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MULTI-AUTHOR REVIEW

Cis–trans interactions of cell surface receptors: biological roles and structural basis

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Abstract Cell surface receptors bind ligands expressed on other cells (in *trans*) in order to communicate with neighboring cells. However, an increasing number of cell surface receptors are found to also interact with ligands expressed on the same cell (in *cis*). These observations raise questions regarding the biological role of such *cis* interactions. Specifically, it is important to know whether *cis* and *trans* binding have distinct functional effects and, if so, how a single cell discriminates between interactions in *cis* versus *trans*. Further, what are the structural features that allow certain cell surface receptors to engage ligand both on the same as well as on an apposed cell membrane? Here, we summarize known examples of receptors that display *cis–trans* binding and discuss the emerging diversity of biological roles played by these unconventional two-way interactions, along with their structural basis.

Keywords NK cell receptor · Ly49 · LILRB/PIR-B · MHC · Notch · *Cis* interaction · *Trans* interaction · NK cell education

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Abbreviations

BCR	B cell receptor
BTLA	B and T lymphocyte attenuator
HVEM	Herpesvirus entry mediator
Ig	Immunoglobulin
LILRB	Leukocyte immunoglobulin-like receptor B
MHC-I	Major histocompatibility complex class I
PIR-B	Paired immunoglobulin-like receptor-B
Siglec	Sialic acid-binding immunoglobulin-like lectin

Introduction

One of the first cell surface receptors shown to bind ligand present in the plane of the same cell membrane was CD22, a sialic acid-binding immunoglobulin-like lectin (Siglec) expressed by B cells. Most CD22 was found to be "masked" by binding sialic acids attached to galactose (Sia α2–6Gal) on N-linked glycans of glycoproteins expressed on the B cell itself [1]. Sialidase treatment of B cells was sufficient to unmask CD22 and allow binding of exogenous sialoside probes. The preferential binding in *cis* is explained by the high concentration of sialic acid on the B cell surface (25–30 mM), which is around 100-fold higher than the K_d of CD22 for sialic acid (0.1–0.3 mM) [2]. Indeed, CD22 binding of higher-affinity multivalent sialoside probes does not require prior unmasking, and *trans* ligand can compete with *cis* ligand for CD22 binding in cell–cell conjugates [3]. These data suggest a dynamic equilibrium, in which CD22 can switch between *cis* and *trans* interactions, depending on the relative affinity/avidity of the respective ligands. However, the nature of the relevant sialic acid-modified *cis* ligand has remained elusive, although CD22 is associated with the B cell receptor

(BCR), CD45 and CD22 itself [4]. Functional consequences of CD22 *cis* binding were proposed based on an analysis of human B cell lines. Separation of CD22 from the BCR in the B cell membrane by sequestration improved B cell activation [5]. Similarly, B cells expressing a CD22 mutant that lacks sialic acid-binding function were hyperresponsive to stimulation through the BCR [6]. *Cis* binding is thus thought to set a signaling threshold, which prevents overstimulation of B cells after BCR cross-linking by antigen. This notion has been confirmed in several (but not all) mouse models, in which CD22 or sialic acid modifications have been disrupted completely or partially (for recent reviews, see [7, 8]). The work on Siglecs has provided a framework to investigate the role of *cis–trans* interactions in other receptor–ligand systems.

Renewed interest in the fact that certain cell surface receptors bind ligands expressed on the same membrane has stemmed from the analysis of natural killer (NK) cell receptors specific for major histocompatibility complex class I (MHC-I) molecules [9]. Such receptors were originally identified based on their ability to inhibit the effector function of NK cells in response to specific allelic variants of MHC-I molecules expressed on target cells [10, 11]. Some of these NK receptors were subsequently found to constitutively bind to the same MHC-I ligand expressed by the NK cell itself. This represented one of the first instances where *cis–trans* binding of a cell surface receptor was based on protein–protein interactions and where the *cis* and *trans* ligands were identical (for a review, see [12]).

Additional examples of receptors that can bind ligand in both *cis* and *trans* have since been reported [13–17], suggesting that such two-way interactions are more common than initially thought. In addition, it has emerged that *cis* interactions can serve distinct purposes and that there may be multiple structural solutions to the problem of how a cell surface receptor can engage the identical ligand on the same as well as on an apposed cell. Here, we summarize recent examples of receptor–ligand systems where evidence for *cis* and *trans* binding has been obtained. We also discuss the structural basis for this unconventional type of interaction and compare the biological roles of *cis* binding in these different systems. We reserve the term "*cis* interaction" for receptors that can bind the identical ligand expressed on the same and on an apposed cell, as schematically shown in Fig. 1.

Biological roles of *cis–trans* interactions of cell surface receptors

Cis association by inhibitory NK cell receptors specific for MHC-I

One of the first cell surface receptors shown to bind ligand in both *cis* and *trans* was the C-type lectin-like receptor Ly49A [9], an inhibitory receptor specific for multiple H-2D alleles expressed by NK cells [10]. Ly49A is the founding member of a small receptor family, which allows mouse NK cells to detect diseased host cells with non-physiologically low levels of MHC-I molecules. Recognition of such cells is based on a dual receptor system consisting of activating and MHC-I-specific inhibitory receptors. Inhibitory receptors interrupt NK cell activation signaling when a cell's MHC-I levels are normal. When MHC-I levels are low, inhibitory signaling does not occur, which allows NK cell activation signaling to induce target cell lysis. This recognition strategy is classically known as "missing-self recognition" [18].

In addition to binding H-2D^d expressed by potential target cells (in *trans*), Ly49A is associated with H-2D^d expressed on the NK cell itself (in *cis*) [9]. To inhibit NK cell-mediated lysis, Ly49A binds H-2D^d on target cells

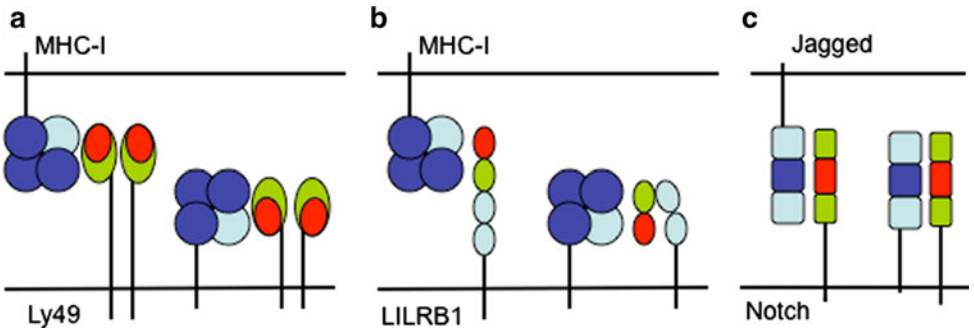

Fig. 1 Schematic representation of the structural basis for *cis–trans* interaction by cell surface receptors. **a** Ly49 receptors reverse the orientation of their ligand-binding domains relative to the NK cell membrane using a long flexible stalk region. **b** LILRB1 has a shorter stalk but is composed of four Ig domains, whereby MHC-I binding is mediated by the two membrane-distal Ig domains. *Cis* binding, which is likely based on a reversal of the orientation of the two distal Ig domains, may be enabled by flexibility of the short hinge between Ig domains 2 and 3. **c** Notch and Delta are rigid EGF repeat-containing receptors. Symmetric binding sites allow anti-parallel (*trans*) and parallel (*cis*) binding without a need for reversal of the ligand-binding domains

Table 1 Cell surface receptors that interact with ligand expressed in *trans* and in *cis*

Receptor	Structure	Cell type	Species	Receptor function	Ligand	Role of *cis* binding	Ref.
Ly49A, C, I	CTLL	NK	Mouse	Inh.	MHC-Ia	Comp. Inh.	[9]
Ly49Q		pDC	Mouse	Act.		?	[27], [29]
LILRB2	Ig	Mast, DC	Human	Inh	MHC-Ia/b	Inh	[14]
LILRB1		T, B, NK					[28]
PIR-B	Ig	Mast, DC	Mouse	Inh	MHC-Ia/b	Inh	[14], [35]
HVEM	TNFR	T	Human	Act	BTLA	Comp. Inh.	[16]
					HSV env	Act	[16]
EphA4		Neuron	Mouse	Act	Ephrin-A2	Comp. Inh.	[13]
Plexin-A4	GAP, Ig	Neuron	Mouse	Act	Sema6a	Comp. Inh.	[17]
Notch1	EGF		Human	Act	Delta-like1	Comp. Inh.	[47]
					Jagged1		[15]

CTLL C-type lectin like, *Ig* immunoglobulin-like, *TNFR* tumor necrosis factor related, *EGF* epidermal growth factor related, *GAP* GTPase activating protein domain, *NK* natural killer cells, *pDC* plasmacytoid dendritic cells, *DC* dendritic cell, *Inh* inhibitory, *Act* activating, *Comp. inh.* competitive inhibition of *trans* binding, *MHC-Ia* classical major histocompatibility complex class I molecules, *MHC-Ib* non-classical MHC-I molecules, *BTLA* B- and T-lymphocyte attenuator, *HSV env* herpes simplex virus envelope

using a binding site beneath the peptide-binding platform of H-2D^d [19–21]. This same binding site allows the association of Ly49A with H-2D^d in *cis* [9]. Indeed, the majority (>70%) of Ly49A molecules is bound in *cis* [22, 23] and only a minority remains available to bind ligand in *trans*. Besides a high affinity for H-2D^d, Ly49A binds other H-2D alleles with variable affinities [24]. Interestingly, the affinity of Ly49A for a specific H-2D allele directly correlates with the number of receptors bound in *cis*. Consequently, the number of free receptors available to bind MHC-I in *trans* decreases when the affinity of Ly49A for ligand increases [25]. The association of Ly49A with H-2D^d in *cis* is stable and, unlike Siglecs, there is no evidence that *cis*-bound Ly49A can switch to *trans* ligand during the formation of an immune synapse [22]. Finally, it is important to point out that NK cells expressing a *cis*-engaged Ly49 receptor are fully functional, which implies that *cis* association does not result in constitutive inhibitory signaling that renders NK cells non-functional. Collectively, therefore, *cis* association of Ly49A with MHC-I stably reduces the number of receptors that are available for functional interaction with MHC-I on target cells [22]. The fact that the number of available receptors is reduced when the affinity for H-2D is increased raises the possibility that NK cell inhibition via Ly49A is relatively independent of whether a given mouse strain expresses a high or a low affinity H-2D allele. Notwithstanding, in a given mouse strain, *cis* association lowers the threshold at which NK cell activation exceeds inhibition during target cell encounter [9]. Since *cis* association is stable, the function of NK cells is constitutively improved. This does not exclude the possibility that changes in the expression levels of MHC-I or Ly49 on the surface of NK cells could further modify the responsiveness of these effector cells.

Like Ly49A, most other inhibitory Ly49 receptors specific for MHC-I have the capacity to interact with MHC-I molecules in *cis* [26, 27] (Table 1). Besides Ly49 family receptors, NK cells use additional inhibitory receptors specific for MHC-I. These include heterodimeric C-type lectin-like CD94/NKG2 receptors, which are used by human and rodent NK cells, as well as structurally unrelated killer immunoglobulin-like receptors (KIRs) and leucocyte immunoglobulin-like receptor B1 (LILRB1) (also termed LIR-1 or ILT2) expressed by human NK cells. In contrast to Ly49 receptors, there is no evidence for *cis* binding in the case of the mouse CD94/NKG2A receptor or the human KIR family receptor KIR2DL1 (unpublished data). However, *cis* association has been reported for LILRB1 [28], which will be discussed below.

Certain receptors specific for MHC-I are not actually expressed by NK cells and some activate rather than inhibit cellular functions. For example, the MHC-I-specific Ly49Q receptor improves the response of plasmacytoid dendritic cells (pDC) to microbial infection. PDCs lacking Ly49Q produce low amounts of type I interferon in response to Toll-like receptor 9 (TLR9) stimulation by unmethylated microbial cytosine guanine dinucleotide (CpG) DNA [29]. Similarly, the response is also low in mice devoid of classical MHC-I molecules [29], suggesting that Ly49Q improves the response of TLR9 in connection with MHC-I binding. Indeed, Ly49Q co-localizes with MHC-I at the cell surface [30] and most, but not all, Ly49Q is physically associated with MHC-I in *cis* [27]. However, it is not formally known whether *cis* binding of MHC-I by Ly49Q

plays an essential role. Moreover, it is not clear how Ly49Q improves the function of endosome-resident TLR9. One way in which TLR9 function can be improved was discovered based on an analysis of the human inhibitory NK cell receptor KIR3DL2 (p140), which was known to be specific for a small group of HLA-A alleles. This cell surface receptor directly binds CpG DNA and delivers it to endosomes, which improves cytokine production by NK cells in response to microbial DNA [31]. Therefore, in addition to MHC-I binding, members of structurally distinct human and mouse receptor families are also involved in TLR signaling. It will be interesting to see whether recognition of MHC-I and of CpG DNA are separate functions, or whether MHC-I and CpG recognition is instead coordinated. If so, the physiological function of MHC-I recognition in this innate immune response to microbes remains to be defined.

Subsequent to C-type lectin-like Ly49 receptors and KIR, immunoglobulin-like human LILRB1 and LILRB2 receptors and the homologous mouse PIR-B receptor were identified as a third type of inhibitory MHC-I receptor that is relatively broadly expressed by lymphoid, as well as myeloid, cells. Further, these receptors bind a wide range of classical and non-classical MHC-I molecules with low affinity and with limited allele specificity (reviewed in [32]). LILRB1 (ILT2) inhibits NK cell effector function in response to target cells expressing MHC-I [33], and LILRB/PIR receptors generally dampen the stimulation of immune responses upon binding to MHC-I molecules on antigen-presenting cells [33]. Subsequently, LILRB/PIR-B receptors were found to associate with MHC-I molecules in *cis* [14, 28]. This extended ligand binding in *cis* by C-type lectin-like receptors to structurally unrelated Ig-like receptors suggested that *cis* binding of MHC-I impacts numerous hematopoietic cell types and is not restricted to NK cells.

Similarly to Ly49, the association between MHC-I and LILRB/PIR-B receptors in *cis* is constitutive [14]. However, in contrast to Ly49, the ITIMs of LILRB/PIR-B receptors are constitutively phosphorylated and *cis* association results in constitutive inhibition signaling [14]. In mast cells, the suppressive effect increases the threshold for degranulation upon activation through the Fc receptor for IgE, FcεRI [14]. The interaction of LILRB2/PIR-B with MHC-I in *cis* reduces activation signaling and maturation in additional cell types. The expression of a specific HLA-B allele (HLA-B35x), for which LILRB2 has increased affinity, is associated with impaired maturation and cytokine production by dendritic cells (DC). It has been proposed that recognition of the DC's own HLA-B35x by LILRB2 contributes to this impairment and that this correlates with accelerated disease in HIV patients who carry the HLA-B35x allele [34]. Further, the activation of T cells by DCs is reduced due to competition between the CD8 co-receptor (on the T cell) and PIR-B (on the DC) for binding to MHC-I on the DC [35]. Collectively, therefore, *cis* association of LILRB/PIR-B receptors with MHC-I mediates a suppressive effect, which is further increased upon engagement of MHC-I in *trans*. Even though it is not known whether *cis* association of LILRB/PIR-B receptors is stable, it results in an increased threshold for cellular activation, similar to the role of *cis* binding by CD22. For LILRB/PIR-B receptors, it is not clear how the *cis* complex couples to the various activation receptors. In contrast, *cis* binding by Ly49 receptors mainly serves as a competitive inhibitor for *trans* binding, which lowers the activation threshold in NK cells. Together, these findings suggest that *cis* binding of MHC-I molecules can play distinct biological roles.

NK cell education

In addition to dampening the effector function of NK cells during target cell recognition, inhibitory receptors specific for MHC-I impact NK cell function in another way. Engagement of MHC-I, possibly during development, improves the function of activating NK cell receptors [26, 36–39]. This effect is known as NK cell "education" [40]. The improved functionality of activation receptors may be essential for NK cell reactions to cells lacking MHC-I in the absence of acute inflammation. While it is clear that engagement of MHC-I receptors induces functional changes, it is currently not possible to identify educated NK cells based on phenotypic changes. In addition, the precise role of inhibitory receptors for NK cell education is debated. The "licensing" model implies that NK cell activation receptors are by default hypo-active. They are rendered responsive when NK cells express an inhibitory receptor specific for self-MHC-I [26, 38]. Conversely, the "disarming" model [41] implies that activating NK cell receptors are by default active. They are rendered hypo-active due to continuous stimulation. This is prevented by the acquisition of an inhibitory receptor, which is specific for self-MHC [37]. Irrespective of the precise mechanism involved, both models imply that NK cell education depends on an interaction of inhibitory receptors with MHC-I expressed on other cells. However, the fact that most inhibitory Ly49 NK cell receptors also bind MHC-I on the NK cell itself raised the issue of whether NK cell education depends on MHC-I recognition in *trans* or in *cis*. This question was addressed using a Ly49 receptor variant, which binds MHC-I in *trans*, but fails to bind in *cis*. This was achieved by replacing the predicted flexible stalk region of Ly49A with a rigid stalk from another C-type lectin-like receptor, CD72, which should prevent *cis* binding by directing the ligand-binding domains of Ly49A

away from the NK cell membrane. Indeed, this receptor variant failed to bind MHC-I in *cis* while retaining the ability to inhibit NK cell effector function in response to target cells expressing H-2D^{d}. Despite the fact that this engineered receptor was a functional inhibitory NK cell receptor, it was unable to educate NK cells [42]. These data dissociate the classical inhibitory from the educating function of an MHC-I receptor. Such dissociation is not compatible with "disarming", as this model relies exclusively on the inhibitory function of MHC-I receptors. In addition, the data suggest that *cis* interaction is necessary for NK cell education. In agreement with this conclusion, an increasing affinity of wild type Ly49A for MHC-I enhances NK cell education, and, as discussed above, this correlates with the extent to which Ly49A is associated with MHC-I in *cis* [25].

A key question, then, is how *cis* interaction contributes to NK cell education. Once again, sequestration of Ly49 by MHC-I expression in *cis* seems to play a role. We noted that unengaged Ly49 receptors (i.e.. Ly49 receptors, which have no MHC-I ligand in a particular mouse strain, such as Ly49A in H-2^{b} mice), dampen NK cell activation. In this situation, unengaged Ly49 receptors may be in proximity to activation receptors, thereby reducing their responsiveness. As a precedent, the proximity of the co-inhibitory CD22 receptor is sufficient to dampen BCR stimulation, despite the fact that CD22 is not engaged by a *trans* ligand. In agreement with this model, the suppressive effect of unengaged Ly49A requires a functional cytoplasmic inhibition motif [43]. Finally, unengaged Ly49A, as well as KIR [43], accumulate at NK cell synapses formed with target cells, which lack the corresponding MHC-I ligand [42]. The presence of MHC-I ligand in *cis* is believed to sequester Ly49A away from activation receptors, which reverses the suppressive effect of unengaged Ly49A and improves the function of NK cell activation receptors.

As discussed above, NK cell education by Ly49A is dependent on *cis* interaction with MHC-I. This raises the question of how receptors, which do not bind MHC-I in *cis* (such as KIR2DL1 or CD94/NKG2A), are able to educate NK cells. One structural difference between these receptors is that KIR2DL1 and CD94/NKG2A contain two immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic domains. By contrast, each subunit of the homodimeric Ly49 receptors has a single ITIM. Perhaps the tandem arrangement of ITIMs can lead to distinct types of signals based on ligand binding in *trans*. Consistent with such a possibility, KIR2DL1 can play a costimulatory or an inhibitory role in CD4^{+} T cell hybridomas [44]. For Ly49 receptors, their sequestration by MHC-I expression in *cis*, rather than signaling induced by *cis* binding, appears to be essential for NK cell education.

Irrespective of the precise mechanism, *cis* association of ITIM-bearing Ly49 receptors improves the function of mouse NK cells in two separate ways: it improves the function of activating NK cell receptors and it reduces the inhibitory capacity of MHC-I receptors. Both effects contribute to lowering the threshold at which NK cell activation will exceed inhibition signals, rendering NK cells more reactive and potentially more useful.

Cis binding by activating receptors

Cis binding was initially observed for inhibitory Ly49 and LILRB receptors, while an activating Ly49 family receptor was not influenced by the presence of its ligand in *cis* [45], raising the question of whether *cis* interaction is restricted to cell surface receptors with inhibitory function. However, this is not the case, as evidence for *cis–trans* binding has now been reported for activating receptors that recognize non-MHC ligands. A recent example includes the TNF family receptor herpes virus entry mediator (HVEM), which is subject to regulation by its ligand, B and T lymphocyte attenuator (BTLA), present on the T cell membrane. HVEM engagement of BTLA expressed on neighboring cells results in bi-directional signaling: the HVEM-expressing cell is strongly co-stimulated while the BTLA-expressing cell is co-inhibited. This raised the question of how naïve HVEM-expressing T cells are kept in check. Recent data show that HVEM is constitutively associated with BTLA in *cis* and acts as a competitive inhibitor, which prevents HVEM clustering by ligand engagement in *trans* [16]. Consequently, HVEM–BTLA *cis* complexes are thought to maintain T cells in a naïve state. Upon T cell activation, BTLA is downregulated, which renders HVEM competent for signaling. These findings suggest that *cis* association makes the activating HVEM receptor functionally unavailable, whereas *trans* binding results in signaling. Similar to Ly49, *cis* binding of HVEM serves as a competitive inhibitor for *trans* binding.

Activating receptors that can bind the equivalent ligand expressed in *cis* and *trans* are not restricted to the immune system. Ephrin receptors (Eph) are involved in the sorting of distinct cell types in various tissues, such as the gut, or in directing the growth of axons. Cell sorting occurs based on cellular repulsion, which is induced when Eph binds ephrin ligands on other cells. Recent data suggest that the activity of certain Eph is influenced by ephrin expression by the same cell [46]. For example, the EphA receptor binds ephrinA ligand expressed in *cis* via the Eph ligand-binding domain [13]. Similar to HVEM, *cis* interaction does not induce Eph tyrosine kinase activity; rather, it reduces the interaction with ephrin on other cells, which dampens the repulsive signaling by Eph [13]. In a functionally related system, plexin receptors on neurons bind to semaphorin

ligands on other cells (*trans* interaction) and transduce signals for regulating neuronal axon guidance. However, expression of Plexin-A4 and Semaphorin6A on the same neuron was found to attenuate the response to semaphorin ligands in *trans* [17]. This effect is mediated by the formation of stable complexes between Plexin-A4 and Semaphorin6A in *cis*, which prevent ligand binding in *trans*. In this way, the Plexin-A4–Semaphorin6A *cis* interaction serves as an inhibitory mechanism to modulate axon guidance.

Finally, recent data suggest that the activity of the Notch receptor is influenced by *cis* binding to its Delta ligand [15]. Notch induces distinct cell fates in groups of initially identical cells, a process referred to as lateral signaling. Delta has two activities: it activates Notch in neighboring cells and it inhibits Notch expressed on the same cell. The negative effect is based on the interaction of the two proteins in *cis*, which prevents *trans* binding by the receptor. Functional data demonstrate that the response of Notch to Delta expressed in *trans* is graded (i.e., it is proportional to the amount of Delta present on a neighboring cell). Delta expression in *cis* serves as a competitive inhibitor for Notch activation by *trans* Delta. Mathematical modeling and functional data showed that this generates a very sharp threshold, above which Notch responds to *trans* Delta [47]. This Notch–Delta switch may explain how very small differences in the relative concentrations of Notch and Delta on the surfaces of two cells can be translated into binary cell fates.

Altogether, there is now ample evidence from multiple distinct receptor–ligand systems that the ability of a cell surface receptor to respond to ligand on a neighboring cell may be subject to control by expression of the same ligand on the same cell. In most of these cases, *cis* binding does not induce receptor signaling. Rather, by competitively inhibiting *trans* binding, *cis* interaction modifies the threshold at which the cell will be activated.

Cis binding and viral infection

HVEM was originally identified as the receptor for herpes simplex virus envelope glycoprotein D (HSV gD) [48]. Based on the formation of a *cis* complex between HVEM and BTLA, the viral envelope protein gD was also tested for association with HVEM in *cis*. This was indeed the case; however, the functional consequence of this interaction was different from the HVEM–BTLA *cis* association. Whereas the HVEM–BTLA complex is functionally inert, the HVEM–gD *cis* complex activates NF-κB signaling [16]. NF-κB activation induces the expression of pro-survival genes and may provide infected cells with a selective survival advantage early during HSV infection. These data suggest that certain viruses exploit the ability of cell surface receptors to bind ligand in *cis* in order to generate cell-autonomous signals that favor infected cells.

Perhaps along the same lines, in addition to binding HLA molecules, LILRB1 recognizes the HLA-like molecule UL18 encoded by human cytomegalovirus (HCMV) [49]. The fact that LILRB1 binds MHC-I in *cis* raises the issue of whether UL18 might also interact with LILRB1 on the surface of HCMV-infected cells and, if so, whether this has functional consequences similar to or distinct from MHC-I binding in *cis*.

Structural basis for *cis* versus *trans* interaction

From a structural perspective, it is not obvious how a cell surface receptor can bind a ligand in both *cis* and *trans* using the identical binding site. In addition, it is not known why *cis* and *trans* binding results in distinct functional outcomes. These issues have been addressed for the interaction of Ly49 receptors with MHC-I ligand [50]. These studies revealed that Ly49 receptors adopt two distinct conformations to mediate *cis*–*trans* recognition.

Ly49 receptors are homodimeric type II glycoproteins, with each chain composed of a ligand-binding C-type lectin-like domain, the so-called natural killer receptor domain (NKD), which is connected by an unusually long stalk of around 70 residues to the transmembrane and cytoplasmic domains [51, 52]. Based on the crystal structure of Ly49L, which was the first to provide structural information regarding the stalk region, we proposed that the NKDs must back-fold onto a specific segment of the stalk region, the so-called α_S3 segment, to bind MHC-I in *trans* (Fig. 2a). In agreement with this model, replacement of the α_S3 segment greatly reduces the ability of the Ly49A receptor to functionally engage MHC-I on other cells [50], validating the proposal that Ly49 *trans* binding is mediated by an unusual receptor conformation in which the NKDs are back-folded onto the stalk.

The crystal structure of Ly49L without the stalk region revealed distinct non-covalent associations of the two NKDs in the absence as compared to the presence of the stalk. These data, together with the earlier co-crystal structure of Ly49A bound to H-2D^d [19], raised the possibility that Ly49 receptors assume an extended conformation, in which the α_S3 segments of the stalk do not contact the NKDs, to bind MHC-I in *cis* (Fig. 2b). Indeed, the α_S3 segment, which is essential for functional *trans* binding, is not required for *cis* binding, providing evidence that the NKDs are disengaged from the stalk during *cis* interactions. In addition, progressive shortening of the stalk region impaired *trans* rather than *cis* binding. This is expected if *cis* binding is mediated by an extended receptor conformation. Based on the model (Fig. 2), it is easy to

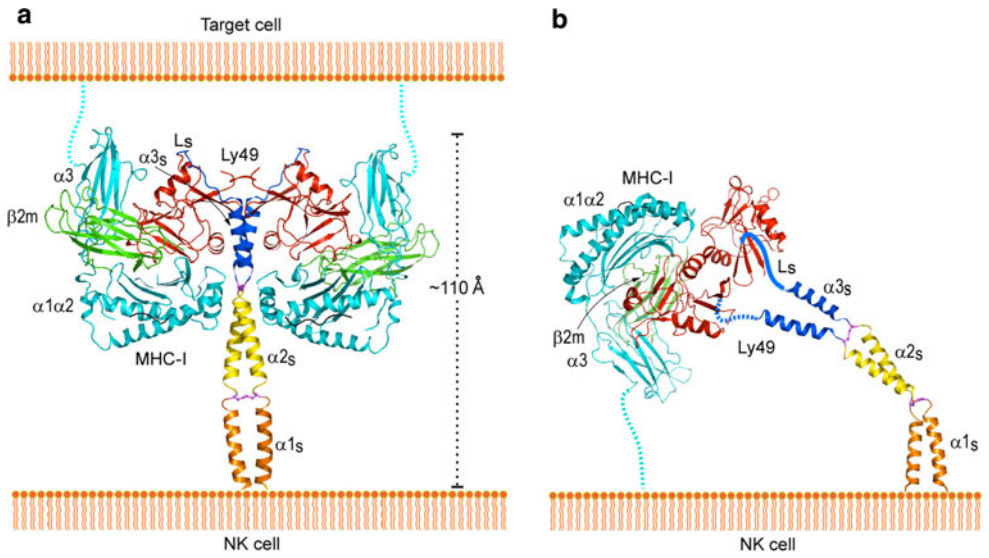

Fig. 2 *Trans* and *cis* interactions of Ly49 receptors with MHC-I ligands. **a** *Trans* interaction of an Ly49 receptor with two MHC-I molecules. The MHC-I heavy chain is *cyan*; β_2m is *green*; the Ly49 NKD is *red*; the $\alpha 3_S$ helix of the Ly49 stalk and loop L_S connecting $\alpha 3_S$ to the NKD are *blue*; the disulfide bond linking the $\alpha 3_S$ helices is *magenta*. The predicted $\alpha 1_S$ and $\alpha 2_S$ helices of the stalk are drawn arbitrarily in *orange* and *yellow*, respectively, with the putative disulfide bond in *magenta*. The ligand-binding domains in the Ly49 homodimer are back-folded onto the stalk to bind two MHC-I molecules on a target cell. **b** *Cis* interaction of Ly49 with a single MHC-I molecule. The Ly49 receptor assumes an extended conformation, which engages a single MHC-I molecule on the NK cell itself. The ligand-binding domains are thereby dissociated from the stalk region. Reproduced with permission from *Immunity* [50]

envisage how *cis* binding by a receptor with a shortened stalk can be accommodated, while *trans* binding is impaired by structural clashes between the MHC-I molecules and the NK cell membrane. Finally, biochemical analyses confirmed that the *cis* complexes consist of an Ly49A homodimer associated with a single D^d molecule, as predicted by the model (Fig. 2b). By contrast, *trans* complexes consist of an Ly49A homodimer associated with two D^d molecules (Fig. 2a) [50].

Cis and *trans* interactions of Ly49 NK cell receptors with MHC-I are thus mediated by two distinct receptor conformations. The two ligand-binding domains of Ly49 are back-folded onto the long stalk region to bind two MHC-I molecules in *trans*. Dissociation of the ligand-binding domains from the stalk and their reorientation relative to the NK cell membrane allow monovalent binding of MHC-I in *cis*. The back-folded and extended configurations define the structural basis for *cis–trans* binding by Ly49 receptors (schematically shown in Fig. 1a).

Cis–trans binding may generally depend on large structural rearrangements that allow cell surface receptors to inverse the orientation of the ligand-binding domain relative to the cell membrane. While *cis–trans* binding of Ly49 receptors depends on an unusually long stalk (>70 amino acids), the stalk regions of LILRB/PIR-B receptors are considerably shorter (42–52 amino acids). In addition, these receptors are composed of four or six extracellular Ig-like domains [33, 49], whereby the membrane distal D1 and D2 domains mediate MHC-I binding. Based on the corresponding specificity of *trans* and *cis* binding [14], it seems likely that LILRB/PIR-B receptors use the same binding site for the two types of interactions. If that is indeed the case, the D1 and D2 domains of LILRB1 must reverse direction with respect to the effector cell surface to bind MHC-I in *cis*. This would require that the receptor bends back on itself to adopt a horseshoe-shaped configuration of the four Ig domains, implying considerable flexibility in the segment connecting the D2 and D3 domains. As a precedent, the four N-terminal Ig-like domains of the *Drosophila* Dscam protein do assume a horseshoe arrangement, which depends on a very short, five-residue hinge between D2 and D3 [53]. In the case of PIB-B, which has six Ig-like domains, the segments connecting D4–D5 or D5–D6 may provide additional flexibility. Therefore, the ability to reverse the orientation of their ligand-binding domains may be a general feature of receptors that bind in *cis* and *trans* (schematically shown in Fig. 1b). Such reversals may be made possible by two distinct structural features: long stalk regions in the case of Ly49s and flexible interdomain hinges in the case of LILRB2 and PIR-B.

Recent data point to an additional structural solution for *cis* binding. As discussed above, Notch interacts with Delta in *cis* and *trans*. However, the extracellular domains of Notch and Delta are composed of EGF-like domains that are thought to form extended and rigid structures. Notwithstanding, structural and functional analyses suggest

that Notch–ligand interactions use the same surfaces for *cis* and *trans* binding. In silico docking of crystal structures of the binding domains of Notch with that of its ligand, Jagged, provided evidence for two distinct receptor–ligand complexes [15]. An anti-parallel engagement of receptor and ligand is thought to mediate a *trans* interaction. Unexpectedly, there is also the possibility of a parallel engagement that would allow *cis* binding. Anti-parallel and parallel binding modes appear to be possible because the respective binding sites are symmetric. Even though this model remains to be further tested, symmetry of binding sites does represent, in principle, an additional solution to the structural problem of how cell surface receptors can bind the same ligand in *cis* and *trans* (schematically shown in Fig. 1c).

Basis for the divergent outcomes of *cis* and *trans* binding

An important unresolved issue is how *cis* and *trans* binding can result in distinct functional outcomes. Receptor engagement in *trans* is normally productive and results in receptor signaling. In contrast, binding of the same ligand in *cis*, using the same binding site, is often non-productive. The engagement of cell surface receptors induces intracellular signaling via two principal mechanisms: ligand-induced structural changes in the receptor and/or oligomerization of receptor–ligand complexes [54]. For Ly49 receptors, it was shown that Ly49C in complex with H-2K^b retains nearly the same structure as the free receptor [55], arguing against MHC-induced conformational changes in Ly49 as a signaling mechanism. On the other hand, monovalent human KIR-HLA-C complexes are thought to multimerize in a zinc-dependent fashion, and this seems to be needed for inhibitory signaling at the NK cell immune synapse [56]. Similarly, Ly49 signaling may depend on clustering of ligand-engaged Ly49 dimers. Although a single Ly49 receptor can associate with two MHC-I molecules to form a bivalent Ly49–MHC-I complex (Fig. 2a), it is not apparent how higher-order clustering of Ly49–MHC-I complexes might assemble. A prominent feature of the Ly49L structure, which contains a portion of the stalk region, is a flexible loop region that connects the $\alpha 3_S$ segment with the NKD. This so-called L_S loop is not needed for MHC-I binding in *trans.* Surprisingly, however, L_S is essential for the inhibitory function of the Ly49A receptor and is required for receptor accumulation at the immune synapse. In the model of the bivalent Ly49–MHC-I *trans* complex (Fig. 2a), the L_S loop is on the exterior of the complex, fully exposed to solvent. It is thus conceivable that L_S mediates lateral interactions with other cell surface molecules or adjacent bivalent Ly49–MHC-I *trans* complexes, and that these lateral associations are required for receptor signaling. Importantly, in the *cis* complex, where the NKDs are dissociated from the stalk, the L_s loop must adopt an extended conformation (Fig. 2b). This structural change may prevent L_S from mediating lateral association of Ly49 receptors that are bound to MHC-I in *cis*. Accordingly, in situations where *cis* complexes are inert, *cis* engagement may be mediated by receptor conformations that cannot cluster.

Concluding remarks

A significant number of cell surface receptors have now been shown to be able to interact with ligands in both *cis* and *trans*. Two general scenarios emerge from these different receptor–ligand systems with regard to the role of *cis* interaction: First, the binding of a ligand to its receptor in *cis* is functionally inert and serves as a competitive inhibitor for receptor binding in *trans*. Second, the binding of a ligand to its receptor in *cis* results in tonic (inhibition) signaling, which is enhanced when the receptor is engaged in *trans*. In both cases, *cis* binding alters the threshold at which cells produce a response to an external stimulus. *Cis* binding may be stable to permanently modify the threshold for a response. Alternatively, *cis* and/or *trans* ligands may be subject to regulation or *trans* ligand may compete with *cis* ligand for receptor binding. In these cases, the threshold response may vary depending on the relative abundance of *cis* and *trans* ligand. Consequently, the various flavors of *cis* binding may allow for diverse ways in which receptor function is not only dependent on the presence of ligand on other cells but can also be modified by the presence of ligand on the same cell.

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