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Invited Review

Human Natural Killer cell receptors: insights into their molecular function and structure

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Abstract

NK cells express receptors characterized by opposite functions that finely regulate their activities. Among inhibitory receptors, some are specific for different groups of MHC class I alleles, while others are still orphan receptors. On the contrary, various activating receptors are involved in the triggering of NK-mediated natural cytotoxicity. In general, their engagement induces human NK cells to kill target cells that are either HLA class I-negative or -deficient. Thus, the process of NK cell triggering mediated by Natural Cytotoxicity Receptors can be mainly considered as a non MHC-restricted mechanism. Here, a brief description of the molecular nature of these receptors, as well as, of their 3D-structures and of the implications for ligand recognition, is given.

Keywords: Natural Killer (NK) - Leucocyte Ig-like Receptor Complex (LRC) - Natural Cytotoxicity Receptors (NCRs) - MHC - Inhibitory Receptors - Immunoglobulin Superfamily (Ig-SF) - Crystal Structure - Activating Receptors - Sialic acid-binding Ig-like lectin (Siglec)

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Introduction

Natural Killer (NK) cells are important effectors of the innate immune response, that have been originally described on a functional basis as capable of killing tumors or virally infected cells without previous stimulation [1,2]. This lymphocyte subpopulation is characterized by the absence of conventional receptors for antigen, *i.e.* surface immunoglobulin or T cell receptor (TCR) and displays a $CD3^-$ $CD16^+$ phenotype. CD16 (FcγRIII), the low affinity receptor for IgG, is involved in the Antibody Dependent Cell-mediated Cytotoxicity (ADCC). CD16 is expressed on the majority of, but not all, human NK cells and on activated monocytes as well as on a T cell subset [3].

Thus, until recently, NK cells appeared to express only a small number of surface receptors. Moreover, these molecules did not explain NK cell functions completely, since they are not responsible for natural cytotoxicity. On the other hand, in the last ten years we have witnessed the discovery of an array of receptors expressed by NK cells. In particular, two distinct families of receptors are responsible for the regulation of NK cell activity by binding to MHC class I molecules or to still unidentified ligands: the immunoglobulin-like NK receptors (KIR, LILR, NCRs, p75/AIRM1, IRp60, 2B4/CD244, NTB-A, DNAM1/CD226 and LAIR) and the C-type lectin-like NK receptors (CD94/NKG2, NKG2D, NKp80, NKRP1 and the rodents Ly49 receptors) (Tab I) [4-10]. These receptors are characterized by either inhibitory or activating properties and are involved in the fine regulation of NK cell function.

Signaling of inhibitory receptors is mediated by immunoreceptor tyrosine-based inhibitory motifs (ITIM) present in their cytoplasmic tail. Upon receptor/ligand interaction, ITIM are tyrosine phosphorylated and associated with Src-homology domain-bearing tyrosine phosphatases (SHP-1, -2); this results in the inhibition of the intracellular activation cascade [4]. Some of these receptors recognize MHC class I molecules (KIR, CD94/NKG2, Ly49 and LILR). The MHC class I-specific inhibitory receptors are expressed by all NK cells in a clonally distributed fashion; their interaction with different groups of HLA class I alleles spares normal cells from NK lysis, since it results in the inhibition of natural cytotoxicity [3,7,9]. Importantly, NK lymphocytes kill cells that have undergone tumor transformation or viral infections, known to have lost, partially or completely, MHC class I expression [11,12]. These concepts are the basis of the "missing self" hypothesis [13] that has been confirmed and extended by experimental data in both mouse and human. While NK cells do not lyse autologous normal tissues that express self MHC class I alleles, NK cell triggering is mediated by non MHC-restricted activating receptors [10].

Activating receptors transduce signals through the association with molecules, such as CD3ζ, FcεRIγ and DAP-12, that contain in their intracytoplasmic region Immunoreceptor Tyrosine Activating Motifs (ITAM) that upon phosphorylation transduce activation via $p72^{syk}$ and ZAP70 cytoplasmic PTK [5,6,14]. On the contrary, other receptors such as NKG2D use the associated DAP-10 polypeptide to trigger via the PI-3 kinase pathway [15].

Natural Cytotoxicity Receptors (NCRs; [14]) and NKG2D [15] are the major receptors involved in NK cytotoxicity; while cellular ligands of NCRs are still unknown, human NKG2D has been shown to recognize different ligands, such as the MHC-class I homologs MICA and MICB and the family of UL16-binding proteins (ULBP1-4) [15-17]; moreover, orthologs of ULBP (Rae-1 and H60) have been identified as the cellular ligands of murine Nkg2d [15,18]. Other NK receptors (NKp80, CD244, DNAM-1 and NTB-A) play a role as activating coreceptors in NK-mediated cytolysis. CD244 (2B4) recognizes CD48 molecule [19], DNAM-1 has been recently shown to bind Polio Virus receptor (PVR/CD155) and Nectin-2 (PRR2/CD112) [20], while NKp80 and NTB-A are still orphan receptors [5,6].

NK cells also express non-MHC specific inhibitory receptors, such as IRp60, p75/AIRM-1 and leukocyte adhesion inhibitory receptor (LAIR)-1 [5,6]. The sialoadhesin p75/AIRM1 binds sialic-acid oligosaccharide moieties [21,22], while IRp60 and LAIR-1 [23] are two inhibitory receptors involved in the regulation of NK cell activity recognize still unidentified cellular ligands. Finally, although the natural ligand of human NKRP-1 is still unknown, some members of murine Nkrp1 have been recently shown to recognize C-type II lectins belonging to the Clr family [24].

Natural Cytotoxicity Receptors (NCRs)

NK cells express three different receptors directly involved in natural cytotoxicity, termed NCRs [14].

Table 1 Functions and ligands of human Natural Killer (NK) cell receptors.

Receptor name	Other name	Function	Cellular ligand(s)	Viral Ligand(s)
NKp46	Ly94/NCR1	activating	unknown	SV-HA; IV-HA
NKp30	1C7/NCR3	activating	unknown	
NKp44	Ly95/NCR2	activating	unknown	SV-HA; IV-HA
NKG2D	D12S2489E	activating	MICA, MICB, ULBPs	
2B4	CD244	activating*	CD48	
NTB-A	KALI	activating*	unknown	
NKp80	KLRF1	activating	unknown	
CD16	FcRIIIa	activating	IgG	
CD2	LFA-2	activating	CD58; LFA-3	
DNAM-1	CD226	activating	PVR/CD155, Nectin-2/CD112	
p58.1	KIR2DL1	inhibitory	HLA-Cw2, 4, 5, 6	
p58.2	KIR2DL2	inhibitory	HLA-Cw1, 3, 7, 8	
p70	KIR3DL1	inhibitory	HLA-Bw4	
p140	KIR3DL3	inhibitory	HLA-A3, -A11	
p49	KIR2DL4	inhibitory (?)	HLA-G	
LIR1/ILT2	LILRB1	inhibitory	HLA-G, other alleles	HCMV-UL18
LIR2/ILT4	LILRB2	inhibitory (?)	HLA-F	
CD94†	KLRD1	#	HLA-E	
NKG2A†	KLRC1	inhibitory	HLA-E	
p50.1	KIR2DS1	activating	HLA-Cw2, 4, 5, 6	
p50.2	KIR2DS2	activating	HLA-Cw1, 3, 7, 8	
p50.3	KIR2DS4	activating	unknown	
NKG2C†	KLRC2	activating	HLA-E	
NKG2E†	KLRC3	activating	HLA-E	
p40	LAIR1	inhibitory	unknown	
IRC1	IRp60/CMRF35H	inhibitory	unknown	
CMRF35	CMRF35	?	unknown	
p75/AIRM1	Siglec-7	inhibitory	sialylated sugars moieties	

*These molecules have been shown to display inhibitory rather that activating functions in NK cells derived from XLP patients. †CD94 forms heterodimers with NKG2A, NKG2C and NKG2E specific for HLA-E. #CD94 lacks signaling motifs in the cytoplasmic tail.

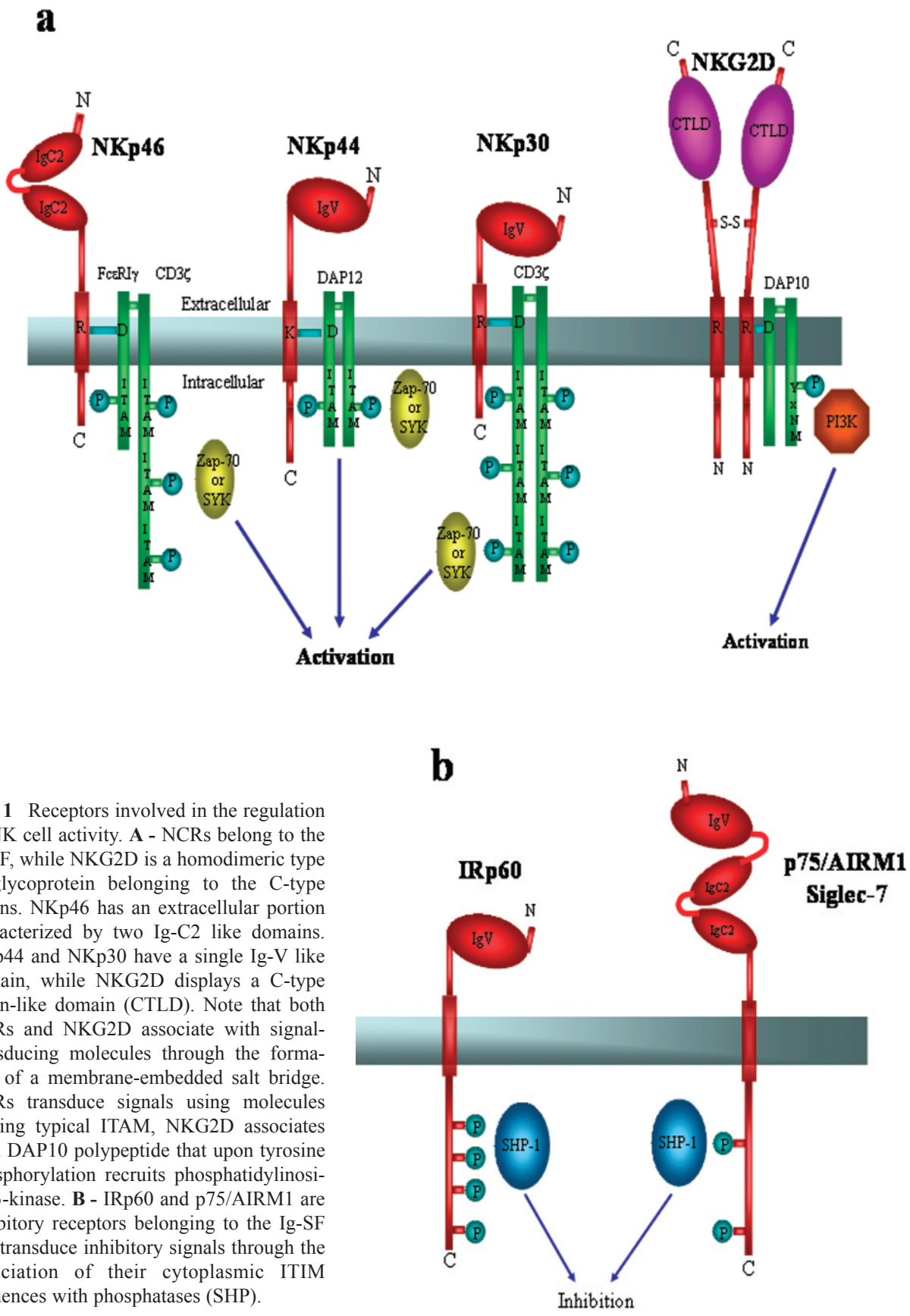

Fig. 1 Receptors involved in the regulation of NK cell activity. **A -** NCRs belong to the Ig-SF, while NKG2D is a homodimeric type II glycoprotein belonging to the C-type lectins. NKp46 has an extracellular portion characterized by two Ig-C2 like domains. NKp44 and NKp30 have a single Ig-V like domain, while NKG2D displays a C-type lectin-like domain (CTLD). Note that both NCRs and NKG2D associate with signal-transducing molecules through the formation of a membrane-embedded salt bridge. NCRs transduce signals using molecules bearing typical ITAM, NKG2D associates with DAP10 polypeptide that upon tyrosine phosphorylation recruits phosphatidylinositol 3-kinase. **B -** IRp60 and p75/AIRM1 are inhibitory receptors belonging to the Ig-SF and transduce inhibitory signals through the association of their cytoplasmic ITIM sequences with phosphatases (SHP).

These receptors expressed by NK cells are NKp46, NKp30 and NKp44 (Fig 1A). Although the cellular ligands recognized by NCRs have not been characterized yet, NKp46 and NKp44 were described to recognize viral proteins such as haemagglutinin of influenza virus or haemagglutinin-neuraminidase of parainfluenza virus [25,26].

Resting and activated NK cells express NKp46 and NKp30, while NKp44 is present only on the surface of activated NK cells [10,14]. NCRs belong to the immunoglobulin superfamily (Ig-SF) [5,6], display one or two extracellular Ig-like domains and transduce activating signals through ITAM-bearing molecules associated via the formation of a salt bridge in their transmembrane region [5,6,14]. In particular, it has been shown that crosslinking of each NCRs results in three early events: activation of $p56^{lck}$ and $p59^{fyn}$, tyrosine phosphorylation of the NCR-associated polypeptides and activation of $p72^{syk}$ and ZAP70 cytoplasmic PTK [27]. The existence of a functional "cross-talk", is also supported by the recent finding that the engagement of a single NCR is able to activate the signal transduction pathway used by the other NCR leading to the amplification of NCR-mediated activating signals [27].

NKp46 is characterized by two extracellular Ig-like domains of the C2 type [5,6] and is associated with CD3ζ and FcεRIγ that, upon receptor engagement, become tyrosine-phosphorylated (Fig 1A). The gene coding for NKp46 has been mapped on human chromosome 19q13.42 in a region called Leukocyte Receptor Complex (LRC), telomeric to the KIR multigene family [28]. The NKp46 gene is conserved in rodents (both mouse and rat) and in primates, such as chimpanzees and macaques [5,6,29].

NKp30 is a 30 kDa glycoprotein characterized by a single extracellular Ig-like domain of the V type and associates with CD3ζ polypeptides (Fig 1A). The NKp30 gene maps on human chromosome 6p21.32, at the telomeric extremity of the HLA class III region between the LTB (lymphotoxin beta or TNF superfamily member 3) and AIF1 (allograft inflammatory factor 1) genes [5,6]. NKp30 gene is conserved in macaques and rats [29,30], while only a pseudogene has been identified in mice, suggesting that NKp30 gene appeared more recently than NKp46 during evolution.

Recently, it has been found that transforming growth factor β1 (TGFβ1) may modulate the surface expression of receptors involved in the NK-mediated cytolysis suggesting a tumor escape mechanism to the NK surveillance. In particular, TGFβ1, that may be released by tumors of different histotypes, including melanomas, neuroblastomas, carcinomas, and leukemias, is able to induce a strong down-regulation of surface expression of triggering NK receptors, such as NKp30 and, at least in part, of NKG2D [31]. On the other hand, NKp30 is the major receptor responsible for the recognition and killing of dendritic cells (DC), suggesting important interdependence between native and adaptive immune responses and in the generation of optimal cytotoxic T lymphocyte responses [32].

NKp44 displays a single extracellular Ig-like V domain and a transmembrane portion containing the charged residue (Lysine), likely involved in the association with KARAP/DAP12 molecules (Fig 1A) [5,6]. Gene coding for NKp44 maps on human chromosome 6p21.1 close to TREM-1 (triggering receptor expressed on myeloid cells-1) and TREM-2 genes coding for receptors that also associate with KARAP/DAP12 molecules, but expressed by neutrophils and monocytes or macrophages and dendritic cells, respectively [33]. Differently from other NCRs, homologous genes for NKp44 in species different from humans have not been identified so far. Moreover, in the synthenic region of mouse chromosome 17, no NKp44 gene has been mapped [33], indicating that the latter gene evolved in human, but not in mouse genome and suggesting that NKp44 is the last NCR gene appeared in phylogenesis.

NKG2D

It is well established that NCRs and NKG2D receptor cooperate in NK-mediated cytolysis; however, the role of NKG2D is better appreciated in NK-tumor cell killing by NK cells expressing low NCRs surface densities (NCR^{dull} phenotype) [34]. NKG2D is a C-type II lectin-like receptor, distantly related to the other NKG2 family members; differently from them, it does not associate with CD94 and is expressed at the cell surface as a homodimer [15]. NKG2D triggers NK-mediated cytolysis through the associated DAP10/KAP10 polypeptides, displaying an YxxM motif that, upon tyrosine

phosphorylation, recruits phosphatidylinositol 3-kinase (PI3-kinase) (Fig. 1A) [15]. NKG2D gene is localized in a locus, termed NK gene complex (NKC) that maps on human chromosome 12p13.2, where other genes encoding members of the C-type II lectin family are located. NKG2D is constitutively expressed on the surface of all human NK, TCR γ/δ and $CD8^+$ T lymphocytes, while in mice it is expressed by all NK cells, but only in a subset of TCR γ/δ and $CD8^+$ T lymphocytes.

Interestingly, several NKG2D-specific cellular ligands have been identified so far. In human, they are represented by the stress-inducible molecules MICA and MICB [15,35], encoded within the human MHC complex, and by the cell surface proteins ULBP1-4 [16,17]. MICA and MICB molecules are normally expressed by the gastrointestinal epithelium, but they can be expressed also by some epithelial, lung, breast, kidney, ovary, prostate, colon tumors [35], and melanomas [34]. Their expression is upregulated in response to cellular stress and following certain pathogen infections. Structurally, they are transmembrane molecules containing the α1, α2 and α3 MHC class I-like domains, but they do not associate with β2-microglobulin and do not bind any peptide. More recently, at least four additional NKG2D ligands known as human cytomegalovirus UL-16 binding proteins (ULBP) have been described [16,17]. ULBP-1, -2 and -3 are glycosylphosphatidyl inositol (GPI)-linked surface molecules, whereas ULBP-4 displays a transmembrane and a cytoplasmic portion. ULBP are expressed in a variety of cell lines, normal tissues and tumors and are characterized by the α1 and α2 MHC class I domains. It is of note that ULBP share sequence homology with the products of the retinoic acid-inducible gene family Rae1α–ε that represents one of the two types of ligands for murine NKG2D; together with the transmembrane surface molecule H60 [15,18]. Rae1α–ε is expressed in the early embryogenesis and are usually absent in normal mature tissues, but their expression is found on several murine tumor cell lines. On the contrary, H60 molecule is differentially expressed in different mouse strains. Although, it is intriguing that a single receptor may bind to different ligands sharing a low degree of similarity, an induced-fit mechanism, based on crystallographic observations, has been proposed to address this issue [36].

NKG2D signal transduction depends on its association with DAP10 adapter molecule that triggers multiple signal transduction pathways in an ITAM-independent way. Differently from other NK cell triggering receptors, NKG2D/DAP10 recruits, the p85 subunit of the PI3-kinase and Grb2, resulting in downstream phosphorylation of the ERK1/2 MAP kinase pathway. Engagement of NKG2D by soluble ULBP was also shown to trigger JAK2/STATs signaling pathway and Ca^{++} flux [15,16].

It has been proposed that NKG2D may override the inhibition mediated by MHC class I-specific inhibitory receptors, in consideration of the stress-inducible nature of its ligands and of their specific expression on different tumor cell lines [15]. Although, this is still an open question, different experimental models suggest that NKG2D plays a role in the fine regulation of NK cell function under the control of MHC class I molecules. Thus, these data, together with the findings that ULBPs may be expressed on normal tissues are in conflict with the proposed mechanism of independence of NKG2D signaling from the inhibitory action of MHC class I-specific inhibitory receptors [15,34]. Thus, stress-induced upregulation of NKG2D ligands in tumor cells may be an additional triggering signal towards activation and induction of NK-mediated cytolysis. To this end, it is important to stress that NKG2D use a signaling pathway distinct from the one used by other activating receptors, this may results in a different sensitivity to the inhibitory signals delivered by KIR and C-type lectins or, on the contrary the induction of multiple activating pathways may alter the dynamic equilibrium between inhibitory versus activating signals resulting in an apparent insensitiveness to inhibitory receptor [15,34].

IRp60 and the gene cluster on chromosome 17q25 linked to the psoriasis susceptibility (PSORS2) locus

Among the non-MHC specific inhibitory receptors, IRp60 has been identified as a surface molecule expressed by all NK cells and displaying inhibitory function on NK-mediated cytotoxicity induced via different triggering receptors. Actually, IRp60

may play a more general role in the regulation of the immune response since it is expressed also on cell types other than NK cells, including T lymphocyte subsets, monocytes and granulocytes [5].

IRp60 is a 60 kDa surface molecule belonging to the Ig-SF, displaying one extracellular Ig-like domain of the V type, a hydrophobic transmembrane portion and a cytoplasmic tail containing three classical, and a fourth "non classical", ITIM motifs involved in the inhibitory function mediated by IRp60 (Fig. 1B). Thus, following receptor crosslinking, IRp60 becomes tyrosine phosphorylated and, subsequently, SHP1 and SHP2 phosphatases are recruited (Fig. 1B).

A protein called CMRF35-H has been identified that displays 98% sequence identity with IRp60 likely representing an allelic isoform. Moreover, IRp60 IgV domain displays a high degree of sequence identity with similar Ig-SF domains, such as the one displayed by NKp44, poly Ig receptor for IgA and IgM and CMRF35-A. In particular, IRp60 and CMRF35-A Ig-V domains share 90% of identity, but differently from IRp60, CMRF35-A protein contains a negatively charged residue in the transmembrane region and does not display any ITIM in its cytoplasmic portion, although its surface expression and functional properties are still uncharacterized.

The gene encoding IRp60 is located on human chromosome 17q25, in a gene cluster characterized by other five CMRF35-A-like genes [5,37]. Intriguingly, another IRp60-related cluster of genes maps on human chromosome 6 containing NKp44, Triggering Receptor Expressed on Myeloid cells (TREM)-1 and TREM-2, suggesting a common evolution of these two gene clusters from a common ancestor by gene, or segmental, duplication events [33,38]. Recently, a non-HLA locus linked to psoriasis susceptibility (PSOR2) has been localized in the gene cluster region 17q24-25 described above [37]. In particular, two SNP-based haplotypes from this region showed some evidence for association with psoriasis: one consists of a non-synonymous polymorphism within CMRF35-H (R111Q), while the second one lies within the first intron of CMRF35-A2 gene (also termed TREM-5) [37]. One of these associations suggests a role for IRp60/CMRF35-H in the susceptibility to psoriasis due to a possible deficit in the inhibitory regulation dependent from this receptor.

p75/AIRM1 is an inhibitory receptor belonging to sialoadhesin

p75/AIRM1 is a type I transmembrane glycoprotein, belonging to the Ig-SF and sharing sequence homology with the Sialic acids binding Ig-like lectins (Siglec)[5,6,39]. Eleven Siglec members have been described so far in humans; all characterized by an amino terminal V-set Ig domain followed from 1 to 16 C2-set Ig domains [39]. The p75/AIRM-1, also known as Siglec-7, belongs to the CD33-related Siglec subgroup, is the major Siglec expressed by NK cells, where it has been characterized as an inhibitory receptor and it is characterized by three extracellular Ig-like domains, an amino-terminal V-domain, and two C2-set domains (Fig. 1B) [5,6,39]. The p75/AIRM-1 gene maps in the extended LRC region on chromosome 19q13.3-4 where all Siglec genes, belonging to the CD33 subgroup, are located [5,6,39,40]. Other Siglec genes coding for CD22 (Siglec-2) and myeloid-associated glycoprotein (Siglec-4) map on human chromosome 19q13.1, while the one for Siglec-1 is located on human chromosome 20p [39].

The cytoplasmic tail of p75/AIRM-1 is characterized by tyrosine residues, one of them in the context of a classical ITIM that upon tyrosine phosphorylation recruits the SHP-1 phosphatase responsible for the inhibition of the molecular events involved in triggering pathways (Fig. 1B) [5,6,39].

Interestingly, p75/AIRM1 is not only expressed on NK cells, but also on monocytes, and in particular on acute (AML) or chronic myeloid leukemic cells (CML) of the M4 and M5 subtypes where its engagement results in strong inhibition of leukemic cell proliferation, either in CML or AML [41]. This regulatory role on myeloid cell proliferation is also exerted by CD33, another sialoadhesin expressed by myeloid cells, but not by other inhibitory receptors present on the surface of such cells, such as IRp60 [41]. This is intriguing since all of these molecules have been shown to transduce inhibitory signals through SHP recruitment. Moreover, p75/AIRM-1/Siglec-7 and CD33/Siglec-3 may play a role on normal hematopoiesis [41].

All Siglec mediate protein-carbohydrate interactions, they bind terminal sialic acids on glycoconjugate moieties of glycoproteins and glycolipids. Siglec are expressed by specific cell types

and have a particular preference for glycosidic linkage of sialic acid to adjacent sugar [22,39]. In particular, p75/AIRM-1/Siglec-7 binds preferentially to α(2,8)-linked disialic acid, and at a lesser extent to α(2,6)- or α(2,3)-linked sialic acid [22,39,40]. Interestingly, a small region in the IgV domain of Siglec-7 (Asn^{70}-Lys^{75}) appears to be responsible for this preferential binding [40].

Structure of NCRs and p75/AIRM1: implication for ligand recognition

Further progress in understanding the function of NK cells in innate immunity is in part due to the knowledge of their three-dimensional structure determined by X-ray crystallography. Structurally, two distinct families of receptors are responsible for the regulation of NK cell activity by binding to MHC class I or to still unidentified ligands: the C-type lectin like and the immunoglobulin-like NK receptors [8]. Both the Ig-like and the C-type lectin-like families include inhibitory and activating members. Here, in the following paragraphs, the structural aspects and implications for ligand recognition by NCRs and p75/AIRM1 are summarized. This takes advantage of the recent published X-ray crystal structure of NKp44, NKp46 and p75/AIRM1 [22,42-45].

Crystal structure of the IgV-like domain of NKp44

The X-ray crystal structure of this domain, likely implicated in ligand recognition and binding has been determined at 2.2 Å resolution [42] (Fig. 2A). NKp44 extracellular domain adopts a standard IgV-like fold, which is characterized by a β sandwich of eight β strands joined together in two disulfide-linked antiparallel β sheets (Fig. 2A). The structure revealed two unique features. First, this IgV like-domain contains an additional disulfide bond connecting residues Cys37 and Cys45 (Fig. 2A). This disulfide bridge is important to constrain the C-C' β-strands and the interconnecting loop; this loop region has been shown to be important in mediating ligand selectivity for p75/AIRM1 (or Siglec-7) and Siglec-9 in experiments of loop grafting [40]. This disulfide bridge has not been described before and is not present in other standard IgV domains for which the crystal structures are known; consequently, this disulfide bridge is a distinctive feature of NKp44 extracellular domain. Second, and more attractive, concerning the putative ligand binding site, the extracellular domain of NKp44 possesses an asymmetrical surface charge distribution [42], where a positively charged interior surface in a groove may constitute a potential binding site for anionic ligands such as sialylated sugars. To this regard p75/AIRM1, which binds sialylated sugars [22], possesses a topologically similar groove that is approximately superimposible with the NKp44 positively charged groove (Fig. 2A,C). This site could be the potential binding site for sialylated sugars on NKp44. A lot of efforts are presently directed towards the identification of cellular and viral ligands for the NCRs. The first and yet only published data on NCR ligands have shown that NKp46 and NKp44 recognize viral hemagglutinin in a sialylation-dependent fashion [25,26]. Along this line, NKp44 IgV like-domain displays structural and sequence similarities with the N-terminal IgV like-domain of sialoadhesins, such as p75/AIRM1 (Fig. 2C) or Siglec-1. The crystal structure of Siglec-1 in complex with 3' sialyllactose [46] has confirmed the critical role played by the Arg97 in the sugar recognition and binding (Fig. 2C). This Arg97 has a close structural equivalent in NKp44 Arg92 (Fig. 2A] that could potentially mediate the principal binding to sialylated sugars. However, no experimental evidence has been described for direct binding to sialic acid or to sialic acid derivatives for NKp44 IgV like-domain; much more work is needed to carefully address the significance of sugars or sugar modifications in the interaction of NKp44 with its ligand(s).

Crystal structure of the extracellular Ig-like domains of NKp46

The crystal structure of the two extracellular Ig like-domains of NKp46 is the latest NCR structure solved at 1.93 Å resolution (Fig. 2B) [43,44]. The overall structural arrangement of the two IgC2-domains of NKp46 resembles that of Leukocyte

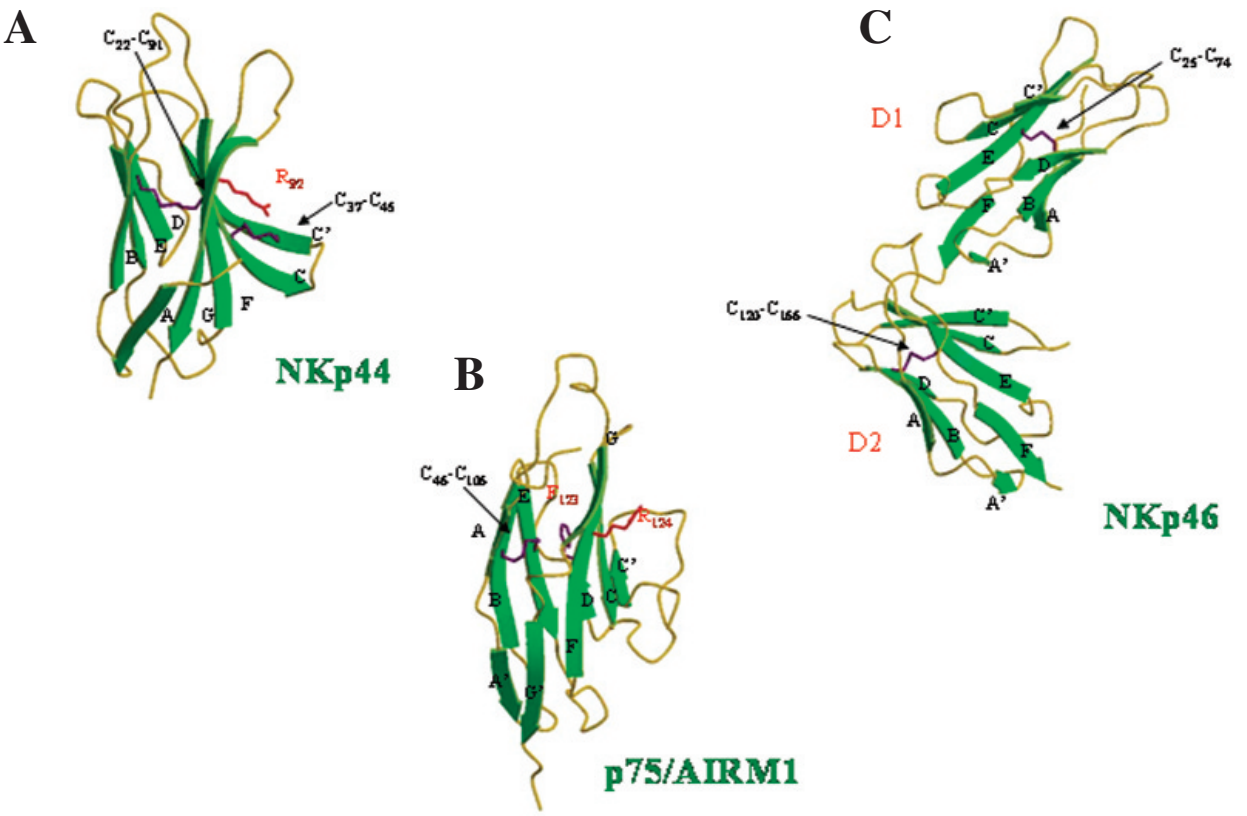

Fig. 2 **A -** Ribbon diagram of the extracellular N-terminal IgV like-domain of NKp44 (PDB: 1HKF). β-strands are labeled according to the standard IgV nomenclature. The disulfide bond Cys22-Cys91 and the disulfide bond Cys37-Cys45 unique of NKp44 are shown in magenta. The position of Arg92, which is the structural homolog of Arg97 in Siglec-1 and Arg124 in p75/AIRM1, is shown in magenta. **B -** Structure of the two IgC2 like-domain of NKp46 (PDB: 1OLL). β-strands are labeled according to the standard IgV nomenclature. The D1 and D2 domains are labeled; disulfide bridges are shown in magenta. **C -** N-terminal IgV like-domain of p75/AIRM1. (PDB: 1NKO). β-strands are labeled according to the standard immunoglobulin nomenclature. The intra-sheet disulfide bond between Cys46-Cys106 and the Phe123 replacing a Cys in standard Ig-like folds are shown in magenta. The position of Arg124 that is responsible for the primary binding to the N-terminal sialic acid in sialoadhesins is shown in magenta.

Immunoglobulin Receptor-1 (LIR-1 also termed ILT2 or LILRB1) and KIR2D immunoreceptors [9]. Even if the structural arrangement in NKp46, LIR-1 and KIR2D is very similar, the amino acids that are responsible for the HLA binding in LIR-1 and KIR2D are not conserved in NKp46. For example, KIR2DL2 binds HLA-Cw3 in a region that is located between the D1D2 interdomain hinge [43]. It is of note that, the amino acids in the corresponding region of NKp46 are not conserved [43]. This suggests that the putative ligand-binding site in NKp46 may be located in an area of the molecule distinct from the interdomain hinge region. Moreover, this could be a further structural explanation of the experimental evidence that NKp46, like the other NCRs, does not bind classical MHC class I molecules. Thus, the only NKp46 ligands identified so far, are the hemagglutinin of influenza virus and the hemagglutinin-neuroaminidase of parainfluenza virus [25], suggesting a role for sugars also in NKp46 ligand recognition.

Crystal structure of the saccharide-binding domain of p75/AIRM1

The X-ray crystal structure of the saccharide-binding domain of p75/AIRM1 has been determined at 1.45Å resolution [22,45] (Fig. 2C). p75/AIRM1

structure shows a classical Ig-V like domain organization characterized by a β-sandwich formed by β-sheets ABED and A'GFCC' (Fig. 2C), respectively. A feature of p75/AIRM1 structure is the presence of an intra-sheet disulfide bond between Cys46 and Cys106 (Fig. 2C) that is common to all sialoadhesin family members. This disulfide bond replaces the inter-sheet disulfide bridge commonly observed in other Ig-like domains [46], since in p75/AIRM1 the Cys in strand F, is replaced by Phe123 (Fig. 2C). Thus, the absence of the inter-sheet disulfide bridge, results in opening up and extending the two β-sheets, that could expose residues to facilitate ligand recognition or give conformational plasticity to the binding site needed to accommodate oligosaccharide ligands. A marked difference between p75/AIRM1 and sialoadhesin structures occurs in the loops connecting the β-strands, in particular loop connecting β-strands B-C and C-C' (Fig. 2C). As for Siglec-1 [46], the putative carbohydrate-binding site in p75/AIRM1 could be located between strands A and G (Fig. 2C). Of particular note is that the partial opening-up and widening of the p75/AIRM1 β-sandwich caused by the absence of the inter-sheet disulfide as discussed before, provides a very large flat surface that perfectly matches the surface area in the 3'-sialyllactose/siglec-1 complex structure [46]. In addition to a conserved arginine at position 124 in p75/AIRM1 (Fig. 2C), which forms a salt bridge with the carboxylate group of sialic acid in the 3' sialyllactose/siglec-1 complex [46], the putative binding site of p75/AIRM1 contains a number of hydrophobic (Tyr26, Trp132) and basic (Arg23, Arg120, Lys135) residues, that may contribute to the binding specificity.

Conclusion

The past ten years have witnessed dramatic progress in our understanding of how natural killer (NK) cells function. We believe that the characterization of the ligands involved in the NK-mediated cytolysis and their tissue distribution, in normal *versus* pathological samples, together with the elucidation of the fine mechanisms of receptor/ligand interaction, will further contribute to the possible usage of NK cells in therapeutic approaches and they will open exciting new avenues of investigation in tumor immunology and viral pathogenesis. It is important to stress that the recent knowledge of NK cells alloreactivity are under clinical evaluation in hematopoietic transplantation. Thus, KIR/HLA class I mismatches, in human leukocyte antigen (HLA) haplotype-mismatched transplants, may drive NK-cell alloreactions mediating strong graft versus leukemia (GVL) effects, producing higher engraftment rates, and do not causing graft versus host disease (GVHD) and might lead to a true revolution in bone marrow transplantation.

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