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# The role of SAP and SLAM family molecules in the humoral immune response

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**Effective B cell–mediated immunity, including the formation of germinal centers and the generation of high-affinity memory B cells and long-lived plasma cells, is dependent on CD4$^+$ T cells. Immunodeficiencies that present with defects in the antibody response have provided insights into the molecular mechanisms of B cell responses and the provision of T cell help. One such immunodeficiency is X-linked lymphoproliferative disease (XLP), which results from mutations in *SH2D1A*, the gene encoding SLAM-associated protein (SAP). Patients with XLP present with humoral defects characterized by hypogammaglobulinemia. We now know that SAP, through its signaling downstream of multiple members of the signaling lymphocytic activation molecule (SLAM) family of cell surface receptors, plays a crucial role in many aspects of this immune response. Here, we discuss the role of SAP in the generation of humoral immunity, particularly T cell–dependent antibody responses and the generation of germinal centers.**

**Keywords:** SAP; SLAM family; germinal center; T follicular helper cell; antibody

## Defects in SAP expression cause X-linked lymphoproliferative disease

Despite earlier reports of immunodeficiency associated with infectious mononucleosis,[1,2] Purtilo *et al.*[3] are generally credited for the first description of the rare immunodeficiency that is now referred to as X-linked lymphoproliferative disease (XLP). In 1975, they characterized an immunodeficiency in the Duncan kindred in which affected individuals displayed extreme susceptibility to Epstein-Barr virus (EBV) infection that was often associated with a clinical triad consisting of fulminant infectious mononucleosis, acquired hypogammaglobulinemia, and B cell lymphoma.[3] Subsequent studies of XLP patients have revealed that additional, but less prevalent, manifestations of this condition include aplastic anemia, necrotizing vasculitis, virus-associated hemophagocytosis, pulmonary lymphoid granulomatosis, psoriasis, and eosinophilia.[4,5] However, it is following infection with EBV that affected individuals exhibit uncontrolled polyclonal expansion of the B cell, T cell, and macrophage lineages, as well as subsequent mortality due to hepatic necrosis and/or bone marrow failure.[3,4]

This disease was only apparent in male individuals, with female family members spared; thus, an X-linked mode of inheritance was anticipated. Even with this hypothesis, it took another 23 years before the identification of the first XLP gene, *SH2D1A*, was mapped to a single locus at the q25 band of the X chromosome. *SH2D1A* encodes a 128 amino acid, 14-kDa molecule referred to as SLAM-associated protein (SAP).[6–8] Interestingly, SAP was found to be highly nonpolymorphic and extremely conserved between species.[8,9] Consequently, disruptions to the primary sequence are deleterious in that most missense mutations that result in single amino acid substitutions cause a loss of SAP expression.[10–12]

Because SAP consists almost entirely of a single src homology 2 (SH2) domain, its predicted role was as an adaptor protein in intracellular signaling cascades. In fact, as the name implies, one of the original studies that lead to the discovery of SAP found that

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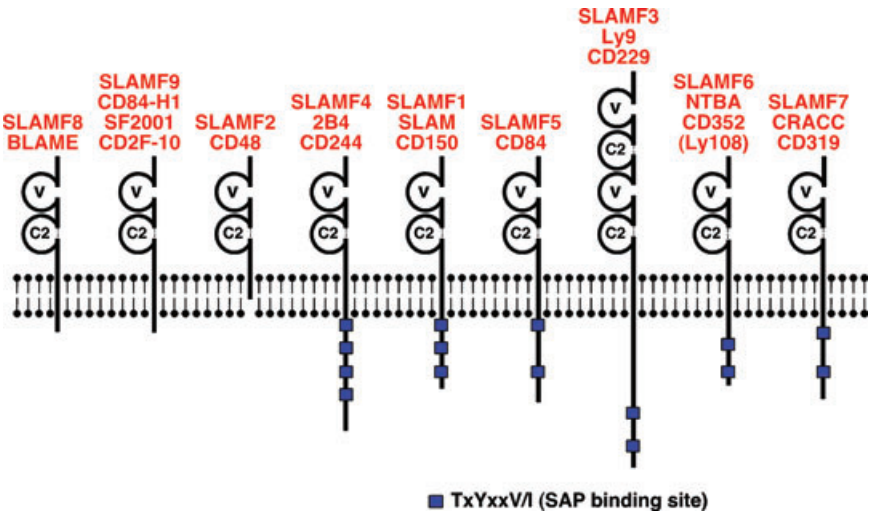


**Figure 1.** SLAM family of cell surface receptors. The SLAM family of cell surface receptors is composed of nine members. These surface glycoproteins are structurally characterized by an amino-terminal Ig variable (V)–like domain that lacks the typical disulfide bond, and by a carboxyl-terminal Ig constant-2 (C2) domain. CD48 lacks a cytoplasmic domain, while the cytoplasmic domain of BLAME and CD84-H1/SF2001/CD2F-10 do not contain any signaling motifs. The cytoplasmic domains of 2B4, SLAM, CD84, Ly9, NTBA, and CRACC contain one or more tyrosine-based motifs, TxYxxV/I (where x represents any amino acid), that act as docking sites for SAP and other SH2-domain containing proteins.

it associated with the cytoplasmic domain of the cell surface receptor SLAM (signaling lymphocyte activation molecule).[8] Subsequent studies revealed that SAP could bind to six CD2-like immunoglobulin (Ig) receptors, which now constitute the SLAM family of cell surface receptors(Fig. 1).[13–15] The expression of SAP is primarily confined to the immune system, specifically in T cells, NK cells, NKT cells, and eosinophils.[5–8,16,17] Although SAP is expressed in some transfected B cell lines and malignant B cells,[18,19] most studies have found that it is not present in normal primary B cells.[4,20,21]

## SAP deficiency leads to lymphocyte defects

Initially, lymphocyte development appeared to be independent of SAP, as mature T cell, B cell, and NK cell compartments could be observed in XLP patients.[22,23] However, more recent studies have shown a requirement for SAP expression in the development of NKT cells.[16,24,25] In addition, functional defects in NK[23,26,27] and $CD8^+$ T cells[26,28] have been reported in XLP patients. This breakdown in the cytotoxic arm of the immune response in the form of defects in NK cells and $CD8^+$ T cells, coupled with an NKT cell deficiency, are likely to be responsible for the underlying susceptibility to EBV infection exhibited by XLP patients.

With regard to the humoral immune response, SAP expression has been shown to be required for the generation of memory B cells[20,29,30] and the function of effector $CD4^+$ T cells.[29] This defect in memory B cells and effector $CD4^+$ T cells is likely to account for the underlying hypogammaglobulinemia in XLP patients. The focus of this review is the role of SAP in the formation of an intact humoral immune response in the context of $CD4^+$ T cells and germinal center (GC) formation.

## XLP2 due to defects in the expression of XIAP

Defects in *SH2D1A* account for the majority but not all cases of XLP. A second genetic lesion, *BIRC4*, which encodes X-linked inhibitor of apoptosis (XIAP), has recently been identified as a cause of a similar lymphoproliferative syndrome characterized by EBV susceptibility and hemophagocytosis.[31] Because *BIRC4* is also on the X-chromosome, this disease has been named XLP2. XIAP is a member of the inhibitor of apoptosis (IAP) family and is widely expressed in the immune system. XIAP functions as an antiapoptotic protein,[32] and has also been

**Table1. SLAM family of cell surface receptors**

| Receptor | SLAM family designation | Ligand | Expression |
|---|---|---|---|
| SLAM (CD150) | SLAMF1 | SLAM, measles receptor | Thymocytes, HSCs, B cells, T cells, $T_{FH}$ cells, mature DCs, platelets, macrophages |
| CD48 | SLAMF2 | 2B4 | B cells, T cells, NK cells, monocytes |
| Ly9 (CD229) | SLAMF3 | Ly9 | Thymocytes, B cells, T cells, $T_{FH}$ cells, NKT cells, NK cells, DCs, macrophages |
| 2B4 (CD244) | SLAMF4 | CD48 | $CD8^+$ T cells, γδ T cells, NK cells, macrophages, basophils, eosinophils, mast cells |
| CD84 | SLAMF5 | CD84 | Thymocytes, HSCs, B cells, T cells, $T_{FH}$ cells, NKT cells, mast cells, monocytes, macrophages, DCs, neutrophils, basophils, eosinophils, platelets |
| NTBA (Ly108 in mice; CD352) | SLAMF6 | NTBA in humans and Ly108 in mice | Thymocytes, B cells, T cells, $T_{FH}$ cells, NK cells, NKT cells, DCs, eosinophils, neutrophils |
| CRACC (CD319) | SLAMF7 | CRACC | B cells, T cells, NK cells, mature DCs, macrophages |
| BLAME | SLAMF8 | ? | Monocytes, DCs |
| CD84H1, SF2001, CD2F-10 | SLAMF9 | ? | B cells, T cells, monocytes, macrophages, DCs |

implicated in the Smad, NF-κB, and JNK signaling pathways.[33]

The clinical manifestations of XIAP-deficient XLP2 patients are similar to that of SAP-deficient XLP patients, in that both cohorts often present with EBV-associated hemophagocytic lymphohistiocytosis (HLH) and hypogammaglobulinemia.[4,31] However, key phenotypic differences reported to date of XIAP-deficient patients compared to SAP-deficient patients include the lack of lymphoma, the less severe or absent NKT cell defect,[31,34] the lower incidence of EBV-associated symptoms, and the higher prevalence of HLH.[6–8,31,35] Furthermore, the presentation of splenomegaly is diagnostic in XIAP-deficient patients, but it is not a common clinical manifestation in SAP-deficient patients.[31,35] From a functional perspective, NK cell-mediated cytotoxicity is defective in SAP-deficient XLP patients[23,26,27] although it is intact in the absence of XIAP.[31,35] Taken together, the evidence points to XLP due to an absence of SAP or the absence of XIAP as clinically distinct diseases. Indeed, XIAP deficiency has been recently proposed to be an X-linked familial HLH rather than an XLP.[35]

## SLAM family of cell surface receptors

The SLAM family consists of nine members (Fig. 1) and is structurally characterized by an amino-terminal Ig variable (V)–like domain that lacks the typical disulfide bond, and a carboxyl-terminal Ig constant-2 (C2) domain within the extracellular region of the receptors.[36] In general, the SLAM family members are homophilic receptors and can thus function as self-ligands.[37–42] The only known exception to this is 2B4, which interacts with CD48,[43] and is the only SLAM family member that exists as a glycosyl phosphotidyl inositol-linked membrane protein without a cytoplasmic domain.[44] However, the lesser-characterized members of the SLAM family members, BLAME (SLAMF8) and CD84-H1/SF2001/CD2F-10 (SLAMF9), have short cytoplasmic tails that lack signaling motifs, and are thus unlikely to mediate downstream signaling events.[45–48]

SAP can interact with six of the nine SLAM-family cell surface receptors, namely SLAM, Ly9, 2B4, CD84, natural killer, T, B cell antigen (NTBA in humans and Ly108 in mice), and CD2-like receptor activating cytotoxic cells (CRACC; Table 1; Fig. 1).[4]

The interactions between SAP and the SLAM family members are facilitated by a conserved arginine at position 32 (R32) within the SH2 domain of SAP and the tyrosine-based motif, TxYxxV/I (where x represents any amino acid) in the cytoplasmic domain of SLAM family members.[4,8] This tyrosine-based motif is often referred to as an immunoreceptor tyrosine-based switch motif (ITSM), owing to its ability to regulate downstream signaling by differential binding of SH2 domain-containing proteins such as SAP and the related molecule EAT2.[14,18,49] Although SAP can constitutively bind to SLAM, its interaction with other SLAM family members requires phosphorylation of the ITSM present in the cytoplasmic tail.[8,49–51]

A considerable amount of work has contributed to our understanding of how SAP mediates downstream signaling of the SLAM receptor family. The best characterized is that of SLAM-mediated signaling. Following the association of SAP with the ITSM in the cytoplasmic domain of SLAM, SAP selectively recruits the protein tyrosine kinase Fyn. The associations between the SH2 domain of SAP, the ITSM of SLAM receptor family members, and the SH3 domain of Fyn are facilitated via residues R32 and R78 in SAP, respectively.[11,51–53] The recruitment of Fyn enables phosphorylation of tyrosine residues in the cytoplasmic domain of SLAM, which in turn acquire the potential to act as docking sites for several proteins, thereby initiating different downstream signaling cascades. One such pathway consists of SH2 domain-containing inositol phosphate (SHIP-1), Dok1, Dok2, Shc, and Ras GTPase-activating protein, which can lead to suppression of IFNγ production.[51] Another pathway directly associated with T cell receptor signaling involves protein kinase C-theta (PKC-θ), Bcl-10, IκB, and NF-κB. The SLAM-mediated downstream effects of this pathway include increased T cell activation and Th2 (IL-4) cytokine production.[54] However, the ability of SAP to recruit Fyn to the cytoplasmic domain of SLAM is not unique. This sequence of events, following receptor ligation, has been also observed for 2B4[55] and Ly108.[56] In both situations, engagement of 2B4 and Ly108 results in SAP-mediated recruitment of Fyn and subsequent receptor phosphorylation, followed by the activation of downstream mediators such as Vav-1 and, to a lesser extent, c-Cbl.[55,56] The outcome of these events in NK cells can be cytotoxicity and cytokine (IFNγ) production.[55] This signaling downstream of Ly108 may play a role in enhancing T cell responsiveness.[56]

Despite the discovery that the clinical presentation of XLP resulted from mutations in *SH2D1A*,[6–8] the mechanisms by which loss of SAP expression and/or function results in an immunodeficiency was not immediately apparent. The opacity of these mechanisms was compounded by the observation that both SAP and the SLAM family members are widely expressed in the immune system. However, the contribution of numerous research group over the last decade has significantly improved our understanding of the function of SAP and SLAM receptors in the immune system. One example of these contributions is the initial proposal that SAP may be involved in the development of NKT cells, which was based on the observation that defects in Fyn, PKC-θ, Bcl-10, NF-κB, all downstream effectors of the SAP-SLAM signaling pathway, ablated NKT cell development.[57–60] Consistent with this, expression of SAP was found to be required for the development of NKT cells in both humans and mice,[16,24,25] thereby providing a molecular link between NKT cell development and SLAM/SAP signaling. However, mice that were rendered deficient for SLAM, Ly9, or Ly108 (NTBA in humans) had normal or only mildly decreased NKT cell numbers.[61,62] These observations suggested there is substantial redundancy in the functioning of the SLAM receptor family members that are involved in the generation of NKT cells. Consistent with this, it was subsequently shown that both SLAM and Ly108 contributed to the development of the NKT cell lineage.[62] A similar approach has also been applied to understanding the molecular basis of the hypogammaglobulinemia in XLP. The remainder of this review will focus on what is now known about the role of SAP and the SLAM receptor family in the generation of an intact humoral immune response in the context of GC formation.

## Antibody responses in the absence of SAP

The finding of hypogammaglobulinemia in many XLP patients suggests a central role for SAP in the generation of antibody (Ab) responses. Importantly, reduced levels of serum Ab are observed in both EBV positive and negative patients, indicating that dysregulation of Ab responses is not secondary to EBV infection.[63,64] The humoral defects in XLP patients

were subsequently examined more closely, revealing that these patients also had severely reduced numbers of memory B cells.[20,29,30] Furthermore, the few memory cells that were generated had not undergone class switching and still expressed IgM.[20,30] Immunohistology of spleens from XLP patients also demonstrated a lack of GCs.[20]

To further study the mechanisms of defective immune function in XLP, several groups generated SAP-deficient mice, which generate normal responses to T independent (TI) antigens (Ag) such as TNP-Ficoll and TNP-LPS.[65,66] However, when SAP-deficient mice were immunized with T dependent (TD) Ag, Ab responses were greatly decreased.[65–67] These defects were associated with decreased B cell proliferation, which could be observed as early as four days after immunization,[66,67] as well as an absence of GCs and decreased generation of memory B cells and bone marrow plasma cells.[65–68] Thus, SAP-deficient mice recapitulate the humoral defects observed in XLP patients, while at the same time allowing greater dissection of the processes involved.

SAP-deficient mice also displayed decreased Ab responses to viral infections, such as lymphocyte choriomeningitis virus, influenza virus, and murine gammaherpes virus-68.[19,67–70] Interestingly, in some of these studies, the early Ab response (IgM) was relatively normal, probably reflecting an intact TI component of the Ab response.[67,68]

## SAP in humoral immunity

Although the data show that SAP plays an essential role in the generation of TD Ab responses, it was not immediately clear which cell type required SAP expression. Much work has been carried out on this topic, however, controversy still exists on whether SAP plays any cell-intrinsic role in B cells. Thus, while some papers detected SAP in B cells,[18,19,71] others were unable to confirm these observations.[20,21,72] There are several possible explanations for these discrepancies, including differences in the method and sensitivity of detection, variation between different mouse strains and humans, and the possibility that SAP is upregulated in B cells only following very specific activation conditions.

Consistent with the expression of SAP in B cells, some studies have also showed B cell-intrinsic defects in Ab production.[19,71] However, the majority of studies concur that SAP-deficient B cells respond normally to stimulation both *in vitro* and *in vivo*.[30,66–68,72] For example, B cells from XLP patients showed no intrinsic defect in their response to TD stimuli such as CD40L and IL-4 with respect to proliferation and Ig isotype switching.[30] Similarly, multiple *in vivo* mouse studies using adoptive transfer of SAP-deficient B cells[66–68] or conditional deletion of SAP in different lymphocyte populations[72] demonstrated no requirement for SAP in B cells. Furthermore, the absence of SAP in $CD4^+$ T cells is sufficient to ablate the TD Ab response.[66–68,72,73] Thus, although the possibility remains that under some rare conditions SAP may play a role in B cells, the primary role of SAP during TD Ab responses occurs in $CD4^+$ T cells to enable them to provide help to B cells.

## T–B cell interactions in Ab response development

Effective long-lived humoral immune responses are dependent on the help provided by $CD4^+$ T cells.[74] This T cell help supports the development of GCs, which in turn allow for affinity maturation and the generation of high affinity memory B cells and long-lived plasma cells. It is clear, however, that T cell help is required before GCs are generated. Following an encounter with their cognate Ag, B cells become activated, internalize the Ag, and present it on their surface in the context of MHC class II complexes and migrate toward the T cell zone (Fig. 2). Similarly, naive $CD4^+$ T cells become activated following an encounter with Ag presented on dendrite cells (DCs) within the T cell zone and move toward the B cell follicle. This movement brings activated T and B cells within proximity of each other, consequently allowing the T cells to interact with B cells presenting cognate Ag at the border of the T cell zone and B cell follicle.[75–77] Following activation, B cells either develop into short-lived plasmablasts or enter a GC where they can undergo somatic hypermutation, affinity maturation, and develop into effector B cells in the form of memory cells or long-lived plasma cells (Fig. 2). Which of these fates B cells follows is determined by factors such as the affinity of the BCR for Ag and costimulatory signals provided by T cells.[76,78,79] The requirement for T cell help, even at this early phase, is demonstrated by the lack of B cell expansion and reduced plasmablast formation in the absence of effective T cell help.[75,80,81]

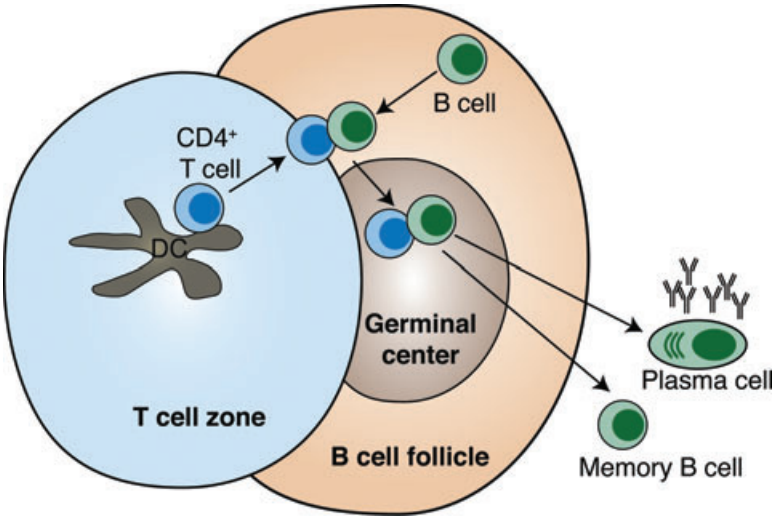


**Figure 2.** Orchestration of TD B cell responses within the spleen. Naive $CD4^+$ T cells that recognize Ag presented by DCs become activated and upregulate CXCR5 and CCR7, allowing them to migrate to the B cell follicle. Correspondingly, B cells bind and internalize Ag, become activated, and migrate to the T–B cell border. Here, the internalized Ag is presented by B cells in the context of MHC class II to activated $CD4^+$ T cells. Sustained T–B cell interactions are required at this point to enable B cells to receive the signals required to support proliferation, differentiation, and GC formation. Within the GC, interactions between $T_{FH}$ cells and B cells are required to maintain the GC response, allowing affinity maturation to occur and the generation of memory B cells and long-lived plasma cells. In the absence of SAP, B cells do not receive sufficient help at the T–B cell border, and the B cell response fails.

T cell help is provided by a specialized subset of $CD4^+$ T cells termed T follicular helper ($T_{FH}$) cells.[82,83] $T_{FH}$ cells are identified by expression of high levels of CXCR5 and PD1, as well as by their localization within the follicle and GC.[84] This upregulation of CXCR5, combined with downregulation of CCR7, is required for the migration of activated $CD4^+$ T cells from the T cell zone into the B cell follicle.[85–87] $T_{FH}$ cells also express costimulatory molecules such as CD40L, ICOS, and OX40 and the cytokines IL-10, IL-21, and IL-4, which enable these cells to promote B cell responses (Fig. 3).[84,88,89] The regulation of $T_{FH}$ cell fate is controlled by expression of the transcription factor Bcl-6[90–92] in a similar manner to the control of other T helper lineages, e.g., Th1, Th2, Th17, and $T_{reg}$ by transcription factors T-bet, GATA3, RORγt, and FoxP3, respectively.[93]

## Mechanisms underlying defective help to B cells

Given the severe block in TD Ab responses in the absence of SAP, there has been much interest in determining the underlying molecular mechanisms that contribute to the breakdown in provision of T cell help. Initial studies on $CD4^+$ T cells from XLP patients and SAP-deficient mice did not identify any major defect in activation. Studies then turned to examining more closely the effect of SAP-deficiency on expression of the surface molecules and cytokines that have known roles in the provision of T cell help to B cells.

As noted above, $T_{FH}$ cells express high levels of CD40L, which binds CD40 expressed on B cells. CD40L is a potent inducer of B cell proliferation and differentiation, and disruption of CD40–CD40L interactions ablates TD Ab responses, including GC formation and memory responses.[80,94,95] This phenotype closely resembles what is seen in SAP-deficient mice; however, studies revealed that CD40L expression was normal, or even enhanced, on SAP-deficient $CD4^+$ T cells following activation.[66,68,96]

Similarly, disruption of ICOS–ICOSL interactions, both in mice and humans, inhibits the generation of GC and TD B cell responses.[97–101] Several studies reported that $CD4^+$ T cells isolated from the blood of XLP patients[30] or SAP-deficient mice[66,102] had defective ICOS upregulation. However, other studies did not report

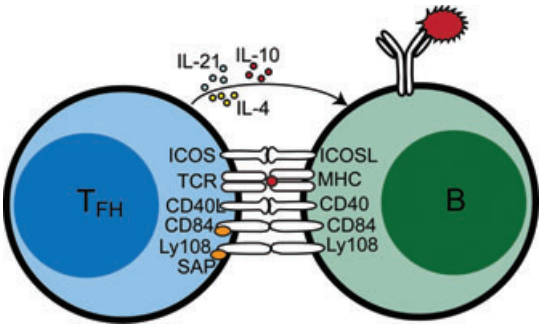


**Figure 3.** Molecules involved in T–B cell collaboration. Multiple cell surface receptors and soluble factors, such as cytokines, are involved in the cross talk between $T_{FH}$ cells and B cells. B cells present Ag they have acquired via their BCR on MHC class II molecules, which stimulates T cells through the TCR. B cells also express ICOSL, which interacts with ICOS expressed on the surface of activated $CD4^+$ T cells, providing costimulatory signals to the T cell. In turn, the $T_{FH}$ cells express high levels of CD40L, which is critical for stimulating B cells through surface CD40 and thus inducing their activation and differentiation. In addition, $T_{FH}$ cells secrete cytokines such as IL-4, IL-10, and IL-21, which support proliferation and differentiation of B cells. CD84 and Ly108 (NTBA) contribute to this interaction by signaling through SAP to allow stable, long-lived T–B cell conjugate formation, thereby facilitating the delivery of all the other signals.

a defect in ICOS upregulation[96,103–105] even under conditions where B cell responses were severely diminished. Thus, although SAP may play a role in ICOS upregulation under some conditions this cannot explain the severe defect in TD B cell responses. Regulation of other cell surface markers associated with $CD4^+$ T cell help to B cells such as CCR7 downregulation[104] and OX40 upregulation[66,105] also appeared normal in the absence of SAP.

Although examination of the cell surface molecules associated with T cell help have not been identified, major defects in the absence of SAP expression and some alterations in cytokine production have been observed. Ma *et al.* showed that $CD4^+$ T cells isolated from the blood of XLP patients produced less IL-10 than $CD4^+$ T cells from healthy donors.[30] IL-10 is able to induce proliferation and differentiation of human B cells,[106] and *in vitro* XLP $CD4^+$ T cells were less proficient at providing help to B cells than $CD4^+$ T cells from healthy donors.[30] However, whether this decrease in IL-10 plays a role *in vivo* during TD Ab responses remains unclear because IL-10 has little effect on mouse B cells, making it difficult to investigate this *in vivo*.[107]

$CD4^+$ T cells from SAP-deficient mice exhibited decreased IL-4 production in response to anti-CD3 and anti-CD28 Ab stimulation, although they are able to make normal levels of IL-4 in response to Th2 polarizing conditions.[54,70,108,109] Interestingly, a mutant version of SAP unable to bind FynT could not rescue this defect in IL-4 production in response to TCR and costimulation, indicating that FynT signaling downstream of SAP is crucial for the induction of IL-4.[66,109] However, the Th2 defect cannot account for the defect in TD Ab responses because rescuing Th2 polarization did not restore GC formation or Ab responses.[66,96] The same decrease in IL-4 is not observed in cells from XLP patients, suggesting that the role of SAP in cytokine induction may differ between the two species.[30]

Other studies sought to determine whether decreased production of IL-21, which is another potent modulator of B cell differentiation,[110–112] could account for the lack of helper function in the absence of SAP. Although some studies reported an approximately threefold decrease in IL-21 produced from SAP-deficient $CD4^+$ T cells,[102,103] under other conditions IL-21 production was relatively normal.[104] Thus, SAP seems to play at most a minor role in controlling IL-21 production. Furthermore, while some studies show severe defects in B cell responses in the absence of IL-21 signaling,[113–115] others have observed much milder defects.[115–117] Work by Bessa *et al.* suggests that these discrepancies may be explained in part by the ability of TLR signals to compensate for the absence of IL-21.[115] Regardless, this shows that IL-21, unlike SAP, is not critical for the development of TD B cell responses. This in turn makes it unlikely that differences in IL-21 significantly contribute to the defect in help that results from the absence of SAP.

An important insight into the mechanism of defective $CD4^+$ T cell help in the absence of SAP was provided by Qi *et al.* Using two-photon imaging, they tracked the interaction of $CD4^+$ T cells with B cells and demonstrated that SAP-deficient $CD4^+$ T cells were unable to form stable and prolonged interactions with B cells.[105] In contrast, they found that SAP was not required for interaction between T cells and DCs.[105] This accounts for why the initial events of $CD4^+$ T cell activation—such as expansion, upregulation of ICOS and CD40L, and downregulation of CCR7—are intact in the absence of SAP. Moreover, it provided an explanation for why

SAP-deficient T cells are unable to deliver help to the B cells even though they express many of the molecules required to provide help. Importantly, the defect in T–B cell conjugate formation occurred early in the response when T and B cells were interacting at the T–B border, not just within the GC. This is consistent with the observed defect in B cell responses, which manifests during the initial phase of the response[66,67] prior to the generation of GCs.

Interestingly, it has also been shown that SAP-dependent signaling through FynT is not required for the helper function of $CD4^+$ T cells[66,73] or for T–B cell conjugate formation.[105] Therefore, it remains to be determined which signaling pathways are involved in mediating increased stability in T–B cell interactions downstream of SAP.

## Role of SAP in $T_{FH}$ cell development

The defect in T-dependent Ab responses observed in the absence of SAP suggests that there may be a requirement for SAP in the development of $T_{FH}$ cells. Examination of XLP patients revealed normal numbers of circulating $CD4^+CXCR5^+$ T cells, although it is unclear whether these reflect true $T_{FH}$ cells.[104] Several groups have also examined $T_{FH}$ cell development in SAP-deficient mice; however, the results have been somewhat contradictory, with some groups describing normal numbers of $T_{FH}$ cells[96,103,105] while other groups have observed a profound decrease.[102–104,118] Some of these inconsistencies may result from variation in the strategy used to identify $T_{FH}$ cells in the different studies; however, this cannot fully explain the discrepancies observed. Our recent work has clarified the role of SAP in $T_{FH}$ cell development and accounted for these inconsistencies by defining the role of Ag presentation by B cells in the generation of $T_{FH}$ cells.[104]

Previously, it had been thought that $T_{FH}$ cell formation was a two-step process that required initial Ag presentation by DCs followed by additional signals provided by B cells.[74] This model was supported by studies that found dramatically decreased $T_{FH}$ cell numbers in mice lacking B cells,[87,90,119] a scenario in which SAP-deficient T cells would have defective $T_{FH}$-cell generation because of their inability to undergo sustained interactions with B cells. Our results demonstrated that although B cell Ag presentation was important for the generation of $T_{FH}$ cells in many experimental systems this was not because B cells provided a unique signal that was required to drive $T_{FH}$ development[104]; rather it reflected Ag availability. Thus, when Ag was abundant and DCs continued to present Ag they were fully capable of driving $T_{FH}$ cell development in a B-cell independent manner.[104] This requirement for ongoing Ag presentation in the development of $T_{FH}$ cells is consistent with the phenotype of $T_{FH}$ cells (such as heightened expression of PD1, CXCR5, and IL-21, and downregulation of CD127) being associated with strong TCR activation[120,121] and the role for Ag affinity in $T_{FH}$ cell generation.[122] Thus, experimental systems where Ag presentation quickly becomes focused on B cells show a significant defect in $T_{FH}$ cell development in the absence of SAP, while those systems that allow for longer presentation by DCs display only a small reduction in $T_{FH}$ cell numbers. Such a scenario may also explain differences observed in IL-21 production and ICOS upregulation, as it would be expected that these would also be affected by changes in the level of Ag presentation. Indeed, our studies showed increased IL-21 production from both WT- and SAP-deficient $CD4^+$ T cells, following Ag boosting.[104]

Interestingly, a recent report suggested that SAP might be specifically required for the generation of a subpopulation of $T_{FH}$ cells that are $GL7^+$.[103] The authors suggested that this population corresponded specifically to $T_{FH}$ cells that were located in the GC rather than within the B cell follicle. Earlier studies by Qi *et al.* demonstrated that binding to cognate B cells, and thus SAP expression by $CD4^+$ T cells, promotes efficient recruitment and retention of $CD4^+$ T cells in the GC.[105] However, other studies have observed SAP-deficient T cells in the GC,[68,96,104] indicating that GC localization is not absolutely dependent on SAP. Further study will be required to determine whether $GL7^+$ $T_{FH}$ cells are absolutely dependent on SAP expression (and presumably B cell interactions) or whether like $T_{FH}$ cells they are dependent on prolonged Ag presentation. A first step in this process will be determining whether GC localized $T_{FH}$ cells observed in other experimental systems also express GL7.

It should be noted, however, that although $T_{FH}$ cells can be generated in the absence of SAP[104] they are still unable to provide effective help for Ab responses due to their inability to form stable

interactions with B cells to deliver the appropriate helper signals.[105]

## SLAM family members in T–B cell interactions

Although it has been established that SAP plays a critical role in the generation of TD Ab responses, it remains to be determined which of the SAP-associating SLAM family receptors mediate the effects of SAP. As discussed earlier, the SLAM family of receptors is expressed widely on many cells of the immune system including T and B cells (Table 1). Ly108, SLAM, and CD84 are upregulated on B cells following activation *in vitro* or on GC B cells.[73,103,118,123] Similarly, SLAM, Ly108, and CD84 are expressed more highly on activated $CD4^+$ T cells than on naive $CD4^+$ T cells.[73,89,103,104,109,118,123,124] Ly9 is expressed on both $CD4^+$ T cells and B cells regardless of activation state.[104,123,125] In contrast, neither $CD4^+$ T cells nor B cells express significant levels of 2B4.[123] These expression patterns suggest that interactions between one or more of Ly9, Ly108, SLAM, or CD84 expressed on $CD4^+$ T cells with the same molecules expressed on B cells are crucial for successful T–B collaboration and subsequent humoral immune responses.

Mutant mice lacking specific members of the SLAM family of receptors have been generated, allowing the role of these molecules in TD B cell responses to be investigated. Mice deficient in Ly9 showed normal generation of Ag-specific Ab and GCs in response to LCMV, indicating Ly9 interactions are not required for the provision of T cell help to B cells.[61] Similarly, mice deficient in SLAM show no defects in TD B cell responses.[73] However, SLAM-deficient $CD4^+$ T cells, such as SAP-deficient cells or cells displaying a mutant form of SAP that is unable to bind FynT, display a reduction in IL-4 production.[54,109] Thus, although SLAM-SAP-FynT signaling is not required for the provision of help to B cells, it plays a role in the induction of IL-4 in $CD4^+$ T cells.

Mice deficient in CD84 have recently been generated and, like SAP-deficient mice, display normal TI Ab responses but decreased TD B cell responses, including decreased Ag-specific IgG responses and GC formation.[118] CD84-deficient $CD4^+$ T cells also showed decreased adhesion to cognate B cells,[118] supporting a model whereby SAP acts downstream of CD84–CD84 interactions to mediate prolonged binding between T–B cells and the provision of effective help signals. The magnitude of the defects observed in the absence of CD84, however, is not as severe as those observed with SAP-deficient $CD4^+$ T cells, suggesting other SLAM family members may also contribute to effective T–B cell interactions. Consistent with this, disruption of Ly108 in addition to CD84 resulted in a further decrease in T–B cell adhesion such that levels were similar to that observed with SAP deficient T cells.[118]

The involvement of SLAM family molecules in supporting TD B cell responses is mirrored in their support of $T_{FH}$ cell formation. Thus, although CD84-deficient T cells show a defect in the generation of $T_{FH}$ cells under some conditions,[118] SLAM-deficient mice have normal numbers of $T_{FH}$ cells.[103] Again these findings are consistent with a role for CD84 (and possibly Ly108), but not SLAM or Ly9, in promoting adhesion between T–B cells, and thus allowing the ongoing Ag presentation to $CD4^+$ T cells required for $T_{FH}$ cell development.

## Concluding remarks

In the past decade, our understanding of the role of SAP in TD immune responses has increased dramatically. However, some questions remain unanswered. Although it has been demonstrated that SAP is important for stable conjugate formation between T and B cells, it is unknown how SAP, CD84, and Ly108 mediate this process. Furthermore, it is unclear whether this increased adhesion is the sole reason for the requirement of CD84–SAP interactions in TD B cell responses. Alternatively, SAP may initiate additional downstream signaling events that are important for the differentiation and delivery of $CD4^+$ T helper function to B cells. Further studies will shed light on these issues.

By using SAP deficiency, whether in the context of humans or mice, there have been significant insights into the understanding of the T–B cell interactions required for the generation of intact humoral immune responses. Although much work on the role of T cell help focuses on the GC response, SAP-deficiency clearly demonstrates the importance of the signals B cells receive from $CD4^+$ T cells prior to GC formation. Further work clarifying the $CD4^+$ T helper populations that operate at the different stages of B cell responses (i.e., T–B border, extrafollicular, and GC) will serve to increase our

understanding of humoral immunity and ability to manipulate these responses.

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## Conflicts of interest

The authors have no conflicts of interest.

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