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Review

Structure and function of major histocompatibility complex (MHC) class I specific receptors expressed on human natural killer (NK) cells

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Abstract

Natural killer (NK) cells express receptors that are specific for MHC class I molecules. These receptors play a crucial role in regulating the lytic and cytokine expression capabilities of NK cells. In humans, three distinct families of genes have been defined that encode for receptors of HLA class I molecules. The first family identified consists of type I transmembrane molecules belonging to the immunoglobulin (Ig) superfamily and are called killer cell Ig-like receptors (KIR). A second group of receptors belonging to the Ig superfamily, named ILT (for immunoglobulin like transcripts), has more recently been described. ILTs are expressed mainly on B, T and myeloid cells, but some members of this group are also expressed on NK cells. They are also referred to as LIRs (for leukocyte Ig-like receptor) and MIRs (for macrophage Ig-like receptor). The ligands for the KIR and some of the ILT receptors include classical (class Ia) HLA class I molecules, as well as the nonclassical (class Ib) HLA-G molecule. The third family of HLA class I receptors are C-type lectin family members and are composed of heterodimers of CD94 covalently associated with a member of the NKG2 family of molecules. The ligand for most members is the nonclassical class I molecule HLA-E. NKG2D, a member of the NKG2 family, is expressed as a homodimer, along with the adaptor molecule DAP10. The ligands of NKG2D include the human class I like molecules MICA and MICB, and the recently described ULBPs. Each of these three families of receptors has individual members that can recognize identical or similar ligands yet signal for activation or inhibition of cellular functions. This dichotomy correlates with particular structural features present in the transmembrane and intracytoplasmic portions of these molecules.

In this review we will discuss the molecular structure, specificity, cellular expression patterns, and function of these HLA class I receptors, as well as the chromosomal location and genetic organization.

Keywords: NK cells; Inhibitory receptor; KIR; CD94/NKG2; ILT

1. Introduction

NK cells are a heterogeneous lymphoid population defined by a $CD3^-$, $CD16^+$ (FcγRIIIA), $CD56^+$ (N-CAM) surface phenotype and are characterized by their ability to inherently lyse a great variety of cell types (referred to as target cells) that have been transformed or infected with viruses. NK cell lysis of target cells does not require prior sensitization of the host and is not restricted by major histocompatibility complex (MHC) encoded molecules (Moretta et al., 1996; Lanier, 1998); however, target cell lysis often correlates with the downregulation of some or all of the MHC class I molecules expressed by the target cells. This observation led to the "missing self" hypothesis of NK cell recognition (Ljunggren and Karre, 1990), whereby the postulated role of NK cells is to destroy cells that have downregulated expression of self-MHC class I molecules, a common feature of virally-infected and transformed cells.

NK cells can recognize and lyse target cells by two basic mechanisms: natural cytotoxicity and antibody dependent cell cytotoxicity (ADCC). For so called natural cytotoxicity, a large variety of receptors have been identified that can recognize target cells directly. Some of these activating

Abbreviations: CTL, cytotoxic T lymphocyte; HCMV, human cytomegalovirus; ILT, immunoglobulin-like transcript; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; KIR, killer immunoglobulin-like receptor; LRC, leukocyte receptor cluster; LIR, leukocyte immunoglobulin-like receptor; NCR, natural cytotoxicity receptors; NK, natural killer; SH2, Src-homology-domain 2; SHP, SH2-containing tyrosine phosphatase

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receptors are mainly expressed on NK cells, whereas others can be expressed on other cell types. Recently described NK cell specific receptors are NKp46, NKp44 and NKp30, referred to as natural cytotoxicity receptors (NCR) by their discoverers (Moretta et al., 2000). The target cell ligands for these receptors are not known, except for a recent report suggesting that haemagglutinins on virus-infected cells can be recognized by NKp46 (Mandelboim et al., 2001). Cell surface expression of these three NCRs is closely coordinated and the ability of NK cells to kill most target cells is directly related to the level of surface expression. Roda-Navarro et al. (2000) and Vitale et al. (2001) recently described an additional NK cell specific molecule, NKp80, that functions as a coreceptor for cytolysis, thereby enhancing NK cell function stimulated by other receptors. NKp80 is a type II transmembrane protein belonging to the C-type lectin family of receptors and is encoded in the NK complex, whereas the members of the NCR are type I proteins belonging to the Ig superfamily. Another NK cell receptor that is expressed mainly by NK cells and activated $CD8^+$ T cells is 2B4 (CD244), whose ligand is CD48 (Nakajima et al., 1999; Nakajima and Colonna, 2000; Chuang et al., 2001). NTB-A is a very recently described coreceptor that also triggers cytolytic activity, but only by NK cells expressing high surface densities of NCR (Bottino et al., 2001).

KIRs and CD94/NKG2 family members that are specific for MHC class I molecules that function as activating receptors are also apparently limited to expression on NK cells and a subpopulation of T cells (see Fig. 1). NKG2D is a homodimeric activating receptor expressed on NK cells, a fraction of $CD8^+$ $\alpha\beta^+$ T cells, and $\alpha\beta^+$ T cell clones (Wu et al., 1999). The ligands for NKG2D are the stress-induced class I like molecules MICA, MICB (Steinle et al., 2001) and ULBPs (Cosman et al., 2001). For a more detailed review of NK cell activating receptors see recent reviews by Moretta et al. (2001) and Biassoni et al. (2001).

There are several receptors that can activate NK cell lytic activity that are not unique to NK cells. These include CD2 (Nakamura et al., 1991; Vivier et al., 1991b) and CD26 (Madueno et al., 1993) that are also expressed by $CD4^+$ and $CD8^+$ T cells, CD69 (Borrego et al., 1999) is expressed by all leukocytes after activation and β1 integrins (Perez-Villar et al., 1996) are also expressed by many cell types.

The second type of target cell recognition utilized by NK cells is indirect through the CD16 molecule and is referred to as ADCC. CD16 is a receptor specific for the Fc portion

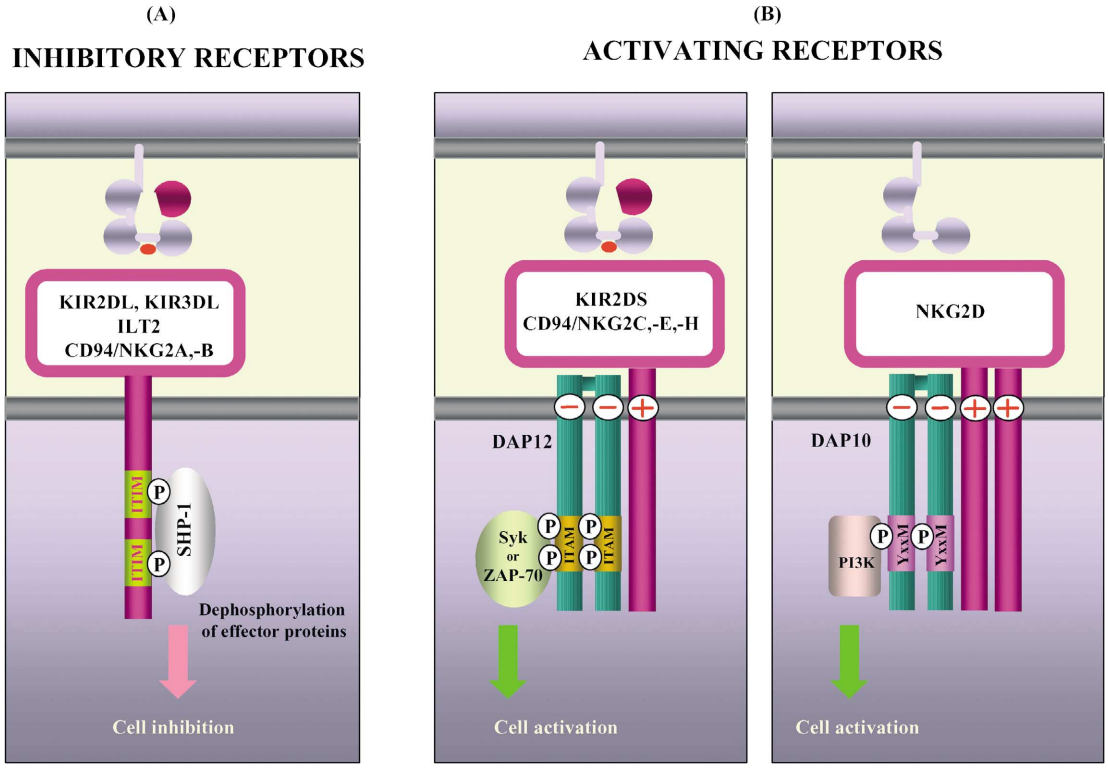


Fig. 1. Mechanism of signal transmission through human NK cell-receptors by HLA class 1 molecules. (A) The recruitment and activation of SHP-1 by inhibitory receptors leads to the dephosphorylation of proteins whose phosphorylation is necessary for conveying activating signals. Although SHP-2 can bind phosphorylated ITIMs, it is unclear whether it can function in the same context as SHP-1 (Olcese et al., 1996; Barford and Neel, 1998). (B) Activating receptor complexes on NK cells associate with the ITAM bearing adaptor molecule DAP12 through charged residues in their transmembrane regions. The membrane adaptor protein DAP10 does not have an ITAM in its cytoplasmic region, rather it has an YxxM motif that is a potential Src homology 2 (SH2) binding domain. Activation of NK cells through ligation of these complexes leads to recruitment and activation of SH2 domain containing protein tyrosine kinases, such as Syk or ZAP-70, except for NKG2D which recruits PI3-kinase.

of IgG. NK cells can be activated to lyse these target cells by binding antibody molecules that have specificity for ligands on these cells (Vivier et al., 1991a; Stahls et al., 1992).

The ligands recognized by receptors that activate NK cell lytic activity are also present on normal cells. To prevent wanton killing of "normal" cells a mechanism had to evolve that would override the NK cell killing machinery that is always functional in mature NK cells (Lanier, 1998). In order to accomplish this, NK cells express a variety of molecules that inhibit NK cell activation through their recognition of MHC encoded class I molecules. Class I molecules are expressed by virtually all normal cells, but tend to be down-regulated by transformed and virally-infected cells (Ploegh, 1998; Algarra et al., 2000).

In humans, three families of such inhibitory NK receptors have been described. One major group is referred to as killer Ig-like receptors (KIR). They possess two or three immunoglobulin (Ig) domains and each member interacts with a different group of closely related HLA class I molecules (Lanier, 1998) (see Fig. 2). A second group of receptors known as ILT (immunoglobulin-like transcript) are also members of the Ig superfamily. Some of the ILTs react with a variety of HLA class I molecules (Colonna et al., 1999). The third major group of NK cell inhibitory receptors is the heterodimeric CD94/NKG2 C-type lectin proteins that are specific for HLA-E (López-Botet and Bellon, 1999). All of the inhibitory receptors from each group possess immunoreceptor tyrosine-based inhibition motifs (ITIM) in their cytoplasmic tails (Long, 1999) (see Figs. 1 and 2).

Table 1
Common names and CD nomenclature for ILT and KIR molecules[a]

Common names		CD designation
ILT5	LIR3	CD85a
ILT8		CD85b
	LIR8	CD85c
ILT4	LIR2, MIR10	CD85d
ILT6	LIR4	CD85e
ILT11		CD85f
ILT7		CD86g
ILT1	LIR7	CD85h
	LIR6	CD85i
ILT2	LIR1, MIR7	CD85j
ILT3	LIR5	CD85k
ILT9		CD85l
ILT10		CD85m
KIR3DL7	KIRC1	CD158z
KIR2DL2/L3	P58.2/p58.3	CD158b1/b2
KIR2DL1	P58.1	CD158a
KIR2DS6	KIRX	CD158c
KIR2DL4		CD158d
KIR3DL1/S1	P70	CD158e1/e2
KIR2DL5		CD158f
KIR2DS5		CD158g
KIR2DS1	P50.1	CD158h
KIR2DS4	P50.3	CD158I
KIR2DS2	P50.2	CD159j
KIR3DL2	P140	CD158k

[a] The CD nomenclature of the KIRs and ILTs is based in part on the previous CD designation of some members of these receptor families and on the position of the genes on chromosome 19. An alphabetical order has been assigned according to the centromeric–telomeric localization of the genes. This nomenclature also takes into consideration the allelic polymorphism of these gene families. KIR2DL2/KIR2DL3 corresponds to CD158b1/b2 and KIR3DL1/KIR3DS1 corresponds to CD158e1/e2 (Andre et al., 2001).

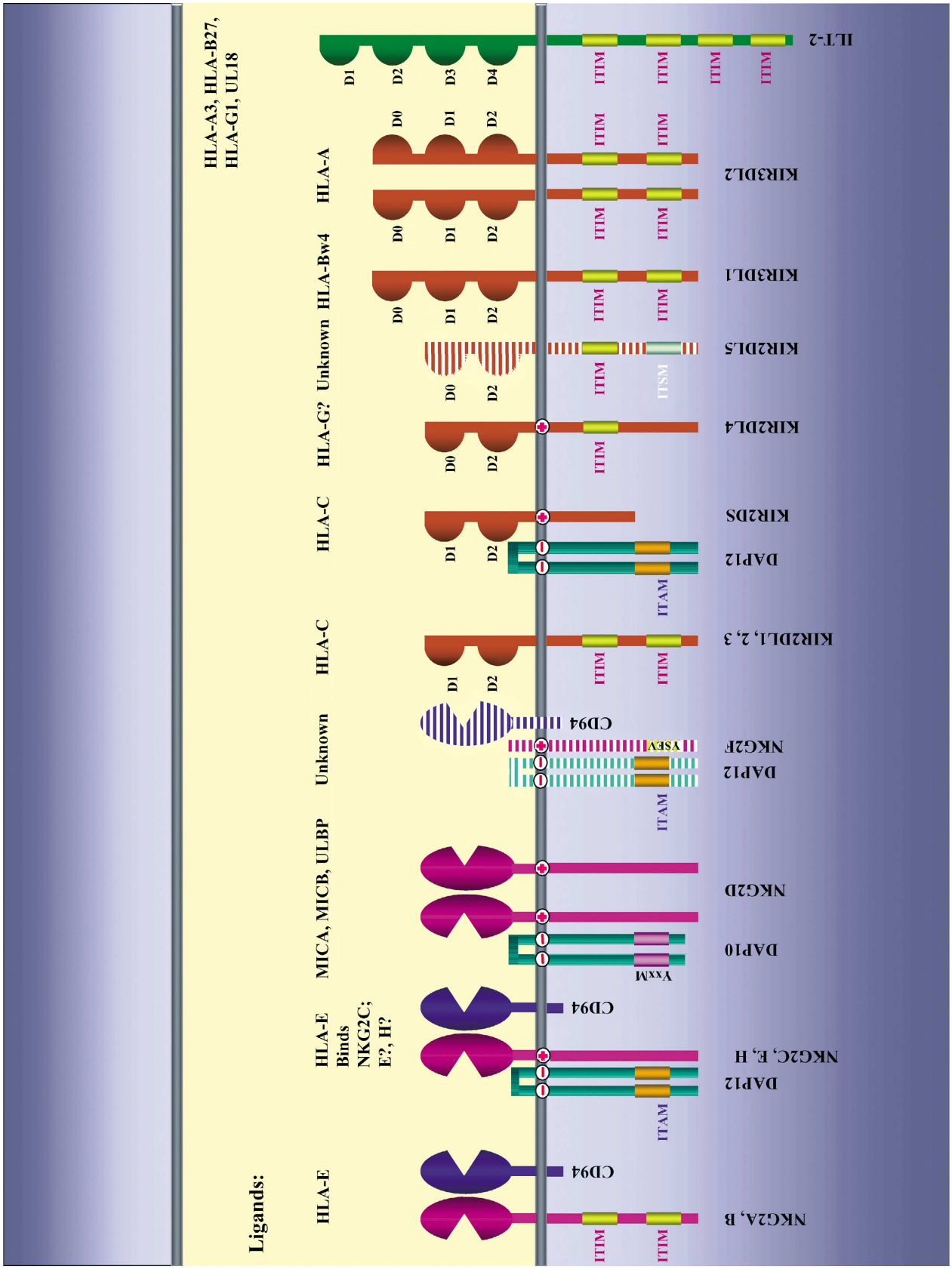
2. Killer immunoglobulin-like receptors (KIR)

KIRs comprise a family of molecules (Fig. 2) that are encoded by multiple loci that (see Fig. 4) vary in certain structural features and ligand specificity. A generally accepted nomenclature has been adopted to categorize these molecules. According to this nomenclature, the acronym KIR is followed by a suffix that describes the molecule. The number of immunoglobulin-like extracellular domains they possess is indicated by either 2D or 3D; the letter L or S designates whether they have a long (L) or short (S) cytoplasmic domain; and finally, a code number is assigned to each gene/molecule. During the 7th Human Leukocyte Differentiation Antigen Workshop, a new CD nomenclature was proposed for the KIR and ILT gene families. It is based on previous CD designation of some members of these families and on the position of the genes on chromosome 19 (Andre et al., 2001) (Table 1). KIRs with long (L) cytoplasmic tails are inhibitory and contain ITIM sequences, while those with short (S) cytoplasmic tails activate NK cell cytotoxicity through interactions with the adaptor molecule, DAP12 (Lanier et al., 1998a) (see Fig. 2). NK cells expressing KIR with ITIM sequences in their cytoplasmic domains (KIR2DL and KIR3DL) are inhibited from lysing target cells that express MHC class I molecules reactive with the expressed KIR molecule (Moretta and Moretta, 1997). This interaction usually also inhibits cytokine production by the effector cells (D'Andrea et al., 1996). In contrast, the expression of KIR without an ITIM (e.g. KIR2DS) by NK cells appears to promote cytolysis against target cells expressing an appropriate MHC class I ligand (Moretta et al., 1995).

2.1. HLA class I ligand specificity

Even though the specificity for class I molecules displayed by different KIR receptors is not as discriminatory as TCR, they are capable of distinguishing among groups of HLA class I molecules that have particular structural features. For example, HLA-C molecules are the ligands for KIR2D receptors, while subsets of HLA-B and HLA-A molecules are the ligands for KIR3D receptors.

Several experimental methods have been used to elucidate the nature of the interaction between KIR receptors and their ligands. Initial studies relied on site-directed mutagenesis of class I molecules and their transfection into class I negative target cells followed by the analysis of their functional recognition with appropriate NK cell clones (Biassoni et al., 1995; Gumperz et al., 1997). Other investigators


Ligands:
HLA-E
HLA-E
Binds
NKG2C;
E?; H?
MICA, MICB, ULBP
Unknown
HLA-C
HLA-G? Unknown
HLA-Bw4
HLA-A
HLA-A3, HLA-B27,
HLA-G1, UL18
NKG2A, B
NKG2C, E, H
NKG2D
KIR2DL4
KIR2DS
KIR2DL1, 2, 3
KIR2DL5
KIR3DL1
KIR3DL2
ILT-2
DAP12
DAP10
CD94
ITIM
ITAM
ITSM
YxxM
YSEX
D0
D1
D2
D3
D4

have examined specificity by doing direct binding studies with soluble forms of recombinant inhibitory receptors on cells expressing specific class I molecules, or, in reverse, by analyzing binding of class I oligomers to cells expressing specific NK cell receptors (Winter and Long, 1997; Winter et al., 1998; Allan et al., 1999). Ultimately, crystal structures of these receptors, some in complex with ligand, have revealed the finer points of these interactions.

The crystal structure of three different KIR2DL receptors, KIR2DL1 (CD158a) (Fan et al., 1997), KIR2DL2 (CD158b1) (Snyder et al., 1999) and KIR2DL3 (CD158b2) (Maenaka et al., 1999b), reveals that their immunoglobulin-like domains (D1 and D2) (Fig. 3A) are positioned at an acute angle that is different for each receptor. The topology of the domains and their arrangement relative to each other reveal a structural relationship of KIR2D with the haematopoietic receptor family (Fan et al., 1997). The differences in the hinge angles observed in the different KIR2D studied, as well as the differences observed in two crystal forms of KIR2DL2, can be explained by different sets of amino acid residue interactions found at the interdomain interfaces. When complexed with class I molecules, the receptors have a very similar interdomain orientation that is dictated by the constraints imposed by ligand binding (Fan et al., 2001). The crystal structure of the complex formed by KIR2DL2 and HLA-Cw3 reveals that KIR2D receptors bind in a nearly orthogonal orientation across the α1 and α2 helices of HLA class I molecules (Boyington et al., 2000). The D1 domain interacts with polymorphic regions of the Class I α1 helix, residues 69–84, and the D2 domain interacts with more conserved regions of the α2 helix, residues 145–151 (Fig. 3A). The KIR-HLA class I interface shows a predominance of charged residue and hydrophilic residue interactions that form an extensive array of hydrogen bonds and salt bridges. Site-directed mutants that disrupt these interface salt bridges substantially diminish binding affinity, supporting the crucial role for charged residues in HLA recognition. The high impact of these mutations on KIR binding affinity suggests that the relatively low affinity KIR receptors achieve their ligand specificity by requiring a high energy threshold for recognition (Boyington et al., 2000).

The "footprint" of KIR2DL2 on HLA-Cw3 involves six loops interacting with conserved HLA-C residues (Fig. 3A). Of the 12 HLA-Cw3 residues intimately involved in this interface with KIR, 11 are invariant in all HLA-C molecules (Fig. 3B). Of particular interest is the fact that the divergent residue lies in position 80 of the α1 helix. This residue is Asn in HLA-Cw1, 3, 7 and 8, the ligands for KIR2DL2 and KIR2DL3, and Lys in HLA-Cw2, 4, 5, 6 and 15, the ligand for KIR2DL1. Not surprisingly, of the 16 KIR2DL2 residues that interact with HLA-Cw3, all of them are identical in KIR2DL3, which also reacts with the same HLA-Cw3 and related allotypes. Fourteen of these residues are identical in KIR2DL1, the KIR2DL specific for the other group of HLA-C molecules (Fig. 3B). The two different residues are Lys 44 and Met 70 in KIR2DL2 and KIR2DL3 and Met 44 and Thr 70 in KIR2DL1. Not surprisingly, in the cocrystal structure it is possible to see the hydrogen bond formed between Lys 44 of KIR2DL2 and Asn 80 of HLA-Cw3 (Boyington et al., 2000). In conjunction with the other hydrogen bonds and salt bridges, this hydrogen bond stabilizes the interaction of KIR2DL2 with HLA-Cw3 and is apparently sufficient for determining the receptor-ligand specificity (Winter and Long, 1997). Very recently, the crystal structure of KIR2DL1-HLA-Cw4 complex was published (Fan et al., 2001). The footprint of this KIR receptor on the HLA-Cw4 molecule is very similar to the KIR2DL2 footprint on HLA-Cw3. A major difference relating to specificity is that in KIR2DL1 Met 44 lies in an electronegatively charged pocket that hosts the side chain of Lys 80 from HLA-Cw4, whereas in the KIR2DL2/HLA-Cw3 interaction specificity relies on a hydrogen bond between Lys 44 in KIR2DL2 and Asn 80 in HLA-Cw3. The importance of residue 70 in KIR/HLA-C interactions was shown by substituting Thr 70 in KIR2DL1 (see Fig. 3B) with Lys, which occurs naturally in KIR2DS1. This dramatically decreased the binding of a KIR2DL1/Ig fusion protein to cells expressing HLA-Cw4 (Biassoni et al., 1997). Mutagenesis experiments (Winter et al., 1998) also showed the importance of Phe 45 for KIR2DL2 ligand binding. This residue forms hydrophobic contacts with Arg 75, Val 76 and Arg 79 in HLA-C (Boyington et al., 2000). KIR2DS2 expresses a Tyr residue at position 45 and its binding to cells expressing HLA-Cw3 is almost null, but substitution of this Tyr 45 by Phe was sufficient to permit specific binding of KIR2DS2 to HLA-Cw3.

There is less information about the features of how KIR3D receptors interact with their ligands. KIR3DL1 binds to HLA-B alleles, specifically those of the Bw4

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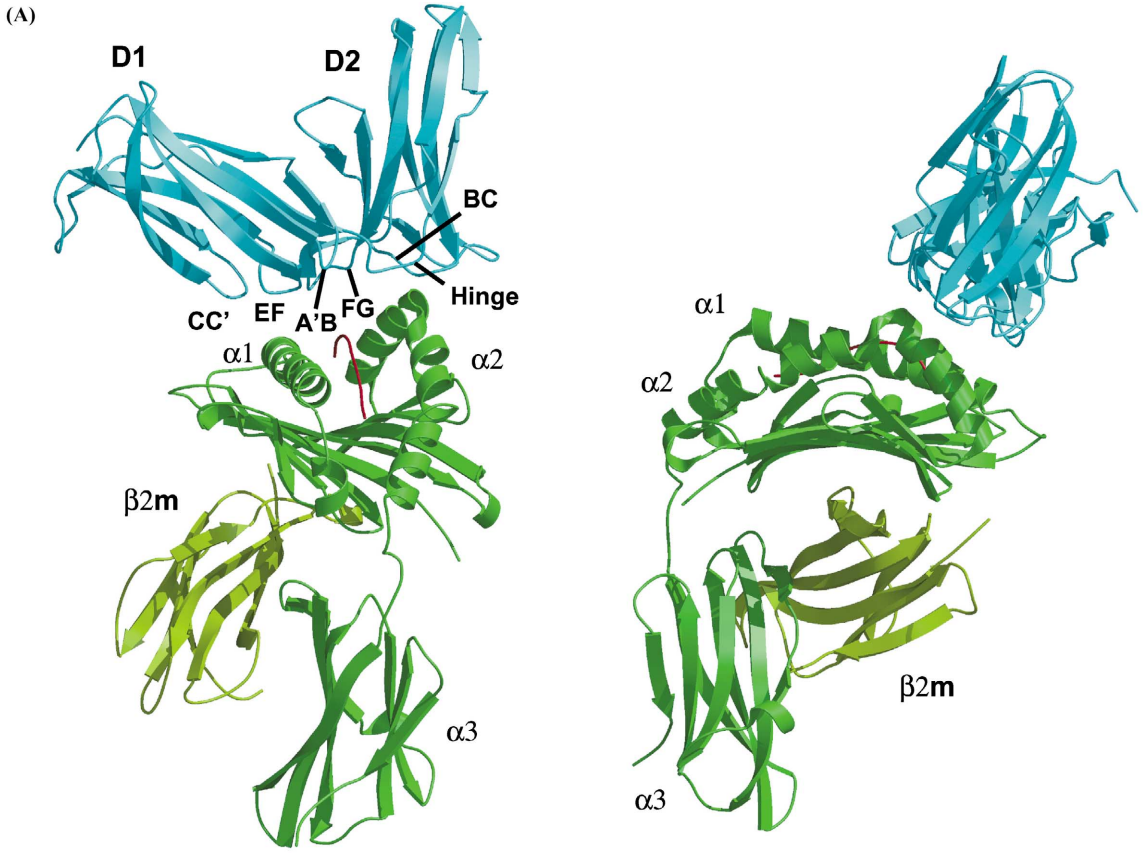
Fig. 2. NK cell activating and inhibitory receptors with their signaling motifs and ligands. Positive and negative signs indicate charged residues in transmembrane regions. The *NKG2F* gene codes for an ITIM like motif (YSEV); whether this sequence functions as an ITIM remains to be determined. NKG2F and KIR2DL5 are depicted with stripes because only transcripts have been reported. If NKG2F is expressed, it is unknown if it partners with CD94 or DAP12 (also striped). The putative KIR2DL5 molecule contains both ITIM and ITSM (immunoreceptor tyrosine-based switch motif) motifs. KIR2DL4 is an activating receptor with an ITIM and a positively charged amino acid in its transmembrane domain. Despite this, evidence from transfection experiments suggests that KIR2DL4 does not partner with FcRγ, DAP12 or DAP10 (Rajagopalan et al., 2001). KIR2DL4 and KIR2DL5 have their extracellular domains in a D0/D2 configuration rather than the typical D1/D2 configuration. We have chosen to depict all KIRs, except KIR3DL2, as monomers even though they may exist as dimers and oligomers especially after interaction with ligand (Boyington et al., 2001). Pende et al. (1996) have demonstrated that KIR3DL2 is expressed as a dimer. Question marks denote that it is not clear that KIR2DL4 is a receptor for HLA-G, and no evidence exists that CD94/NKG2E and CD94/NKG2H bind HLA-E.



(B)

Loop	A'B	CC'	EF	hinge	BC	FG
	20 23	43 46	67 74	103 108	130 135	181 187
KIR2DL2	SEE	GKFK	GPMMQDLA	GLYEKP	SRSSYD	FRDSPYE
KIR2DL1	---	-M-N	SR-T----	------	------	-H-----

	55	90	141	170
HLA-Cw0304	EGPEYW DRETQKYKRQ AQTDRVSLRN LRGYYNQSEA		QITQRKWEAA REAEQLRAYL EGLCVEWLRR	
HLA-Cw0401	------ ---------- --A---N--K ------D		---------- -----R---- --T-------	

Fig. 3. (A) Ribbon drawing showing the crystal structure of HLA-Cw3 bound to KIR2DL2. The β2m domain is yellow, HLA-Cw3 heavy chain is green and the peptide is magenta. The KIR2DL2 molecule bound to HLA-Cw3 is blue. D1 and D2 domains in KIR2DL2 and the six loops (CC′, EF, A′B, FG, BC and the hinge region) interacting with α1 and α2 domains of HLA-Cw3 are pointed out. The right view is rotated 90° from left view along the vertical axis to illustrate that KIR binding is located at the carboxy-terminal end of the peptide and the corresponding region of the HLA-Cw3 α1 and α2 helices. (B) Sequence alignments of the binding regions of KIR2DL1, KIR2DL2, HLA-Cw3 and HLA-Cw4. The conserved residues are indicated by dashes. Blue spirals denote the α1 and α2 helices of HLA class I molecules. KIR2DL1 that align with KIR2DL2 contact residues are blue on a green background. HLA-Cw4 residues that align with HLA-Cw3 contact residues are red on a yellow background (courtesy of Drs. Jeffrey C. Boyington and Peter D. Sun).

serotype (Gumperz et al., 1995) (see Fig. 2). A comparison among HLA-B molecules reveals that all but 3 of the 12 residues corresponding to the HLA-C residues that interface with KIR2DL are conserved across HLA-B and HLA-C loci. The positions that differ are at position 69 (Ala or Thr in HLA-Bw4 and Arg in HLA-C), position 76 (Glu in HLA-Bw4 and Val in HLA-C) and position 80 (Asn, Thr or Leu in HLA-Bw4 and Asn or Lys in HLA-C). So the residues at these three positions, especially those at positions 69 and 76, likely determine KIR specificity for HLA-B and HLA-C molecules. Indeed, the protein encoded by the unusual HLA-B*4601 allele, which is the product of an interlocus recombination, has HLA-Cw*0102 residues 66–76 in its α1 helix and interacts with NK cells that recognize HLA-Cw1 (Barber et al., 1996). Although all three domains of KIR3D are required for binding of a soluble receptor to

HLA-B*5101 (Rojo et al., 1997), it has been suggested that the KIR3DL1/HLA-Bw4 interaction involves mainly the D1 and D2 domains (see Fig. 2) of KIR3D like the D1 and D2 domains in the KIR2DL2/HLA-Cw3 interaction (Boyington et al., 2000).

2.1.1. Role of the peptide

The sequence of the peptide bound to MHC class I molecules influences the interaction of class I complexes with their corresponding KIRs. The crystal structure reveals that KIR2DL2 directly contacts residues p7 and p8 of the HLA-Cw3 associated peptide (Boyington et al., 2000). This observation is corroborated by functional studies showing that the lysis of target cells by NK clones exhibits peptide specificity (Zappacosta et al., 1997). Overt interactions between the HLA-Cw4 peptide and KIR2DL1 are less apparent in the crystal structure (Fan et al., 2001); however, studies with synthetic peptides have indicated that substitution of p8 Lys of the peptide with a negatively charged residue results in the loss of KIR binding (Rajagopalan and Long, 1997). This agrees with the observation that KIR2DL1 has an electronegatively charged polar surface in the area opposing the peptide p8 Lys side chain. As with HLA-C molecules, the peptides bound to HLA-B molecules have been shown to be important for the interaction with KIR3D (Malnati et al., 1995; Peruzzi et al., 1996b) and, likewise, peptide positions p7 and p8 have been shown to be important for NK cell recognition of target cells expressing HLA-B*2705 (Peruzzi et al., 1996a).

2.1.2. Role of carbohydrate moiety

All human class I molecules have a carbohydrate moiety attached to Asn 86. It is not clear if this carbohydrate moiety plays a role in the interaction of HLA class I molecules with KIRs. Based on the fact that cells transfected with HLA-C mutant cDNAs lacking carbohydrate attachment sites are less sensitive to NK cell recognition, Baba et al. (2000) proposed that the N-linked carbohydrates interact with KIRs, either directly or by influencing the conformation of HLA-C at the KIR recognition site. However, it is not clear whether these investigators took into account that class I molecules devoid of carbohydrates often have a reduced efficiency for cell surface expression. KIR inhibitory function is sensitive to the levels of HLA class I cell surface expression (Borrego, unpublished data).

2.1.3. Quantitative binding studies

Surface plasmon resonance and analytical ultracentrifugation studies with soluble proteins obtained from bacterial expression systems have been utilized to examine the kinetics and affinity of the interaction between KIR and class I molecules. HLA-C molecules bind KIR with very fast association and dissociation rate constants, which are more similar to the kinetics of the interactions of adhesion molecules with their ligands than to those of TCR and their ligands (Vales-Gomez et al., 1998a; Maenaka et al., 1999a). As determined by the surface plasmon resonance experiments, the half-life of KIR/HLA-C complexes is less than 1 s. Similarly, the half-life of complexes between adhesion molecules and their ligands is less than 1.5 s, while the half-life of TCR/MHC complexes is greater than 5 s (Vales-Gomez et al., 1998b). KIR binds class I molecules with a favorable binding entropy that is consistent with an optimally fixed binding site, which is unlike TCR/peptide-MHC interactions that are characterized by unfavorable entropic changes, suggesting that binding is accompanied by conformational adjustments (Willcox et al., 1999).

What is the significance of the rapid kinetics of KIR/HLA class I interaction for the biology of NK cells? A primary physiological role of NK cells appears to be surveillance for self-MHC class I proteins. For this function, they must rapidly assess the expression of HLA class I proteins on the surface of a cell as a sign of "normality". Once that is determined, they must dissociate to continue the surveillance of other cells. In this regard, it has been shown that NK cells can interact with target cells expressing an inhibitory HLA class I ligand without affecting the subsequent lysis of susceptible target cells (Lanier, 1998). This observation implies not only a rapid association–dissociation rate for KIR/HLA-C complexes, but also a rapid reversal of the inhibitory signal.

Despite the very high homology of the D1 and D2 domains of corresponding KIR2DS and KIR2DL molecules, KIR2DS bind HLA-C molecules only very weakly (Vales-Gomez et al., 1998a; Winter et al., 1998). This difference is thought to have an underlying functional significance. Viewed simplistically, if activating and inhibitory receptors in similar concentrations are competing for binding to the same HLA-C molecules, then the signals from the inhibitory receptors are more likely to predominate. This belies the more important question of why there are activating receptors for class I molecules (see Section 5.3).

2.1.4. Role of metal ions as binding cofactors

Evidence indicates that KIR can bind certain metal ions, but it is not clear if they have a functional role. The first extracellular domain of KIR has an unusual abundance of histidine residues that are capable of binding zinc ions (Rajagopalan et al., 1995; Rajagopalan and Long, 1998). The simultaneous replacement of six His by Ala residues in the putative zinc binding sites abolished binding of zinc to soluble KIR. Even more striking, the mutational disruption of a single exposed amino-terminal zinc-binding motif alone was sufficient to impair the inhibitory function of KIR. NK cells expressing such mutagenized KIR were impaired in their inhibitory function, but surprisingly their ability to bind HLA-C was apparently not affected. Surface plasmon resonance, analytical ultracentrifugation, and chemical cross-linking experiments have shown that zinc binding to KIR2D molecules induces multimerization of KIR proteins that alters the kinetics of the KIR2D-HLA-C interaction (Vales-Gomez et al., 2001). In the absence of zinc, the binding reaction is a simple first-order interaction

with very fast association and dissociation rates. In contrast, in the presence of zinc, the association and dissociation phases of the KIR2D-HLA-C interaction became a mixture of fast and slow rate components. Cobalt also induces KIR dimerization increasing its affinity to HLA-C. The mutation of the His residue most proximal to the amino terminus to an Ala residue in KIR2D abolished cobalt binding and, as expected, dimerization did not occur (Fan et al., 2000). Within the KIR2DL2/HLA-Cw3 crystal complex, apart from the class I peptide associated binding interface, KIR molecules also make an additional contact with a neighboring related HLA-Cw3 in a peptide-independent manner. Based on these observations and previous work by Davis et al. (1999), Boyington et al. (2000) proposed that this form of receptor-ligand oligomerization resembled the receptor clustering on the surface of NK cells during immune synapse formation.

2.2. KIR2DL4 and KIR2DL5

KIR with two Ig domains exhibit a D1–D2 configuration, while KIR with three Ig domains have an order of D0–D1–D2, but KIR2DL4 is unique among members of the KIR family. cDNA clones encoding KIR2DL4 predict a molecule with two Ig domains in a D0–D2 configuration (see Fig. 2) (Selvakumar et al., 1996; Cantoni et al., 1998). It has both an Arg residue in the transmembrane region and a long cytoplasmic tail with a single ITIM. Therefore, KIR2DL4 molecules have features typical of both activating and inhibitory receptors leaving open the question of what type of signals are transmitted by this receptor. Binding assays with recombinant soluble KIR2DL4 showed that this receptor could bind HLA-G (Rajagopalan and Long, 1999), as well as HLA-A3, -B46 and weakly to -B7 molecules (Cantoni et al., 1998). In contrast, data published by others (Allan et al., 1999; Navarro et al., 1999) suggest that HLA-G binds ILT2 and ILT4, but not KIR2DL4.

Unlike other KIR molecules that show clonal distribution, KIR2DL4 molecules have been reported to be expressed on the surface of all peripheral NK cells (Rajagopalan and Long, 1999). In contrast, others have reported (Ponte et al., 1999) that KIR2DL4 is expressed on a significant proportion of NK cells in the decidua during the first trimester of pregnancy and on all NK cells obtained from the placenta at term, but is absent on NK cells from the mother's peripheral blood. The fact that these studies employed different antibodies for detection of KIR2DL4 may have some bearing on the reported discrepancies. Very recently, it has been reported that KIR2DL4 is an activating receptor with the unique feature of inducing IFN-γ secretion but not cytotoxic potential in resting NK cells. In previously activated NK cells, KIR2DL4 is capable of inducing cytotoxicity (Rajagopalan et al., 2001).

Recently transcripts for another type of KIR gene, called KIR2DL5, have been detected in subpopulations of NK cells and T cells, but as yet, no evidence for protein expression is available (Vilches et al., 2000a). KIR2DL5 molecules would have an extracellular configuration of the D0–D2 type, like KIR2DL4 (Fig. 2). Although similar in extracellular structure, KIR2DL4 has a single ITIM in its cytoplasmic region, whereas KIR2DL5 has two ITIM motifs separated by 24 residues. The additional ITIM differs from the consensus motif by having a Thr residue instead of the I/V at position -2. This TxYxxV/I motif has been called ITSM (immunoreceptor tyrosine-based switch motif) and is present in a broad range of receptors, for example CD150. Protein structures with this motif have been shown to bind the SH2-containing protein tyrosine phosphatase SHP-2 and the inositol phosphatase SHIP, as well as the adaptor, SH2 domain containing, protein 1A (SH2D1A/DSHP/SAP) (Shlapatska et al., 2001). KIR2DL5 lacks the Arg residue present in the transmembrane region of KIR2DL4 and, unlike KIR2DL4, transcripts are clonally expressed by NK cells within an individual and expression is limited to the "B" KIR haplotype (Vilches et al., 2000b).

2.3. Genetic organization and evolution of KIR receptors

The genes encoding the KIR are located on human chromosome 19q13.42 in a region called the leukocyte receptor cluster (LRC) (Fig. 4). Other members residing in the LRC are the ILTs, leukocyte-associated inhibitory receptors (LAIR), FcαR and NKp46 (Barten et al., 2001). All the genes encoding two-Ig-domain KIRs, with the exception of KIR2DL4 and KIR2DL5, have a pseudo exon 3 that has remarkable similarity to the exon encoding the first Ig domain (D0) of the three Ig domain KIRs (Selvakumar et al., 1997; Wilson et al., 1997, 2000; Vilches et al., 2000b). These data are consistent with the hypothesis that genes encoding the two-Ig-domain KIRs have evolved from a gene encoding a three-Ig-domain KIR through disablement of an exon (Barten et al., 2001).

The KIR region is plastic in its organization and the genes contained within exhibit isotypic and allotypic variation (Wilson et al., 2000; Barten et al., 2001). Over 100 highly homologous KIR sequences have been deposited in databases and there are several alleles for each particular KIR loci (Selvakumar et al., 1997). There is a high degree of diversity in KIR gene expression that arises from haplotypic differences in gene number and allelic polymorphism (Uhrberg et al., 1997). There are a minimum of 15 different haplotypes, which based on the presence or absence of particular loci, allow for many different genotypes (Uhrberg et al., 1997; Barten et al., 2001). There are three loci (KIR2DL4, KIR3DL2 and KIR3DL7, previously known as KIR3DL3) that are present in all haplotypes (Barten et al., 2001). The conservation of gene structures and sequence homologies between the different KIR receptor haplotypes indicates that the LRC evolved by extensive gene duplication and recombination with insertion and deletion mechanisms

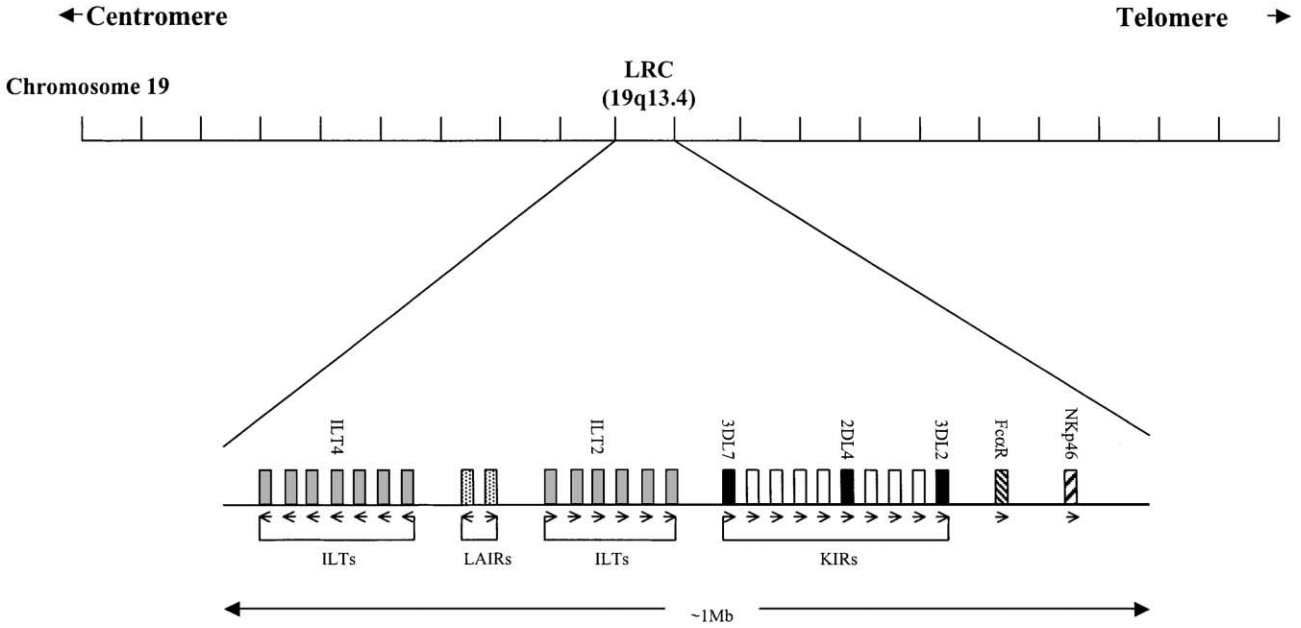


Fig. 4. The human leukocyte receptor cluster (LRC) is a ~1 mb region located on chromosome 19q13.42. Within this complex lie the *ILT*s, *LAIR*s, *KIR*s, *FcαR* and *NKp46* genes. *KIR3DL7*, *KIR2DL4* and *KIR3DL2* (in black) are framework loci present in all haplotypes. Note: this figure is not drawn to scale.

leading to KIR gene diversification (Shilling et al., 1998; Kwon et al., 2000). The frequencies with which certain combinations of KIR loci are found in different individuals exceeded levels expected from random association (Uhrberg et al., 1997) and is indicative of linkage disequilibrium of alleles in haplotypes.

MHC class I molecules are characterized by their rapid evolution and divergence. Khakoo et al. (2000) have shown that, as would be expected, KIRs have also evolved very rapidly. A comparison between human and chimpanzees showed that their KIR families are very divergent, with only three KIR families conserved between chimpanzees and humans. Relating to this divergence is the observation that KIRs that recognize the orthologous human and chimpanzee MHC ligands HLA-B and -C and Patr-B and Patr-C, respectively, are recognized by nonorthologous KIRs in these species. As discussed in Section 2.1, KIR specificity for human MHC-C molecules is largely determined by the amino acid residue at position 44 of the KIR-D1 domain; consistent with this finding, the human and chimpanzee KIR that have identical MHC-C specificity also have the identical residues at this position. These observations show that KIR receptors have evolved relatively rapidly, and that "catching up" with class I molecules is not the only mechanism driving their evolution (Khakoo et al., 2000).

3. Immunoglobulin-like transcripts (ILT)

In a search for new Ig superfamily members, several investigators isolated multiple cDNA clones that code for proteins with Ig-like domains and that had distinct expression patterns in leukocytes (Samaridis and Colonna, 1997; Wagtmann et al., 1997). Those receptors are called ILT (also LIR or MIR) and they can be categorized into three groups: those containing ITIM motifs, those with short cytoplasmic tails and a charged amino acid residue within the transmembrane region, and one member that contains no transmembrane segment and that is presumably a secreted molecule (Borges et al., 1997). These receptors are expressed in a broad array of cells including monocytes, macrophages, dendritic cells, B, T and NK cells (Colonna et al., 1999). Of interest for this review is ILT2 (LIR-1, MIR7, CD85j), the only member of this family that is expressed on some NK cells (Colonna et al., 1997; Cosman et al., 1997).

ILT2 has four extracellular Ig domains and four ITIMs in its intracytoplasmic tail and it can bind a broad panel of HLA class I molecules (Colonna et al., 1997; Cosman et al., 1997), including HLA-G (Allan et al., 1999; Navarro et al., 1999) (Fig. 2). In addition, ILT2 binds the human cytomegalovirus class I homolog UL18 (Cosman et al., 1997; Vitale et al., 1999). ILT2 interacts with the α3 domain of the class I and UL18 proteins (Chapman et al., 1999), which probably explains its broad range of reactivity, as the α3 domain of class I molecules shows the highest sequence conservation. Interestingly, ILT2 binds UL18 with higher affinity than class I molecules. This supports the hypothesis that UL18 may function to inhibit recognition by ILT2 bearing immune cells of HCMV infected cells that have downregulated expression of classical class I molecules (Chapman et al., 1999). The crystal structure of domains 1 and 2 (D1D2) of ILT2 has been solved and they are arranged at an acute angle that resembles the structure of KIRs, but in contrast to KIR the binding site of ILT2 to UL18 is located in a portion of D1

distant from the hinge region between D1 and D2 (Chapman et al., 2000). It has been proposed that the binding of ILT2 to UL18 is similar to the interaction between the D1 domain of CD4 and the β2 domain of class II MHC proteins (Chapman et al., 1999).

HLA-F is a nonclassical MHC encoded class I molecule that has been shown to be expressed intracellularly, but not as yet on the cell surface of restricted group of tissue types. Recently, Lepin et al. (2000) have shown that HLA-F tetramers can stain cells expressing ILT2 and ILT4, and surface plasmon resonance studies demonstrated a direct molecular interaction of soluble forms of ILT2 and ILT4 with HLA-F. These findings lead them to speculate that cells expressing HLA-F on their surfaces, perhaps in the presence of a specific peptide, can reach the cell surface and interact with cells expressing ILT2 and ILT4 thereby modulating the activation threshold of such immune effector cells.

ILT genes are clustered in the LRC centromeric to the KIR (Fig. 4). In contrast to KIR genes, the ILT genes are more stable in number, except for ILT6, which is present in only one haplotype (Wilson et al., 2000). There are two clusters of ILT genes that are separated by about 200KB and that are transcribed in opposite directions. Two LAIR genes are encoded between the two clusters of ILT genes (Wende et al., 1999) (Fig. 4). ILT2 is expressed on most myelomonocytic cells, B cells, dendritic cells, and subsets of T cells and NK cells. One study indicated that all T cells express ILT2, at least intracellularly, but only a fraction may express it on the cell surface (Saverino et al., 2000). Like other inhibitory receptors, the interaction of ILT2 with HLA class I molecules on target cells inhibits killing of these cells, including CD16-mediated activation by the NK cells and T cell cytotoxicity induced by the TCR. It also inhibits B cell and monocyte activation (Colonna et al., 1997).

4. C-type lectin receptors

The third family of human NK receptors for HLA class I antigens are C-type lectin family members. Most members of this family are expressed as heterodimers with CD94 covalently associated with a member of the NKG2 family (Lazetic et al., 1996; Brooks et al., 1997; Carretero et al., 1997; Bellon et al., 1999). The sole identified homodimer in this family is the activating receptor NKG2D, a distantly related member of the NKG2 family (Wu et al., 1999).

4.1. CD94/NKG2

Similar to KIRs, the CD94/NKG2 family of receptors has individual members with activating and inhibitory features that correlate with structural characteristics in their transmembrane and intracytoplasmic tails (López-Botet and Bellon, 1999) (see Figs. 1 and 2). The inhibitory members of this family are NKG2A and an isoform of NKG2A generated by alternative spliced pre-mRNA referred to as NKG2B. In accordance with their inhibitory function, they have intracytoplasmic tails with two ITIMs. The activating forms are NKG2C, -E and -H with NKG2E and -H being generated by alternative splicing of the same pre-mRNA transcript. These molecules have intracellular tails without ITIM motifs and a charged amino acid in the transmembrane region necessary for the association with DAP-12, an ITAM-bearing adaptor molecule (Bellon et al., 1999; López-Botet and Bellon, 1999) (see Fig. 1). An interesting member of the NKG2 family is NKG2F. So far, only the transcripts of this gene have been detected, but they are present in most NK clones (Kim and Coligan, unpublished data). The putative NKG2F protein has a charged residue in the transmembrane region, an ITIM-like motif in the cytoplasmic tail and does not contain any C-type lectin domain. However, a conserved 24-amino acid sequence, present in all members of the NKG2 family, suggests that NKG2-F could form heterodimers with CD94 (Plougastel and Trowsdale, 1997).

The ligand of CD94/NKG2 receptors is the nonclassical class I molecule HLA-E (Borrego et al., 1998; Braud et al., 1998; Lee et al., 1998). In general, HLA-E cell surface expression depends on specific peptides derived from positions 3–11 of the signal sequence of classical HLA class I molecules and HLA-G (Braud et al., 1997). (A*V***M***APRTLVL*LLSGALALTQTWA is the sequence for the signal peptide of HLA-A2 molecules with the HLA-E binding peptide shown in italics.) Thus, NK cells can monitor HLA-A, -B, -C and -G class I expression on cells directly through their recognition by KIR and indirectly through the recognition of HLA class I-derived peptides complexed with HLA-E. HLA-A, -B, -C and -G signal sequences can be grouped according to whether they have a Met residue (shown in bold) at position p2 of the resulting HLA-E binding peptide or a Thr residue (see review Posch et al., 1998). If present in sufficient levels (added exogeneously), either p2 Met or Thr containing peptides can stabilize HLA-E surface expression; however, the affinity of Thr containing peptides is significantly lower (Brooks et al., 1999). Because of this lower affinity, class I molecules that contain Thr at p2 of their signal sequence do not support the production of sufficient endogenous peptide to affect the stable expression of HLA-E on the cell surface. Once the HLA-E/peptide complex is stable on the cell surface, it must be recognized by CD94/NKG2A. As exemplified by EBV protein BZLF-1, there are nonsignal sequence derived peptides that stabilize HLA-E cell surface expression but that are not recognized by CD94/NKG2A (Ulbrecht et al., 1998; Brooks et al., 1999; López-Botet et al., 2000). While CD94/NKG2C clearly can bind HLA-E (Braud et al., 1998; Vales-Gomez et al., 1999), no such evidence has yet been reported for CD94/NKG2E or CD94/NKG2H. Moreover, it has been suggested that the ligand of CD94/NKG2H is not HLA-E (Bellon et al., 1999).

Like the binding of KIRs to HLA-C, the binding of CD94/NKG2A to HLA-E has very fast association and dissociation rates and the binding affinity is relatively

low, about 10 μM (Vales-Gomez et al., 1999; López-Botet et al., 2000). The CD94/NKG2C activating receptor binds to HLA-E with at least a 10-fold lower affinity than does CD94/NKG2A. This mimics the binding relationship of KIR activity and inhibitory receptors for HLA-C (see Section 2.1.4). The only peptide complexed with HLA-E that was recognized with relatively high affinity (~10 μM) by CD94/NKG2C was the leader peptide from HLA-G. This finding is consistent with functional results reported by Llano et al. (1998). These investigators examined the ability of NK clones expressing CD94/NKG2C to lyse 721.221 target cells that express HLA-E with different exogeneously loaded peptides. They found that the HLA-G derived signal sequence peptide, but not the equivalent p2 Met containing peptides derived from HLA-C molecules, was capable of sensitizing the 721.221 target cells for lysis.

The crystal structure of the extracellular portion of CD94 revealed a unique variation of the classical C-type lectin fold (Boyington et al., 1999). In the CD94 crystal structure, the carbohydrate-binding site is significantly altered and the Ca^{2+} binding site appears nonfunctional. Since C-type lectins require both of these sites to bind carbohydrate (Day, 1994), it seems unlikely that CD94 can do this. The fact that CD94 crystalizes as a dimer gave licence to Boyington et al. (1999) to predict the structure of CD94/NKG2 hetrodimers and the location of the putative HLA-E binding site.

4.2. NKG2D

NKG2D is distantly related to the other members of the NKG2 family and does not dimerize with CD94, rather it is expressed as a homodimer (Wu et al., 1999). It is an activating receptor expressed not only on NK cells, but also on all TCRγδ$^+$ T cells and activated CD8$^+$ TCRαβ$^+$ T cells (Bauer et al., 1999). The surface expression of NKG2D requires the association with an adaptor protein termed DAP10 (Wu et al., 1999) (see Figs. 1 and 2). The ligands of NKG2D are MICA and MICB, which are encoded within the human MHC (Bauer et al., 1999). MICA and MICB are stress-induced class I like molecules with three extracellular domains that neither associate with β_2-microglobulin nor bind peptides. They are commonly expressed on tumors of epithelial origin (Groh et al., 1996, 1999; Li et al., 1999). Considerable polymorphism has been observed with 54 alleles of MICA and 16 alleles of MICB so far identified (Bahram, 2000). At least one allele, MICA009, has been shown to be strongly associated with disease, in this case Behcet's disease (Mizuki et al., 1997). It is not known whether all allelic gene products of MICA/B bind to NKG2D; however, work by Cerwenka and Lanier (2001) suggest that the affinity of binding is quite variable. Recently Cosman et al. (2001) have shown that the ULBP (1, 2, and 3) class I-like molecules are additional ligands for NKG2D. These molecules have the class I related α1 and α2 domains but lack an α3 domain, and have a glycosylphosphatidylinositol (GPI) linkage to the plasma membrane. ULBPs show only low homology (23–26% identity) to other members of the MHC class I family. ULBP transcripts are detected in many normal tissues, and are upregulated in colon and stomach tumors, but were shown to be downregulated in a kidney tumor (Cosman et al., 2001). Very interestingly, UL16, a human cytomegalovirus glycoprotein, binds ULBPs and MICB, and a soluble form of UL16 can block the interaction of ULBPs or MICB with NKG2D suggesting a mechanism for viral interference of NKG2D recognition (Cosman et al., 2001).

The engagement of NKG2D by MICA or MICB activates the cytolytic response of NK cells, TCRγδ$^+$ T cells, as well as some CD8$^+$TCRαβ$^+$ T cells (Wu et al., 1999). Similarly, the expression of ULBPs by NK cell-resistant target cells confers susceptibility to NK cell cytotoxicity and stimulates cytokine production by NK cells (Cosman et al., 2001; Kubin et al., 2001). NKG2D has been shown to complement the other activation receptors expressed on NK cells, especially the functionally important NCR (see Section 1). The killing of certain target cells has been shown to be exclusively dependent on NCR expression by NK cells, other target cells are only susceptible to NK cells expressing NKG2D, and some target cells can be lysed by either NCR or NKG2D bearing NK cells. This pattern of target-cell susceptibility to killing is likely dependent on the ligands that they express (Pende et al., 2001).

It has been shown that fibroblast and endothelial cells substantially increase MIC cell surface expression following infection by human cytomegalovirus (HCMV) (Groh et al., 2001). MIC engagement of NKG2D augmented TCR-dependent cytolytic and cytokine responses by HCMV-specific CD28$^-$ CD8$^+$αβ$^+$ T cells, thereby overcoming the downregulation of MHC class I molecules induced by the virus. The ability of NKG2D to potently enhance TCR mediated production of IL-2 and T cell proliferation suggest that it can function as a costimulatory molecule on T cells. This was confirmed by showing that it could substitute for CD28 costimulation by hyperactive CD28$^-$, CD8$^+$ αβ$^+$ T cells.

The crystal structure of the NKG2D-MICA complex revealed a NKG2D homodimer bound to a MICA monomer in an interaction that is analogous to that seen for T cell receptor-MHC class I protein complexes (Li et al., 2001). Similar surfaces on each NKG2D monomer interact with large and highly complementary areas of the α1 or α2 domains of MICA. NKG2D itself is more closely related to CD94 than previously described members of the C-type lectin family and like CD94, does not contain a putative carbohydrate binding site or any of the features that are characteristic of the Ca^{2+} binding sites of C-type lectins. By surface plasmon resonance analysis, it was shown that the equilibrium dissociation constant (K_d), 1 mM at 37 °C, was one to two orders of magnitude stronger than NK cell receptors previously analyzed, as well as many TCR interactions with MHC proteins. Kinetic analysis indicated that

the NKG2D-MICA interaction might be more stable than TCR-ligand and other NK receptor-ligand complexes (Li et al., 2001).

A mouse NKG2D has been described that is expressed on NK cells, activated macrophages and activated $CD8^+$ T cells. The ligands for mouse NKG2D are H60 and Rae1 (Cerwenka et al., 2000; Diefenbach et al., 2000). In common with MICA and MICB, Rae-1 and H60 appear to be up-regulated in a number of tumors. The over-expression of Rae-1b and H60 in mouse tumors that do not naturally express them resulted in NK-mediated immune rejection of the tumors in vivo that was NKG2D dependent (Cerwenka et al., 2001; Diefenbach et al., 2001). Sometimes NKG2D tumor rejection requires $CD8^+$ αβ T cells (Diefenbach et al., 2001) and Girardi et al. (2001) have shown that $NKG2D^+$ γδ T cells are crucial for immune surveillance against malignant epidermal cells. The expression of Rae-1 and H60 increases in skin treated with the carcinogens. The killing of Rae-1- and H60-positive skin cells by γδ intraepithelial lymphocytes requires both NKG2D and γδ TCR, suggesting that NKG2D provides a costimulatory signal for the TCR. The chromosomal location of ULBPs on human chromosome 6 is syntenic with that of the RAE1 and H60 genes on mouse chromosome 10 supporting the idea that they are functional homologs (Cosman et al., 2001). Recently, O'Callaghan et al. (2001) showed that H-60 competes with a 25-fold higher affinity compared to RAE-1 for the same NKG2D binding site.

4.3. NK complex: gene organization and evolutionary perspective

The human NK complex and adjacent regions of chromosome 12 contain genes coding for C-type lectins, many of which are highly relevant in NK cell biology (Renedo et al., 2000; Hofer et al., 2001). The 28 genes identified within the 2.5 Mb defined as the NK complex code for C-type lectins, as well as proteins unrelated to immune function (Fig. 5). The telomeric boundary is defined by the C-type lectin gene DLEC/CLECSF11 expressed by monocyte derived dendritic cells (Arce et al., 2001). In the NK complex lies the dendritic cell immunoreceptor (*DCIR*), which codes for a C-type lectin found on blood monocytes and granulocytes but not T or NK cells. The next C-type lectin centromeric to *DCIR* is *MAFA-L*, an inhibitory C-type lectin found on peripheral blood NK cells as well as basophils. Located next to *MAFA-L* are the α2-macroglobulin (*A2M*) and the pregnancy zone protein (*PZP*) genes. These two genes share 70% identity, are functional homologs, and act as potent proteinase inhibitors (Bonacci et al., 2000). Eighteen genes within the human NK complex code for C-type lectin receptors, of which 13 are transcribed by NK cells (*MAFA-L*, *NKR-P1A*, *LLTI*, *CD69*, *AICL*, *KLRF1*, *CLEC-2*, *CD94*, *NKG2A/B*, *NKG2C*, *NKG2D*, *NKG2E/H* and *NKG2F*). Four genes have been identified that code for C-lectin type proteins, which are not expressed by NK cells. These include the aforementioned DCIR, CLEC-1,

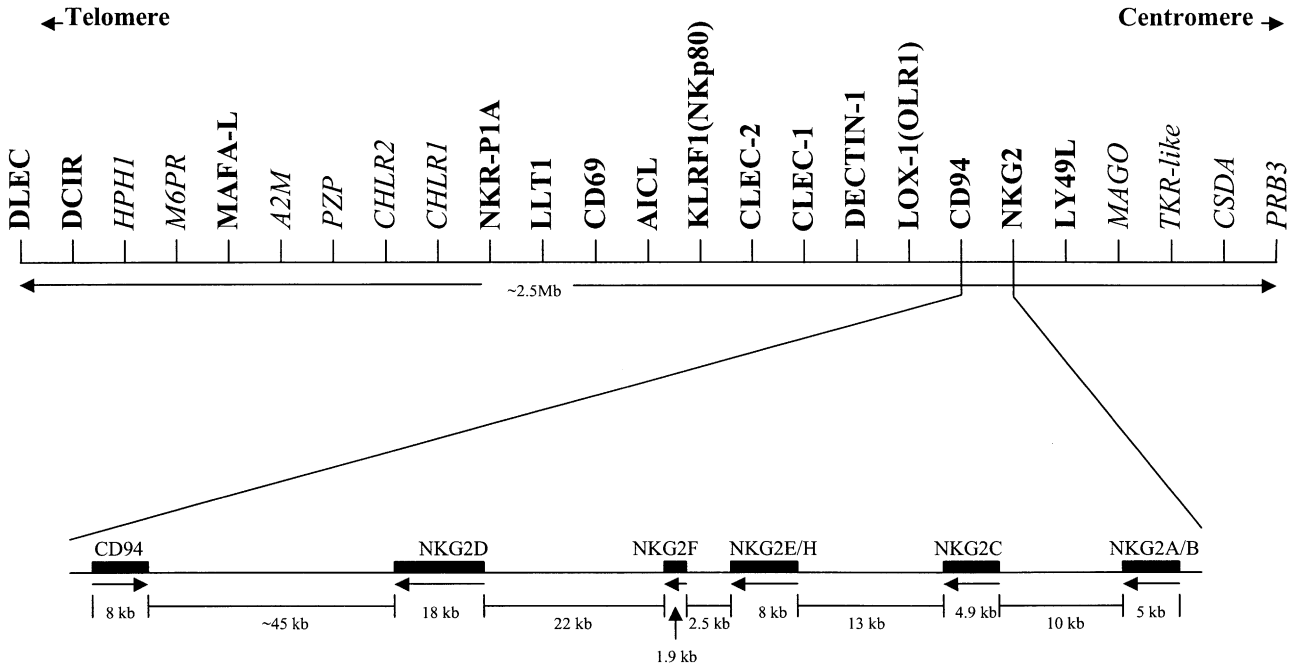


Fig. 5. The human NK complex is a ~2.5 Mb region on chromosome 12p12–p13 with boundaries defined by DLEC/CLECSF11 on the telomeric side and PRB3 on the centromeric side. The genes in bold code for C-type lectins, the genes in italics do not. Two of the *NKG2* genes generate multiple proteins through alternative splicing of pre-mRNA. *NKG2A* codes for the A and B protein isoforms which differ by the presence or absence of the protein sequence encoded by exon 4, respectively. Likewise, *NKG2E* codes for both the E and H protein isoforms. The NKG2H transcript lacks the terminal exon 7 of *NKG2E*. The magnified view of *CD94* and the *NKG2* family shows distances between genes and the direction of transcription. Note: this figure is not drawn to scale.

DECTIN-1 and LOX-1. CLEC-1 and Dectin-1 are expressed primarily on dendritic cells and LOX-1 is expressed in vascular endothelial cells (Yamanaka et al., 1998). In addition, Ly-49L codes for a C-type lectin but most likely does not produce an expressed protein (see below). PRB3 defines the centromeric boundary of the NK complex and codes for a salivary, proline-rich protein. Examination of the NK receptor mapping data indicates that the central region of the NK complex, from *NKR-P1a* to *LY49L* seems to contain exclusively C-type lectin receptors. Flanking this region are several unrelated genes, which code for non-lectin proteins. These include the previously mentioned *A2M*, *PZP* and *PRB3* as well as *HPH1*, *M6PR*, *CHLR1*, *CHLR2*, *MAGO*, *TKR-like* and *CSDA*. On the telomeric part of the NKC, near DCIR, lies *HPH1*, the human homolog of the *D. melanogaster* polyhomeotic gene, which plays a role in maintenance of the transcriptional repression state of *HOX* genes. *M6PR* codes for the calcium dependent mannose 6-phosphate receptor, which is involved in the intracellular transport of lysosomal enzymes. The *CHLR1* and *2* genes code for helicases. Centromeric to *Ly-49* is *MAGO*, the human homolog of mago nashi, a *Drosophilia* embryo germ plasm development protein (Zhao et al., 2000). Next lies the heretofore-uncharacterized putative tyrosine kinase receptor gene, *TKR-like.* The gene for cold shock domain protein A (CSDA), a putative repressor of GM-CSF transcription, follows the *TKR-like* gene (Cole et al., 1996).

DNA and protein sequence homology comparisons show *NKG2A*, *-C*, *-E*, and *-F* to be a closely related cluster of genes whereas *NKG2D* is no more similar to other members of the *NKG2* family than to *CD94* (Glienke et al., 1998). Transcriptional start sites for *NKG2D* have been identified within exon IV of *NKG2F* as well as closer to exon I of *NKG2D* (Houchins et al., 1991; Plougastel and Trowsdale, 1997). This suggests that *NKG2D* may be under the control of dual promoters. The *NKG2A*, *-C*, *-E* and *-F* genes are highly similar in genomic organization (Fig. 6) and sequence and have identical transcriptional orientation (Fig. 5). Sobanov et al.

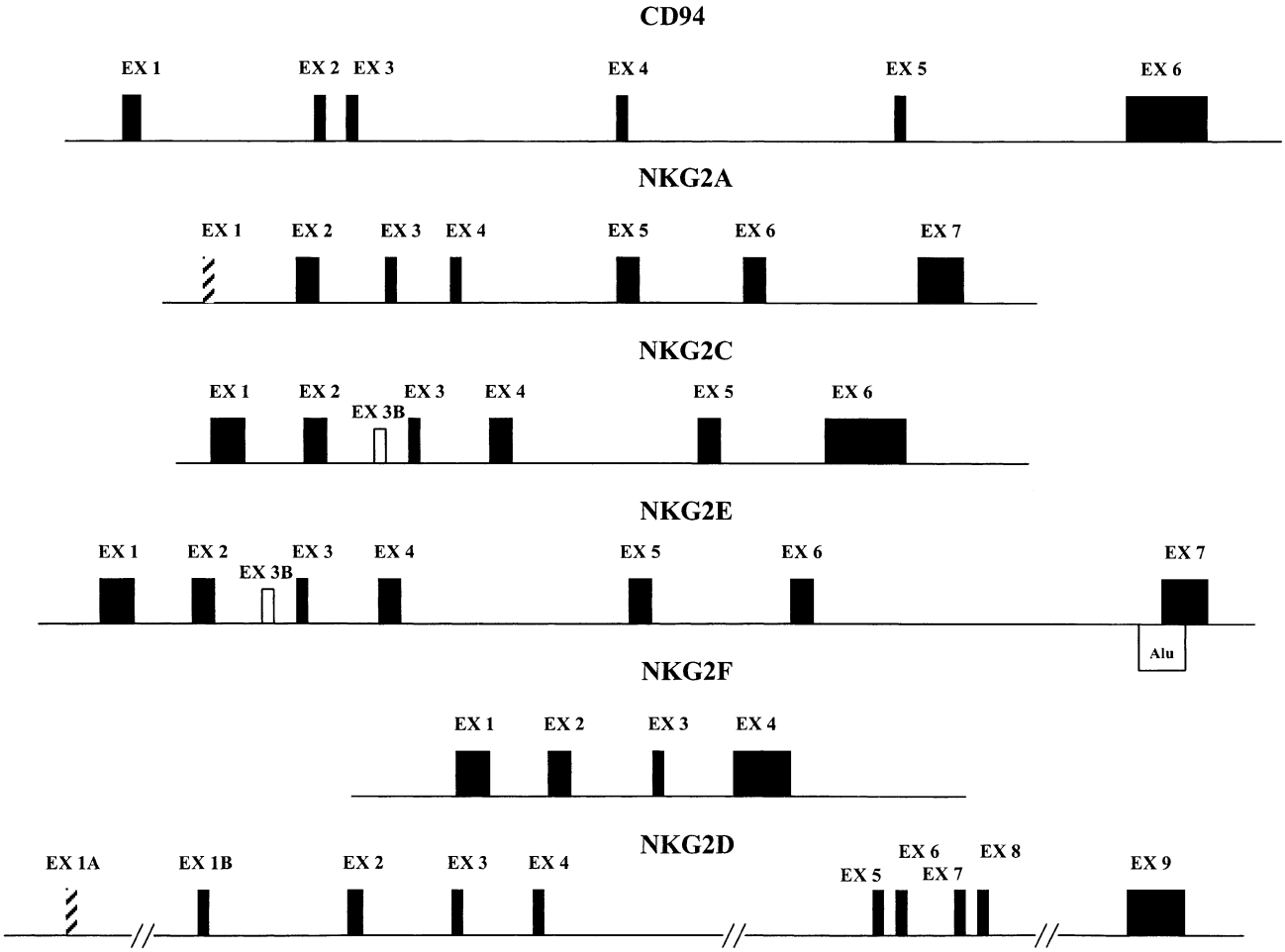


Fig. 6. This shows the relative exon/intron structure of the *CD94* gene and the genes of the *NKG2* family. The *CD94* gene is telomeric to the *NKG2A* gene and has low homology with *NKG2* gene family members. *NKG2A* has a non-coding initial exon represented by the diagonal lines. *NKG2C* and *E* have a duplicated exon 3B. *NKG2E* also has an Alu sequence which crosses the intron 6-exon 7 junction. *NKG2D* is a large gene with several long introns which are represented by '//'. Some *NKG2D* transcripts contain 131 noncoding nucleotides from *NKG2F* exon 4 at their 5′ end. Note: this figure is not drawn to scale.

(1999) proposed that these *NKG2* genes evolved through a mechanism of consecutive gene duplication. It is probable that an initial duplication event gave rise to *NKG2A* and a second gene that underwent subsequent duplication events to give rise to *NKG2F* and finally to *NKG2C* and *-E*. Prior to this last duplication event, there was an internal duplication of exon 3. A two base pair insertion within exon 4 of *NKG2F* lead to a frame shift which causes a premature stop codon to be read and results in a short transcript lacking an extracellular domain. Sequence comparison of *NKG2C* and *-E* revealed that they have a remarkable 98% identity in the 3 kb region upstream from their translation start sites, whereas *NKG2F* shows only a 75% identity (Brostjan et al., 2000). *NKG2C* and *-E* both contain a second version of exon 3 (exon 3b) within intron 2 (Fig. 6), which has yet to be observed in any cDNA (Glienke et al., 1998). *NKG2E* also contains an Alu element within the coding sequence of its 3′ end.

Multiple start sites for *NKG2A* transcripts have been identified ∼2.2 kb upstream from the start site of translation by primer extension analysis and 5′ RACE mapping. All NKG2A transcripts include a 5′ untranslated exon (Plougastel and Trowsdale, 1998; Brostjan et al., 2000). In addition to transcripts for *NKG2A*, the *NKG2A* locus codes for a pre-mRNA splice variant NKG2B that lacks an exon 4 encoded sequence. Similarly, *NKG2E* codes for both the -E and -H protein isoforms. The NKG2H transcript lacks the terminal exon sequence present in NKG2E transcripts. *CD94* lies to the telomeric side of *NKG2D* in the NK complex. *CD94* contains six exons, has a TATA less promoter, and multiple transcription start sites (Rodriguez et al., 1998). The human *Ly49L* gene is centromeric to NKG2A and fails to properly splice exon 5 at its 3′ end leading to termination of transcription at a premature stop codon in intron 6, plus it appears to have a defective polyadenylation site (Mager et al., 2001). The low abundance of this truncated mRNA and the unlikelihood that a translated protein would be functional suggests that the *Ly49L* gene is an evolutionary remnant (Barten and Trowsdale, 1999).

Comparison of the MHC I receptors of mouse and human NK cells indicates that the Ly49, CD94 and the NKG2 families existed before the divergence of the rodent and primate lineages around 100 million years ago (Kumar and Hedges, 1998). Functional CD94/NKG2 receptors are found in both mouse and human NK cells. However, *Ly49* genes, while functional in rodents, do not produce a detectable product in humans (see above). The presence of the KIR genes in primates, but not in rodents, suggests that the KIR genes evolved after the two lineages diverged and in humans, the KIR gene products assumed the functions executed by Ly49 proteins in mice. However, unlike humans, baboons and possibly other old world monkeys, express Ly49L as a secreted protein (Mager et al., 2001), but it is difficult to envision a functional role for a soluble Ly49 molecule. As in humans, it seems more likely to be an evolutionary remnant. The genes for *CD94* and the *NKG2* family are highly conserved between humans and primates with fewer interspecies differences than the genomic average (Khakoo et al., 2000). Chimpanzees have a species-specific duplication of *NKG2C*, which is not observed in humans or rhesus monkeys (Khakoo et al., 2000; LaBonte et al., 2000). *NKG2B* transcripts exist as an alternative splice product in both chimpanzees and rhesus monkeys. In addition to the canonical transcripts, rhesus monkeys also have alternatively spliced pre-mRNAs for NKG2C and -D that result in shorter transcripts (LaBonte et al., 2000).

The most highly conserved primate NK cell receptors for class I molecules are those that interact with the non-classical MHC class I molecules, i.e. members of the CD94/NKG2 family. This is consistent with the fact that the HLA-E gene sequence is highly conserved between humans and chimpanzees whereas the genes for the classical MHC class I molecules (KIR ligands) show much greater divergence between these same species (Arnaiz-Villena et al., 1997; Knapp et al., 1998).

5. Signal transduction coupled to HLA class I specific receptors

As previously stated, NK cells express both activating and inhibitory cell surface receptors that interact with MHC class I molecules (see Fig. 1). Inhibitory signaling receptors all possess cytoplasmic ITIMs, whereas the activating receptors lack ITIMs and associate with adaptor molecules, which are responsible for the transmission of the triggering signal (Ravetch and Lanier, 2000). It has become apparent that the effect or function of NK cells is regulated by a balance between opposing signals delivered by the MHC class I specific inhibitory receptors and by the activating receptors responsible for NK cell triggering. Upon ligation of activating receptors, NK cells can undergo blastogenesis, develop lytic capacity, produce cytokines, and show enhanced migration. However, the simultaneous ligation of activating receptors and inhibitory receptors with target cell ligands usually results in a dominance of inhibitory effects that downregulates the signals initiated via the activating pathways. The ligation of inhibitory receptors is characterized by tyrosine phosphorylation of ITIMs and subsequent recruitment and activation of phosphatases (SHP-1 and SHP-2), which leads to the inhibition of various NK cell-mediated effector functions (Burshtyn et al., 1996; Long, 1999; Tomasello et al., 2000a; Moretta et al., 2001).

5.1. Inhibitory receptors: the ITIM and SHP-1/SHP-2 phosphatases

Initially, ITIMs were characterized by the amino acid sequence YxxL/V, which was extended to I/V/L/SxYxxL/V (Vivier and Daeron, 1997; Ravetch and Lanier, 2000; Tomasello et al., 2000a). After ligation of inhibitory

receptors by MHC class I molecules, the first step in the transmission of the inhibitory signal is the phosphorylation of the ITIMs by a Src tyrosine kinase. Using a somatic genetic model, Binstadt et al. (1996) showed a requirement for $p56^{lck}$ in mediating KIR3DL tyrosine phosphorylation, but others (Burshtyn et al., 1996) have shown that other Src tyrosine kinases can also phosphorylate the ITIMs.

The phosphorylated ITIMs create the SH2 domain docking sites for recruiting and activating phosphatases (Burshtyn et al., 1996) (Fig. 1). The conserved aliphatic Y-2 residue (position 2 residues upstream of the Tyr) has been shown to be important for phospho-ITIM containing peptides to bind SHP-1 and SHP-2 in "in vitro" studies (Burshtyn et al., 1996, 1997; Vely et al., 1997). In vivo studies have shown that the membrane-proximal ITIM of KIR2DL (Fig. 1) has an important role in the transmission of the inhibitory signal, while the membrane-distal ITIM has a less relevant role. In addition, substitution of the conserved Y-2 residue in the membrane-proximal ITIM with Ala weakened the function of the receptor (Burshtyn et al., 1999). All phospho-ITIMs studied so far have an affinity for SHP-1 and/or SHP-2 protein tyrosine phosphatases (Binstadt et al., 1996; Burshtyn and Long, 1997; Leibson, 1997; Ono et al., 1997). After pervanadate treatment of appropriate cells, both phosphatases can be detected in immunoprecipates of KIR and CD94/NKG2A (Campbell et al., 1996; Le Drean et al., 1998; Bruhns et al., 1999). The importance of SHP-1 in KIR signaling is highlighted by the observation that the overexpression of a catalytically inactive form of SHP-1 (dominant negative) can reverse the inhibitory effect of KIR ligation for both ADCC and natural cytotoxicity (Burshtyn et al., 1996). On the other hand, since SHP-2 has been shown to be preferentially involved in activating signaling cascades (Huyer and Alexander, 1999; Qu et al., 1999), the relevance for its recruitment by the KIR and NKG2A ITIMs is unclear. The fact that SHP-2 has been implicated in signal transmission by CTLA4, a receptor whose engagement depresses T cell activation, suggests SHP-2 can play a role in negative signaling events (Thompson and Allison, 1997).

Inhibitory KIRs and NKG2A molecules each contain two ITIMs in their cytoptasmic tails. The membrane proximal motif starts about 30 residues from the transmembrane segment. All inhibitory receptors, whether they contain one or more ITIMs, have similar distances between the plasma membrane and the membrane proximal ITIM (Bléry et al., 2000). Although inhibitory KIRs and NKG2A molecules have two very similar ITIM motifs that are spaced similarly in the intracytoplasmic domains, they are orientated in opposite orientations. This is because KIRs and NKG2A are type I and type II integral membrane proteins, respectively. For KIR molecules, the membrane proximal motif appears to be functionally more important (Burshtyn et al., 1999) whereas the opposite is true for NKG2A (Kabat and Coligan, unpublished observations). This difference appears to be more related to the directional orientation of the molecules than to the structure of the individual ITIMs. The amino acid length between the two ITIMs in KIR and NKG2A is about 25 amino acid residues; a distance that is thought to be optimal for the recruitment of tandem SH2 containing phosphatases (Bléry et al., 2000). The simultaneous engagement of SHP-1/-2 SH2 domains is required for maximal phosphatase catalytic activity (Pluskey et al., 1995; Pei et al., 1996; Burshtyn et al., 1997).

The targets of catalytically activated SHP-1/-2 phosphatases are only beginning to be elucidated. The fact that the simultaneous ligation of 2B4 (CD244) and KIR2DL1 or CD94/NKG2A completely abrogates phosphorylation of 2B4 indicates that inhibition begins with the earliest steps in the activation process (Watzl et al., 2000). Little data exists in humans, but for mice several apparent targets of SHP-1 have been described and may explain the inhibitory capabilities of KIRs and CD94/NKG2A. Analysis of *Motheaten* mice, which are genetically deficient in SHP-1, revealed an increase in the phosphorylation state of Src family kinases upon TCR stimulation of T cells (Lorenz et al., 1996), as well as in the ITAM-containing, tranducing molecules CD3ζ and CD3ε. Also in *Motheaten* mice the adaptor protein linker (LAT) for T cell activation is hyperphosphorylated and it can be dephorphorylated by SHP-1 in vitro. In vivo, this dephosphorylation induces the dissociation of LAT and PLCγ in NK cells (Valiante et al., 1996). The SLP-76 adaptor protein has also been shown to be a target for SHP-1 in T and NK cells (Binstadt et al., 1998) and SHP-1 can dephosphorylate ZAP-70 and Syk (Plas et al., 1996; Dustin et al., 1999). These results indicate that SHP-1 has the potential to terminate activating signals by dephosphorylating molecules that function early in activation signalling pathways. However, a caveat to these results is that SLP-76 (−/−) (Peterson et al., 1999) and LAT (−/−) (Zhang et al., 1999) mice appear to have normal NK cytolytic function indicating that there is either redundancy in activation pathways or that SHP-1 dephosphorylation of these molecules is not involved in deactivation signals. Schematic models depicting the potential sites where inhibitory signals may interfere with NK cell activation signals are shown in reviews by Bléry et al. (2000) and Long et al. (2001).

When NK cells form conjugates with sensitive tumor cells, receptor containing rafts become polarized to the site of target recognition (Lou et al., 2000). This redistribution of lipid rafts requires the activation of both Src and Syk family protein tyrosine kinases. In contrast, the engagement by inhibitory KIRs on NK cells of HLA class I molecules on resistant, MHC-bearing tumor targets blocks raft redistribution. This inhibition is dependent on the catalytic activity of SHP-1, and supports a role for SHP-1 in inhibiting signal transmission through inhibition of raft aggregation. Recently it has been shown that the recruitment of SHP-1 to rafts and its association with LAT was dramatically increased after TCR engagement suggesting that SHP-1 is also involved in regulating raft-mediated T cell activation (Kosugi et al., 2001).

5.2. Activating receptors: involvement of DAP12 and DAP10

Although the first MHC class I specific NK receptors described were inhibitory receptors, soon afterwards highly homologous receptors were found that functioned as NK activating receptors (Biassoni et al., 1996). These receptors have short cytoplasmic tails and do not have an intracellular motif that explains their triggering capacity. An adaptor protein, called DAP12 or KARAP, was described that associates noncovalently with these short-tail NK receptors (Olcese et al., 1997; Lanier et al., 1998a) (see Figs. 1 and 2). A positively charged residue in the transmembrane region of the receptor associates with a negatively charged residue in the transmembrane region of DAP12 serving to stabilize the interaction between these two proteins (Lanier et al., 1998b). Cell surface expression of KIR2DS and CD94/NKG2C is greatly enhanced by the association with DAP12 (Lanier et al., 1998a,b). Crosslinking of DAP12 containing NK cell receptors results in cellular activation, as demonstrated by tyrosine phosphorylation of cellular proteins and upregulation of early-activation antigens (Lanier et al., 1998a,b). DAP12 is also expressed in peripheral blood monocytes, macrophages, and dendritic cells, suggesting association with other receptors present in these cell types (Lanier et al., 1998b).

DAP12 is expressed as a homodimer and contains a classical ITAM motif in its cytoplasmic domain (Fig. 1). The ITAM contains two of the consensus YxxL amino acid sequences spaced by 7 amino acids. Receptor clustering results in a rapid and transient phosphorylation of Tyr residues within the ITAMs, thereby creating binding sites for several SH2-domain containing cellular proteins such as protein tyrosine kinases and adaptor molecules coupling to downstream process (Isakov, 1997). The phosphorylated DAP12 initiated activation pathway is similar to that of T- and B-cell antigen receptors. Studies of the binding of signal transducing molecules to the ITAMs of the CD3-ζ chain showed that ZAP70 bound specifically to biphosphorylated but not to the mono- or unphosphorylated peptides (Zenner et al., 1996) Evidence that the two YxxL in each ITAM sequence are functionally distinct, emerged from mutations of the Tyr or Leu residues in the N-terminal YxxL segment of the membrane proximal ITAM of CD3-ζ which abolished all signal transduction functions for this molecule. In contrast, mutations of Tyr or Leu in the C-terminal YxxL of the ITAM amino sequence abrogated signals for IL-2 production, but did not prevent phosphorylation of the N-terminal Tyr of the ITAM nor did it interfere with other ITAM mediated functions (Sunder-Plassmann et al., 1997).

DAP12 (−/−) mice have normal numbers of NK cells and the repertoire of inhibitory MHC class I receptors is intact (Bakker et al., 2000). As expected, the class I specific Ly49 activating receptors were functionally impaired resulting in a diminished killing capacity for xenogenic tumor cells; however, killing of several mouse tumor cell lines was not affected indicating that DAP12 independent pathways are involved in these instances. Other features presented by DAP12 (−/−) mice include a resistance to peptide-induced experimental autoimmune encephalomyelitis. Resistance was associated with a strongly diminished production of IFN-γ by myelin peptide-reactive $CD4^+$ T cells due to inadequate T cell priming in vivo. These data suggest that DAP12 signaling may be required for optimal antigen-presenting cell (APC) function or inflammation (Bakker et al., 2000). Similar results were obtained by Tomasello et al. (2000b) using loss-of-function (dominant negative) mutant mice. Human polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), also known as Nasu–Hakola disease, is a recessively inherited disease characterized by a combination of psychotic symptoms rapidly progressing to presenile dementia and bone cysts restricted to the wrists and ankles. Genetic analyses of patients with this disease showed loss-of-function mutations in DAP12. Curiously, no abnormalities in NK cell function were detected in PLOSL patients homozygous for a null allele of DAP12 (Paloneva et al., 2000).

Similar to the activating KIR and CD94/NKG2 receptors, NKG2D does not have an intracellular motif that accounts for the transmission of the triggering signal, but instead of DAP12, NKG2D associates with an adaptor protein called DAP10 (Fig. 2) that is predominantly expressed in hematopoietic cells. In its cytoplasmic domain, each DAP10 has the amino acid sequence YxxM that binds the SH2 domain of the p85 subunit of PI 3-kinase (Wu et al., 1999). The sequence differs from the canonical YxxI/L sequence present in tandem in ITAM motifs (Gergely et al., 1999).

Recent studies by Warren et al. (2001) demonstrated that activating receptors are capable of stimulating NK cell lytic function in the presence of ligated inhibitory receptors. This was shown by using CD158b mAb that is equally reactive with activating KIR2DS2 and inhibitory KIR2D2 and KIR2DL3 receptors. These investigators showed that the activating KIR2DS2 receptor could be stimulated without interference from the inhibitory KIR2DL2 and KIR2DL3 receptors by using low concentrations of CD158b mAb and $Fc\gamma RII^+$ P815 target cells. High concentrations of CD158b resulted in the inhibition of the killing of the P815 target cells. The authors postulated that recognition of the same HLA ligand by co-expressed NK cell receptors may function as a fail-safe mechanism for activating NK cells in situations whereby HLA concentrations on target cells are below that capable of inhibiting NK cell function. This mechanism could be effective if no other NK cell-activating receptors and their appropriate target cell ligands are present. It will be important to determine if these results using CD158b mAb are applicable to responses to a natural ligand such as HLA-Cw3 and to rationalize these results with the fact activating receptors have significantly lower affinity than inhibitory receptors (see Section 2.1.4).

5.3. Possible role of activating receptors

The purpose of inhibitory receptors with class I specificity on NK cells seems rather clear, but, as yet, it is not so obvious why for each inhibitory receptor there is a corresponding activating receptor of similar specificity, albeit apparently of significant lower affinity. If they have the same ligand, and the inhibitory receptor has a significantly higher affinity, inhibition of activation would presumably prevail. Moreover, why would it be necessary to have activating receptors that recognize "normally" expressed class I molecules? It has been proposed (Tomasello et al., 2000a) that, by interacting with the same ligand, the activating receptors might serve to recruit the Src tyrosine kinase that phosphorylates the ITIMs in the inhibitory receptors. This seems unlikely since Ly49 inhibitory receptors work properly in mice deficient of DAP12 (Bakker et al., 2000). A more intriguing possibility is that KIR, Ly49, and NKG2 activating receptors have a low affinity for intact classical MHC class I molecules because these are not the prescribed ligands for these receptors. It is possible that the activating receptors recognize: (1) MHC class I type molecules associated with specific peptides, perhaps related to some pathological condition(s), (2) MHC class I type molecules like MICA/B expressed by cells under stress, and yet to be identified, and/or (3) MHC class I type molecules not encoded within the MHC that could even be pathogen encoded. A good example supporting the latter possibility is data (Daniels et al., 2001; Brown et al., 2001) showing that the expression of the murine Ly49H activating receptor correlates with resistance to the MCMV infection. Results by Ryan et al. (2001) suggest that Ly49H recognizes a viral encoded protein instead of a classical MHC class I molecule. Thus, it is possible that inhibitory receptors bind self-MHC to prevent autoimmunity, while the activating receptors might have evolved to recognize bacterial or viral MHC class I like proteins or unique pathogen-derived peptides presented by a classical or nonclassical MHC class I molecule(s) (Lanier, 2001). In this context, autoreactive $CD4^+CD28^-$ T cells with cytolytic capabilities are expanded in patients with vasculitic rheumatoid arthritis (RA). These cells have been shown to express the KIR2DS2 activating receptor, usually in the absence of KIR inhibitory receptors (Namekawa et al., 2000). Expression of the KIR2DS2 gene was significantly increased in patients with rheumatoid vasculitis compared with normal individuals and in patients with RA but no vasculitis. Also, the distribution of HLA-C allotypes, which are putative ligands for KIR2DS2, was significantly different in patients with rheumatoid vasculitis in comparison with control individuals. These data suggest that $CD4^+CD28^-$ T cells expressing KIR2DS2 receptors may regulate vascular damage in RA vasculitis through recognition of particular HLA-C ligands (Yen et al., 2001) perhaps in complex with a particular peptide.

6. Expression of NK cell receptors

The expression of most class I specific NK cell receptors are clonally distributed. Each NK cell expresses at least one inhibitory receptor specific for a self-MHC class I molecule, and they may or may not express an activating receptor (Valiante et al., 1997). NK cell receptors can also be expressed by subpopulations of human T cells. The molecular mechanisms that regulate the clonally diverse expression of NK cell receptors on NK and T cells are unknown. Unlike the TCR, the expression of NK cell receptors is not dependent on gene recombination events, indicating that their expression is largely transcriptionally regulated.

6.1. Regulation of expression in NK cells

The mechanism that generates and selects for NK cell receptor gene expression in humans is not well defined. Activated NK cell populations and NK clones isolated from single donors have been shown to display different patterns of cytolytic activity against a panel of allogeneic cells, indicating that an NK cell repertoire exists (Moretta et al., 1994). Receptors are clonally distributed and many NK cells in an individual share the same repertoire of receptors (Long, 1999). Work in the mouse indicates that the expression of NK cell inhibitory receptors involves a stochastic process with the final repertoire shaped by educational processes based on MHC class I molecules expressed by the host (Lanier, 1998; Raulet et al., 2001). Once an NK cell receptor gene is activated for transcription, KIR and CD94/NKG2 receptor expression is stably maintained in the clonal expansion of the cells in long-term NK clone cultures (Moretta et al., 1990).

A recent study looking into human NK cell receptor ontogeny made use of stem cells derived from umbilical cord blood cells to show the developmental stages at which CD94 and KIR receptor expression is acquired (Miller and McCullar, 2001). Contact-dependent ligand stimulation afforded by stromal feeder cells, along with the presence of IL-15 and IL-2 in the culture, were important for optimal NK cell differentiation leading to receptor expression. When cultures were devoid of the stimulating cytokines or contact with the feeder cells, the NK cell progeny were KIR and CD94 receptor negative. From single progenitor cells, NK cell receptor acquisition was shown to be polyclonal for both CD94 and KIR. The frequency of $CD94^+$ NK cells was greater than KIR and CD94 expression occurred earlier, as shown by the number of $CD56^+/CD94^+$ cells in cultures at different time points. Other studies have shown similar results using stroma-free cultures of progenitor cells (Jaleco et al., 1997; Yu et al., 1998; Muench et al., 2000). However, experiments by Mingari et al. (1997) using thymic precursors showed that IL-15 in the absence of stroma cells provides an appropriate stimulus for the expression of CD94/NKG2A, but not for KIR receptors in the maturing of NK cells. The dependency on stroma cell presence

for complete human NK cell differentiation and receptor acquisition agrees with the development of Ly49 receptor expression by mouse NK cells (Roth et al., 2000). These studies agree on the importance of IL-15 for NK cell development and receptor acquisition; however, whether stromal cells are also necessary needs to be resolved, especially since IL-15 can be made by stroma and macrophages.

The co-expression of KIR and CD94/NKG2 inhibitory molecules within the NK cell population is well established. Although, at first hand, the expression of these two families of molecules appears to be somewhat redundant, their combined presence appears to be advantageous to the host. The NK cells expressing the CD94/NKG2A receptor utilize HLA-E as a sentinel to reflect the global status of class I expression. Cells are deemed abnormal only if they down-regulate synthesis of most, if not all, class I molecules. While the elegance of this system is that it allows an invariant NK cell receptor to detect the expression of a highly divergent group of class I genes, the loss in the expression of individual class I molecules can be overlooked. Therefore, it seems that *KIR* genes in humans and *Ly49* genes in the mouse may have evolved subsequent to the *NKG2A* gene family to monitor the loss of expression of single class I molecules (Lanier, 1998; Long, 1999).

6.2. Regulation of expression on T cells

This topic has recently been reviewed by McMahon and Raulet (2001). About 5% of human peripheral blood $CD8^+$ T cells express KIR and/or CD94/NKG2 family members (Mingari et al., 1996a; Speiser et al., 1999b). Both stimulatory and inhibitory KIRs are expressed (Andre et al., 1999; Mandelboim et al., 1998) and, like NK cells, $CD8^+$ T cells are clonally distinct in their receptor expression pattern (Uhrberg et al., 2001). KIR molecules expressed on T cells can regulate effector functions such as cytokine release and target cell cytolysis (Mingari et al., 1998; Ugolini and Vivier, 2000), as can CD94/NKG2A molecules (Noppen et al., 1998; Speiser et al., 1999a).

KIR and CD94/NKG2 are not detectably expressed by naïve $CD8^+$ T cells or by thymocytes. KIR expression is restricted to $CD8^+$ T cells bearing a memory phenotype (Mingari et al., 1996b). It is not clear what leads to the induction of KIR expression, perhaps stimulation by particular types of antigen. T cells specific for tumors, particularly melanomas (Speiser et al., 1999b; Huard and Karlsson, 2000b), viral antigens (Noppen et al., 1998) or self-antigens (Huard and Karlsson, 2000a) have been shown to express KIRs. Despite these examples, the majority of antigen specific CTLs do not express KIR. Unlike KIRs, CD94/NKG2A is upregulated on $CD8^+$ T cells under certain culture conditions. This was demonstrated by culturing T cells with superantigen or anti-TCR Ab in the presence of IL-15 and IL-10 (Mingari et al., 1998; Galiani et al., 1999) or TGF-β (Bertone et al., 1999). Recent data indicates that CD94/NKG2A expression is not limited to $CD8^+$ T cells. Romero et al. (2001) showed that in the presence of TGF-β and IL-10 CD3 activated $CD4^+$ T cells can express CD94/NKG2A. Coligation of the TCR and CD94/NKG2A on these cells resulted in an inhibition of TNFα and IFN-γ secretion suggesting that CD94/NKG2A may at times regulate $CD4^+$ T cell responses. Thus, current data indicate that KIR expression is restricted to specific conditions of activation, perhaps antigen regulated, whereas CD94/NKG2A expression is more generalized.

A number of hypotheses have been put forward to explain why $CD8^+$ T cells express NK receptors (McMahon and Raulet, 2001). One possibility is that inhibitory KIR expressed by mature CTLs are reactive with peripheral self antigens as a means of preventing autoimmunity. This could allow class I specific inhibitory receptors to prevent destruction of normal cells while allowing lysis of tumor cells that have down-regulated expression of a particular class I allele (Ikeda et al., 1997). A second possibility is that KIR^+ CTL may arise from chronic stimulation. This is supported by the fact that almost no CTL express KIR during acute viral infections, but chronic viral infections like CMV or HIV give rise to oligoclonal KIR^+ CTL populations (Mingari et al., 1996a). Obviously such KIR expression would lead to a weakened host response to chronic infection and could contribute to viral pathogenesis (De Maria and Moretta, 2000). To reconcile this, it has been suggested that inhibitory receptor expression provides a mechanism for chronically stimulated CTLs to avoid over-stimulation leading to activation-induced apoptosis. Subsequent down modulation of KIR by surviving cells may allow them to respond later to antigenic restimulation. Indeed Huard and Karlsson (2000a) have shown that, in the absence of antigen, KIR^+ CTL down-regulate KIR expression to non-functional levels. Lastly it has been proposed that inhibitory receptors, by preventing activation induced apoptosis of CTL during immune response, may aid in the formation of memory CTLs (Ugolini et al., 2001).

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