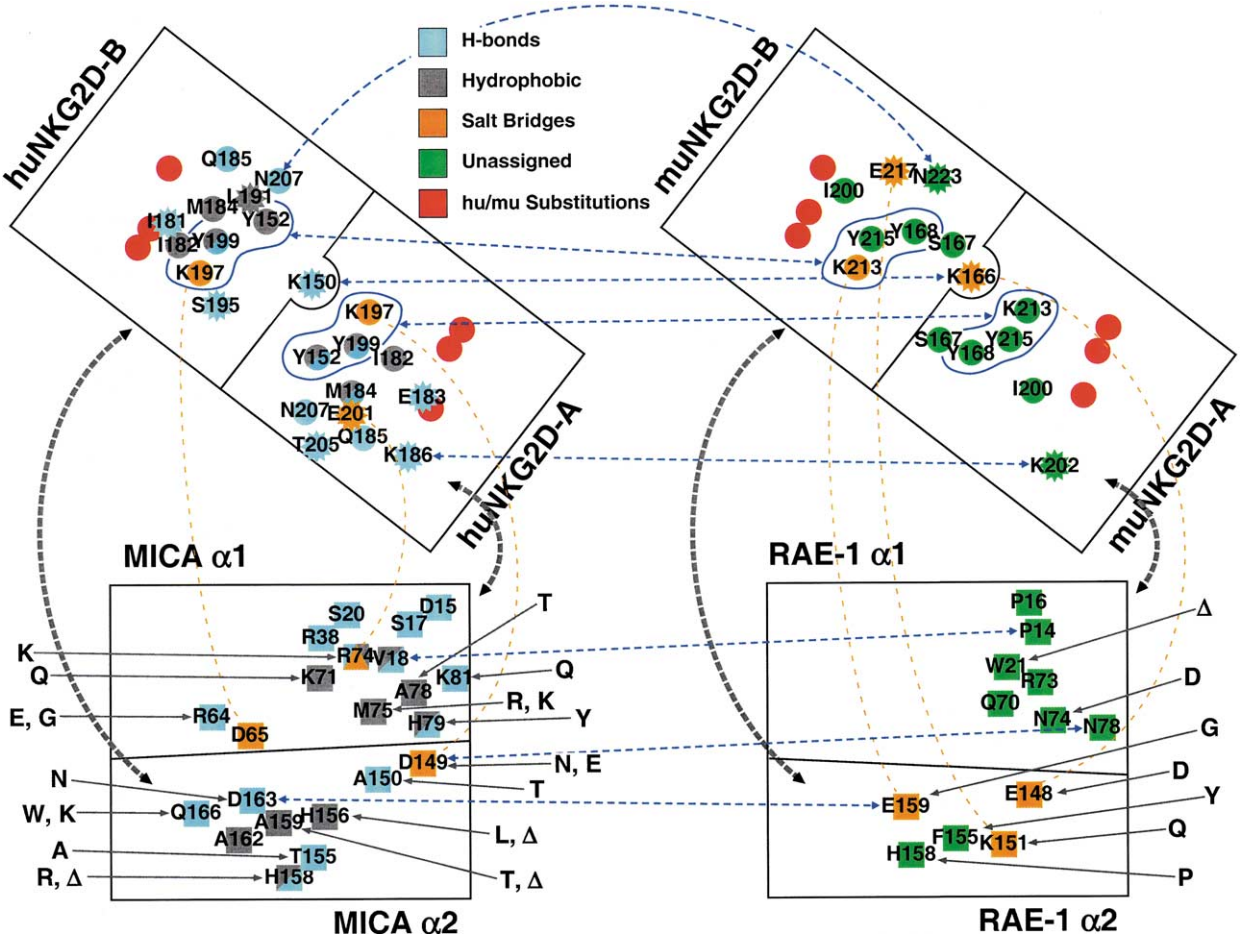

Fig. 4. Contact maps of the huNKG2D–MICA and muNKG2D–RAE-1 interfaces. Schematic representations of the position and identity of contact residues at the MICA–huNKG2D (left) and RAE-1–muNKG2D (right) interfaces. In these views, the proteins are represented as boxes, labeled, and shown in orientations looking down onto the two binding surfaces, as if the receptors had been peeled away from the ligands in the complexes. Contact residues are labeled and represented as circles (receptors) or squares (ligands) and colored by the type of bond mediating the contact: light blue, hydrogen bonds; gray, hydrophobic contacts; and orange, salt bridges. Aside from salt bridges, RAE-1–muNKG2D contacts are unassigned (green) due to the lower resolution of this structure. Salt bridges are connected by orange dotted lines to help orient the views. Positions of residues in the β5′β5 stirrup loops of human and muNKG2D where sequence substitutions occur are indicated by red circles. In the receptor binding sites, ligand contact residues unique to one homodimer site (NKG2D-A or -B) are shown as crenellated circles. Receptor contact residues in common between human and muNKG2D are linked by blue arrows and circles when clustered; residues situated in similar positions on the ligands, and that share similar bonding interactions, are also linked by blue arrows. The relatively fewer murine contact residues is a reflection of the less extensive muNKG2D–RAE-1 interface. Sequence substitutions between RAE-1 isoforms and of MIC residues that contact NKG2D among the human MICA, MICB and non-human primate sequences known to bind to human NKG2D are indicated (Groh et al., 1998; Steinle et al., 1998); deletions: Δ.

to other MHC class I homologs. Thus, NKG2D would not accommodate the platform domains of other MHC class I proteins or homologs, and other Ly49A and NKG2–CD94 hetero-dimers would be a poor match for MICA and RAE-1. MuNKG2D will also bind the huNKG2D ligand MICB, but huNKG2D does not bind to RAE-1 with appreciable affinity (Li et al., 2002). This is explained by changes in the conformations of the "stirrup" loop (β5′β5) of NKG2D, the largest difference between the structures of huNKG2D and muNKG2D (Figs. 3 and 4). The slightly narrower saddle of huNKG2D clashes with RAE-1, but not vice versa.

RAE-1 sits in the muNKG2D binding saddle slightly differently from the orientation of MICA in the complex with huNKG2D. When murine and huNKG2D in the two complexes are superimposed, RAE-1 is displaced from the position of the MICA platform domain by a rotation of 20–25° and a translation of approximately 7 Å (Fig. 3). The effect of this relative movement in the complexes is to bring certain ligand structural elements closer into alignment, such as helices in the α2 domain that provide multiple contacts to NKG2D-B, while moving many other elements apart, such as the α1 domain H2 helix and the H1 helix in the α2 domain (Fig. 3). The MICA platform loop that deviates most dramatically from other MHC class I structures, the first loop in the α1 domain ($\beta1\beta_2$), provides several key NKG2D-A stirrup loop contacts in the MICA–huNKG2D complex. The same loop in RAE-1 is much more similar in conformation to other MHC class I structures, but due to the

reorientation of the ligand in the NKG2D binding saddle, continues to provide comparable contacts to the corresponding muNKG2D-A stirrup loop (Fig. 3). However, despite these minor structural coincidences, the overall impression drawn from the superposition of the NKG2D ligands between the two complexes is that NKG2D uses the same surface to bind to two very different ligand surfaces. Based on these observations, H60, despite limited sequence similarity and the expectation that its structure is likely to be as novel an example of an MHC class I fold as RAE-1, would also be predicted to interact with muNKG2D in a very similar manner. Models of NKG2–CD94–HLA-E complexes, some of which are quite detailed, also predict a similar arrangement of domains, and use of similar binding surfaces, to the NKG2D complex crystal structures (Boyington et al., 1999; Li et al., 2001).

4. Recognition plasticity of the NKG2D binding site

The contacts at the NKG2D–MICA and –RAE-1 interfaces are mapped in Fig. 4 and consist of a mixture of polar, hydrophobic and ionic interactions (the resolution of the murine complex structure is insufficient to confidently assign bonds other than salt bridges). The Ly49A–H-2D^d and KIR2DL–HLA-C interfaces, in contrast, are dominated by ionic interactions. The binding sub-sites on NKD2D-A and -B in both complexes involve the same surface on NKG2D monomers. Therefore, not only has NKG2D evolved a binding surface that is capable of specific recognition of *two* distinct ligands (MICA and RAE-1), but a surface capable of recognizing *four* very different surfaces: the α1 and α2 domains of MICA and RAE-1 separately. Binding is achieved by utilizing a core set of residues in common between the NKG2D-A and -B sub-sites (huNKG2D or muNKG2D residues represented by non-crenellated circles in Fig. 4). Specificity is achieved because these core residues are capable of making different contacts to different residues in either the α1 and α2 domains of the ligands (Fig. 4). For example, Lys197 in huNKG2D, equivalent to Lys213 in muNKG2D, makes contacts to ligand residues in both NKG2D-A and -B. In the huNKG2D–MICA complex, Lys197 (NKG2D-A) forms a salt bridge to Asp149 in the α2 domain of MICA and Lys197 (NKG2D-B) forms another salt to Asp65 in the α1 domain of MICA. But in the muNKG2D–RAE-1 complex, Lys213 (NKG2D-A) contacts Asn78 in the α1 domain of RAE-1, likely forming a hydrogen bond, while Lys213 (NKG2D-B) forms a salt bridge to Glu159 in the α2 domain (Fig. 4). When complexes are superimposed, the side-chain of Asp149 in MICA does come very close to the side-chain of Asn78 in RAE-1 in essentially the only example of a conserved interaction in the four sub-site structures. But the bonding partner of Lys213 in RAE-1, Glu159, superimposes on Asp163 in MICA, not Asp65. Only very small rearrangements of side-chains are necessary to alter these bonding patterns—and there are few significant conformational changes between the four NKG2D binding site structures. The largest example is Tyr152 in huNKG2D, where the side-chains in the two huNKG2D sub-sites have moved approximately 6.5 Å at the hydroxyl group.

To this core set of residues, asymmetrical binding interactions are added from residues not shared between NKG2D-A and -B sub-sites (residues marked with crenellated circles in Fig. 4). The ability to make such asymmetrical interactions is likely key for utilizing a single NKG2D surface to bind to a series of different ligand surfaces with minimal spatial rearrangements. For example, Lys150 in huNKG2D-B (Lys166 in muNKG2D-B) makes a hydrogen bond to the backbone carbonyl oxygen of Alα150 in MICA, but Lys150 in huNKG2D-A does not make any MICA contacts. Lys166 in muNKG2D-B makes a salt bridge to Glu159 in RAE-1, but Lys166 in muNKG2D-A also does not make any RAE-1 contacts. This remarkable binding plasticity is essentially built into the NKG2D binding site since all the residues at or near the NKG2D binding surface are conserved between human and murine proteins except for three residues in the stirrup loops (Fig. 4).

Even more remarkable, though, is that this single binding site on NKG2D has evolved the ability to tolerate dramatic substitutions and deletions of ligand contact residues. HuNKG2D has been reported to bind to various human MICA and MICB alleles and non-human primate MIC proteins, though with varying relative affinities (Groh et al., 1998; Steinle et al., 1998). MuNKG2D binds to all the of RAE-1 isoforms, over an approximately two-fold range of affinity (Table 1). When these sequence substitutions and deletions are mapped onto the MICA and RAE-1 protein structures, many contact residues are affected (Fig. 4). While some changes are quite conservative, and would not be predicted to directly affect affinity, many are quite dramatic and would have been predicated as likely having larger effects on affinity than observed. Considering only human MICA and MICB allelic differences, the positions of the substitutions are fairly evenly distributed over the surface of the platform domain, though cluster somewhat to the α2 domain in MICA and to the helix side of the platform domain in MICB (Holmes, M.A., and Strong, R.K., unpublished results). When all human and non-human NKG2D-binding MIC sequences are considered, the substitutions clearly cluster on the NKG2D-binding side of the platform domain, leaving two "patches" of well conserved residues on the underside, one of which corresponds to the β_2-m-binding binding surface of classical MHC class I proteins. Prior to the determination of the huNKG2D–MICA complex structure, these patches had been proposed as possible receptor binding sites—a possibility since this surface is not blocked by β_2-m association and is quite accessible due to the inter-domain flexibility (Li et al., 1999). It remains formally possible that these sites represent additional MIC interaction surfaces with other molecules.

However, some substitutions distant from the interaction surfaces may also affect binding, presumably through indirect conformational changes. The methionine-to-valine substitution at position 129 in the α2 domain of MICA, a conservative substitution which has no atom closer than 21 Å to any atom of NKG2D, has been experimentally shown to have a 30-fold affect on the affinity for NKG2D (Steinle et al., 2001 and Holmes, M.A., Willcox, B.E., Li, P., and Strong, R.K., unpublished results). Also, some of the characterized *MIC* allelic differences are known to dramatically affect folding and cell-surface expression of MICA. The arginine-to-proline substitution at position six in *MICA010* is an example, where introduction of a proline would obviously disrupt the platform β-sheet, with the result that this allele is essentially null (Li et al., 2000).

It remains unclear exactly how NKG2D can tolerate such plasticity in ligand binding sites while retaining specificity and significant affinities. Further crystallographic, binding and mutagenesis studies will be required to parse the rules of molecular recognition for this singular receptor system. But the question remains: what is the functional role of NKG2D ligand polymorphisms? One speculation is that by varying the affinity (and therefore the half-life) of NKG2D–ligand interactions through expression of different ligands allows for NKG2D-mediated activation signals with a range of strengths. This would then allow greater flexibility in the decision of an effector cell to activate or not, depending upon the activation threshold set by the constellation of other stimulatory and inhibitory signals received through engagement of other cell-surface receptors. However, there is no experimental evidence that supports differential expression of *MICA* versus *MICB*, or of one allele versus the other, in response to any form of cellular stress. Nor is there yet any compelling reason to support the observed *MIC* polymorphism present in the population.

Acknowledgements

The author thanks those colleagues who have participated in or collaborated on these studies: Pingwei Li, Daniel L. Morris, Gerry McDermott, Alexander Steinle and Thomas Spies. This work was supported by the NIH (R01 AI48675) and the Pendleton Fund.

References

Bahram, S., Spies, T.A., 1996. Nucleotide sequence of a human MHC class I MICB cDNA. Immunogenetics 43, 230–233.

Bahram, S., Bresnahan, M., Geraghty, D.E., Spies, T.A., 1994. A second lineage of mammalian major histocompatibility complex class I genes. Proc. Natl. Acad. Sci. U.S.A. 91, 6259–6263.

Bahram, S., Mizuki, N., Inoko, H., Spies, T.A., 1996. Nucleotide sequence of the human MHC class I MICA gene. Immunogenetics 44, 80–81.

Bakker, A.B., Wu, J., Phillips, J.H., Lanier, L.L., 2000. NK cell activation: distinct stimulatory pathways counterbalancing inhibitory signals. Hum. Immunol. 61, 18–27.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., Spies, T., 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 285, 727–729.

Bjorkman, P.J., Parham, P., 1990. Structure, function and diversity of class I major histocompatibility complex molecules. Ann. Rev. Biochem. 90, 253–288.

Boyington, J.C., Riaz, A.N., Patamawenu, A., Coligan, J.E., Brooks, A.G., Sun, P.D., 1999. Structure of CD94 reveals a novel C-type lectin fold: implications for the NK cell-associated CD94/NKG2 receptors. Immunity 10, 75–82.

Boyington, J.C., Motyka, S.A., Schuck, P., Brooks, A.G., Sun, P.D., 2000. Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand. Nature 405, 537–543.

Cerwenka, A., Bakker, A.B., McClanahan, T., Wagner, J., Wu, J., Phillips, J.H., Lanier, L.L., 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. Immunity 12, 721–727.

Cerwenka, A., Baron, J.L., Lanier, L.L., 2001. Ectopic expression of retinoic acid early inducible-1 gene (*RAE-1*) permits NK cell-mediated rejection of a MHC class I-bearing tumor in vivo. Proc. Natl. Acad. Sci. U.S.A. 98, 11521–11526.

Chapman, T.L., Heikema, A.P., Bjorkman, P.J., 1999. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL-18. Immunity 11, 603–613.

Cosman, D., Mullberg, J., Sutherland, C.L., Chin, W., Armitage, R., Fanslow, W., Kubin, M., Chalupny, N.J., 2001. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 14, 123–133.

Davis, M.M., Boniface, J.J., Reich, Z., Lyons, D., Hampl, J., Arden, B., Chien, Y., 1998. Ligand recognition by alpha beta T cell receptors. Ann. Rev. Immunol. 16, 523–544.

de Vos, A.M., Ultsch, M., Kossiakoff, A.A., 1992. Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science 255, 306–312.

Diefenbach, A., Jamieson, A.M., Liu, S.D., Shastri, N., Raulet, D.H., 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat. Immunol. 1, 119–126.

Ding, Y.H., Smith, K.J., Garboczi, D.N., Utz, U., Biddison, W.E., Wiley, D.C., 1998. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/tax peptide complex using different TCR amino acids. Immunity 8, 403–411.

Fan, Q.R., Mosyak, L., Winter, C.C., Wagtmann, N., Long, E.O., Wiley, D.C., 1997. Structure of the inhibitory receptor for human natural killer cells resembles haematopoietic receptors. Nature 389, 96–100.

Fan, Q.R., Long, E.O., Wiley, D.C., 2001. Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex. Nat. Immunol. 2, 452–460.

Gao, G.F., Tormo, J., Gerth, U.C., Wyer, J.R., McMichael, A.J., Stuart, D.I., Bell, J.I., Jones, E.Y., Jakobsen, B.K., 1997. Crystal structure of the complex between human CD8αα and HLA-A2. Nature 387, 630–634.

Garcia, K.C., Degano, M., Speir, J.A., Wilson, I.A., 1999. Emerging principles for T cell receptor recognition of antigen in cellular immunity. Rev. Immunogen. 1, 75–90.

Germain, R.N., Margulies, D.H., 1993. The biochemistry and cell biology of antigen processing and presentation. Ann. Rev. Immunol. 11, 403–450.

Groh, V., Bahram, S., Bauer, S., Herman, A., Beauchamp, M., Spies, T., 1996. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc. Natl. Acad. Sci. U.S.A. 93, 12445–12450.

Groh, V., Steinle, A., Bauer, S., Spies, T., 1998. Recognition of Stress-induced MHC molecules by intestinal epithelial γδ T cells. Science 279, 1737–1740.

Groh, V., Rhinehart, R., Secrist, H., Bauer, S., Grabstein, K.H., Spies, T., 1999. Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. Proc. Natl. Acad. Sci. U.S.A. 96, 6879–6884.

Kern, P.S., Teng, M.K., Smolyar, A., Liu, J.H., Liu, J., Hussey, R.E., Spoerl, R., Chang, H.C., Reinherz, E.L., Wang, J.H., 1998. Structural basis of CD8 coreceptor function revealed by crystallographic analysis of a murine CD8αα ectodomain fragment in complex with H-2Kb. Immunity 9, 519–530.

Kern, P., Hussey, R.E., Spoerl, R., Reinherz, E.L., Chang, H.C., 1999. Expression, purification, and functional analysis of murine ectodomain fragments of CD8αα and CD8αβ dimers. J. Biol. Chem. 274, 27237–27243.

Lanier, L.L., Corliss, B.C., Wu, J., Leong, C., Phillips, J.H., 1998. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature 391, 703–707.

Lanier, L.L., 2000. Turning on natural killer cells. J. Exp. Med. 191, 1259–1262.

Lenschow, D.J., Walunas, T.L., Bluestone, J.A., 1996. CD28/B7 system of T cell costimulation. Ann. Rev. Immunol. 14, 233–258.

Li, P., Willie, S.T., Bauer, S., Morris, D.L., Spies, T., Strong, R.K., 1999. Crystal structure of the MHC class I homolog MIC-A, a γδ T cell ligand. Immunity 10, 577–584.

Li, Z., Groh, V., Strong, R.K., Spies, T., 2000. A single amino acid substitution causes loss of expression of a MICA allele. Immunogenetics 51, 246–248.

Li, P., Morris, D.L., Willcox, B.E., Steinle, A., Spies, T., Strong, R.K., 2001. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. Nat. Immunol. 2, 443–451.

Li, P., McDermott, G., Strong, R.K., 2002. Crystal structures of RAE-1β and its complex with the NK cell receptor NKG2D.

Maenaka, K., Juji, T., Stuart, D.I., Jones, E.Y., 1999. Crystal structure of the human p58 killer cell inhibitory receptor (KIR2DL3) specific for HLA-Cw3-related MHC class I. Structure 7, 391–398.

Malarkannan, S., Shih, P.P., Eden, P.A., Horng, T., Zuberi, A.R., Christianson, G., Roopenian, D., Shastri, N., 1998. The molecular and functional characterization of a dominant minor H antigen, H60. J. Immunol. 161, 3501–3509.

Malarkannan, S., Horng, T., Eden, P., Gonzalez, F., Shih, P., Brouwenstijn, N., Klinge, H., Christianson, G., Roopenian, D., Shastri, N., 2000. Differences that matter: major cytotoxic T cell-stimulating minor histocompatibility antigens. Immunity 13, 333–344.

O'Callaghan, C.A., Cerwenka, A., Willcox, B.E., Lanier, L.L., Bjorkman, P.J., 2001. Molecular competition for NKG2D: H60 and RAE1 compete unequally for NKG2D with dominance of H60. Immunity 15, 201–211.

Snyder, G.A., Brooks, A.G., Sun, P.D., 1999. Crystal structure of the HLA-Cw3 allotype-specific killer cell inhibitory receptor KIR2DL2. Proc. Natl. Acad. Sci. U.S.A. 96, 3864–3869.

Steinle, A., Groh, V., Spies, T., 1998. Diversification, expression, and gamma delta T cell recognition of evolutionarily distant members of the MIC family of major histocompatibility complex class I-related molecules. Proc. Natl. Acad. Sci. U.S.A. 95, 12510–12515.

Steinle, A., Li, P., Morris, D.L., Groh, V., Lanier, L.L., Strong, R.K., Spies, T., 2001. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics 53, 279–287.

Tormo, J., Natarajan, K., Margulies, D.H., Mariuzza, R.A., 1999. Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. Nature 402, 623–631.

Trinchieri, G., 1989. Biology of natural killer cells. Adv. Immunol. 47, 187–376.

Vales-Gomez, M., Reyburn, H.T., Mandelboim, M., Strominger, J.L., 1998. Kinetics of interaction of HLA-C ligands with natural killer cell inhibitory receptors. Immunity 9, 337–344.

Vales-Gomez, M., Reyburn, H.T., Erskine, R.A., Lopez-Botet, M., Strominger, J.L., 1999. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2-A and the activating receptor CD94/NKG2-C to HLA-E. EMBO J. 18, 4250–4260.

Weis, W.I., Taylor, M.E., Drickamer, K., 1998. The C-type lectin superfamily in the immune system. Immunol. Rev. 163, 19–34.

Willcox, B.E., Gao, G.F., Wyer, J.R., Ladbury, J.E., Bell, J.I., Jakobsen, B.K., van der Merwe, P.A., 1999. TCR binding to peptide-MHC stabilizes a flexible recognition interface. Immunity 10, 357–365.

Wingren, C., Crowley, M.P., Degano, M., Chien, Y.-H., Wilson, I.A., 2000. Crystal structure of a γδ T cell receptor ligand T22: a truncated MHC-like fold. Science 287, 310–314.

Wu, J., Song, Y., Bakker, A.B., Bauer, S., Spies, T., Lanier, L.L., Phillips, J.H., 1999. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 285, 730–732.

Wu, J., Cherwinski, H., Spies, T., Phillips, J.H., Lanier, L.L., 2000. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. J. Exp. Med. 192, 1059–1067.

Wyer, J.R., Willcox, B.E., Gao, G.F., Gerth, U.C., Davis, S.J., Bell, J.I., van der Merwe, P.A., Jakobsen, B.K., 1999. T cell receptor and coreceptor CD8 alphaalpha bind peptide-MHC independently and with distinct kinetics. Immunity 10, 219–225.

Zou, Z., Nomura, M., Takihara, Y., Yasunaga, T., Shimada, K., 1996. 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. 119, 319–328.