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## SLAM family receptors and the SLAM-associated protein (SAP) modulate T cell functions

### Cynthia Detre,

BIDMC Division of Immunology, Harvard Center for Life Sciences, Rm. CLS 938, 3 Blackfan Circle, Boston MA 02115 USA

### Marton Keszei,

BIDMC Division of Immunology, Harvard Center for Life Sciences, Rm. CLS 938, 3 Blackfan Circle, Boston MA 02115 USA

### Xavier Romero,

BIDMC Division of Immunology, Harvard Center for Life Sciences, Rm. CLS 938, 3 Blackfan Circle, Boston MA 02115 USA

### George C. Tsokos, and

BIDMC Division of Rheumatology, Harvard Center for Life Sciences, Rm. CLS 937 3 Blackfan Circle, Boston, MA 02115, USA

### Cox Terhorst

BIDMC Division of Immunology, Harvard Center for Life Sciences, Rm. CLS 938, 3 Blackfan Circle, Boston MA 02115 USA, Harvard Medical School, Boston MA USA

Cynthia Detre: cdetre@bidmc.harvard.edu; Cox Terhorst: cterhors@bidmc.harvard.edu

## Abstract

One or more of the signaling lymphocytic activation molecule (SLAM) family (SLAMF) of cell surface receptors, which consists of nine transmembrane proteins, i.e., *SLAMF1-9*, are expressed on most hematopoietic cells. While most SLAMF receptors serve as self-ligands, SLAMF2 and SLAMF4 use each other as counter structures. Six of the receptors carry one or more copies of a unique intracellular tyrosine-based switch motif, which has high affinity for the single SH2-domain signaling molecules SLAM-associated protein and EAT-2. Whereas SLAMF receptors are costimulatory molecules on the surface of CD4+, CD8+, and natural killer (NK) T cells, they also involved in early phases of lineage commitment during hematopoiesis. SLAMF receptors regulate T lymphocyte development and function and modulate lytic activity, cytokine production, and major histocompatibility complex-independent cell inhibition of NK cells. Furthermore, they modulate B cell activation and memory generation, neutrophil, dendritic cell, macrophage and eosinophil function, and platelet aggregation. In this review, we will discuss the role of SLAM receptors and their adapters in T cell function, and we will examine the role of these receptors and their adapters in X-linked lymphoproliferative disease and their contribution to disease susceptibility in systemic lupus erythematosus.

## Keywords

XLP; SAP; SH2D1A; SLAM family; Systemic lupus erythematosus

## The nine SLAM family receptors

Signaling lymphocytic activation molecule (SLAM) family (SLAMF) receptors are type I transmembrane glycoproteins, except SLAMF2, which has a glycosylphosphatidylinositol membrane anchor [1–5]. While the ectodomains of eight SLAMF receptors are comprised of an N-terminal V-Ig domain and a C-terminal C2-Ig domain, SLAMF3 consists of two V-Ig/C2-Ig sets.

SLAMF2 is a receptor for SLAMF4, but the remaining SLAMF (1, 3, 5, 6, 7, 8, and 9) represent self-ligands and their ectodomains interact in a homophilic fashion ([6,7] and unpublished). Interestingly, SLAMF1 also serves as one of the two known receptors for measles virus, and SLAMF2 interacts with CD2 and FimH (Table 1). The specificity of the self-ligand interactions of most of the SLAMF receptors is explained by the known structures of their extracellular domains [8,9]. For example, at the interface of the two V-Ig in the SLAMF6 homodimer, the  $\beta$ -sheets contain multiple residues, which contribute to the stability of the interaction. Similarly, the structure of SLAMF5 explains the orthogonal association of two SLAMF5 molecules but precludes an interaction with SLAMF6. The orthogonal association of two SLAMF5 or of two SLAMF6 molecules enables these complexes to localize to the immunological synapse [8,9]. Specific SLAMF members are thought to function as costimulatory molecules in the conventional T cell, natural killer (NK) T (NKT) cell, innate CD8+ T cell, or NK cell synapse through their homophilic or heterophilic interactions [10–12] (Fig. 1).

The cytoplasmic tails of SLAMF1 and SLAMF3 through SLAMF7 contain several intracellular tyrosine-based switch motifs (ITSM) as well as a number of other phosphotyrosine (pY) residues. Thus, SLAM-associated protein (SAP) or EAT-2-dependent and EAT-2-independent recruitment of intracellular molecules leads to the inhibitory and stimulatory signals, which ensue upon engagement of these receptors. Integration of signaling networks determines the outcome of several effector functions depending on the cell type and state of activation. But, SAP/EAT-2-independent signal transduction events have also been observed, e.g., Grb-2 binds to the most C-terminal pY of SLAMF3 and plays a role in endocytosis of this receptor in conjunction with TCR/CD3 [13,14]. In addition, some receptors may play a dominant role in a given cell, e.g., SLAMF6 appears to be a major signaling entity in activation-induced cell death (AICD) and/or restimulation-induced cell death (RICD) of CD8+ T cells, while other SLAMF receptors are expressed in the same cell. The notion that both SLAMF receptors and their protein isoforms are often co-expressed in the same cell and exert partially overlapping functions remains one of the challenges of this field.

## The SLAM-associated protein and the X-linked lymphoproliferative syndrome

X-linked lymphoproliferative syndrome (XLP) is an extremely rare primary immunodeficiency, which affects only males and is at first recognized as a defective immune response to Epstein–Barr virus (EBV) [15]. The majority (60%) of XLP patients infected with EBV develop fatal or fulminant infectious mononucleosis, which is caused by a dysregulated immune response, resulting in a large polyclonal proliferation of T cells and B cells. This leads to lymphoid infiltration of several organs such as the liver, kidney, thymus, and bone marrow causing organ failure. The extensive destruction of liver and bone marrow often results in fulminant hepatitis and virus-associated hemophagocytic syndrome [6]. Patients often exhibit low serum levels of IgG and increased levels of IgA and IgM. Almost all *lymphomas* found in XLP patients are of B cell origin and are located along the gastrointestinal tract. Of them, 53% are Burkitt's, 18% immunoblastic, 12% large noncleaved, 12% small cleaved or mixed cell, and 5% unclassifiable lymphomas [16–18]. XLP patients are usually asymptomatic prior to virus exposure, but after recurrent viral infections, they develop *dysgammaglobulinemia* [18–20] (Table 2).

### Mutations in the gene encoding the *SAP* gene cause XLP

Ten years ago, the *SH2D1A* gene, which encodes SAP, was found to be defective in XLP patients with macro/microdeletions, splice-site mutations, nonsense mutations, or missense mutations [21–23]. Whereas most XLP patients have mutations in the *SH2D1A* (*SAP*) gene, more than 20% express intact SAP but have a mutation in the X-linked inhibitor of apoptosis (*XIAP*) gene [24–26]. Interestingly, these patients lack NKT cells, a phenotype which was not found in the *XIAP*-deficient mouse [27].

Missense mutations that disrupt the binding of SAP to SLAM family receptors interfere (a) with the interactions involving the N-terminal amino acids in the ITSM motif, e.g., T53I, (b) binding in the pY pocket, e.g., R32T, C42W, or (c) interactions with the Y+3 amino acid in the hydrophobic cleft, E67D, T68. Interestingly, the mutant T53I binds to the phosphorylated SLAM but fails to bind to SLAMF3 and SLAMF4 [28,29]. A number of the missense mutations that decrease the half-life of SAP are conserved amino acids in SAP isolated from human, mouse, and other species, e.g., Y7C, S28R, Q99P, P101L, V102G, Y54C, F87S, and I84T [28,30]. Surprisingly, mutations Y54C and F87S also affect SAP binding to some, but not all the SLAM family receptors, suggesting that the requirement of SAP binding might differ between receptors.

### *SAP*, a single SH2-domain protein, binds with high affinity to SLAM family receptors and is an adapter, which recruits Fyn

Although SH2 domains are highly conserved noncatalytic modules, they exhibit considerable flexibility in the way they bind to their respective pY ligands. SH2 domains associate with the pY motif in a “two-pronged fashion”: a pY-binding pocket and a “specificity determining” region that interacts with an amino acid C-terminal of the pY. By contrast, the SH2 domain of SAP interacts with the ITSM motif in a “three-pronged binding mode”, which results in a high affinity binding to the phosphorylated ITSM-based peptide SLAMF1 Y281 (Fig. 2) [21]. This SH2 domain is also unique in that it also binds to the nonphosphorylated ITSM-based peptide SLAMF1 Y281 [21,31–33]. This “three-pronged binding mode” is caused by the additional interactions involving three residues N-terminal to the SLAMF1 Y281 residue [21,33].

Because of its high affinity for phosphorylated ITSM motifs, SAP should outcompete SH2 domain proteins that bind to the same phosphotyrosine motif with lesser affinity. Although support for the concept that SAP is an inhibitor of the recruitment of the other SH2 proteins to SLAMF receptors exists [31,34] (Fig. 4), further experiments are required. The second SLAMF adapter EAT-2 binds to the ITSM motifs using a similar three pronged interaction [35]. However, in cells that express both adapters, SAP seems to outcompete EAT-2.

The second unique and somewhat surprising property of the single SH2 domain protein SAP is that it is an adapter, which couples the protein tyrosine kinase Fyn to the SLAMF receptors. Biochemical studies and structural analyses unveiled the remarkable mechanism of interaction between the SH2 domain of SAP and the SH3 domain of Fyn that directly couples Fyn to SLAM receptor [36,37]. The structure of the SAP/FynSH3 complex reveals that SAP binding to Fyn domain does not involve canonical SH3 interactions. Instead at the point of interaction, the positively charged SAP surface interacts with negatively charged Fyn SH3 surface at a dissociation constant of 3.45  $\mu$ M for the entire complex. On the SAP SH2 domain, R78 appears to be particularly critical for the interaction, as it forms dual salt-bridge hydrogen bonds with D100 in Fyn, and its guanidinium moiety stacks with the side chain of W119 in the Fyn SH3 domain. Indeed the R78 mutation of SAP almost abolishes the interaction with Fyn, and conversely, mutations of key residues of Fyn reduce binding to SAP [36,38]. The site of the SAP SH2 domain, which interacts with Fyn, does not overlap with the SLAMF binding site, and, in fact, SAP mutants that are unable to bind to the SLAMF cytoplasmic tail still bind to

Fyn. Consequently, the SH2 domain of SAP is able to bind simultaneously to SLAM and Fyn [36,38]. Importantly, structural considerations predict that the SAP/FynSH3 complex cannot be formed by the auto-inhibitory form of Fyn. In vitro experiments indicate that SAP can activate the inactive form of Fyn [39,40]. Thus, the interaction of SAP SH2 with its ligands may control two key events: the tyrosine site location of signaling molecules within the cell and the switch to the inactive/active configuration of Src kinases. These two events allow the activation of Src-like kinases at the right time and in the correct location. It is conceivable that SAP bound to SLAM could bind to a Fyn molecule that has been activated by other mechanisms. The association of the negatively charged surface of Fyn with the positively charged surface of SAP is unique because most of the amino acids involved in the SH3 binding surface of Fyn are not conserved in other Src kinases. As the EAT-2 SH2 domain does not contain the positively charged residues that are requisite for the interaction with the Fyn SH3 domain, the role of EAT-2 in enhancing the phosphorylation of SLAMF receptors [35] requires further clarification.

SAP may also bind directly to Lck, as suggested by the results from in vitro studies and yeast two-hybrid analyses [39]. Although the putative binding of SAP to the kinase domains of Fyn and Lck remains ill-defined, Lck phosphorylates SLAMF1 and SLAMF3-5. The fact though that SLAMF1 phosphorylation is reduced, but not eliminated, in Fyn<sup>-/-</sup> T cells supports the notion that other Src kinases are involved in SLAMF1 phosphorylation [39]. Comparative studies with SAP-deficient and Fyn-deficient mice clearly indicate that the two proteins control distinct, yet overlapping mechanisms [41–44].

### SAP binds to proteins other than SLAMF receptors

Whereas the “three-pronged binding mechanism” increases substrate specificity and the affinity of the SAP SH2 domain for its phosphorylated ITSM ligand, it is otherwise a conventional SH2 domain characterized by a central  $\beta$ -sheet flanked by two alpha helices ( $\alpha$ A and  $\alpha$ B) and a small  $\beta$ -sheet. Consequently, the SAP SH2 domain could in principle bind to other pY containing peptides. The association of SAP with the SH3 domain of the PAK-interacting protein ( $\beta$ -PIX), a guanine nucleotide exchange factor specific for Rac/Cdc42 GTPase, was recently demonstrated. Similar to the SAP/Fyn interaction, the binding of SAP to  $\beta$ -PIX is mediated by the C-terminal region of the SAP SH2 domain and the  $\beta$ -PIX SH3 domain [45]. The mutant SAPR78A, but not SAPR32Q, fails to bind  $\beta$ -PIX, suggesting that  $\beta$ -PIX and Fyn might compete with each other for SAP binding [45]. SAP recruits  $\beta$ -PIX to SLAMF4, suggesting that  $\beta$ -PIX may mediate some biological functions of the SLAMF receptors [45].

In spite of major progress in our understanding of the functions of SAP and of SLAMF receptors in adaptive immunity, the mechanisms involved in the pathogenesis of XLP need to be better understood. We have only begun to understand the role of SAP in signaling networks, which prevent the extreme proliferation of CD8 cells in response to viruses (“Aberrant CD8+ responses in the absence of SAP” section), the role of SAP in CD4 T cells, which partake in the germinal center (GC) reaction (“Defective humoral response in SAP<sup>-/-</sup> mice” section), NKT cell development and function (“The role of SLAMF and SAP in the development and function of T cells selected on thymocytes, e.g., NKT cells and innate CD8+ T cells” section) and NK cell killing. Furthermore, as no genotype/phenotype correlation has been observed in XLP patients, modifying genes and environmental factors could very well play a significant role in the diverse disease manifestations, which are caused by mutations in the SH2D1A gene.

## Aberrant CD8<sup>+</sup> responses in the absence of SAP

### Responses of SAP<sup>-/-</sup> mice to viral infections

CD8<sup>+</sup> T cell responses are aberrant in XLP patients, in particular during the fatal infectious mononucleosis, in which EBV-specific CD8 cells proliferate without a contraction of the response. Although EBV, of course, does not infect mouse cells, the study of the response to lymphocytic choriomeningitis virus (LCMV), murine gammaherpesvirus (MHV)-68, and influenza virus in SAP<sup>-/-</sup> mice has helped us to understand cell-specific defects and in elucidating the molecular mechanisms that are altered in the absence of the adapter. Increased numbers of antigen-specific CD8<sup>+</sup> T cells, IFN- $\gamma$  secreting CD8<sup>+</sup> T cells, and elevated production of IFN- $\gamma$  per cell occur upon infection with LCMV. This response subsides after 2 weeks, as it does in control mice. However, whereas SAP<sup>-/-</sup> mice were able to survive acute infection, they failed to resolve chronic infection and died most likely because of a lack of antibody responses. [46,47].

Upon infection with MHV-68, SAP<sup>-/-</sup> mice also respond by increasing the number of virus-specific CTL to resolve the acute infection as effectively as *wt* mice. By contrast to an LCMV infection, the chronic infection with MHV-68 is more efficiently controlled by SAP<sup>-/-</sup> CTL than by *wt* CTL, as judged by the increased number of virus-specific CD8<sup>+</sup> T cells [48,49]. Strikingly, mouse SAP<sup>-/-</sup> CD8<sup>+</sup> T cells display greater cytotoxicity toward MHV68-infected cells than their *wt* counterparts. The observations made in SAP<sup>-/-</sup> mice are in contrast to some analyses of CD8<sup>+</sup> T cells from XLP patients, which exhibit defective lysis of EBV-infected B cells and a decrease in numbers of IFN- $\gamma$ -producing cells [6]. However, as shown in the next section, the general principle that CD8<sup>+</sup> T cells proliferate more due to the absence of SAP has been found in human cells as well.

Naïve human and mouse CD8<sup>+</sup> T cells express several SLAMF receptors, i.e., SLAMF2, 3, 5, and 6, and upon activation, human and mouse SLAMF1 and SLAMF4 are upregulated. Because CD8<sup>+</sup> T cells co-express these receptors, it was not surprising that the functions of CD8 cells isolated from individual SLAMF receptor knockout mice were not different from those of *wt* mice [50–52]. As discussed in “The role of SLAMF and SAP in the development and function of T cells selected on thymocytes, e.g., NKT cells and innate CD8<sup>+</sup> T cells” section, downregulation of both SLAMF1 and SLAMF6 affected NKT cell development, while only minor defects were detected in single knockout mice.

### The role of SAP in CD8<sup>+</sup> T cell apoptosis

Recent experiments provide direct evidence that the hyperproliferation of SAP-deficient CD8<sup>+</sup> T cells is related to impaired apoptosis and RICD.

Chen et al. found that ovalbumin-peptide-activated SAP<sup>-/-</sup> OT-I CD8<sup>+</sup> T cells have lower AICD compared to their *wt* counterparts [53]. Furthermore, the induction of p73, a key mediator of TCR-induced apoptosis through the mitochondrial apoptotic pathway, was significantly reduced at both the mRNA and protein levels in the activated SAP<sup>-/-</sup> cells. Meanwhile, a reduced level of active caspase 9, the initiator caspase of the mitochondrial apoptotic pathway, was observed in the mutant cells. These observations led to the conclusion that impaired p73 induction in the mitochondrial apoptotic pathway is mainly responsible for the reduction of AICD in activated SAP<sup>-/-</sup> CD8<sup>+</sup> T cells, which may explain the hyperresponsiveness of CD8<sup>+</sup> T cells in SAP<sup>-/-</sup> mice. Although the precise underlying mechanism is still unclear, one can hypothesize that defective Fyn activation in the absence of SAP results in decreased activity of Itch, a ubiquitin ligase shown to be essential in the regulation of p73 protein stability (Fig. 3).

SAP also plays a “pro-apoptotic” role in human T and B lymphocyte-derived lines. In clones of a T-ALL tumor cell line, high SAP levels correlate with succumbed AICD. Importantly, retroviral expression of SAP into lymphoblastoid cell lines established from XLP patients leads to elevated apoptotic response to DNA damage. Surprisingly, similar results were obtained upon ectopic expression of SAP in an osteosarcoma line. Because SAP binds to the anti-apoptotic protein valosin-containing protein, a SLAMF receptor-independent mechanism could be involved [54].

A more detailed analysis by Snow et al. showed that the lack of susceptibility of pre-activated T cells to RICD after re-encountering the antigen is a substantial problem in XLP derived CD8 + T cells [55]. In this process, SAP is suggested to deliver SLAMF6 signaling in a Fyn-independent manner, facilitating TCR signal strength for reaching the required threshold for the induction of apoptosis in normal T cells. Indeed, downmodulation of SAP and SLAMF6 expression were found to block TCR-induced upregulation of RICD execution molecules FasL and Bim. In contrast, in XLP-derived T cells where SAP-mediated dissociation of SHP-1 from SLAM receptors does not occur SLAMF6 and SHP-1 contribute to RICD resistance [55].

Taken together, the SAP mutation of XLP patients or mice may abolish multiple pathways of the homeostatic regulation of T cells at different stages of the immune response. As EAT-2 has been detected in a subset of CD8 cells, its role in viral infections must be examined as well.

### Defective humoral response in *SAP*<sup>-/-</sup> mice

Defects in primary and secondary humoral responses and an aberrant germinal center formation were observed in *SAP*<sup>-/-</sup> mice upon infections with LCMV, influenza virus, MHV68, or after immunization with proteins or haptens [56–61]. As GCs are specific sites for the development of long-term humoral immunity, their absence could account for the significant decrease in circulating memory B cells and long-lived plasma cells resulting in a reduced levels of serum IgG. The absence of SAP-dependent signaling networks impairs T helper functions and consequently germinal center formation, as shown by a number of adoptive transfer experiments [56–58].

The role of SAP in T cell function is not yet fully understood. While the strongly reduced IL-4 secretion has an impact, the contribution of dysregulation of other cytokines, e.g., IL-6 and IL-21 or CD40L, are less clear [46,56,57,62,63]. The notion that SAP is dispensable for the priming of naïve CD4+ T cells into a subset of effector cells programmed to help B cells, but critical for the communication with B lymphocytes is supported by different experiments. As judged by intravital fluorescence microscopy, *SAP*<sup>-/-</sup> T cells form conjugates with antigen pulsed DCs as efficiently as their wt counterparts [64]. Further evidence that SAP is not requisite for primary activation comes from experiments in which T cells were retrovirally transfected with SAP 5 days after their APC-mediated priming. These cells become potent B cell activators [65]. Qi et al. provided elegant evidence for the concept that the duration of the interaction between B cells and *SAP*<sup>-/-</sup> T cells is rather short-lived, which in turn results in inefficient B cell proliferation and germinal center development [64,66].

In the last decade, a class of CD4+ T cells, i.e., T follicular helper cells (Tfh), localized in B cell follicles through expression of the chemokine receptor CXCR5 has attracted attention since they appeared to be particularly potent B cell helpers [67–69]. Further investigation revealed that their gene expression profile is distinct from of conventional Th1 and Th2 cells, as they express transcription factor BCL-6, high levels of IL-21, and surface receptors ICOS and PD-1.

In agreement with the SAP-independent nature of normal T-DC interactions, primed *wt* and *SAP*<sup>-/-</sup> CD4+ T cells were found to upregulate CXCR5 and ICOS in a comparable fashion

[64,65]. Curiously, in some studies, attenuated ICOS expression and abnormal CD40L expression have been observed on SAP<sup>-/-</sup> T cells after in vitro or in vivo stimulation [58,62].

According to a current study by Eddahri et al., IL-6/STAT3 promoted IL-21 secretion correlates with the acquisition of B cell help without significantly affecting expression of characteristic surface markers of Tfh cells [70]. Consistent with this model, the prolonged CD40L expression of SAP<sup>-/-</sup> CD4+ T cells is uncoupled from their diminished capacity to help B cells in influenza-infected SAP<sup>-/-</sup> mice [65].

Cytokine responses, the other major factors by which T cells facilitate and shape humoral defense, exhibit a dramatic bias in SAP<sup>-/-</sup> CD4+ T cells, as they display an impaired ability to differentiate into Th2 cells but produce elevated levels of IFN- $\gamma$  upon TCR stimulation in vitro and in vivo [46,71]. Although there were no differences observed in the IL-4 or IFN- $\gamma$  production, IL-10 secretion of CD4+ T cells from XLP patients was also found to be severely impaired [62]. Further biochemical analyses revealed an important role of SAP in the recruitment of PKC $\theta$  to the immune synapse followed by NF- $\kappa$ B activation and induction of GATA-3, the master transcription factor of IL-4 [43] (Fig. 4). However, while these signal transduction pathways are dependent on the Fyn recruiting ability of SAP [72], defective induction of germinal centers could be rescued by retroviral reconstitution of T cells with SAP-R78A, a Fyn binding mutant form of SAP [58].

Since T–B conjugates are bidirectional stimulating units; not only B cells require sustained interaction for their comprehensive activation and subsequent germinal center formation but differentiation of Tfh cells also appears to depend on a prolonged, cognate engagement [64]. Nevertheless, it still remains to be determined whether the development of incompetent Tfh cells participates in defective germinal reactions of SAP-deficient mice. Furthermore, the SAP dependence of suitable contact formation between GC B cells and GC Tfh cells in the second round of T–B interaction also needs further clarification.

Another remaining question is the contribution of the different SLAM family receptors in T cell-mediated B cells responses. Mice carrying single deletions of SLAMF1, SLAMF3, or SLAMF6 did not recapitulate the humoral defect of SAP<sup>-/-</sup> mice ([73], [50] and unpublished observations), supporting the notion that multiple SLAM receptors are implicated.

## **The role of SLAMF and SAP in the development and function of T cells selected on thymocytes, e.g., NKT cells and innate CD8+ T cells**

### **NKT cell development in the thymus**

NKT cells form a separate lineage of unconventional T lymphocytes operating on the border of innate and adaptive immune systems. Unlike conventional T cells, they co-express NK cell-associated receptors and a limited repertoire of TCRs specialized for glycolipid antigen recognition presented in complex with the major histocompatibility complex (MHC)-like molecule CD1d [74–76]. Accordingly to their memory-like phenotype, they mount a rapid cytokine response to TCR stimulation secreting Th1 and Th2 cytokines as well as IL-17 [75, 77,78].

Recent studies have established a crucial role for SAP in NKT cell ontogeny as they were absent in XLP patients and SAP-deficient mice [79–81]. NKT cells arise from common CD4+CD8+ thymic T cell precursors with conventional T cells and the fact that only the development of NKT cells is affected suggested that SAP is crucial for a specific step in their selection process. A partial block of NKT cell maturation was also detected in Fyn<sup>-/-</sup> mice [44,82,83]. As SAP has been shown to couple SLAM family receptors to Fyn in the periphery, SAP–Fyn interaction is a plausible component of signaling pathways involved in NKT cell

development [6,36,39]. However, the recruitment of Fyn to SAP was indeed found to be involved in NKT cell maturation; it is not the exclusive mechanism by which SAP controls the development of NKT cells. Fyn binding mutant of SAP (R78A) can restore the generation of NKT cells, albeit less efficiently than wt SAP, in a OP9-DL1-based in vitro system as well as in SAP-R78A knock-in mice and SAP-R78A competitive bone marrow chimeras [84].

Because the positive selection of NKT cells depends on thymocyte–thymocyte interactions, instead of being selected on cortical epithelial cells [85,86], SLAMF receptors represent obvious candidates to support NKT development because of their SAP-dependent signal transduction and their homotypic cellular interactions [44]. SLAMF1 and SLAMF6 are not only highly expressed on DP thymocytes and immature NKT cells, SLAMF1<sup>-/-</sup> as well as SLAMF6<sup>-/-</sup> mice develop slightly reduced NKT cell compartment. In addition, overlapping functions of these two receptors were observed [51,52]. To investigate the conceivable redundant functions of SLAMF1 and SLAMF6 in NKT cell maturation, pseudodouble knockouts were generated in bone marrow chimeras [42]. As predicted, NKT cell development was severely impaired in SLAMF1/SLAMF6 functional double mutant chimeras. Thus, SLAMF1 and SLAMF6 complement each other in the positive selection of NKT cells, mediated through their homophilic interactions between DP thymocytes.

It is important to note that although the reduction of NKT cells is dramatic in SAP-deficient mice, the reduction of NKT cells in SLAMF1/SLAMF6 double mutant chimeras as in Fyn-deficient mice is not complete, suggesting a possible role of other protein kinases (e.g., Lck or SLAMF members such as SLAMF3 and SLAMF5) [42]. Although the precise signaling mechanism by which SLAMF1/SLAMF6–SAP–Fyn control NKT cell development remains to be elucidated, PKC $\theta$  and possibly NF- $\kappa$ B might be involved [44,87,88].

It is conceivable that XLP disease manifestations can be explained, in part, by an absence of NKT cells. For example, they have been described to promote antiviral responses by stimulating NK cells and CD8<sup>+</sup> T lymphocytes through robust cytokine production or indirectly via enhanced antigen presentation and pDC activation [89,90]. Additionally, CD1d<sup>-/-</sup> mice, which lack NKT cells, recapitulate the same CD8<sup>+</sup> T cell hyperproliferation observed in SAP<sup>-/-</sup> mice after viral infection [90]. By controlling cytotoxic responses, NKT cells have been also implicated in tumor surveillance in human and mouse [91–93]. Furthermore, several recent studies suggest that activated NKT cells enhance multiple levels of antibody responses by cognate and noncognate interactions with CD1d-expressing B cells as well as through dendritic cells and helper T cells [94–96]. Because SAP R78A knock-in mice develop a significant number of NKT cells, they have been exploited to investigate the importance of SAP–Fyn interaction in response of NKT cells following in vivo TCR stimulation. Interestingly, contrary to regular CD4<sup>+</sup> T cells, Fyn binding of SAP is required neither for normal IL-4 and IFN- $\gamma$  production nor the activation of bystander lymphocytes [84]. In another study, the role of SAP in NKT function was examined by generating SAP-deficient NKT cells using SAP<sup>-/-</sup> mice expressing the invariant TCR (V $\alpha$ 14J $\alpha$ 18) encoding transgene [97]. Although these SAP<sup>-/-</sup> NKT cells appeared to have an immature phenotype, the defective cytokine response and corresponding absence of key transcription factors GATA-3 and T-bet suggest a complex function of SAP in NKT cell development, homeostasis, and function [97,98].

### Innate CD8<sup>+</sup> T cells

The selection and differentiation of innate-like CD8<sup>+</sup> T cells differs from those required for conventional T cell populations [99,100]. They are selected by nonclassical MHC class I molecules expressed on hematopoietic cells rather than on thymic epithelial cells. Itk and Rlk, members of the Tec family tyrosine kinases, are necessary for the development of conventional T lymphocytes but are dispensable for the selection of innate-like CD8<sup>+</sup> T cells [101–103].



Consequently, the majority of CD8<sup>+</sup> T cells that develop in *Itk*- and *Itk/Rlk*-deficient thymuses exhibit the characteristic phenotype of innate-like T cells, providing an opportunity to study the specifications of their development as well as their function [102,104,105]. According to the observation by Horai et al., the thymic selection of this group of innate-like CD8<sup>+</sup> T cells also requires SAP-dependent signal transduction [106]; however till date, we still do not have information which SLAM receptors participate.

### **SAP governs development of T-CD4 cells and a subset of innate CD4<sup>+</sup> T cells**

MHC class II-expressing double-positive thymocytes, which are generated from specific class II transgenic mice, induce progression of conventional CD4<sup>+</sup> T cell development as efficiently as cortical thymic epithelial cells [107,108]. These thymocyte-selected CD4<sup>+</sup> T cells, or T-CD4 cells, require the same signaling components as NKT cells, namely SAP, Fyn, and PKC $\theta$ . Furthermore, SAP is critical for IL-4 production by T-CD4 cells, and T-bet is necessary to produce the maximum amount of IFN- $\gamma$  for CD4<sup>+</sup> T cells regardless of the selection pathway. Thus, in contrast to epithelial cell-selected CD4<sup>+</sup> T cells, the two distinct lineages of T cells selected by thymocytes, i.e., T-CD4 and NKT cells, both utilize the SAP–Fyn–PKC $\theta$  pathway for their development and function. Likewise in the case of NKT cells, the lack of SLAMF6 resulted only a slight decrease of T-CD4 cells, suggesting that other SLAM receptors are also involved in their maturation [41]. Although these cells do not occur naturally in the mouse, they exist in humans and they might be absent in XLP patients.

A unique subset of innate-like T cells was identified recently among the thymocytes of *Itk*- and *Itk/Rlk*-deficient mice [109]. Interestingly, these innate CD4<sup>+</sup> T cells, unlike innate CD8<sup>+</sup> T cells described in the same mice, are not selected by hematopoietic cells, but they require MHC class II expression on the thymic epithelium for their development [100]. Surprisingly, they still appear to be dependent on the interactions with the SLAM family receptors present on hematopoietic cells during thymic maturation [100].

According to an elegant interpretation of Berg and colleagues, the balance of TCR and SLAM family signaling pathways determines conventional versus innate T cell lineage fate: Strong TCR signaling restricts the effect of SLAM signals promoting the development of conventional  $\alpha\beta$  CD4<sup>+</sup> and CD8<sup>+</sup> T cells, while NKT cells emerge upon strong signaling of both TCR and SLAM family receptors, whereas weak TCR signaling combined with intact SLAM signals facilitate innate-like CD4<sup>+</sup> and CD8<sup>+</sup> T cell maturation [100].

## **Do SLAMF polymorphisms play a role in the pathogenesis of systemic lupus erythematosus?**

### **SLAMF polymorphisms and isoforms**

SLAMF receptors on chromosome 1 differ in coding and noncoding sequences in inbred mouse strains. Two major haplotypes exist in the region containing the seven SLAMF genes: One is represented by C57BL/6 and the second by 129Sv, NZW, or BALB/c. The ectodomains of SLAMF3 and SLAMF4 differ the most. Less variation is found in the ectodomains of SLAMF2, SLAMF8, and SLAMF9 and none in the other SLAMF ectodomains. Ectodomain amino acid sequence variation has not yet been found in humans, but increasing numbers of cDNA's lacking one of the extracellular domains, part of the cytoplasmic tail, or the transmembrane segment of a SLAM family member have been reported for both mouse and human receptors. These variations are caused by alternative splicing events. The loss of one of the ectodomains will, expectedly, affect the receptor/ligand interactions in the restricted space requirements of the immune synapse [8,110]. Variants of the cytoplasmic tail will affect signal transduction because one of the ITSM motifs involved in binding of SAP and/or EAT-2

will be lacking. Several of the isoforms are expressed as proteins, but the complexity of these SLAMF isoforms is not yet understood.

### **SLAMF gene polymorphisms are likely to be susceptibility factors that contribute to the pathogenesis of SLE**

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease, marked by a range of autoantibodies with a long prodromal phase of autoantibody development and epitope spreading. Although the pathogenesis of this disease and primary mechanisms underlying the loss of tolerance remains largely unknown, it would appear that a multitude of genes are involved, as is the case for other autoimmune diseases [111–113]. A number of studies clearly demonstrate that disease manifestations involve dysregulation of T/B cell interactions, as well as aberrant DC and macrophage functions [114].

Genome-wide linkage scans of families with multiple members affected with SLE have consistently demonstrated the presence of a susceptibility locus on chromosome 1q23, which includes the SLAMF genes [115–121]. In addition, it established a first-generation haplotype map, using 63 single nucleotide polymorphisms (SNPs) across the SLAM region in a set of 254 SLE trios and 160 discordant sibling pairs [122]. These preliminary results suggest that there is variation within the human SLAM region that is associated with SLE.

More recently, a specific association with SLE disease susceptibility was found using the nonsynonymous SNP rs509749 SNP, which is located in exon 8 of SLAMF3 [123]. This SNP encodes a Val/Met change in amino acid 602 in the cytoplasmic domain of the SLAMF3 protein (Table 1; Fig. 2). Only two of the eight tyrosine's in its cytoplasmic tail are embedded in ITSM motifs and have been shown to associate with SAP and/or EAT-2 [35,124]. The C-terminal tyrosine binds Grb2 via its Sh2 domain [13]. Whether the V602M replacement in the –1 position of the first ITSM affects function requires further investigation, especially because the M602 allele is present in a substantial percentage of the human population (NCBI, dbSNP).

### **SLAMF gene polymorphisms as susceptibility factors in murine SLE**

In mice, the region syntenic to human 1q23 has been implicated in three different models of spontaneous lupus: the (NZB × NZW) F2 intercross, the NZM/Aeg2410 New Zealand mouse, and the BXSB mouse [125–127]. The disease phenotype of these mice is similar to that of SLE patients, both in terms of production of autoantibodies as well as severe nephritis and multi-organ involvement. A strong, spontaneous, antinuclear antibody response occurred in congenic mice (*B6.Sle1*) derived from crosses between the lupus-prone mouse strain NZM2410 (NZB × NZW/F1) and C57BL/6. The presence of a small NZW-derived chromosome #1 segment (*Sle1*) on a C57BL/6 background was sufficient to generate these autoantibody responses [128,129]. Fine mapping of the *Sle1* locus determined that three loci within this congenic interval, termed *Sle1a*, *Sle1b*, and *Sle1c*, are implicated cause a loss of tolerance to chromatin [128–132].

The *Sle1b* locus, which mediates a breach in tolerance to nuclear antigens [128,129], is an approximately 0.9-Mb segment that includes the SLAMF locus [129]. Although this locus contains 24 expressed genes, the NZW-based SLAMF variants embedded in the C57BL/6 background are thought to be the major contributors to the pathogenesis of SLE. This notion is supported by studies with congenic strains derived from crosses between 129 and C57BL/6 mice, which develop spontaneous autoimmunity [133,134]. One congenic strain, *B6.129chr1b*, carrying a 129 interval contains the SLAMF locus. Thus, the SLAMF-haplotype II, e.g., derived from NZW or 129, segments *Sle1b* and *129chr1b* in the C57BL/6 background induce loss of tolerance to nuclear antigens. We do, however, not know which C57BL/6 genes

are involved in the putative epistatic pathogenic networks between SLAMF genes and other genes [135].

As judged by sequence and single nucleotide polymorphism analyses, the NZW-derived or 129-derived SLAMF locus embedded in the C57BL/6 genome of *B6.Sle1b* or *B6.129Chr1b* is identical to the SLAMF locus of the 129 mouse strain [129]. However, although *B6.129Chr1b* carries SLAMF haplotype II, the 129 mouse itself does not develop autoimmunity. Thus, an interplay between SLAMF haplotype II variants and the background must be maintained by either “suppressive” 129 genes or by epistatic activating genes in the C57BL/6 genome. While there are marked differences in a variety of immune responses between the C57BL/6 and 129 mouse [136–140], surprisingly, 129 mice are more susceptible to certain autoimmune stimuli than C57BL/6 [141,142]. This might indicate that a specific epistatic interaction exist in C57BL/6 × 129 congenic mice rather than just a generally suppressed autoimmunity in 129. This notion would be consistent with the development of autoimmunity in the other SLAMF-haplotype II mouse, namely *B6.Sle1b*.

While *Sle1b* subcongenic line shows a pronounced B cell phenotype (similar to the one of the whole *Sle1* locus), the effect of congenic region on T cell compartment seems to be more elusive. A broad range of autoimmune manifestations have been observed in the congenic *B6.Sle1* and partially in aged *B6.129Chr1b* T cells (e.g., CD4+ T cell activation, decreased number of T regulatory cells, increased proliferation and cytokine production, presence of histone-specific T cells) [131,134,143]. In contrast, the subcongenic *Sle1b* T cell phenotype is restricted to an increased percentage of activated cells and an elevated calcium flux in response to receptor cross-linking [129,131]. Interestingly, this activation phenotype of T cells could be amplified in an epistatic interaction between *Sle1b* and *Yaa* or *Fas<sup>lpr</sup>* [132], [144]]. This interplay resulted a hyperactivation of PI3K/AKT/mTOR signaling pathway in *Sle1b* × *Fas<sup>lpr</sup>*.

Mohan, Wakeland, and colleagues postulate that among the SLAM family members, *Slamf6* in the context of the C57BL/6 background may be the strongest candidate to be causally correlated with the *B6.Sle1b* phenotype. They find that the long isoform of murine Ly108, i.e., Ly108-1, is upregulated, while the short isoform (Ly108-2) is down-regulated in T and B cells of *B6.Sle1b* mice compared to C57BL/6 [129]. Supporting this hypothesis, transfection of Ly108-2 was able to sensitize the immature B cell line WEHI-231 to undergo apoptosis, suggesting that Ly108 could regulate the stringency with which self-reactive B cells are censored during early development [145].

### The role of SAP in murine SLE

Aberrant T cell functions caused by the SAP mutation have been studied in a few lupus-prone mouse strains. For instance, *SAP<sup>-/-</sup>* animals are resistant to pristane-induced lupus [59] or crossing *SAP<sup>-/-</sup>* mice with *B6.Sle1b* resulted in a decrease in penetrance of autoantibody production upon aging [146] and the SLE phenotype of MRL-*Fas<sup>lpr</sup>* is greatly reduced by a spontaneous SAP mutation [147]. Vinuesa and colleagues have shown recently that SAP null mutation also abrogates the lupus-like phenotype of Roquin<sup>san/san</sup> mice [148]. This finding provides more insight into the role of SAP in T cell-mediated humoral autoimmunity since their studies support the theory that autoimmune phenotype of Roquin<sup>san/san</sup> is mediated by Tfh cells. The authors demonstrated that aberrant expansion of Tfh cells and induction of humoral autoimmunity is T cell intrinsic in Roquin<sup>san/san</sup> mice and SAP deficiency effectively corrected these phenotypes along with the observed spontaneous GC formation. However, the connection between SAP signaling and lupus is likely to be indirect because of the lack of germinal center formation caused by the absence of SAP affects autoantibody production.

Taken together, an increasing body of evidence shows that SAP and SLAM family members are involved in controlling humoral autoimmunity in several murine lupus models by T

lymphocytes, in particular CD4<sup>+</sup> Th cells. Studies of SAP-deficient mice point to a possible role of Tfh population and their role in mediating negative and positive selection of GC B cells. Furthermore, murine SLAM family receptors are distinguished among candidate genes of chromosome 1 lupus congenic models due to their highly polymorphic nature and their generic expression on lymphoid lineage. They have the potential to regulate T cell function in the level of thymic development and cytokine production in periphery. Because of their functional diversity and overlapping signaling, one can assume that their control over self-tolerance is not confined to only one SLAMF receptor.

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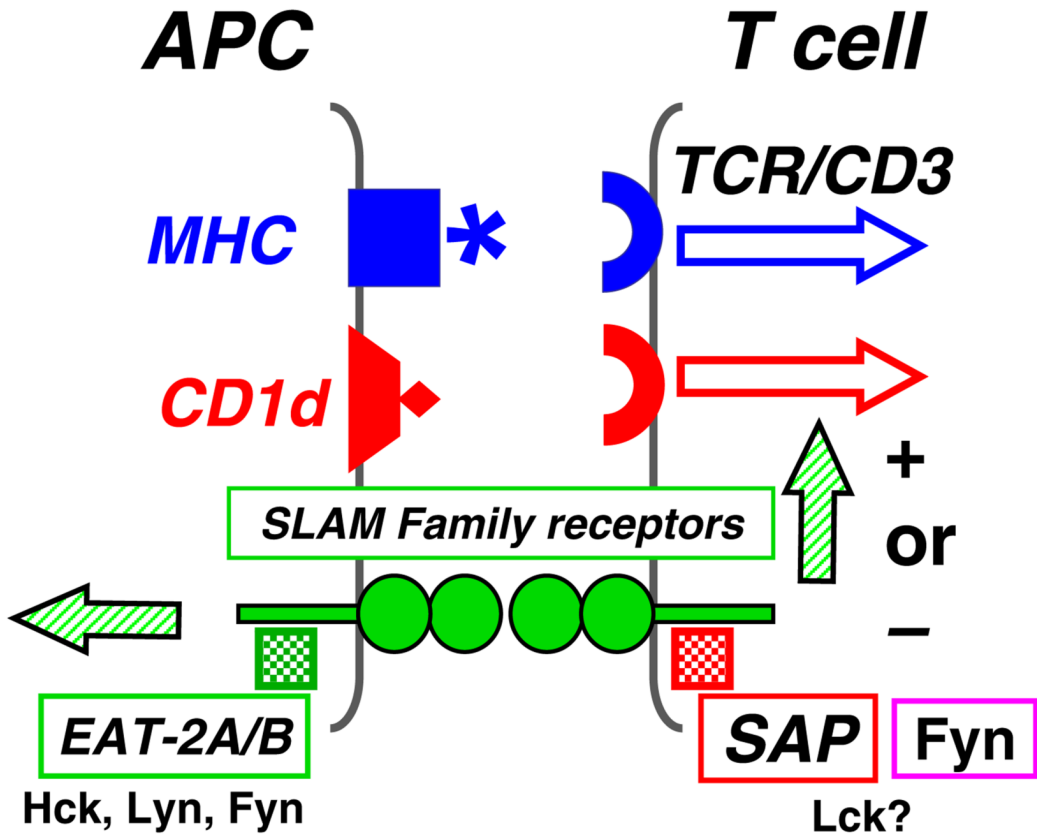


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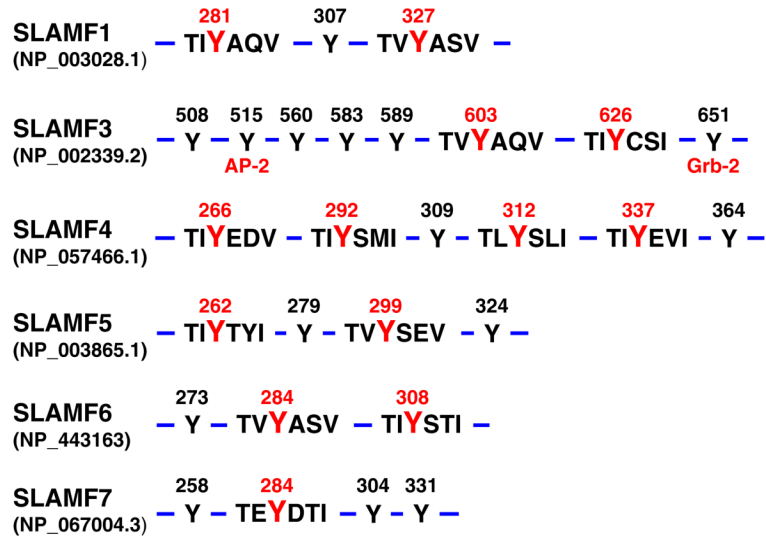
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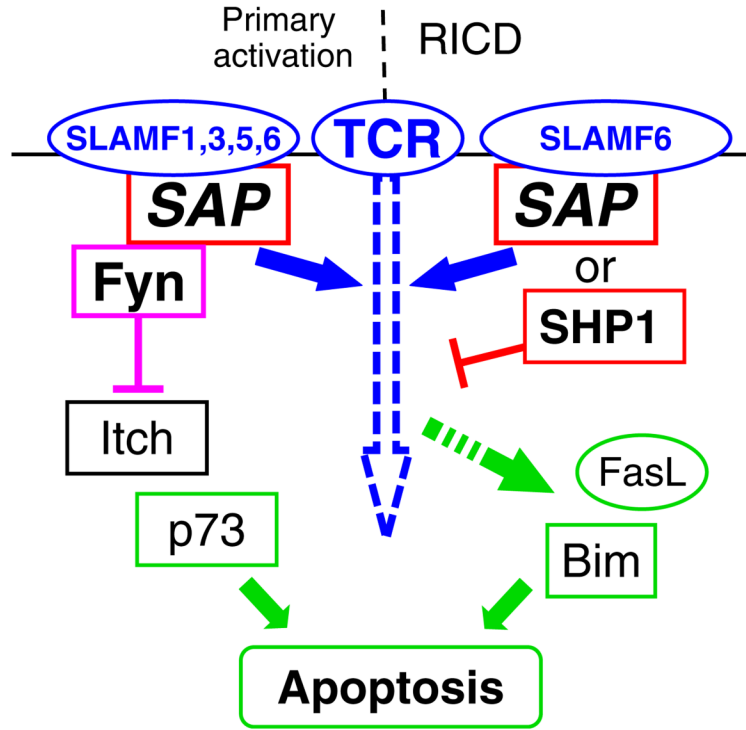
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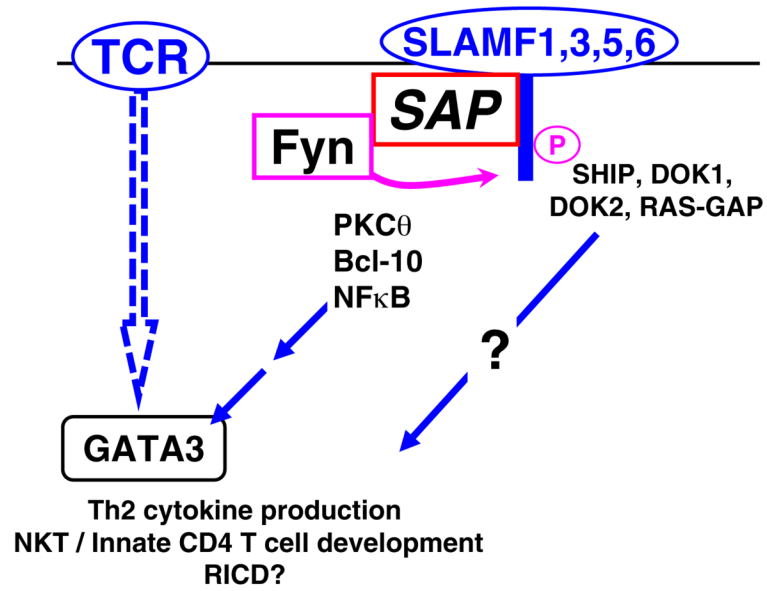
**Fig. 1.** SLAM family receptors in the immune synapse. The mostly homophilic interactions between SLAMF receptors subserve their role as costimulatory molecules. The binding of the SLAMF receptors to their ligands induces tyrosine phosphorylation of ITSM motifs in their cytoplasmic tail to which the adapters SAP or EAT-2 bind subsequently. SAP is mostly expressed by T cells, whereas EAT-2 is primarily expressed in APC



**Fig. 2.** Cytoplasmic tails of human SLAM receptors and the functional ITSM motifs and other docking sites. Cytoplasmic tails of human full-length SLAMF1 and SLAMF3-7 contain several ITSM motifs (tyrosine (Y) in red) which are docking sites for the SAP and EAT-2 adapters. Additional tyrosines in the cytoplasmic tail of the SLAMF molecules (Y in black) are involved in the association with other effector molecules, e.g., SLAMF3 binds to the adaptor molecules AP-2 and Grb-2. Numbers indicate the tyrosine position relative to the molecule's N-terminal amino acid accession numbers between brackets correspond to Ensembl ID



**Fig. 3.** A model depicting how apoptosis in CD8+ T cells is controlled by SAP in a Fyn-dependent and Fyn-independent manner. Signaling of SLAMF receptors through SAP binding and subsequent recruitment of Fyn fine-tunes the strength of the TCR signals and contributes in the p73-mediated way of apoptosis during primary T cell responses. In this model, activated Fyn could block the ubiquitin ligase Itch and therefore inhibit the degradation of p73. During the re-activation phase of T cells, SLAMF receptors, e.g., SLAMF6, might promote apoptosis by a SAP-dependent but Fyn-independent mechanism. In this model, SAP dislodges the phosphatase SHP1 from the cytoplasmic tail of SLAM molecules, which facilitates a stronger level of TCR signals sufficient to induce the transcription of RICD mediators such as FasL and Bim. *Rectangles*: intracellular signaling molecules, *ellipses*: membrane receptors or their secreted forms (FasL)



**Fig. 4.** Signal transduction by SLAM family surface receptors in CD4+ T cells. During formation of the immune synapse, clustering of SLAMF receptors brings SAP to its cytoplasmic tails and mediates recruitment and activation of Fyn. Thus, SLAMF receptors modulate TCR signaling by inducing a sustained recruitment of PKC $\theta$  and Bcl-10, which in turn leads to the activation of NF- $\kappa$ B and consequently to the production of Th2 cytokines and participation in NKT/innate CD4 T cell development. Phosphorylated tyrosines in the SLAMF cytoplasmic tails triggers the association with SHIP, docking protein 1 (DOK1), DOK2, and RAS-GAP, which may participate in these processes



**Table 1**

SLAM family receptors, their ligands, and binding partners

SLAM family	Synonym	Ligands	Binds SAP EAT-2	Effectors
SLAMF1	CD150, SLAM	SLAMF1, measles virus	+	Fyn, Lck, SHP1, Dok1, PKC $\theta$ , Akt
SLAMF2	CD48	SLAMF4, CD2, <i>Enterobac. FimH1</i>	-	Fyn, Lck
SLAMF3	CD229	SLAMF3	+	Fyn, Lck, ERK, AP2, Grb2
SLAMF4	CD244	SLAMF2	+	Fyn, Lck, LAT, PI3K, Vav1, SHP1, cCbl, ERK, p38, SHP1, SHP2
SLAMF5	CD84	SLAMF5	+	Fyn, Lck
SLAMF6	NTB-A (h), Ly108 (m)	SLAMF6	+	Fyn, Lck, PLC $\gamma$ , PI3K, SHP1, cCbl, Vav1
SLAMF7	CD319	SLAMF7	+	Fyn, Lck, PLC $\gamma$ , Vav1, PI3K
SLAMF8	BLAME	SLAMF8	-	?
SLAMF9	SF2001	SLAMF9?	-	?

**Table 2**

Disease manifestations of XLP patients and related phenotypes in SAP<sup>-/-</sup> mice

Disease manifestations in XLP patients	Observations in SAP <sup>-/-</sup> mice
Fatal infectious mononucleosis	
Increased numbers of EBV specific CD8+ T cells	Increased numbers of virus specific CTLs and IFN- $\gamma$ -producing CD8+ cells
Reduced AICD of CD8+ T cells	Decreased apoptosis in CD8+ T cells
Reduced SLAMF-dependent NK cell killing	Reduced SLAMF-dependent NK cell killing
Absence of NKT cells	Absence of NKT cells
B cell lymphomas	
Dys-gammaglobulinemia	Impaired primary and secondary antibody responses
Progressive loss of IgG levels and specific antibody responses	Defective Germinal Center formation due to dysfunctional CD4+ $\leftrightarrow$ B cell interactions