CHAPTER 14

NK Receptors

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Introduction

Analogous to T cell receptors (TCRs) on the surface of T lymphocytes, natural killer (NK) cells function through cell-surface receptors (NCRs) that, unlike TCRs, can be any of a diverse array of molecules, either immunoglobulin-like or C-type lectin-like in structure. NCRs specific for classical and nonclassical major histocompatibility complex (MHC) class I proteins, expressed in complex patterns of inhibitory and activating isoforms on overlapping but distinct subsets of NK cells, play an important role in immunosurveillance against cells that have reduced MHC class I expression as a result of infection or transformation. Another NCR, NKG2D, an activating NCR first identified on NK cells but subsequently found on macrophages and a variety of T-cell types, is implicated in direct, antiviral, and antitumor immune responses. Recent crystallographic analyses of NCRs and NCR/ligand complexes reveal a range of recognition mechanisms that can be either similar to or quite distinct from TCR-mediated events.

Immunoreceptors

Recognition events between αβ T cell receptors (TCRs), expressed on the surface of T cells, and processed peptide fragments of endogenous proteins, presented on target cell surfaces as complexes with major histocompatibility complex (MHC) class I proteins, ultimately mediate activation of T-cell cytotoxic responses by the cellular arm of the adaptive immune system [1]. MHC class I proteins are integral-membrane, heterodimeric proteins with ectodomains consisting of a polymorphic heavy chain, comprising three extracellular domains (α1, α2, and α3), associated with a non-polymorphic light chain, β_2-microglobulin (β_2-m) [2]. The α1 and α2 domains together comprise the peptide- and TCR-binding "platform" domain; the α3 and β_2-m domains have C-type immunoglobulin (Ig) folds. Crystal structures of TCR/MHC complexes show that the TCR variable domains sit diagonally on the MHC platform domain, making contact with the peptide and the MHC α1 and α2 domains [3] (see Fig. 1). Binding studies show that the dissociation constants for these interactions range from one to tens of micromolar (see Table 1). Analysis of the kinetics of binding suggest that TCR/MHC binding is accompanied by a reduction in flexibility at the receptor/ligand interface [4].

Natural Killer Cells

Surveillance against cells undergoing tumorigenesis [5–9] or infection by viruses [10,11] or internal pathogens [12,13] is provided by natural killer (NK) cells, components of the innate immune system, thus helping to provide "covering fire" during the period that responses by the adaptive immune system are gearing up [14]. NK cells also act to regulate innate and acquired immune responses through the release of various immune modulators, chemokines, and cytokines, such as tumor necrosis factor α, interferon γ, MIP-1, and RANTES. Unlike T cells, NK cells function through a diverse array of cell-surface inhibitory and activating receptors.

Many NK cell surface receptors (NCRs) are specific for classical (such as HLA-A, -B, and -C in humans) and nonclassical (such as HLA-E in humans) MHC class I proteins and occur in paired activating and inhibitory isoforms [15–17]. Different NCRs, with different MHC class I specificities, are expressed on overlapping, but distinct, subsets of NK cells in variegated patterns—where the strength of the inhibitory signals may be stronger than stimulatory signals.

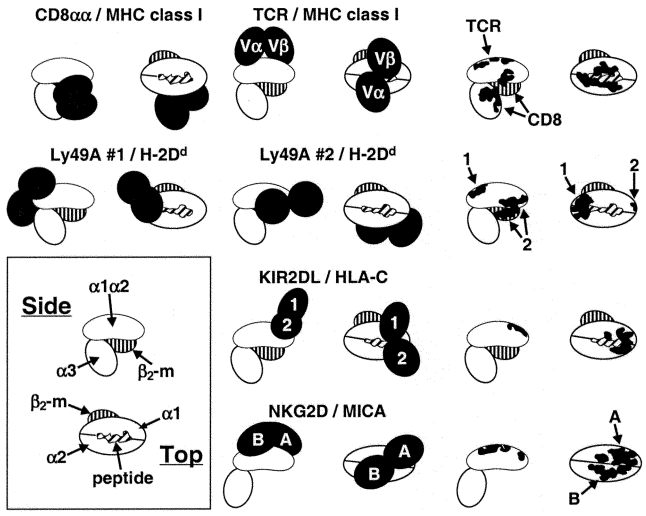

Figure 1 Schematic representations of structurally characterized NK receptor–ligand complexes. Each row shows two views of a receptor–ligand complex, first showing the organization of domains in the complex (receptor domains in black, labeled where a distinction between domains is significant; MHC class I ligand heavy chains in white and β_2-m in vertical stripes). The arrangement of domains in the ligands is detailed in the inset; the approximate solvent-accessible surface area of the bound peptide, if present, is shown as a cross-hatched area. The right-most columns show approximate footprints of receptors and coreceptors on the ligands as black patches, labeled by receptor component, subsite, or domain, as appropriate.

Thus, NK cell effector functions are regulated by integrating signals across the array of stimulatory and inhibitory NCRs engaged upon interaction with target cell surface NCR ligands [16,17], resulting in the elimination of cells with reduced MHC class I expression, a common consequence of infection or transformation [18]. Other NCRs, such as human and murine NKG2D, recognize divergent MHC class I homologs (ULBPs [19], MICA, and MICB in humans [20], and RAE-1 and H60 in mice [21,22] not involved in conventional peptide antigen presentation. Inhibitory receptors transduce signals through recruitment of tyrosine phosphatases, such as SHP-1 and SHP-2, and contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains [23,24]. Activating receptors associate with immunoreceptor tyrosine-based activation motif (ITAM)-bearing adaptor proteins, either DAP12 [25] or DAP10 [26,27], through a basic residue in their transmembrane domain.

Natural killer cell surface receptors can be divided into two groups based on structural homologies [28,29]. The first group includes the killer cell Ig receptors (KIRs) and consists of type I transmembrane glycoproteins with ectodomains containing tandem Ig domains. The second group, including the rodent Ly49 receptor family and the CD94/NKG2 and NKG2D receptor families found in primates and rodents, comprises homo- and heterodimeric type II transmembrane glycoproteins containing C-type lectin-like NK receptor domains (NKDs) [30]. NCR/ligand dissociation constants range from a hundred micromolar to tens of nanomolar (see Table 1). A series of recent results from X-ray crystallographic analyses detail the interactions for a number of NCR/ligand complexes.

Table I Immunoreceptor Affinities

Receptor	Ligand	K_D (μM)	Ref.
TCR	MHC class I	1–90	4,36
NKG2A–CD94	HLA-E	11.23	37
KIR	MHC class I	~10	38
huNKG2D	MICA	0.3	34
muNKG2D	H60	0.0189	39
muNKG2D	RAE-1α, β, γ, δ	0.345–0.726	39

Ig-Type NK Receptors: KIR

Two crystal structures of complexes between inhibitory KIR family NCRs and their MHC class I ligands, KIR2DL2/HLA-Cw3 [31] and KIR2DL1/HLA-Cw4 [32], show that the receptor binds in a 1:1 complex with HLA-C, making contact with both the α1 and α2 platform domains and the carboxy-terminal end of the bound peptide (see Fig. 1). (KIR receptor nomenclature identifies the number of Ig domains [2D(omains) or 3D, specific for HLA-C or HLA-B respectively], and whether the receptor is a long [L] form, containing ITIM repeats, or a short [S] form, interacting with ITAM-containing adaptor proteins.) Both complexes have interfaces showing both significant shape and charge complementarity, with the N-terminal KIR domains interacting primarily with the α1 domains of HLA-C, the C-terminal KIR domains contacting the α2 domains, and additional contacts provided by the interdomain KIR linker peptides (the "elbow"). The kinetics of binding, rapid on and off rates, are consistent with interactions dominated by charge–charge interactions.

Despite a high degree of conservation of binding surface residues between both KIR2DL2 and KIR2DL1, and HLA-Cw3 and -Cw4, few actual intermolecular interactions are conserved. This recognition flexibility is accomplished through altered side-chain conformations. KIR2D receptors distinguish between HLA-C allotypes on the basis of the residue at position 80; KIR2DL1 recognizes lysine and KIR2DL2 recognizes asparagine, and this specificity is conferred by the identity of the residue at position 44 in the receptor. In KIR2DL1, Lys80 is shape and charge matched to a distinct pocket on the surface of the receptor; while Asn80 is sensed through a direct hydrogen bond in the KIR2DL2 complex.

C-Type Lectin-Like NK Receptors: Ly49A

Ly49A is a disulfide-linked, symmetric, homodimeric, NKD-type NCR that is specific for the murine MHC class I protein H-2D^d (the human ortholog is nonfunctional). The crystal structure of the D^d/Ly49A complex [33] shows D^d homodimers binding to two distinct sites on the MHC protein (see Fig. 1). The first binding site positions Ly49A on the D^d platform domain, contacting both α1 and α2 and the

N-terminal end of the bound peptide—the opposite end from where KIR2D binds. The second binding site positions Ly49A in the cleft between the underside of the platform domain (the top being the peptide and TCR binding surface), the α3 domain and β_2-m. The second site is considerably more extensive than the first site, though less shape complementary and less dominated by charge–charge interactions, and is likely to be the immunologically relevant interaction on the basis of subsequent mutagenesis studies. The second site also overlaps the CD8 binding site on MHC class I proteins. As predicted, Ly49A clearly displays a C-type lectin-like fold, though failing to retain any remnant of the divalent cation or carbohydrate binding sites conserved in true C-type lectins. While the simplest binding mode for a symmetric homodimer is to interact with two monomeric ligands through two identical binding sites, each Ly49A interaction with D^d is with a single monomer because binding of ligand at one site sterically blocks binding at the second, homodimer-related site.

C-Type Lectin-Like NK Receptors: NKG2D

NKG2D is an activating, symmetric, homodimeric, NKD-type NCR. While highly conserved between primates and rodents, its ligands include very different molecules, both in humans and rodents. Crystal structures of two complexes, human NKG2D/MICA [34] and murine NKG2D/RAE-1 [35], show that NKG2D interacts with its MHC class I homologous ligands in a manner very similar to the way in which TCRs interact with classical MHC class I proteins (see Fig. 1), even though NKG2D contains NKDs while TCRs contain Ig domains. NKG2D retains the C-type lectin-like fold seen in Ly49A, with few variations, although the binding surface of NKG2D is much more curved than in Ly49A, matching the more curved surface of its ligands (which do not bind peptides), where the Ly49A and NKG2D binding surfaces encompass overlapping surfaces on the receptors. The interaction surfaces bury considerable solvent-accessible surface area and are highly shape complementary, but the human NKG2D/MICA interaction is markedly more so than the murine NKG2D/RAE-1 interaction. The reason that the human complex does not bind considerably more tightly than the murine complex (see Table 1) is likely due to the necessity of ordering a large loop on the surface of MICA concurrent with complex formation, reflected in the unusually slow on-rate for the human complex. Unlike KIR and Ly49A interactions at the first site, the NKG2D binding sites are much less dominated by charge–charge interactions. The stoichiometries of the NKG2D complexes are one homodimer binding to one monomeric ligand; however, unlike Ly49A, both homodimer-related binding sites on NKG2D contribute approximately equally to the interactions in both complexes, reflecting a binding site that has evolved to bind multiple target sites without the degree of side-change rearrangements seen in the KIR interactions. It has also been proposed that the NKG2D/MICA complex is likely a good model for the CD94/NKG2A/HLA-E complex.

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