Clinical Immunology 204 (2019) 37–42

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Review Article

SLAM family receptors in natural killer cells – Mediators of adhesion, activation and inhibition via *cis* and *trans* interactions

Maren Claus, Doris Urlaub, Frank Fasbender, Carsten Watzl*

Department for Immunology, Leibniz Research Centre for Working Environment and Human Factors (IfADo) at TU Dortmund, Dortmund, Germany

ABSTRACT

SLAM family receptors are important for the fine-tuning of immune reactions. Their expression is restricted to cells of hematopoietic origin and most SLAM family receptors are their own ligand. Here we review how these receptors are involved in regulating the functions of Natural Killer (NK) cells. We discuss that promoting cellular adhesion may be a main function of SLAM family receptors in NK cells. The homophilic interactions of SLAM family receptors can not only occur in *trans* between different cells, but also in *cis* on the surface of the same cell. This *cis* interaction additionally modulates the function of the receptors and subsequently affects the activities of NK cells. Finally, SLAM-family receptors can also mediate inhibitory signals under certain conditions. These inhibitory signals can contribute to the functional maturation of NK cells during NK cell education. Therefore, SLAM family receptors are critically involved in many aspects of NK cell functionality.

1. Introduction

The signaling lymphocytic activation molecule (SLAM) is an Ig-like surface receptor on cells of hematopoietic origin that gave its name to a family of six related receptors. These receptors include SLAMF1 (CD150, SLAM), SLAMF3 (CD229, Ly-9), SLAMF4 (CD244, 2B4), SLAMF5 (CD84), SLAMF6 (CD352, NTB-A, Ly108), and SLAMF7 (CD319, CRACC, CS1). The expression of these receptors is restricted to hematopoietic cells and they can be found on NK cells, T cells, B cells, macrophages, monocytes, dendritic cells, platelets, granulocytes, hematopoietic stem cells, and progenitor cells [1–3]. As we will be focusing on the function of SLAM family receptors in NK cells, we will restrict this review to the three receptors expressed on these cells, SLAMF4, SLAMF6 and SLAMF7.

1.1. SLAM family receptor signaling

SLAM family receptors activate signaling pathways via immunoreceptor tyrosine-based switch motifs (ITSMs) in their cytoplasmic domains. Upon phosphorylation the ITSM recruits SLAM-associated protein (SAP) and related adapters such as the Ewing sarcoma-associated transcript (EAT-2) and the EAT-2-related transducer (ERT). These adapters are small SH2-domain containing signaling molecules and they are all expressed in NK cells, with ERT being only found in mouse but not in human cells.

SLAM family receptors are homophilic, which means that they are their own ligand. The exception is SLAMF4, which interacts with CD48, a SLAM family related molecule (therefore it was initially also called SLAMF2) that is expressed by many hematopoietic cells. Engagement of SLAM family receptors by their ligands induces the phosphorylation of the cytoplasmic ITSMs via Src-family kinases such as Fyn [4,5]. This phosphorylation is essential for the recruitment of SAP family adapters, with the exception of SLAMF1, which seem to interact with SAP independently of its phosphorylation [6,7]. Once SAP interacts with the ITSM of SLAM family receptors, it is predicted to change conformation [8]. This enables SAP to recruit Fyn to the receptor via an untypical SH2-SH3 domain interaction which involves an Arginine 78 (R78) motif in SAP [9,10] (Fig. 1). While the initial phosphorylation of SLAM family receptors seems independent of this recruitment [11], Fyn binding via SAP is necessary for the activation of immune cells by SLAM family receptors [9,12].

1.2. Activation of NK cells by SLAM family receptors

SLAMF4 contains four ITSMs in its cytoplasmic domain, which are essential for signaling and receptor function [13]. Engagement of SLAMF4 on human NK cells results in its enrichment in membrane microdomains and the subsequent phosphorylation of the cytoplasmic ITSMs by Src-family kinases [14,15]. This is followed by the binding of SAP and the recruitment of Fyn resulting in the phosphorylation of Vav-1 [4,16]. SLAMF4 can also bind the adapter EAT-2, which does not recruit Fyn, but stimulates PLC-γ, Ca^{2+} flux and ERK activation [17,18] (Fig. 1). Additionally, the adapter 3BP2 can associate with SLAMF4 and stimulate Vav-1, PLC-γ and ERK phosphorylation [19–21]. Ultimately,

* Corresponding author at: Leibniz Research Centre for Working Environment and Human Factors (IfADo) at TU Dortmund, Ardeystrasse 67, 44139 Dortmund, Germany.

E-mail address: watzl@ifado.de (C. Watzl).

https://doi.org/10.1016/j.clim.2018.10.011

Received 29 August 2018; Received in revised form 18 October 2018; Accepted 19 October 2018

Available online 22 October 2018

1521-6616/

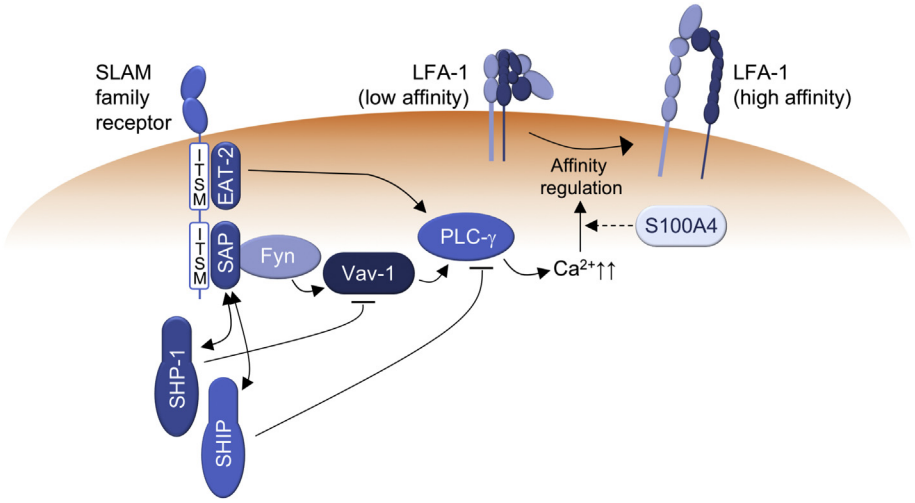

Fig. 1. Inside-out signaling by SLAM family receptors induces high affinity LFA-1. Engagement of SLAM family receptors results in phosphorylation of the ITSMs and the recruitment of SAP or EAT-2, which compete for binding of SHP-1 or SHIP. SAP and EAT-2-mediated signaling cascades induce the affinity maturation of LFA-1. See text for details.

SLAMF4 engagement initiates an activating signaling cascade that results in NK cell cytotoxicity and the release of cytokines such as IFN-γ and TNF-α.

Similar to SLAMF4, engagement of SLAMF6 on NK cells leads to phosphorylation of its three ITSMs, followed by recruitment of SAP and EAT-2 [22–24]. These adapters can then engage similar signaling pathways as described above and mediate NK cell cytotoxicity and cytokine production. In contrast, SLAMF7 does not recruit SAP but only EAT-2 [25,26]. As a result, absence of EAT-2 inhibits SLAMF7-mediated NK cell activation [27], whereas EAT-2 overexpression can enhance the anti-tumor activity of human NK cells [28].

1.3. SLAM family receptors and adhesion

In healthy human NK cells, SLAM family receptors are usually described as typical activating receptors that can stimulate NK cell cytotoxicity and cytokine secretion. However, there are several findings that suggest that one of the main functions of SLAM family receptors may be to stimulate NK cell adhesion. Stimulation of SLAMF4 or SLAMF6 on resting human NK cells can activate the binding affinity and avidity of the integrin LFA-1, which is essential for NK cell adhesion to target cells [29,30]. This activation of LFA-1 is mediated by inside-out signaling and in our hands SLAMF4 and SLAMF6 are some of the few receptors that can induce LFA-1 activation in resting human NK cells [29]. Similarly, measuring the adhesion strength between activated NK cells and their target cells by atomic force microscopy (AFM)-driven single cell force spectroscopy showed that SLAMF4 induces a quick increase in adhesion [31]. This effect was depending on LFA-1 and actin reorganization. Also, SLAMF4 stimulation induced the activation of human NK cells in a real-time cell analyzer assay which is based on changes in impedance due to adhesion and spreading of cells [32]. These data suggest that SLAMF4 and SLAMF6 are particularly good in stimulating NK cell adhesion through LFA-1. Both of these receptors signal via SAP and in mouse NK cells SAP has been shown to be essential for binding of NK cells to tumor cells [16]. In these experiments SLAM family receptor stimulated and LFA-1 mediated adhesion of NK cells to tumor target cells was dependent on Fyn recruitment, Vav, PLC-γ and Ca^{2+} flux.

SLAMF4-mediated inside-out signaling in human NK cells can be enhanced by cytokines [29]. SAP expression is upregulated by proinflammatory cytokine stimulation [33], which could be one explanation for this effect. Changes in the potential to activate integrins and adhere to targets can also be observed during NK cell maturation [29]. S100A4 expression correlates with the better adhesion of mature NK cells and may be involved in cytoskeleton-depending inside-out signals [29]. NK cell adhesion additionally depends on the education of NK cells [34]. The better reactivity of educated NK cells was correlated with higher activation of the Akt/mTOR pathway [35], resulting in enhanced Ca^{2+} flux and LFA-1 activation. These data support a model by which SLAM family receptors can efficiently induce LFA-1 activation through inside-out signaling via SAP, Fyn, Vav, PLC-γ and Ca^{2+} flux, resulting in adhesion to tumor target cells (Fig. 1).

A specific role for SLAM family receptors in mediating cellular adhesion is also well documented for other immune cells. Macrophages can bind to and phagocytose hematopoietic tumor cells when the inhibitory interaction of SIRPα and CD47 is blocked. This interaction is strictly dependent on SLAMF7 [36], which is expressed on macrophages and tumor cells. The effect does not require SAP, consistent with the fact that SLAMF7 does only recruit EAT-2, but not SAP. Interestingly, the interaction between the macrophages and the tumor cells was dependent on the integrin Mac-1, demonstrating that Mac-1 synergizes with SLAMF7 in the interaction with tumor cells. SLAM family receptors and SAP have also been shown to be important for the interaction between B cells and CD4 T cells during germinal center reactions [37] as well as for the adhesion of CD8 T cells to their targets [38].

1.4. Inhibitory functions of SLAM family receptors

The name switch motif for the ITSM is due to the fact that it can mediate positive and negative signals. While binding of SAP and EAT-2 can induce activating signaling cascades as described above, they also prevent the binding of the protein tyrosine phosphatases SHP-1 and SHP-2 as well as the binding of the inositol phosphatase SHIP-1 to the phosphorylated ITSM [6,13,16,39] (Fig. 1). Therefore, in the absence of SAP SLAM family receptors have been shown to mediate inhibitory signals via these phosphatases [6,40]. Interestingly, the importance of SAP for the function of SLAM family receptors is underlined by the phenotype of patients suffering from X-linked lymphoproliferative disease (XLP), a genetic disease with non-functional SAP [41,42]. In NK cells from XLP patients, stimulation of SLAM family receptors is defective and can even be inhibitory [22,43–47]. These and other data support a model by which the strength of receptor stimulation and the intracellular concentration of SAP adapters and phosphatases dictates whether SLAM family receptors mediate activating or inhibitory signals. As these parameters can be quite variable depending on the experimental conditions, this may explain some contradictory results about activating versus inhibitory functions of SLAM family receptors in NK cells.

An inhibitory function of SLAM family receptors in NK cells can have important consequences. SLAMF4-mediated inhibition can prevent the lysis of activated virus-specific T cells by NK cells and can thereby counteract persistent viral infections [48]. In addition,

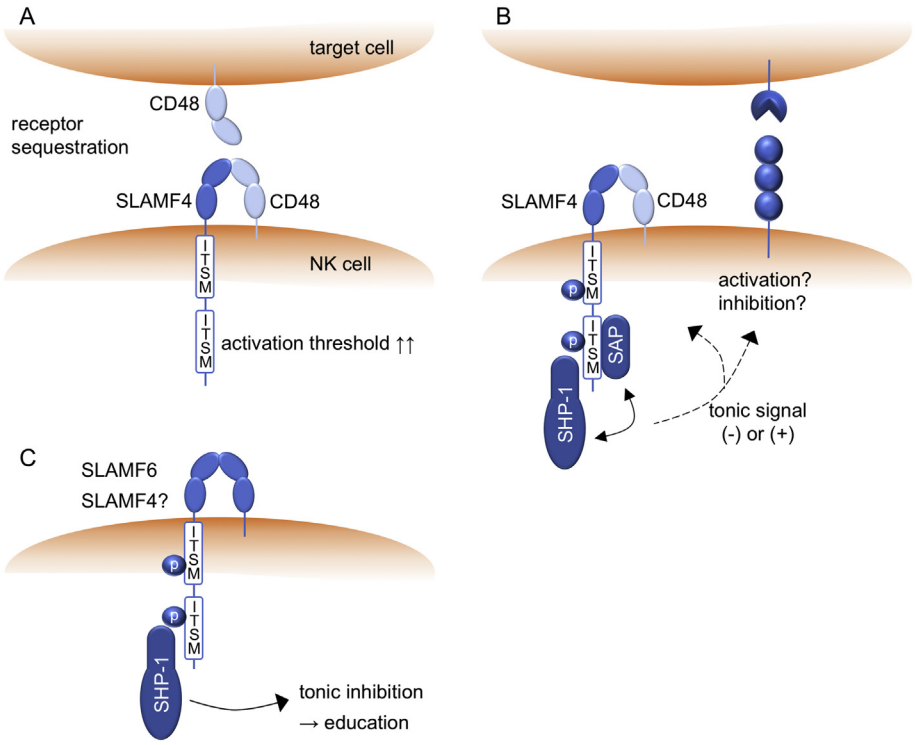

Fig. 2. Consequences of *cis* and *trans* interactions of SLAM family receptors. (A) Stable association of SLAMF4 with its ligand CD48 on the same cell in *cis* reduces binding to CD48 on neighboring cells in *trans*. *Cis* binding thereby increases the threshold for SLAMF4 mediated NK cell activation. (B) *Cis* interaction of SLAMF4 with CD48 induces constitutive low-level phosphorylation of SLAMF4. This allows for the recruitment of SHP-1 or SAP, resulting in tonic inhibitory or activating signals, respectively. These signals may also interfere with activation of neighboring non-SLAM family receptors. (C) In the absence of SAP, interaction of SLAMF6 receptors and possibly also of SLAMF4 with CD48 in *cis* leads to constitutive inhibitory signaling via SHP-1. This tonic inhibition possibly contributes to NK cell education in early NK cell development or in NK cells of XLP patients lacking functional SAP.

inhibitory SLAMF6 can contribute to NK cell education as discussed below [49].

1.5. SLAM family receptors can interact in cis

The extracellular part of SLAM family receptors is comprised of an N-terminal V-type Ig-like domain, which contains the ligand binding interface [50,51], and a membrane-proximal C2-type Ig-like domain. The two Ig-like domains are connected by a short linker and a stalk couples the C2-domain to the transmembrane segment [50,52]. Recently, we could show that SLAMF4 can not only bind to CD48 *in trans*, but it also interacts with CD48 on the surface of the same cell *in cis*. The interaction between SLAMF4 and CD48 *in cis* and *in trans* uses the same binding interface [53]. SLAMF4 is proposed to adopt a rod-like structure during the interaction with CD48 *in trans* [50], which suggests that the binding to CD48 on the same cell *in cis* requires large intramolecular rearrangements. These conformational changes require great structural flexibility of the extracellular part of SLAMF4 and most likely also of CD48.

The structurally related receptors LILR and *Drosophila* Dscam have also been shown to interact with their MHC I ligands on the same cell. Functional analyses and molecular modeling of LILRB2 [54], Dscam [55] and SLAMF4 [53] demonstrated that the short linker between the Ig-like domains provides the structural flexibility to enable *cis* binding to MHC I or CD48 respectively. However, deletion mutants lacking the entire membrane-proximal Ig-like domain of SLAMF4 or CD48 were still able to interact *in cis* [53]. Therefore, the stalk regions of the surface molecules might provide additional flexibility to enable *cis* binding.

1.6. NK cell education and cis-interacting receptors

NK cell activation is controlled by a balance of activating and inhibitory surface receptors [56]. Inhibitory receptors recognizing self-MHC class I molecules, such as the Ly49 receptors in mice or the KIR receptors in humans, are important to ensure the self-tolerance of NK cells. However, inhibitory receptors play an additional role during the functional maturation of NK cells in a process called NK cell 'education' [57]. In essence, only NK cells that express an inhibitory receptor that is specific for self-MHC class I can become functionally mature. NK cells that lack such a self-specific inhibitory receptor and that would therefore be potentially auto-reactive are rendered hypo-responsive [58,59]. This demonstrates that inhibitory signaling is essential for the functional maturation of NK cells [61].

cis-Interactions have been demonstrated for several inhibitory NK cell receptors upon engagement of their MHC ligands in mice [60]. Therefore, the functional relevance of *cis* interaction between Ly49A and its ligand H-2Dd for NK cell function was extensively studied. Studies showed that *cis* interaction is masking the Ly49A receptor for interaction with ligands *in trans*, thereby reducing recruitment of the receptor to the immunological synapse [62,63]. Further, sequestration of Ly49A through *cis* interaction was shown to be necessary for NK cell education by reducing the suppressive effect of unengaged Ly49 receptor during maturation [64,65]. The type of interaction between the Ly49 receptor and the MHC-I molecule during education has been shown to be important but the precise role of *cis* and *trans* binding still remains to be clarified. Using a mutated Ly49A receptor, which is only capable of binding *in trans* to ligands on other cell surfaces but not *in cis*, Chalifour and colleagues found that NK cells from these mice did not appear to be fully educated [65]. Similarly, Bessoles and colleagues' recent work also suggests that *cis* interaction is contributing to NK functionality, though the precise role is not clear [66]. However, there is no evidence for *cis* binding of inhibitory NKG2/CD94 or KIR2DL1 [67], which are necessary for NK cell education in humans [68].

Interestingly, inhibitory signaling via SLAM family receptors can also have an impact on NK cell education. Inhibitory signals via SLAMF6 have been shown to enhance NK cell responses during NK cell education [49]. Adoptive transfer experiments suggest that *cis* interaction of SLAMF6 molecules on the same cell is sufficient to increase NK cell responsiveness towards non-hematopoietic cells [49], demonstrating that also SLAMF6 can interact *in cis*. This enhancement was dependent on SLAMF6 and was only detected in the absence of the signaling adapter SAP, which is necessary for the induction of activating signals via SLAM family receptors. During early NK cell

development, the signaling adapter SAP is absent [69,70] while SLAMF6 and SLAMF4 are already expressed. This would favor inhibitory functions of these receptors during NK cell development [40,49]. It is therefore interesting to speculate that in addition to SLAMF6, also SLAMF4 *cis* interaction may also play a role during NK cell development and education (Fig. 2). However, the contribution of SLAM family receptors to NK cell education in mouse NK cells seems to be strain specific [71], underscoring the dynamic setup of SLAM family signaling.

XLP patients lacking functional SAP were shown to carry high percentages of NK cells lacking self-inhibitory NK receptors [72]. However, these XLP NK cells were not rendered hyporesponsive, but even exhibited increased degranulation capacity towards K562 target cells compared to NK cells from healthy donors [72]. The authors speculate that SLAMF4 signaling is induced by interaction with CD48 on the same cell, suggesting a role for inhibitory SLAMF4 signaling *in cis* for NK cell education in XLP patients. PNH (paroxysmal hemoglobinuria) patients carry a somatic mutation in the phosphatidylinositol glycan-A (PIG-A) gene. Mosaicism of hematopoietic stem cells results in clonal expansion of blood cells lacking GPI-anchored proteins. As CD48 is GPI-anchored, a subset of NK cells from PNH patients lacks CD48, resulting in missing SLAMF4 *cis* interaction on these cells. Interestingly, these NK cells show a skewed repertoire of KIR receptors [73], whereas in functional assays NK cells from PNH patients did not display defects in cytotoxicity [74]. However, these assays were performed with K562 target cells which lack CD48 expression. Therefore, it is unknown how the absence of CD48 and the missing SLAMF4 *cis* interaction affects SLAMF4-mediated functions in these NK cells.

1.7. Regulation of SLAM family receptor signaling by cis interactions

Cis binding of Ly49A to MHC I does not induce receptor phosphorylation [75], but instead reduces Ly49A accessibility for binding to MHC I ligand *in trans* by masking the receptor [63,75]. Besides its role in education, this sequestration of inhibitory Ly49A accessible *in trans* is thought to lower the threshold for activating signals. A growing number of receptors were shown to encounter their ligands *in cis*. Examples are the NK cell receptors NKp44 [76] and Siglec7 [77] as well as HVEM (herpesvirus entry mediator) on T cells [78]. Similar to Ly49 receptors, *cis* binding does not induce phosphorylation or downstream signaling of these receptors, suggesting that it modulates the receptors' activation threshold only by competitively blocking encounter of activating ligands *in trans*. Likewise, masking of SLAMF4 by CD48 on the same NK cell abolishes binding of soluble CD48 and interferes with SLAMF4-mediated signaling during the encounter of CD48 expressing target cells [53] (Fig. 2A).

On B cells, *cis* binding of CD22 to sialic acids induces constitutive phosphorylation of CD22 ITIMs and association with SHP-1 [79,80]. This tonic inhibitory signaling is thought to increase the threshold for B cell activation and to prevent unwanted and spontaneous B cell activation [81,82]. Similarly, binding of mouse PIRB to MHC I on the same cell was shown to dampen spontaneous mast cell activation by constitutive association with SHP-1, thereby generating tonic inhibitory signaling and increasing the threshold for activating signals by counteracting the activating FcεRI [54,83,84]. These findings raise the question whether *cis* interaction of SLAMF4 can influence NK cell functions in a similar fashion. In NK cells, the ITSMs of SLAMF4 are constitutively phosphorylated to a low extent. This phosphorylation is dependent on the presence of CD48 and *cis* binding is sufficient to induce this baseline phosphorylation [53] (Fig. 2B). SLAMF4 is activation-dependently recruited to membrane microdomains and this recruitment is essential for the phosphorylation of the receptor [85]. CD48 contains a GPI anchor and is therefore constitutively associated with membrane microdomains. Its *cis* interaction with SLAMF4 might help to localize SLAMF4 to these specialized membrane domains and thereby induce receptor phosphorylation. The *cis* interaction between SLAMF4 and CD48 might also be important for the function of CD48, as cross-linking of CD48 on mouse NK cells was shown to induce IL-13 production by co-clustering of SLAMF4 and subsequent SLAMF4-mediated signaling [86].

2. Conclusion

SLAM family receptors have important functions for the fine tuning of lymphocyte responses. The data mentioned in this review suggest that one important function of these receptors is the regulation of cellular adhesion. Additionally, SLAM family receptors can interact in *trans* and in *cis*. Therefore, future studies should take the different modes of interaction into account when carefully examining under which conditions SLAM family receptors mediate activating signals and under which conditions they can inhibit cellular responses. This will be essential to gain a complete understanding of these important receptors.

References

[1] N. Wu, A. Veillette, SLAM family receptors in normal immunity and immune pathologies, Curr. Opin. Immunol. 38 (2016) 45–51.
[2] J.L. Cannons, S.G. Tangye, P.L. Schwartzberg, SLAM family receptors and SAP adaptors in immunity, Annu. Rev. Immunol. 29 (2011) 665–705.
[3] M. Claus, S. Meinke, R. Bhat, C. Watzl, Regulation of NK cell activity by 2B4, NTB-A and CRACC, Front Biosci 13 (2008) 956–965.
[4] C. Watzl, C.C. Stebbins, E.O. Long, NK cell inhibitory receptors prevent tyrosine phosphorylation of the activation receptor 2B4 (CD244), J. Immunol. 165 (2000) 3545–3548.
[5] M. Simarro, A. Lanyi, D. Howie, F. Poy, J. Bruggeman, M. Choi, J. Sumegi, M.J. Eck, C. Terhorst, SAP increases FynT kinase activity and is required for phosphorylation of SLAM and Ly9, Int. Immunol. 16 (2004) 727–736.
[6] J. Sayos, C. Wu, M. Morra, N. Wang, X. Zhang, D. Allen, S. van Schaik, L. Notarangelo, R. Geha, M.G. Roncarolo, H. Oettgen, J.E. de Vries, G. Aversa, C. Terhorst, The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM, Nature 395 (1998) 462–469.
[7] S.C. Li, G. Gish, D. Yang, A.J. Coffey, J.D. Forman-Kay, I. Ernberg, L.E. Kay, T. Pawson, Novel mode of ligand binding by the SH2 domain of the human XLP disease gene product SAP/SH2D1A, Curr. Biol. 9 (1999) 1355–1362.
[8] S. Samanta, S. Mukherjee, Microscopic insight into thermodynamics of conformational changes of SAP-SLAM complex in signal transduction cascade, J. Chem. Phys. 146 (2017) 165103.
[9] S. Latour, R. Roncagalli, R. Chen, M. Bakinowski, X. Shi, P.L. Schwartzberg, D. Davidson, A. Veillette, Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation, Nat. Cell Biol. 5 (2003) 149–154.
[10] B. Chan, A. Lanyi, H.K. Song, J. Griesbach, M. Simarro-Grande, F. Poy, D. Howie, J. Sumegi, C. Terhorst, M.J. Eck, SAP couples Fyn to SLAM immune receptors, Nat. Cell Biol. 5 (2003) 155–160.
[11] S. Meinke, C. Watzl, NK cell cytotoxicity mediated by 2B4 and NTB-A is dependent on SAP acting downstream of receptor phosphorylation, Front. Immunol. 4 (2013) 3.
[12] C. Bloch-Queyrat, M.-C. Fondanèche, R. Chen, L. Yin, F. Relouzat, A. Veillette, A. Fischer, S. Latour, Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn, J. Exp. Med. 202 (2005) 181–192.
[13] P. Eissmann, L. Beauchamp, J. Wooters, J.C. Tilton, E.O. Long, C. Watzl, Molecular basis for positive and negative signaling by the natural killer cell receptor 2B4 (CD244), Blood 105 (2005) 4722–4729.
[14] C. Watzl, E.O. Long, Natural killer cell inhibitory receptors block actin cytoskeleton-dependent recruitment of 2B4 (CD244) to lipid rafts, J. Exp. Med. 197 (2003) 77–85.
[15] S.A. Gutgemann, M.M. Sandusky, S. Wingert, M. Claus, C. Watzl, Recruitment of activating NK-cell receptors 2B4 and NKG2D to membrane microdomains in mammalian cells is dependent on their transmembrane regions, Eur. J. Immunol. 45 (2015) 1258–1269.
[16] Z. Dong, D. Davidson, L.A. Perez-Quintero, T. Kurosaki, W. Swat, A. Veillette, The adaptor SAP controls NK cell activation by regulating the enzymes Vav-1 and SHIP-1 and by enhancing conjugates with target cells, Immunity 36 (2012) 974–985.
[17] L.A. Perez-Quintero, R. Roncagalli, H. Guo, S. Latour, D. Davidson, A. Veillette, EAT-2, a SAP-like adaptor, controls NK cell activation through phospholipase Cgamma, ca++, and Erk, leading to granule polarization, J. Exp. Med. 211 (2014) 727–742.
[18] M. Morra, J. Lu, F. Poy, M. Martin, J. Sayos, S. Calpe, C. Gullo, D. Howie, S. Rietdijk, A. Thompson, A.J. Coyle, C. Denny, M.B. Yaffe, P. Engel, M.J. Eck, C. Terhorst, Structural basis for the interaction of the free SH2 domain EAT-2 with SLAM receptors in hematopoietic cells, EMBO J. 20 (2001) 5840–5852.
[19] I. Saborit-Villarroya, J.M. Del Valle, X. Romero, E. Esplugues, P. Lauzurica, P. Engel, M. Martin, The adaptor protein 3BP2 binds human CD244 and links this receptor to Vav signaling ERK activation, and NK cell killing, J Immunol 175 (2005) 4226–4235.
[20] D. Jevremovic, D.D. Billadeau, R.A. Schoon, C.J. Dick, P.J. Leibson, Regulation of

NK cell-mediated cytotoxicity by the adaptor protein 3BP2, Journal of immunology (Baltimore MD) 166 (2001) (1950) 7219–7228.
[21] I. Saborit-Villarroya, A. Martinez-Barriocanal, I. Oliver-Vila, P. Engel, J. Sayos, M. Martin, The adaptor 3BP2 activates CD244-mediated cytotoxicity in PKC- and SAP-dependent mechanisms, Mol. Immunol. 45 (2008) 3446–3453.
[22] C. Bottino, M. Falco, S. Parolini, E. Marcenaro, R. Augugliaro, S. Sivori, E. Landi, R. Biassoni, L.D. Notarangelo, L. Moretta, A. Moretta, NTB-A, a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease, J. Exp. Med. 194 (2001) 235–246.
[23] P.A. Valdez, H. Wang, D. Seshasayee, M. van Lookeren Campagne, A. Gurney, W.P. Lee, I.S. Grewal, NTB-A, a new activating receptor in T cells that regulates autoimmune disease, J. Biol. Chem. 279 (2004) 18662–18669.
[24] P. Eissmann, C. Watzl, Molecular analysis of NTB-A signaling: a role for EAT-2 in NTB-A-mediated activation of human NK cells, J. Immunol. 177 (2006) 3170–3177.
[25] I. Tassi, M. Colonna, The Cytotoxicity Receptor CRACC (CS-1) Recruits EAT-2 and Activates the PI3K and Phospholipase C{gamma} Signaling Pathways in Human NK Cells, J. Immunol. 175 (2005) 7996–8002.
[26] A. Bouchon, M. Cella, H.L. Grierson, J.I. Cohen, M. Colonna, Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family, J. Immunol. 167 (2001) 5517–5521.
[27] M.E. Cruz-Munoz, Z. Dong, X. Shi, S. Zhang, A. Veillette, Influence of CRACC, a SLAM family receptor coupled to the adaptor EAT-2, on natural killer cell function, Nat. Immunol. 10 (2009) 297–305.
[28] Y.A. Aldhamen, S.S. Seregin, C.F. Aylsworth, S. Godbehere, A. Amalfitano, Manipulation of EAT-2 expression promotes induction of multiple beneficial regulatory and effector functions of the human innate immune system as a novel immunomodulatory strategy, Int. Immunol. 26 (5) (2013) 291–303, https://doi.org/10.1093/intimm/dxt061.
[29] D. Urlaub, K. Hofer, M.L. Muller, C. Watzl, LFA-1 Activation in NK Cells and their Subsets: Influence of Receptors, Maturation, and Cytokine Stimulation, J Immunol 198 (2017) 1944–1951.
[30] Y.T. Bryceson, H.G. Ljunggren, E.O. Long, Minimal requirement for induction of natural cytotoxicity and intersection of activation signals by inhibitory receptors, Blood 114 (2009) 2657–2666.
[31] S.C. Hoffmann, A. Cohnen, T. Ludwig, C. Watzl, 2B4 engagement mediates rapid LFA-1 and actin-dependent NK cell adhesion to tumor cells as measured by single cell force spectroscopy, J. Immunol. 186 (2011) 2757–2764.
[32] F. Fasbender, C. Watzl, Impedance-based analysis of Natural Killer cell stimulation, Sci. Rep. 8 (2018) 4938.
[33] J. Endt, P. Eissmann, S.C. Hoffmann, S. Meinke, T. Giese, C. Watzl, Modulation of 2B4 (CD244) activity and regulated SAP expression in human NK cells, Eur. J. Immunol. 37 (2007) 193–198.
[34] L.M. Thomas, M.E. Peterson, E.O. Long, Cutting edge: NK cell licensing modulates adhesion to target cells, J. Immunol. 191 (2013) 3981–3985.
[35] A. Marcais, M. Marotel, S. Degouve, A. Koenig, S. Fauteux-Daniel, A. Drouillard, H. Schlums, S. Viel, L. Besson, O. Allatif, M. Blery, E. Vivier, Y. Bryceson, O. Thaunat, T. Walzer, High mTOR activity is a hallmark of reactive natural killer cells and amplifies early signaling through activating receptors, Elife 6 (2017).
[36] J. Chen, M.C. Zhong, H. Guo, D. Davidson, S. Mishel, Y. Lu, I. Rhee, L.A. Perez-Quintero, S. Zhang, M.E. Cruz-Munoz, N. Wu, D.C. Vinh, M. Sinha, V. Calderon, C.A. Lowell, J.S. Danska, A. Veillette, SLAMF7 is critical for phagocytosis of haematopoietic tumour cells via Mac-1 integrin, Nature 544 (2017) 493–497.
[37] H. Qi, J.L. Cannons, F. Klauschen, P.L. Schwartzberg, R.N. Germain, SAP-controlled T-B cell interactions underlie germinal Centre formation, Nature 455 (2008) 764–769.
[38] F. Zhao, J.L. Cannons, M. Dutta, G.M. Griffiths, P.L. Schwartzberg, Positive and negative Signaling through SLAM Receptors Regulate Synapse Organization and Thresholds of Cytolysis, Immunity 36 (6) (2012) 1003–1016, https://doi.org/10.1016/j.immuni.2012.05.017.
[39] S.G. Tangye, S. Lazetic, E. Woollatt, G.R. Sutherland, L.L. Lanier, J.H. Phillips, Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP, J. Immunol. 162 (1999) 6981–6985.
[40] S. Sivori, M. Falco, E. Marcenaro, S. Parolini, R. Biassoni, C. Bottino, L. Moretta, A. Moretta, Early expression of triggering receptors and regulatory role of 2B4 in human natural killer cell precursors undergoing in vitro differentiation, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4526–4531.
[41] A.J. Coffey, R.A. Brooksbank, O. Brandau, T. Oohashi, G.R. Howell, J.M. Bye, A.P. Cahn, J. Durham, P. Heath, P. Wray, R. Pavitt, J. Wilkinson, M. Leversha, E. Huckle, C.J. Shaw-Smith, A. Dunham, S. Rhodes, V. Schuster, G. Porta, L. Yin, P. Serafini, B. Sylla, M. Zollo, B. Franco, A. Bolino, M. Seri, A. Lanyi, J.R. Davis, D. Webster, A. Harris, G. Lenoir, G. de St Basile, A. Jones, B.H. Behloradsky, H. Achatz, J. Murken, R. Fassler, J. Sumegi, G. Romeo, M. Vaudin, M.T. Ross, A. Meindl, D.R. Bentley, Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene, Nat. Genet. 20 (1998) 129–135.
[42] K.E. Nichols, D.P. Harkin, S. Levitz, M. Krainer, K.A. Kolquist, C. Genovese, A. Bernard, M. Ferguson, L. Zuo, E. Snyder, A.J. Buckler, C. Wise, J. Ashley, M. Lovett, M.B. Valentine, A.T. Look, W. Gerald, D.E. Housman, D.A. Haber, Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13765–13770.
[43] L. Benoit, X. Wang, H.F. Pabst, J. Dutz, R. Tan, Defective NK cell activation in X-linked lymphoproliferative disease, J. Immunol. 165 (2000) 3549–3553.
[44] H. Nakajima, M. Cella, A. Bouchon, H.L. Grierson, J. Lewis, C.S. Duckett, J.I. Cohen, M. Colonna, Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity, Eur. J. Immunol. 30 (2000) 3309–3318.
[45] S. Parolini, C. Bottino, M. Falco, R. Augugliaro, S. Giliani, R. Franceschini, H.D. Ochs, H. Wolf, J.Y. Bonnefoy, R. Biassoni, L. Moretta, L.D. Notarangelo, A. Moretta, X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells, J. Exp. Med. 192 (2000) 337–346.
[46] S.G. Tangye, J.H. Phillips, L.L. Lanier, K.E. Nichols, Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome, J. Immunol. 165 (2000) 2932–2936.
[47] P. Engel, M.J. Eck, C. Terhorst, The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease, Nat Rev Immunol 3 (2003) 813–821.
[48] S.N. Waggoner, R.T. Taniguchi, P.A. Mathew, V. Kumar, R.M. Welsh, Absence of mouse 2B4 promotes NK cell-mediated killing of activated CD8+ T cells, leading to prolonged viral persistence and altered pathogenesis, J. Clin. Invest. 120 (2010) 1925–1938.
[49] N. Wu, M.C. Zhong, R. Roncagalli, L.A. Perez-Quintero, H. Guo, Z. Zhang, C. Lenoir, Z. Dong, S. Latour, A. Veillette, A hematopoietic cell-driven mechanism involving SLAMF6 receptor, SAP adaptors and SHP-1 phosphatase regulates NK cell education, Nat. Immunol. 17 (2016) 387–396.
[50] C.A. Velikovsky, L. Deng, L.K. Chlewicki, M.M. Fernández, V. Kumar, R.A. Mariuzza, Structure of natural killer receptor 2B4 bound to CD48 reveals basis for heterophilic recognition in signaling lymphocyte activation molecule family, Immunity 27 (2007) 572–584.
[51] J.B. Ames, V. Vyas, J.D. Lusin, R.A. Mariuzza, NMR structure of the natural killer cell receptor 2B4 (CD244): implications for ligand recognition, Biochemistry (Mosc) 44 (2005) 6416–6423.
[52] S.J. Davis, P.A. van der Merwe, The structure and ligand interactions of CD2: implications for T-cell function, Immunol. Today 17 (1996) 177–187.
[53] M. Claus, S. Wingert, C. Watzl, Modulation of natural killer cell functions by interactions between 2B4 and CD48 in cis and in trans, Open Biol. 6 (2016).
[54] A. Masuda, A. Nakamura, T. Maeda, Y. Sakamoto, T. Takai, Cis binding between inhibitory receptors and MHC class I can regulate mast cell activation, J. Exp. Med. 204 (2007) 907–920.
[55] R. Meijers, R. Puettmann-Holgado, G. Skiniotis, J.H. Liu, T. Walz, J.H. Wang, D. Schmucker, Structural basis of Dscam isoform specificity, Nature 449 (2007) 487–491.
[56] C. Watzl, How to trigger a killer: modulation of natural killer cell reactivity on many levels, Adv. Immunol. 124 (2014) 137–170.
[57] S. Kim, J. Poursine-Laurent, S.M. Truscott, L. Lybarger, Y.J. Song, L. Yang, A.R. French, J.B. Sunwoo, S. Lemieux, T.H. Hansen, W.M. Yokoyama, Licensing of natural killer cells by host major histocompatibility complex class I molecules, Nature 436 (2005) 709–713.
[58] N.C. Fernandez, E. Treiner, R.E. Vance, A.M. Jamieson, S. Lemieux, D.H. Raulet, A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules, Blood 105 (2005) 4416–4423.
[59] N. Anfossi, P. Andre, S. Guia, C.S. Falk, S. Roetynck, C.A. Stewart, V. Breso, C. Frassati, D. Reviron, D. Middleton, F. Romagne, S. Ugolini, E. Vivier, Human NK cell education by inhibitory receptors for MHC class I, Immunity 25 (2006) 331–342.
[60] W. Held, R.A. Mariuzza, Cis interactions of immunoreceptors with MHC and non-MHC ligands, Nat. Rev. Immunol. 8 (2008) 269–278.
[61] J.E. Boudreau, K.C. Hsu, Natural killer cell education in human health and disease, Curr. Opin. Immunol. 50 (2018) 102–111.
[62] D. Ito, Y.-M. Iizuka, M.P. Katepalli, K. Iizuka, Essential role of the Ly49A stalk region for immunological synapse formation and signaling, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 11264–11269.
[63] J. Back, A. Chalifour, L. Scarpellino, W. Held, Stable masking by H-2Dd cis ligand limits Ly49A relocalization to the site of NK cell/target cell contact, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3978–3983.
[64] A.H. Jonsson, L. Yang, S. Kim, S.M. Taffner, W.M. Yokoyama, Effects of MHC class I alleles on licensing of Ly49A+ NK cells, J. Immunol. 184 (2010) 3424–3432.
[65] A. Chalifour, L. Scarpellino, J. Back, P. Brodin, E. Devèvre, F. Gros, F. Lévy, G. Leclercq, P. Höglund, F. Beermann, W. Held, A Role for cis Interaction between the Inhibitory Ly49A receptor and MHC class I for natural killer cell education, Immunity 30 (2009) 337–347.
[66] S. Bessoles, G.S. Angelov, J. Back, G. Leclercq, E. Vivier, W. Held, Education of murine NK cells requires both cis and trans recognition of MHC class I molecules, J. Immunol. 191 (2013) 5044–5051.
[67] W. Held, R.A. Mariuzza, Cis-trans interactions of cell surface receptors: biological roles and structural basis, Cellular and molecular life Sci. 68 (2011) 3469–3478.
[68] C. Fauriat, M.A. Ivarsson, H.-G. Ljunggren, K.-J. Malmberg, J. Michaëlsson, Education of human natural killer cells by activating killer cell immunoglobulin-like receptors, Blood 115 (2010) 1166–1174.
[69] M. Vitale, J. Zimmer, R. Castriconi, D. Hanau, L. Donato, C. Bottino, L. Moretta, H. de La Salle, A. Moretta, Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor(s) that prevent lysis of normal autologous cells, Blood 99 (2002) 1723–1729.
[70] S. Sivori, M. Falco, E. Marcenaro, S. Parolini, R. Biassoni, C. Bottino, L. Moretta, A. Moretta, Early expression of triggering receptors and regulatory role of 2B4 in human natural killer cell precursors undergoing in vitro differentiation, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4526–4531.
[71] H. Guo, S.A. Cranert, Y. Lu, M.C. Zhong, S. Zhang, J. Chen, R. Li, S.E. Mahl, N. Wu, D. Davidson, S.N. Waggoner, A. Veillette, Deletion of Slam locus in mice reveals inhibitory role of SLAM family in NK cell responses regulated by cytokines and LFA-

1, J. Exp. Med. 213 (2016) 2187–2207.
[72] R. Meazza, M. Falco, S. Marcenaro, F. Loiacono, P. Canevali, F. Bellora, C. Tuberosa, F. Locatelli, C. Micalizzi, A. Moretta, M.C. Mingari, L. Moretta, M. Arico, C. Bottino, D. Pende, Inhibitory 2B4 contributes to NK cell education and immunological derangements in XLP1 patients, Eur. J. Immunol. 47 (2017) 1051–1061.
[73] E. Cosentini, L. Gargiulo, P. Bruno, S. Lastraioli, A. Risitano, R. Camerlingo, V. Luongo, M. Serra, M. Sica, C. Garzillo, U. Giani, R. Notaro, F. Alfinito, G. Ruggiero, G. Terrazzano, Killer immunoglobulin-like receptors (KIR) and their HLA-ligands in Italian paroxysmal nocturnal haemoglobinuria (PNH) patients, Tissue Antigens 80 (2012) 322–327.
[74] Y.M. El-Sherbiny, G.M. Doody, R.J. Kelly, A. Hill, P. Hillmen, G.P. Cook, Natural killer (NK) cell function in paroxysmal nocturnal hemoglobinuria: a deficiency of NK cells, but not an NK cell deficiency, Blood 125 (2015) 1351–1352.
[75] M.-A. Doucey, L. Scarpellino, J. Zimmer, P. Guillaume, I.F. Luescher, C. Bron, W. Held, Cis association of Ly49A with MHC class I restricts natural killer cell inhibition, Nat. Immunol. 5 (2004) 328–336.
[76] M. Brusilovsky, O. Radinsky, L. Cohen, R. Yossef, A. Shemesh, A. Braiman, O. Mandelboim, K.S. Campbell, A. Porgador, Regulation of natural cytotoxicity receptors by heparan sulfate proteoglycans in -cis: a lesson from NKp44, Eur. J. Immunol. 45 (2015) 1180–1191.
[77] T. Avril, S.J. North, S.M. Haslam, H.J. Willison, P.R. Crocker, Probing the cis interactions of the inhibitory receptor Siglec-7 with alpha2,8-disialylated ligands on natural killer cells and other leukocytes using glycan-specific antibodies and by analysis of alpha2,8-sialyltransferase gene expression, J. Leukoc. Biol. 80 (2006) 787–796.
[78] T.C. Cheung, L.M. Oborne, M.W. Steinberg, M.G. Macauley, S. Fukuyama, H. Sanjo, C. D'Souza, P.S. Norris, K. Pfeffer, K.M. Murphy, M. Kronenberg, P.G. Spear, C.F. Ware, T cell intrinsic heterodimeric complexes between HVEM and BTLA determine receptivity to the surrounding microenvironment, J. Immunol. 183 (2009) 7286–7296.
[79] S. Kelm, J. Gerlach, R. Brossmer, C.P. Danzer, L. Nitschke, The ligand-binding domain of CD22 is needed for inhibition of the B cell receptor signal, as demonstrated by a novel human CD22-specific inhibitor compound, J. Exp. Med. 195 (2002) 1207–1213.
[80] L. Jin, P.A. McLean, B.G. Neel, H.H. Wortis, Sialic acid binding domains of CD22 are required for negative regulation of B cell receptor signaling, J. Exp. Med. 195 (2002) 1199–1205.
[81] B.E. Collins, O. Blixt, A.R. Desieno, N. Bovin, J.D. Marth, J.C. Paulson, Masking of CD22 by cis ligands does not prevent redistribution of CD22 to sites of cell contact, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6104–6109.
[82] G.M. Doody, L.B. Justement, C.C. Delibrias, R.J. Matthews, J. Lin, M.L. Thomas, D.T. Fearon, A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP, Science 269 (1995) 242–244.
[83] T. Uehara, M. Blery, D.W. Kang, C.C. Chen, L.H. Ho, G.L. Gartland, F.T. Liu, E. Vivier, M.D. Cooper, H. Kubagawa, Inhibition of IgE-mediated mast cell activation by the paired Ig-like receptor PIR-B, J. Clin. Invest. 108 (2001) 1041–1050.
[84] L.H. Ho, T. Uehara, C.C. Chen, H. Kubagawa, M.D. Cooper, Constitutive tyrosine phosphorylation of the inhibitory paired Ig-like receptor PIR-B, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 15086–15090.
[85] C. Watzl, E.O. Long, Natural killer cell inhibitory receptors block actin cytoskeleton-dependent recruitment of 2B4 (CD244) to lipid rafts, J. Exp. Med. 197 (2003) 77–85.
[86] S.K. Sinha, N. Gao, Y. Guo, D. Yuan, Mechanism of Induction of NK Activation by 2B4 (CD244) Via its Cognate Ligand, Journal of Immunology (Baltimore, Md : 1950), (2010).