Regulation of Cellular and Humoral Immune Responses by the SLAM and SAP Families of Molecules

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Abstract

SAP (SLAM-associated protein) was identified in 1998 as an adaptor molecule involved in the intracellular signaling pathways elicited through the cell surface receptor SLAM and as the protein defective in the human immunodeficiency X-linked lymphoproliferative disease (XLP). During the past eight years, it has been established that the SLAM family of cell surface receptors (SLAM, 2B4, NTB-A, Ly9, CD84) and the SAP family of adaptors (SAP, EAT-2, ERT) play critical roles in lymphocyte development, differentiation, and acquisition of effector functions. Studies of these proteins have shown unexpected roles in cytokine production by T cells and myeloid cells, T cell–dependent humoral immune responses, NK cell–mediated cytotoxicity, and NKT cell development. This review highlights recent findings that have improved our understanding of the roles of the SLAM and SAP families of molecules in immune regulation and discusses how perturbations in the signaling pathways involving these proteins can result in different disease states.

TD: T cell–dependent

SLAM: signaling lymphocytic activation molecule

SAP: SLAM-associated protein

XLP: X-linked lymphoproliferative disease

EBV: Epstein-Barr virus

INTRODUCTION

Lymphocyte activation is strictly regulated by signals delivered through a vast array of cell surface molecules, such as antigen (Ag) receptors, costimulatory molecules, cytokine and chemokine receptors, inhibitory receptors, and Toll-like receptors (TLR). Dysregulation of these signaling processes can cause catastrophic diseases of the immune system, such as autoimmunity and immunodeficiency. The study of human immunodeficiencies caused by single gene mutations, such as severe combined immunodeficiency (SCID), hyper IgM syndrome, agammaglobulinemia, and common variable immunodeficiency, has identified molecules required for B cell development (e.g., *Btk*, *BLNK*, *Igα*, *λ5*, *Rag-1/2*), T cell development (*CD3δ*, *CD3ε*, *CD45*, *ZAP70*, *IL-2γc*, *IL-7R*, *Jak3*, *Rag-1/2*), B cell activation (*CD19*), somatic hypermutation and class switch recombination (*AID*, *UNG*), and T cell–dependent (TD) B cell differentiation (*CD40*, *CD40L*, *ICOS*) (1–3).

Another immunodeficiency for which the genetic lesion has been identified is X-linked lymphoproliferative disease (XLP). XLP is caused by mutations in *SH2D1A* (4–6), which encodes SLAM-associated protein (SAP), an adaptor molecule involved in intracellular signaling elicited through several immune cell receptors belonging to the SLAM family (7–9). In contrast to a disease such as SCID, which is caused by mutations in *γc*, *IL-7R*, or *Jak3* (1–3), the identification of *SH2D1A* as the gene defective in XLP patients did not provide any obvious insights into the function of SAP and how mutations in this gene might contribute to an immunodeficiency. However, since its identification, our understanding of the critical role played by SAP in various aspects of TD immune responses, a central defect in XLP, has exponentially increased. Furthermore, it has become clear that SAP- and SLAM-related receptors have important functions in innate immunity and antiviral responses and the development of autoimmune conditions.

X-LINKED LYMPHOPROLIFERATIVE DISEASE

X-linked lymphoproliferative disease (XLP), or Duncan's disease, was described in 1975 by David Purtilo as an X-linked recessive immunodeficiency affecting 1 in 500,000 to 1 million Caucasian males (10). In over 90% of cases, Epstein-Barr virus (EBV), a member of the human γ-herpesvirus family, is believed to be the cause for the clinical presentation of XLP (11, 12). XLP patients do not display the same degree of vulnerability toward other members of the human herpesvirus family, specifically herpes simplex virus and cytomegalovirus, or viruses such as varicella zoster, which can cause life-threatening infections in individuals with other T cell immunodeficiencies (13, 14). This highlights the unique role of EBV in the pathogenesis of XLP patients. Interestingly, four cases of fatal fulminant infectious mononucleosis (FIM) in males from a single family were initially reported in 1974 (15). These cases were most likely the first description of what later became known as XLP.

EBV infects mature B cells via the complement receptor CD21 and MHC class II. Infected cells proliferate, and some undergo transformation. Following acute EBV infection, NK cells lyse some of the virus-infected cells. Subsequently, an Ag-specific MHC-restricted cytotoxic $CD8^+$ T cell response ensues, which limits the proliferation of EBV-infected B cells (16). In the general population, EBV is a relatively innocuous virus, as demonstrated by the detection of EBV-specific antibodies (Abs) in often asymptomatic healthy individuals. Although latent virus-infected B cells persist for life, they are kept under control by EBV-specific $CD8^+$ T cells and other cytotoxic lymphocytes.

XLP patients are typically asymptomatic prior to EBV exposure, but, in the setting of acute infection, they mount a dysregulated immune response, resulting in the uncontrolled polyclonal expansion of B cells, T cells,

macrophages, and monocytes, causing severe splenomegaly (17, 18). Activated cells disseminate, causing extensive tissue damage by producing inflammatory cytokines. Patients eventually succumb to hepatic necrosis and/or bone marrow failure (7, 8, 13, 17–19). Up to 60% of XLP patients develop FIM, and the majority of them die within 1–2 months of infection. Patients surviving primary EBV infection commonly develop hypogammaglobulinemia (~35%), malignant lymphoma, and/or lymphoproliferation (~30%). Other manifestations, although less common, include aplastic anemia, necrotizing vasculitis, and nephritis. The mean age of manifestation is less than 5 years, with the mortality rate close to 100% by age 20 (7, 8, 13, 18–20).

Several cases of XLP have been documented in patients presenting with lymphoproliferation, B cell lymphoma, and/or hypogammaglobulinemia in the absence of EBV infection (5, 17, 21–24). Indeed, up to 40% of XLP patients do not develop FIM despite exposure to EBV (23). Thus, although EBV initiates disease progression, it is not the sole cause of disease in XLP. This suggests that the gene defective in XLP is involved not only in the immune response against EBV, but also immune homeostasis in general.

Because EBV is an intracellular pathogen, functional $CD4^+$, $CD8^+$, and NK cell responses are crucial for the control of infection (16). The dysregulated anti-EBV immune response in XLP patients has been attributed to an inability of SAP-deficient lymphocytes to eliminate infected B cells (16, 25). It is speculated that EBV-infected B cells provide a constant stimulus for cytotoxic lymphocytes that results in their uncontrolled proliferation and cytokine production. Consistent with this hypothesis, XLP patients with FIM display elevated serum levels of IFN-γ (26), which may be produced by proliferating $CD8^+$ T cells and contribute to the tissue damage seen in patients with acute EBV infection. Furthermore, XLP patients who survive EBV infection do not mount appropriate humoral immune responses because anti-EBV Abs are either present at very low titers, or are absent, in these patients (21).

Defects in Lymphocyte Function in XLP

Since the recognition of XLP as a distinct disease entity, many studies have been performed in an attempt to identify defects in lymphocyte function that may underlie its pathogenesis. Early studies indicated that development of mature B cells, T cells, and NK cells was grossly normal in XLP patients (27, 28). In fact, most XLP patients had increased frequencies of NK cells (28), in parallel with the increased numbers of NK cells in normal individuals with acute EBV infection (29). This result indicated that, unlike immunodeficiencies such as SCID (1–3), lymphocyte development is relatively intact in XLP. However, NK cells from XLP patients have reduced cytotoxic function compared with normal donors (25, 28, 30). Similarly, the frequencies of EBV-specific $CD8^+$ T cells in XLP patients were reduced, and their ability to lyse EBV-infected autologous B cells was also impaired (25, 31) (**Figure 1**). Taken together, these studies showed defects in the cytotoxic arm of the immune system that were proposed to be responsible for the exquisite susceptibility of XLP patients to EBV infection.

XLP patients have reduced humoral responses to infection with EBV and are unable to produce a class-switched immune response following booster vaccinations (12, 15, 32, 33) (**Figure 1**). This reduced immune response is consistent with the general reductions in serum Ig levels (i.e., hypogammaglobulinemia) in XLP patients both before and after EBV infection (17, 21). In vitro assays of blood mononuclear cells showed reduced Ig production in response to B cell mitogens, suggesting immunoregulatory defects (27). However, serum from vaccinated XLP patients contains increased titers of affinity-matured Ag-specific IgM (34).

In recent years, lymphocyte development and differentiation in XLP have been

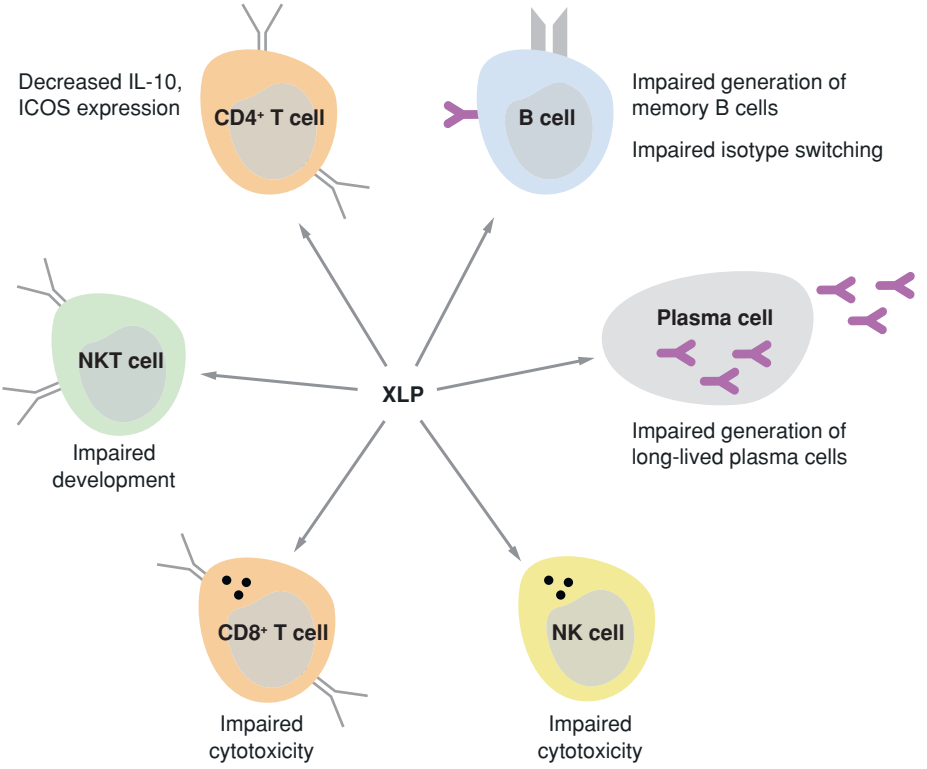


Figure 1

Cellular defects in XLP patients. XLP patients have been found to have impaired development of memory B cells, long-lived plasma cells, and NKT cells, as well as defects in the function of $CD4^+$ T cells (reduced production of IL-10 and expression of ICOS), $CD8^+$ T cells (cytotoxicity against EBV-transformed B cells), and NK cells (reduced cytotoxicity). The molecular mechanisms underlying these defects are incompletely characterized. Similar defects have also been reported in *sap*$^{-/-}$ mice.

reexamined, and the results not only confirm data obtained nearly two decades ago, but also allow for their refined interpretation. First, although B cell development is intact in XLP, there is a severe deficiency in the number of memory B cells in peripheral blood and lymphoid tissues (35–37) and an increase in circulating transitional B cells (38). Second, $CD4^+$ T cells are impaired in their ability to differentiate into effector cells that provide help to B cells, evidenced by reduced IL-10 production (35), reduced expression of inducible costimulator (ICOS) (35), and diminished frequencies of Th2-type memory cells (36). The deficit in $CD4^+$ effector cells and memory B cells, coupled with increased numbers of functionally immature B cells, could underlie the onset of hypogammaglobulinemia in XLP. This may also explain the low amounts of Ig secreted by XLP B cells in vitro compared with normal donors, because Ig production in such assays is dominated by memory, rather than naive, B cells (39). It is also possible that Ag-specific IgM observed following vaccination (34) was derived from the few IgM^+ memory B cells present in XLP patients (35, 37). Third, although T cells and NK cells develop normally in XLP patients, NKT cell development is abrogated (40) (**Figure 1**). This deficiency in NKT cells may have repercussions on several aspects of the immune system of XLP patients, such as impaired B cell function (41), susceptibility to EBV infection, and the development of lymphoma (42).

ICOS: inducible costimulator

IDENTIFICATION OF *SH2D1A*, THE MOLECULAR LESION IN XLP

In 1998, three groups independently identified the gene mutated in XLP (4–6). Coffey et al. (4) and Nichols et al. (5) both used positional cloning to isolate the gene within the XLP disease locus at Xq25. It was found to encode a 128 amino acid src homology 2 (SH2) domain–containing protein and was named SH2 domain protein 1A (*SH2D1A*) by the Coffey group and Duncan's SH2 domain protein (*DSHP*) by the Nichols group. In contrast, while investigating the signaling lymphocytic activation molecule (SLAM), Sayos et al. (6) identified a protein that constitutively bound its cytoplasmic domain, and thus they named it SLAM-associated protein (SAP).

Expression of SAP

SAP mRNA and/or protein expression have been detected in human thymocytes, T cells, NK cells, and, more recently, NKT cells, eosinophils, and platelets, but not in neutrophils, monocytes, or monocyte-derived dendritic cells (DCs) (4–6, 40, 43–49). It is controversial as to whether SAP is expressed in B cells. Some studies have detected SAP in a minor population of germinal center (GC) B cells (50, 51), whereas others failed to detect SAP in activated B cells (45) or in human B cells corresponding to distinct stages of differentiation, including GC B cells (37) (see **Table 1**). The disparity between these studies may be due to the differences in the methods used for SAP detection, i.e., immunofluorescence (50, 51) versus Western blotting (37, 45), respectively. Overall, the predominant cell types expressing SAP appear to be T cells, NK cells, and NKT cells, suggesting a prominent role for SAP in immune cells. SAP mRNA was recently found in neuronal cells during brain development (52), implying that SAP may also function outside the immune system.

Expression of SAP is modulated following lymphocyte activation. For instance, expression of SAP increases in human T cells following stimulation in vitro with anti-CD3 mAb or mitogens (5, 44, 45, 53) or in vivo during EBV infection (44, 54). In contrast, mouse T cells rapidly downregulate SAP expression following anti-CD3 stimulation (55). Thus, the temporal function of SAP in T cells may differ between murine and human T cells. NK cells also upregulate SAP in vivo following viral infection and in vitro in response to IL-2 stimulation (56).

SAP Functions as an Intracellular Adaptor Protein

Biochemical analyses indicate that SAP participates in signal transduction by mediating two complementary protein-protein interactions; i.e., SAP associates with SLAM and the protein tyrosine kinase Fyn.

Association of SAP with SLAM. A conserved arginine at position 32 (R32) within its SH2 domain allows SAP to bind to a tyrosine-based motif, TxYxxV/I (where x represents any amino acid), present in the cytoplasmic domain of SLAM (**Figure 2**) (6, 57). This motif is distinct from the immunoreceptor tyrosine-based activation motif (ITAM) and immunoreceptor tyrosine-based inhibitory motif (ITIM) in activating and inhibitory receptors, respectively (58). This motif has been termed an immunoreceptor tyrosine-based switch motif (ITSM) (59) because of its ability to modulate downstream signaling based on the differential binding of SAP or other SH2 domain–containing molecules. SAP, unlike other SH2 domain–containing proteins, interacts with SLAM independently of tyrosine phosphorylation of the SLAM ITSM (6). Thus, SAP is constitutively associated with SLAM (6).

Biochemical studies using transfected cells demonstrated that ITSMs in the cytoplasmic domain of SLAM could be phosphorylated by the kinases Fyn or Lck. These

SH2: src homology 2

ITSM: immunoreceptor tyrosine-based switch motif

Table 1 Expression and function of SLAM and SAP family of molecules in hemopoietic cells[a]

SLAM family molecule	Cellular distribution	Function
SLAM (CD150 IPO-3)	Thymocytes, naive B cells, memory T cells, in vitro activated T and B cells, mature DCs, platelets, HSCs	• IL-4 secretion by $CD4^+$ T cells • IL-12, TNF-α production by macrophages • MV receptor • Self-ligand
2B4 (CD244)	NK cells, γδ T cells, memory $CD8^+$ T cells, monocytes, basophils, eosinophils	• NK cell cytokine secretion, cytotoxicity • Immune synapse formation in $CD8^+$ T cells • May also signal through its ligand CD48
NTB-A (Ly108 SF2000)	NK cells, T cells, NKT cells, T_{FH} cells, B cells, eosinophils	• Human NK cell cytokine secretion, cytotoxicity • IL-4 secretion by $CD4^+$ T cells • Neutrophil function • Self-ligand
Ly9 (CD229)	Some thymocytes, T cells, T_{FH} cells, B cells, NKT cells, NK cells (dim)	• ? Negative regulator of TcR signaling • Self-ligand • Minimal phenotype of $ly9^{-/-}$ mice
CD84	Most thymocytes, HSCs, B cells, T cells, T_{FH} cells, NKT cells, mast cells, monocytes, macrophages, DCs, neutrophils, basophils, eosinophils, platelets	• ? T cell proliferation, cytokine secretion • Platelet spreading • Self-ligand
CRACC (CS1 mouse novel Ly9)	NK cells, $CD8^+$ T cells, some $CD4^+$ T cells, B cells, mature DCs	• NK cytotoxicity (? SAP-independent) • Self-ligand
SAP	T cells, NK cells, NKT cells, (? B cells), neuronal cells	• TD humoral immune responses (IL-4 secretion, GC formation) • Human NK cell activation • NKT cell development • Regulator of $CD8^+$ T cell activation
EAT-2	NK cells, $CD8^+$ T cells, B cells (?)	• Negative regulator of murine NK cells
ERT	NK cells (only in rodents, pseudogene in humans)	• Negative regulator of murine NK cells

[a]The expression data apply largely to human cells, with the exception of ERT, which is a pseudogene in humans. Analysis of expression of SLAM family receptors in mouse has not been as exhaustive as in human, and thus there may be some species-specific differences. For instance, in contrast to human NK cells, murine NK cells express CD84 but not NTB-A. The indicated functions of Ly9 and CD84 were derived from in vitro experiments using human T cells.

phosphorylated residues facilitated recruitment of SHP-2 (6, 56, 60–62), a protein tyrosine phosphatase that can either promote or inhibit immune cell signaling depending on the receptor involved (58, 63, 64). The SH2 domain of SAP competitively inhibited SHP-2 recruitment by binding the same ITSM in SLAM (6, 62). These studies concluded that because SAP is essentially a single SH2 domain, it could act as a natural inhibitor of interactions between SLAM and other SH2 domain–containing proteins. Thus, it was speculated that SAP could mediate positive signaling, and therefore lymphocyte activation, through SLAM by alleviating inhibitory signals induced by the recruitment of SHP-2 (6). Consistent with this speculation, overexpression of SAP in a human T cell line increased the costimulatory activity of anti-SLAM mAb (6). Based on these data, it was believed that loss of SAP function in XLP might allow SHP-2 to bind SLAM, which would result in the inhibition of SLAM-induced signal transduction and therefore in an ineffective T cell response toward EBV infection (6).

SHP: SH2 domain–containing protein tyrosine phosphatase

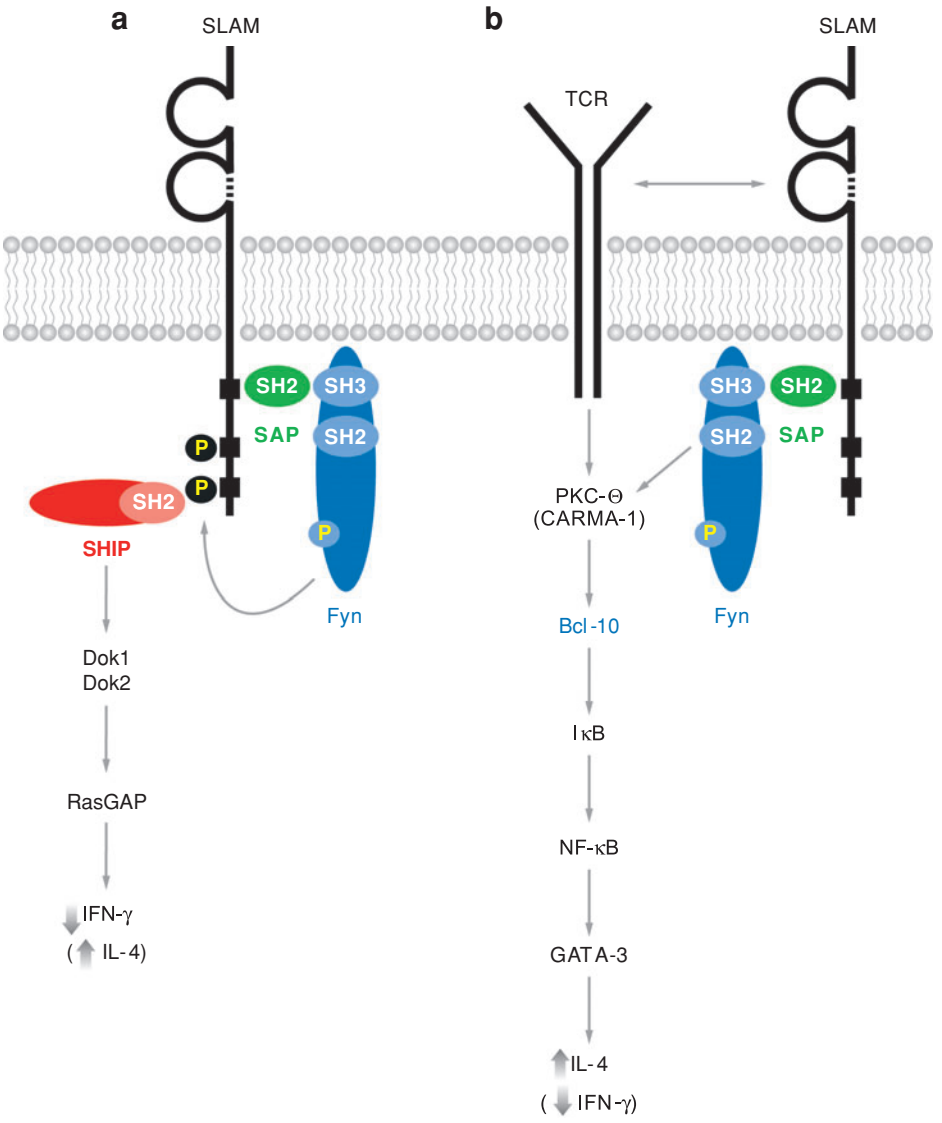


Figure 2

SAP-mediated signal transduction. (*a*) SAP associates with the ITSM in the cytoplasmic domain of SLAM. This interaction is mediated by tyrosine 281 (Y281) in SLAM and arginine 32 (R32) in SAP. Furthermore, arginine 78 (R78) of SAP binds the SH3 domain of Fyn and recruits Fyn to the SLAM/SAP complex. Fyn then phosphorylates tyrosine residues in the cytoplasmic domain of SLAM. These tyrosine-phosphorylated residues act as docking sites for SHIP (SH2 domain–containing inositol phosphatase), which becomes phosphorylated and binds the adaptor proteins Dok1 and Dok2. Tyrosine-phosphorylated Dok proteins bind the SH2 domain of RasGAP. This pathway suppresses production of IFN-γ. (*b*) SAP also contributes to signaling through the TCR by regulating activation of PKC-θ, Bcl-10, and NF-κB. The outcome of this pathway is increased expression of the transcription factor GATA-3 and optimal production of IL-4 by anti-CD3-stimulated $CD4^+$ T cells.

SHIP: SH2 domain–containing inositol phosphatase

PKC-θ: protein kinase C-theta

PIX: PAK-interacting exchange factor

Although this was an attractive hypothesis, there are two caveats. First, SHP-2 can function as a positive regulator of signal transduction pathways elicited through a variety of cell surface receptors, including the T cell receptor (TCR) (63, 64). Thus, it was also conceivable that the tissue destruction characteristic of XLP resulted from dysregulated activation of $CD8^+$ T cells through the inability of SAP to displace SHP-2 from SLAM. Second, and perhaps more significantly, other investigators have been unable to show that SHP-2 is recruited to SLAM in the absence of SAP. These latter data suggested that the altered responsiveness of lymphocytes from XLP patients results from the inability of SAP to couple SLAM to downstream signaling pathways (65).

Recruitment of FynT and activation of intracellular protein tyrosine phosphorylation. Several elegant studies (reviewed in Reference 9; see also 55, 65–68) have shown that SAP regulates intracellular signaling by functioning as an adaptor protein through its ability to bind the SH3 domain of Fyn (**Figure 2**). The residues in the SH2 domain of SAP responsible for binding Fyn (R78) are distinct from those involved in its association with SLAM (R32). Consequently, SAP can simultaneously associate with SLAM and Fyn to form a trimolecular complex (62, 66, 67).

When SLAM is cross-linked on T cells, it becomes tyrosine phosphorylated through a SAP- and Fyn-dependent mechanism (62, 65–67). The phosphorylated ITSM on SLAM provides docking sites for SH2 domain–containing inositol phosphatase (SHIP), which is subsequently phosphorylated and binds the adaptor proteins Dok1, Dok2, and Shc (65). Tyrosine-phosphorylated Dok2 proteins bind the SH2 domain of Ras GTPase-activating protein (RasGAP, **Figure 2*a***) (9, 65). The requirement for SAP and Fyn in eliciting signals downstream of SLAM was underscored by the finding that the recruitment of Fyn and tyrosine phosphorylation of SLAM and intracellular substrates, including SHIP, Dok1, and Dok2, were dramatically reduced in cells expressing SAP^{R78A}, a mutant of SAP that is unable to bind to Fyn (55, 66). Taken together, these data indicate that SAP regulates immune functions through SLAM by recruiting active Fyn. One aspect of the SLAM/SAP/Fyn interaction that was unclear was the mechanism by which SAP, which constitutively binds SLAM, mediates the recruitment and activation of Fyn in response to specific signals through SLAM. Recent data have been presented indicating that the association between SAP and Fyn is inducible, rather than constitutive, and is dependent on a conformational change induced in SAP that is bound to SLAM following engagement of SLAM (67a).

SAP also has important roles in intracellular signaling pathways elicited through the TCR, such as regulating the activation of protein kinase C-theta (PKC-θ) and its recruitment to the immune synapse, and activation of NF-κB (68). As a result, phosphorylation of Bcl-10, a substrate of PKC-θ, is reduced in activated $sap^{-/-}$ T cells (68) (**Figure 2*b***). Many of these defects were also evident in activated $fyn^{-/-}$ T cells (68), confirming the involvement of Fyn in a SAP-mediated pathway of T cell activation. SAP has recently been found to associate with the guanine nucleotide exchange factor PIX (PAK-interacting exchange factor) in T cells, and SAP/PIX complexes could be recruited to 2B4 (68a). Similar to Fyn, the SH3 domain of PIX interacted with SAP through R78 in the SH2 domain of SAP, suggesting that PIX and Fyn may compete with one another for binding to SAP (68a). Importantly, the SAP/PIX interaction appeared to contribute to T cell activation, at least in transfected cell lines (68a). Collectively, evidence suggests that SAP underpins signaling through SLAM and also contributes to TCR-mediated signaling. Defects in these processes would be compromised in XLP.

Effect of Mutations in *SH2D1A* on the Expression and Function of SAP

Since the identification of *SH2D1A* as the genetic lesion in XLP, a broad range of mutations has been identified in >200 patients. Many XLP patients have deletions in individual exons or the entire *SH2D1A* gene. On the other hand, mutations have been detected in exon/intron splice sites, while nonsense mutations causing premature stop codons, or missense mutations resulting in amino acid substitutions within conserved residues of the SH2 domain of SAP, have also been frequently detected (4–6, 8, 23, 69–71). Most missense mutations shorten the half-life of SAP protein and cause a dramatic reduction in its expression (70–72), most likely because of amino acid replacements that change the tertiary structure of SAP and affect protein folding (72). Missense mutations in *SH2D1A* also reduce the affinity of its interaction with SLAM, thus compromising the ability of these proteins to associate (61, 70–72). Some missense mutations do not prevent SAP binding to SLAM, but impair the ability of SLAM to recruit Fyn and activate downstream signaling cascades (71, 72). Collectively, therefore, missense mutations in *SH2D1A* are likely to impair SLAM-mediated signaling in lymphocytes from XLP patients by reducing the following: (*a*) the half-life and therefore expression of SAP, (*b*) the binding of SAP to SLAM, and/or (*c*) the ability to activate signal transduction downstream of the SLAM/SAP complex.

IMMUNE RESPONSES ARE IMPAIRED IN SAP-DEFICIENT MICE

To understand the role of SAP in normal immune responses, three groups independently generated $sap^{-/-}$ mice (73–75). Although $sap^{-/-}$ mice are viable and fertile and lymphocyte development is largely normal, several defects have been noted in the function of different populations of lymphocytes.

In Vivo Responses to Pathogens

Because mice are not susceptible to EBV infection (16), $sap^{-/-}$ mice have been challenged with pathogens such as lymphocytic choriomeningitis virus (LCMV) (73, 74) or murine gammaherpesvirus-68 (γHV-68) (75, 76). These mice have been used as models of infection in XLP because LCMV induces a brisk $CD8^+$ T cell response in mice similar to that of EBV in humans, and γHV-68, which is closely related to EBV, establishes lytic infection in the oropharynx and respiratory epithelium, followed by life-long latency in B cells (77, 78). Infection of mice with γHV-68 also leads to a lymphoproliferation similar to EBV (77, 78). However, LCMV does not infect B cells, and neither pathogen induces B cell transformation.

In a model of acute infection with LCMV, both wild-type (WT) and $sap^{-/-}$ mice cleared the pathogen within one week. However, $sap^{-/-}$ mice showed an increased expansion of Ag-specific $CD4^+$ and $CD8^+$ T cells (73, 74). The cytokine response was skewed toward a Th1 phenotype, as indicated by increases in the frequencies of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells, IL-2-producing $CD4^+$ T cells, and TNF-α-producing $CD8^+$ T cells (73, 74). $CD8^+$ T cells from $sap^{-/-}$ mice effectively eliminated viral load, and their cytotoxic T cell activity was intact. Although $sap^{-/-}$ mice survived acute LCMV infection, during chronic infection, these animals developed an overwhelming $CD8^+$ T cell–mediated response and died (73). When $sap^{-/-}$ mice were exposed to γHV-68, they resolved acute infection as efficiently as WT mice (75). However, the percentage of splenic $CD8^+$ T cells postinfection was higher in $sap^{-/-}$ mice than in WT mice (75, 76). Furthermore, there was more severe and prolonged T cell infiltration of the lungs and liver that was associated with increased tissue damage in γHV-68-infected

LCMV: lymphocytic choriomeningitis virus

TI: T cell–independent

$sap^{-/-}$ mice (75). Defects in cell-mediated immunity in $sap^{-/-}$ mice were not restricted to responses against viruses, as the mice also developed altered T cell responses when challenged with *Leishmania major* or *Toxoplasma gondii* (73, 74). These results indicate that $sap^{-/-}$ T cells become aberrantly activated and cannot appropriately control pathogen infection, thus exhibiting similar increased sensitivity to that displayed by XLP patients to EBV infection and tissue damage caused by activated T cells.

$sap^{-/-}$ $CD4^+$ T Cells Are Impaired in Their Ability to Produce IL-4

When the function of $CD4^+$ T cells from $sap^{-/-}$ mice was examined, the most striking finding was their decreased production of IL-4, IL-10, and IL-13 following in vitro activation (55, 68, 73, 74). Reduced production of Th2 cytokines was accompanied by a mild (i.e., ~20%) increase in IFN-γ production (55, 68, 79). When $sap^{-/-}$ $CD4^+$ T cells were activated in the presence of IL-4, secretion of IL-4, IL-10, and IL-13 was comparable to that of WT T cells (68, 73, 74), indicating that $sap^{-/-}$ T cells can differentiate to a Th2 phenotype, and defects in IL-10 and IL-13 production result from an intrinsic IL-4 deficiency.

Subsequent studies showed that expression of GATA-3, a master regulator of Th2 differentiation, was reduced in activated $sap^{-/-}$ $CD4^+$ T cells compared with WT $CD4^+$ T cells, and that restoration of GATA-3 expression increased IL-4 production by $sap^{-/-}$ $CD4^+$ T cells (55, 68). The involvement of Fyn in SAP-mediated activation of $CD4^+$ T cells was confirmed because $CD4^+$ T cells from both $fyn^{-/-}$ and SAP^{R78A} "knock-in" mice showed defective IL-4 production and GATA-3 activation in vitro (55). Taken together, these observations suggest that the SLAM-SAP-Fyn pathway is important for inducing Th2 cytokine production in response to activation through the TCR.

SAP Is Required for the Generation of T Cell–Dependent Humoral Immune Responses

$sap^{-/-}$ mice have normal amounts of serum IgM, IgG, and IgA prior to infection or immunization (73–75). In contrast, they are unable to produce normal amounts of Ag-specific IgG following infection with different pathogens (51, 73, 75, 79) or after immunization with a TD Ag (51, 80). Serum IgE levels remained low or undetectable both before and after antigenic challenge (55, 74, 75). Thus, similar to XLP patients, $sap^{-/-}$ mice developed hypogammaglobulinemia following infection.

$sap^{-/-}$ mice are unable to form GCs (51, 79–81). This is likely to contribute to the altered TD humoral immune responses. Consequently, there is a paucity of Ag-specific memory B cells and long-lived Ab-secreting plasma cells (PCs) following infection or immunization of $sap^{-/-}$ mice (73, 79). Although this finding is indisputable, there is disagreement as to whether the GC defect results from a requirement for expression of SAP in $CD4^+$ T cells, B cells, or both. Using a series of adoptive transfer experiments, long-term humoral immunity to LCMV could be achieved when $sap^{-/-}$ B cells plus WT T cells, but not WT B cells plus $sap^{-/-}$ T cells, were transferred into irradiated mice (79). This result led to the notion that SAP expression by $CD4^+$ T cells was required for them to provide sufficient "help" for B cell differentiation in vivo, and that B cells from $sap^{-/-}$ mice were intrinsically normal (79). This conclusion was further supported by several independent lines of investigation. First, T cell–independent (TI) B cell responses are intact in $sap^{-/-}$ mice, and serum levels of the TI isotype IgG3 were normal following infection or immunization (51, 75, 80, 81). Second, humoral immune responses following immunization with TD Ag or influenza infection, including the formation of GCs, could be restored to WT levels in immunodeficient mice by reconstituting them with WT $CD4^+$ T cells and $sap^{-/-}$ B cells,

but not *sap*$^{-/-}$ CD4^{+} T cells and WT B cells (80, 80a). Third, B cells from XLP patients respond as well as normal B cells with respect to proliferation and Ig secretion when activated in vitro with either TD or TI stimuli (35, 37). Fourth, some studies failed to detect expression of SAP in B cells (37, 45).

The hypothesis that defects in CD4^{+} T cells are responsible for impaired humoral immunity in *sap*$^{-/-}$ mice has been challenged by the finding that, using a different line of *sap*$^{-/-}$ mice, SAP-sufficient B cells were required for an intact Ag-specific Ab response (51). Similarly, GC formation was impaired in chimeric mice established with B cells from *sap*$^{-/-}$ mice and WT CD4^{+} T cells; in fact, in this study GCs did not develop in the presence of *sap*$^{-/-}$ B cells irrespective of the genotype of cotransferred CD4^{+} T cells (51). Although these results contrast with those from other studies (79, 80, 80a), the proposal that an intrinsic B cell defect contributes to humoral immunodeficiency in *sap*$^{-/-}$ mice is supported by the inability of B cells from these mice to undergo Ig class switch recombination and secrete normal amounts of Ig in vitro (82), and by the reported expression of SAP in some murine B cells (51, 82).

Molecular Defects Underlying Impaired Humoral Immunity in *sap*$^{-/-}$ Mice

Several mechanisms were recently proposed to explain the impaired humoral immune responses observed in *sap*$^{-/-}$ mice (80). Consistent with data reported for human SAP-deficient CD4^{+} T cells (8, 35), Cannons et al. (80) found that *sap*$^{-/-}$ CD4^{+} T cells exhibited reduced expression of the costimulatory molecule ICOS following Ag-specific activation. Mice and humans deficient in ICOS or its ligand have impaired formation of GCs and fail to develop class-switched Ig isotypes and memory B cells (reviewed in 8, 83). *sap*$^{-/-}$ mice also exhibited aberrant expression of CD40L, such that it was induced earlier and on a greater proportion of CD4^{+} T cells from *sap*$^{-/-}$ mice relative to WT mice (80). Although CD40L is critical for formation of GCs and TD immunity (reviewed in 83), sustained engagement of CD40 on Ag-specific B cells prevents the development of GCs and memory B cells, thereby curtailing humoral immune responses (84). Thus, the decrease in ICOS expression coupled with exaggerated expression of CD40L may work synergistically to abrogate TD immune responses in *sap*$^{-/-}$ mice.

The study by Cannons et al. (80) made additional intriguing observations that potentially excluded the contribution of other effector molecules to the impaired humoral responses in *sap*$^{-/-}$ mice. Because CD4^{+} T cells from these mice are unable to produce IL-4, IL-10, or IL-13 in vitro (55, 68, 73, 74), it was tested whether this defect had an effect in vivo. When a potent Th2 response was elicited in *sap*$^{-/-}$ mice by challenge with the helminth *Schistosoma mansoni*, such that their CD4^{+} T cells produced normal amounts of IL-4, IL-5, IL-10, and IL-13, serum levels of Ag-specific IgG remained dramatically reduced and GCs failed to form (80). This demonstrated that the defect in cytokine production by *sap*$^{-/-}$ CD4^{+} T cells could be uncoupled from their inability to provide "help" for B cell responses in vivo (80). Cannons et al. (80) further found that the humoral defect in *sap*$^{-/-}$ mice could be rescued by reexpression of either WT SAP or SAP^{R78A} in CD4^{+} T cells. Thus, although the association between SAP and Fyn is critical for IL-4 production by CD4^{+} T cells (55), a Fyn-independent pathway may be involved in the SAP-mediated regulation of TD B cell responses (80). This suggestion is supported by the observations that GCs can form in *fyn*$^{-/-}$ mice (80), and, although serum levels of IgE are undetectable in *sap*$^{-/-}$ mice (55, 73, 74, 80), IgE can be detected in SAP^{R78A} and *fyn*$^{-/-}$ mice, albeit at levels (~20%) lower than in WT mice (55). These findings add further complexity to the modus operandi of SAP in TD responses.

EAT-2: EWS-Fli1-activated transcript 2

SAP Is Required for the Development of Murine NKT cells

Because SAP can recruit Fyn to SLAM (65, 66), and development of NKT cells is reduced in *fyn*$^{-/-}$ mice (42, 85), the consequence of SAP-deficiency on NKT cell development was examined. *sap*$^{-/-}$ mice were devoid of NKT cells (40), mirroring the defect in development of these cells in XLP patients (8, 40). This was a surprising result because development of NK cells and T cells is intact in the absence of functional SAP (8, 27, 28, 40, 73, 74), and most genetic aberrations that abolish NKT cell development affect CD8^{+} T cells and/or NK cells (42). The exception, however, is Fyn (42, 85), which provides further evidence of a potential SAP/Fyn axis in NKT cell development.

The effect of an NKT cell deficiency on immune dysfunction in *sap*$^{-/-}$ mice and XLP patients is currently unknown. In some situations, NKT cells can prevent development of autoimmune diseases (42). However, there does not appear to be an increased incidence of autoimmunity in *sap*$^{-/-}$ mice. In fact, SAP deficiency can protect susceptible animals from the development of autoimmune diseases, such as experimental allergic encephalomyelitis (EAE) and murine lupus (81, 86). Occasionally, XLP patients exhibit signs of autoimmunity, such as psoriasis, colitis, and pulmonary cerebral vasculitis (K.E. Nichols, unpublished data). Therefore, the NKT cell deficiency could possibly enhance susceptibility to autoimmunity in some XLP patients. Because NKT cells may play a role in the establishment of humoral immunity and cytotoxic antitumor immune responses (41, 42), the absence of this lineage may contribute to hypogammaglobulinemia and lymphoma in XLP.

Comparisons Between XLP Patients and *sap*$^{-/-}$ Mice

Since the first descriptions of *sap*$^{-/-}$ mice, several studies have shown striking similarities between the phenotype and in vivo responses of these mice and XLP patients. For instance, XLP patients and *sap*$^{-/-}$ mice (*a*) lack NKT cells and memory B cells, (*b*) exhibit defects in cytokine production (mice: IL-4; humans: IL-10) and ICOS expression by CD4^{+} T cells, and (*c*) have CD8^{+} T cells that are hyperresponsive to pathogen challenge, resulting in cell-mediated tissue damage (see **Figure 1**). These studies have demonstrated the utility of *sap*$^{-/-}$ mice as a model to study the human disease. However, some important differences between *sap*$^{-/-}$ mice and XLP patients must be considered. First, EBV, the main trigger for the onset of XLP, does not infect or transform murine B cells (16). Second, *sap*$^{-/-}$ mice have not been reported to develop lymphoma (73–75), a common clinical manifestation of XLP. For these reasons, it is important to examine cellular and molecular responses in XLP patients in parallel with those in *sap*$^{-/-}$ mice.

EAT-2 AND ERT: NEW MEMBERS OF THE SAP FAMILY OF SH2 DOMAIN–CONTAINING PROTEINS

EAT-2 (*Sh2d1b*)

When SAP was identified, it was found to have greatest homology with EWS-Fli1-activated transcript 2 (EAT-2; *Sh2d1b*), a 132 amino acid protein also composed essentially of a single SH2 domain (87). Consequently, it was proposed that SAP and EAT-2 may have functional similarities (88). EAT-2 transcripts have been detected in murine NK cells, macrophages, and B cells (89, 90) and in human B cell lines (89) and activated T cells (91), whereas EAT-2 protein has only been detected in human NK cells and CD8^{+} T cells, but not in CD4^{+} T cells, B cells, or DCs (92) (**Table 1**). In contrast to SAP, the gene encoding human *EAT-2* is located on chromosome 1q22 (87).

Similar to SAP, EAT-2 can be recruited to the conserved ITSM in SLAM (66, 89). However, EAT-2 cannot bind the SH3

domain of Fyn because it lacks the arginine residue responsible for the association of SAP with Fyn (66). Thus, SAP and EAT-2 likely have divergent effects on intracellular signaling pathways. Indeed, NK cells from *eat-2/sh2d1b*$^{-/-}$ mice displayed increased cytotoxicity and IFN-γ production (90), suggesting that SAP and EAT-2 have opposing roles in lymphocyte activation, with EAT-2 acting as a negative regulator of NK cell function. The biochemical mechanism by which EAT-2 negatively regulates lymphocyte activation is unknown, but it appears to depend on the phosphorylation of two tyrosine residues in its C terminus (9, 90).

ERT (*Sh2d1c*)

ERT (EAT-2-related transducer) represents the third, and most recently identified, member of the SAP family of cytoplasmic adaptor proteins, exhibiting 82% amino acid identity to EAT-2 and ~40% identity to SAP (90, 93). The gene encoding ERT is located ~25 kB from *eat-2/sh2d1b* on murine chromosome 1 and has the same genomic organization as *eat-2/sh2d1b*, suggesting that these genes arose from gene duplication (90, 93). In contrast to EAT-2, expression of ERT is restricted to NK cells (90, 93) (**Table 1**). NK cells from *ert/sh2d1c*$^{-/-}$ mice display increased killing of target cells, indicating that ERT can also negatively regulate NK cell function (9, 90).

The molecular mechanism underlying the inhibitory effect of ERT is likely to be similar to that of EAT-2 because it also has the conserved tyrosines necessary for EAT-2's inhibitory function and lacks the arginine required to recruit Fyn (90, 93). EAT-2 and ERT can both bind to src tyrosine kinases. However, unlike SAP, which binds to the SH3 domain of Fyn, EAT-2 and ERT directly bind the catalytic domain of protein tyrosine kinases (93). This property of EAT-2 and ERT suggests that they couple SLAM to downstream signaling pathways via a mechanism distinct from that employed by SAP (90).

Although *ert* is a functional gene in rodents, human *ERT* is a pseudogene. Thus, the dual functions of ERT and EAT-2 in mice may be performed by EAT-2 in humans (90). Overall, it appears that SAP, EAT-2, and ERT are members of a new family of small SH2 domain–containing proteins that play important, and perhaps opposing, roles in regulating lymphocyte activation (9, 90, 93).

ERT: EAT-2 related transducer

CRACC: CD2-like receptor-activating cytotoxic cells

THE CD2/SLAM SUBSET OF THE Ig SUPERFAMILY OF CELL SURFACE RECEPTORS

SAP was identified by its ability to bind to the cytoplasmic domain of SLAM (6), which belongs to the CD2 subset of the Ig superfamily of cell surface receptors (57). These molecules are characterized by the presence of an N-terminal Ig variable (V)-like domain and a C-terminal Ig constant-2 (C2) domain within the extracellular portion of the receptor (94). Other members of this family include CD2, CD48, CD58 (reviewed in 88, 94), 2B4 (CD244) (95, 96), CD84 (97), Ly9 (CD229) (98, 99), NTB-A [also named Ly108 or SLAM family (SF) 2000] (100–102), CRACC (CD2-like receptor-activating cytotoxic cells, also named CS-1 or mouse novel Ly9) (103, 104), CD84-H1 (also named SF2001) (105), and BLAME (B lymphocyte activator macrophage expressed) (106) (**Figure 3**). The genes encoding these molecules are all located on human chromosome 1, with *CD2* and *CD58* at band 1p13, and *BLAME*, *CD84-H1*, *NTB-A*, *CD84*, *SLAM*, *CD48*, *CRACC*, *Ly9*, and *2B4* clustered on the long arm at 1q21–24 (7, 59, 88, 94). Murine *cd2* is on a syntenic region of mouse chromosome 3, whereas the genes encoding the other members are present on a syntenic region of mouse chromosome 1 [note that *cd58* does not exist in mice (88, 94, 107)]. Interestingly, the murine and human *EAT-2* genes are located near the *SLAM* locus on chromosome 1q22 (87).

One or more copies of the ITSM that recruits SAP and EAT-2 to SLAM are also in 2B4, CD84, Ly9, NTB-A, and CRACC

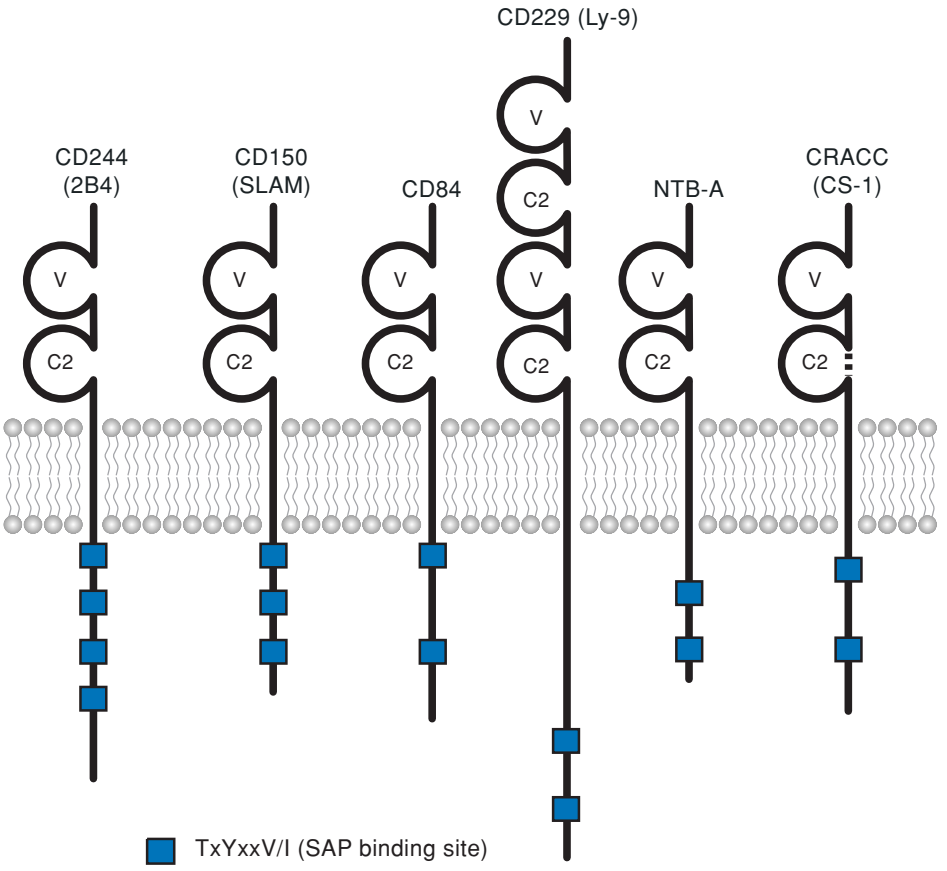


Figure 3

SLAM family of cell surface receptors. The SLAM family of surface receptors comprises six members. A typical receptor consists of an Ig V- and C2-like domain. The cytoplasmic domains of 2B4 (CD244), SLAM (CD150), CD84, Ly9 (CD229), and NTB-A contain ITSMs that mediate recruitment of SAP, as well as other SH2 domain–containing proteins such as EAT-2. Although CRACC (CS-1) also contains this motif, SAP does not associate with CRACC (103).

(**Figure 3**) (7–9, 59, 88), and SAP and EAT-2 also associate with their cytoplasmic domains (89, 91, 92, 96, 101, 104, 108, 109). This observation, together with the fact that these ITSM-containing receptors are genetically linked and presumably arose from an ancestral gene, has prompted them to be grouped into the SLAM family of receptors (**Figure 3**) (7, 59). Although SAP constitutively associates with SLAM (6), it can only bind to phosphorylated ITSMs of 2B4 (56, 61, 96, 110), CD84 (91, 108, 109), Ly9 (108), and NTB-A (101).

SLAM (CD150)

Expression. Human SLAM was first described in 1993 as IPO-3 (111) and cloned two years later by the lab of Jan de Vries (57). Expression of SLAM on human and murine leukocytes is similar, with it being detected on thymocytes, platelets, mature DCs, naive B cells, and memory T cells and with expression increasing after in vitro activation (46–48, 57, 60, 111–114). SLAM is not expressed on NK cells, monocytes, immature DCs, or granulocytes (see **Table 1**).

Functions on leukocytes. SLAM demonstrates homotypic binding and functions as a costimulatory molecule (112, 115). Initial reports found that anti-SLAM mAb enhanced proliferation and cytokine production by activated $CD4^+$ T cells (57, 60, 116) and cytotoxicity of $CD8^+$ T cells (113). A striking effect of anti-SLAM mAb was increasing IFN-γ production by human T cells and redirecting Th2 immune response to a Th0/Th1 phenotype (57, 116), suggesting an important role in regulating $CD4^+$ T cell differentiation. In contrast, homotypic SLAM interactions on murine thymoma cells reduced IFN-γ production in response to TCR engagement (65). This discrepancy in the reported role of SLAM in regulating IFN-γ production could be explained if the anti-SLAM mAb used in the initial studies acted as a blocking Ab, thereby masking, rather than providing, an activating signal. This hypothesis is supported by the findings that Fab fragments of anti-SLAM Ab were as efficient as intact divalent mAbs at inducing IFN-γ production by T cells (57, 116).

Proliferation and Ig production by anti-CD40 mAb-stimulated human B cells could also be augmented by SLAM-expressing transfectants (112). In contrast, using a similar culture system, the production of IL-6, IL-12, and TNF-γ by human DCs following stimulation by CD40L-expressing transfectants was abrogated when transfectants coexpressed SLAM (49). This study suggested that SLAM-SLAM interactions could modulate the behavior of DCs by suppressing secretion of inflammatory mediators.

Analysis of *slam*$^{-/-}$ mice reveals important roles in T cell activation and macrophage function. The generation of SLAM-deficient mice has helped clarify the role of SLAM in regulating cytokine production by $CD4^+$ T cells. IL-4 production by *slam*$^{-/-}$ $CD4^+$ T cells was reduced following in vitro stimulation with anti-CD3 mAb, whereas IFN-γ secretion was mildly increased (117), suggesting that SLAM is required for IL-4 production. This requirement of SLAM in IL-4 production by $CD4^+$ T cells is supported by the finding that *slam*$^{-/-}$ mice fail to exhibit features of an allergic response, namely eosinophilia, increased allergen-specific serum IgE, and increased airway response (118). Thus, SLAM is not only required for production of IL-4, but may also be critical for eliciting other effector mechanisms of an allergic response, which most likely involve production of cytokines such as IL-5 and IL-13. The reduced production of IL-4 by *slam*$^{-/-}$ $CD4^+$ T cells was similar to that of *sap*$^{-/-}$ T cells (55, 117), implying that impaired SAP-dependent signaling through SLAM is predominantly responsible for reduced IL-4 production in *sap*$^{-/-}$ mice.

Although SLAM deficiency had only a modest effect on IFN-γ production by $CD4^+$ T cells, it may still affect the generation of a Th1 response via signals delivered to Ag-presenting cells (APCs). This is supported by the observation that *slam*$^{-/-}$ macrophages were defective in producing IL-12 in response to LPS (117). Reduced IL-12 production by APCs in vivo could lead to decreased IFN-γ production by $CD4^+$ T cells. Indeed, C57/BL6 *slam*$^{-/-}$ mice exhibited heightened susceptibility to, and impaired immunity against, infection with *L. major* (117), which requires IL-12 production by APCs for the generation of a protective response (119). The enhanced susceptibility to *Leishmania* and defects in cytokine production by myeloid cells were not detected in *sap*$^{-/-}$ mice because SAP is not expressed in such cells. Thus, SLAM may function on macrophages independently of SAP, perhaps by using another adaptor protein such as EAT-2, which, in contrast to SAP, is expressed in murine macrophages (90).

The findings from *slam*$^{-/-}$ mice that implicate a requirement for SLAM-SLAM interactions for optimal production of cytokines by macrophages (117) appear to contradict data demonstrating that engagement of SLAM on human DCs inhibits production of a similar array of cytokines (49). However,

TLR: Toll-like receptor

HSC: hematopoietic stem cell

several possible explanations may account for these discrepancies. First, only macrophages from *slam*$^{-/-}$ mice have been studied (117). Thus, SLAM may have a different function on macrophages than on DCs. Second, engagement of SLAM on human DCs reduced cytokine production in response to CD40L (49), whereas cytokine production by *slam*$^{-/-}$ macrophages was impaired in response to LPS. Thus, SLAM may differentially modulate cytokine production depending on the stimulating agent, i.e., T cell help through CD40 or microbial stimuli through TLR ligands. Indeed, when human DCs were stimulated with LPS, costimulation through SLAM slightly improved production of IL-12 and TNF-α compared with stimulation with LPS alone (49). Collectively, the data suggest that SLAM regulates cytokine production by CD4$^+$ T cells, macrophages, and DCs.

SLAM is a cellular receptor for measles virus. CD46, which is on all nucleated cells, was initially identified as a receptor for most laboratory strains of measles virus (MV) (reviewed in 120, 121). However, several strains of MV could infect cells independently of CD46 (120, 121). This suggested the existence of an alternative receptor for MV and led to the identification of SLAM as the principal MV receptor (122).

Infection with MV causes profound immunosuppression (121, 123); however, the mechanism by which this is achieved is unknown. Immunosuppression may result from MV-induced apoptosis of DCs and T cells (124). Alternatively, MV infection may impair the function of APCs. One consequence of SLAM-mediated MV infection of DCs is the modulation of DC phenotype, such that expression of the costimulatory molecules CD40, CD80, CD86, and MHC class II is downregulated (125). Downregulation of these molecules would compromise the ability of DCs to stimulate MV-specific T cells (123) and may explain the impaired induction of adaptive immune responses following MV infection. MV-infected DCs also have decreased expression of SLAM (120, 121) and a reduced capacity to produce IL-12 (124). The reduced expression of SLAM on MV-infected human DCs, therefore, may directly contribute to their inability to produce IL-12, analogous to *slam*$^{-/-}$ murine macrophages, with a resulting diminution in a DC-mediated Th1 anti-MV immune response. Such a response would be consistent with the shift in production of Th1- (i.e., IFN-γ) to Th2- (i.e., IL-4) type cytokines during MV infection (126).

The contribution of SAP-dependent SLAM signaling in response to infection with MV is unknown. Because MV paralyzes the function of DCs (123, 124, 127), and these cells do not appear to express SAP, SAP may not be involved in this aspect of the immune response. However, some XLP patients do exhibit perturbed anti-MV immunity (17), suggesting that the function of SLAM on MV-infected T cells is compromised in the absence of SAP. The role of SAP in response to MV infection could potentially be addressed by crossing human SLAM transgenic mice (127), which have been used as a model to examine MV infection, with *sap*$^{-/-}$ mice.

Expression of SLAM identifies primitive hematopoietic stem cells. SLAM is expressed on hematopoietic stem cells (HSCs) present in bone marrow and fetal liver (128). Expression of the related molecules 2B4 and CD48 can also be used to resolve discrete stages of hematopoiesis. The most primitive HSCs are SLAM$^+$2B4$^-$CD48$^-$, whereas multipotent progenitors are SLAM$^-$2B4$^+$CD48$^-$, and progenitors that yielded more restricted populations are SLAM$^-$2B4$^+$CD48$^+$ (128). Thus, SLAM represents a novel surface marker whose expression can be exploited to identify, purify, and examine the repopulation potential of HSCs. This simplifies the study of hematopoiesis as it obviates the need to use complex combinations of differentially

expressed surface molecules to characterize distinct subsets of HSCs (129).

slam$^{-/-}$ mice have intact hematopoiesis, suggesting that SLAM is not required for the development or function of HSCs (128), consistent with the normal development of most hematopoietic lineages in XLP patients and *sap*$^{-/-}$ mice (8, 73, 74). SLAM may have a redundant function during hematopoiesis that is compensated by another molecule in *slam*$^{-/-}$ mice. Similarly, as expression of SAP has not been examined in HSCs, SLAM may function in a SAP-independent manner on these cells. Despite the uncertainty of how SLAM functions in hematopoiesis, the recognition that differential expression of SLAM family members identifies HSCs and other hematopoietic subsets represents a substantial advance in this field of study.

2B4 (CD244)

Expression. Murine 2B4, the first member of the SLAM family identified, was characterized by Vinay Kumar's group in 1993 (95, 130). A molecule on human lymphocytes recognized by the c1.7 mAb was also described that same year (131), but it was not recognized as the human homolog of 2B4 until 1999 (96). 2B4 is expressed on NK cells, γδ T cells, monocytes, basophils, eosinophils, and some thymocytes (48, 53, 114, 130–133) (see **Table 1**). Approximately 50% of human CD8$^+$ T cells are 2B4$^+$, and these CD8$^+$2B4$^+$ T cells lack expression of CD45RA, CD62L, CD28, and CCR7 and acquire expression of perforin, granzyme B, and IFN-γ, suggesting that 2B4 identifies memory CD8$^+$ T cells (131, 134, 135). Consistent with this finding, 2B4$^+$CD8$^+$ T cells are absent from cord blood, and expression of 2B4 can be induced on human and murine CD8$^+$ T cells activated in vitro (130, 134, 135). 2B4 is absent from most CD4$^+$ T cells and neutrophils (48, 53, 114, 131, 132). Although human B cells do not express 2B4 (53, 114, 132), ∼10% of mouse splenic B cells are 2B4$^+$ (133).

2B4 functions as an activating receptor on NK cells. 2B4 was initially described as a receptor on mouse NK cells that, when engaged by a specific Ab, could activate cytotoxicity and IFN-γ production (95, 130, 133). Cytotoxicity and cytokine secretion by human NK cells is also increased by anti-2B4 mAb (53, 110, 131, 132). The natural ligand for 2B4 is CD48 (136), and engagement of 2B4 by CD48 induces cytotoxicity and cytokine secretion by human and mouse NK cells (53, 110, 131, 132, 137, 138). Furthermore, 2B4/CD48 interactions between NK cells during their expansion appear to be necessary for acquisition of optimal cytolytic effector function (139). Thus, 2B4 has multiple important roles during the development of lytic effector cells and induces effector functions in NK cells.

2B4-mediated activation of human NK cells is impaired in the absence of SAP. The importance of SAP binding to 2B4 was highlighted by the finding that NK cells from XLP patients failed to be activated through 2B4, demonstrating a functional requirement for SAP in 2B4-mediated activation of human NK cells (53, 110, 140). Studies by the Moretta group found that engaging 2B4 on SAP-deficient human NK cells not only failed to induce cytotoxicity but also delivered a negative signal, such that basal killing of target cells was reduced (110). Thus, depending on expression levels of SAP, 2B4 can function as a positive or negative regulator of human NK cell function.

Optimal functioning of 2B4 on human NK cells requires coengagement of other activating receptors. Most studies that investigated 2B4 signaling in human NK cells used in vitro–activated NK cells (53, 131, 132, 137, 141). Some of these initial reports noted variable effects of engaging 2B4; i.e., cytotoxicity was enhanced in some, but not all, experiments (132, 141). It was subsequently found that the ability of NK cell clones to respond to ligation of 2B4 was dependent on

coengagement of the natural cytotoxicity receptor NKp46 (141). These findings led to the concept that 2B4 may act as a coreceptor on human NK cells (132, 141, 142). This was supported by the observation that concomitant engagement of 2B4 with another activating receptor on resting NK cells, which are refractory to the stimulatory effects of anti-2B4 mAb, led to significant cytokine secretion and cytotoxicity (142). Taken together, these data suggest that resting NK cells require signals from multiple stimulatory receptors linked to diverse signaling pathways in order to ensure appropriate activation. This requirement would also prevent inappropriate activation of resting NK cells, which is theoretically possible given the broad expression of ligands for molecules such as 2B4. It is unknown what changes are induced in stimulated versus resting NK cells such that the former can respond to engagement of 2B4 alone (142). Because SAP is required for 2B4-mediated activation of human NK cells (53, 110, 140), expression of SAP in resting NK cells may be insufficient to endow 2B4 with an activating function, and the increased expression observed following cytokine stimulation (43) may convert 2B4 from a coreceptor to a bona fide activating receptor.

Function of 2B4 on CD8$^+$ T cells. Although initial studies indicated that 2B4 did not activate CD8$^+$ T cells (132, 134, 137), more recent studies have provided evidence that 2B4 plays an important role in the cytotoxicity of CD8$^+$ T cells toward EBV-infected target cells. Dupre et al. (143) observed that, following interactions between normal CD8$^+$ T cells and CD48-bearing target cells, 2B4 and perforin polarize to the site of contact in a lipid raft. This process is dependent on 2B4 signaling and is defective in XLP CD8$^+$ T cells (143). Because CD8$^+$ T cells from XLP patients exhibit defective lysis of EBV-infected target cells (143), SAP is likely to enable localization of 2B4 and perforin to the immunological synapse, thereby facilitating destruction of EBV-infected target cells. This process is consistent with the redistribution of 2B4 and SAP to the immune synapse following interactions between NK cells and CD48-bearing target cells (144). Presumably, this process of 2B4 relocalization (143, 144) is also impaired in XLP NK cells.

Function of 2B4 on eosinophils. Cross-linking 2B4 on eosinophils induces cytokine secretion and target cell lysis (48). Although SAP is expressed in eosinophils (48), it is unknown whether SAP is recruited to 2B4 in these cells, or whether 2B4-mediated activation of eosinophils is impaired in XLP patients. This could be determined by examining eosinophil function in *2b4*$^{-/-}$ and *sap*$^{-/-}$ mice. Regardless of these uncertainties, since 2B4 is functional on granulocytes (48), it likely plays important roles in immune cells beyond NK cells and CD8$^+$ T cells.

Gene targeting identified 2B4 as an inhibitory receptor on mouse NK cells. Recently, mice deficient for 2B4 were generated (145). In contrast to in vitro experimental data demonstrating that 2B4 functions as a stimulatory receptor, *2b4*$^{-/-}$ NK cells exhibited enhanced IFN-γ production and increased cytotoxicity toward CD48$^+$ target cells both in vivo and in vitro (145, 146). Furthermore, restoration of 2B4 expression inhibited the ability of *2b4*$^{-/-}$ NK cells to kill CD48$^+$ target cells (145). Activated WT NK cells lysed CD48$^+$ target cells less efficiently than CD48$^-$ target cells, and blocking the 2B4-CD48 interaction restored killing of CD48$^+$ target cells (145, 146). In additional experiments, CD48$^-$ target cells transfected with CD48 were protected against NK cell–mediated cytotoxicity (145). These combined findings suggest that in mice 2B4 predominantly functions as an inhibitory receptor. One report indicated that the inhibitory function of mouse 2B4 is independent of SAP, as the same effects of signaling through 2B4 were evident in *sap*$^{-/-}$ and WT mice (146). However, other groups have shown that 2B4 is an activating receptor on mouse NK cells and

that it functions in a SAP-dependent manner. Specifically, *sap*$^{-/-}$ mice exhibited impaired cytokine production and cytotoxicity following stimulation with either anti-2B4 mAb or CD48-expressing cells (138, 147), a scenario reminiscent of XLP NK cells (53, 110, 140, 148). One possible explanation for these findings is that there may be a threshold for SAP expression that determines whether 2B4 functions as an activating or inhibitory receptor (147). In situations in which SAP is abundantly expressed, 2B4 may function as an activating receptor, whereas when SAP is limiting, EAT-2 and/or ERT may bind to 2B4 and initiate inhibitory signaling. Alternatively, since there are two isoforms of mouse 2B4 that differ in their cytoplasmic domains and signaling capacities (149), the relative amounts of these isoforms could dictate the responsiveness of NK cells to 2B4 ligation. Thus, 2B4 has an important role in regulating the effector functions of human and mouse NK cells. However, the mechanisms by which this occurs may differ between the two species.

2B4-mediated signaling. 2B4 contains four ITSMs that are phosphorylated following receptor cross-linking (148, 150). SAP can be recruited to all four ITSMs, although binding to the membrane proximal ITSM is sufficient for NK cell activation (151). SAP can also recruit Fyn to 2B4, which results in increased phosphorylation of 2B4 as well as recruitment and/or tyrosine phosphorylation of downstream effector molecules including Vav-1, SHIP, and c-Cbl (**Figure 4*a***) (148, 151). Tyrosine phosphorylation of intracellular substrates is defective following stimulation through 2B4 on NK cells from *sap*$^{-/-}$ and *fyn*$^{-/-}$ mice and from XLP patients, leading to the hypothesis that 2B4-mediated activation of NK cells is dependent on recruitment of Fyn by SAP (147, 148). In further support of this possibility is the finding that cross-linking 2B4 on *fyn*$^{-/-}$ NK cells leads to defective IFN-γ production and an inability to lyse target cells (147).

Additional studies have shown that 2B4 constitutively associates with the transmembrane adaptor linker for activation of T cells (LAT) (152, 153). Cross-linking 2B4 induces LAT phosphorylation and the subsequent recruitment of PLCγ and Grb2, intracellular proteins involved in the activation of the Ras/MAPK signal transduction pathway (152). Although NK cells from *lat*$^{-/-}$ mice exhibited normal killing of the NK-sensitive target cell line YAC-1, 2B4-mediated cytotoxicity is defective (153). Thus, both *sap*$^{-/-}$ and *lat*$^{-/-}$ NK cells display defective 2B4-mediated cytotoxicity, suggesting that these proteins may function downstream of 2B4 through a common pathway.

Several scenarios may explain how 2B4 inhibits NK cell functions. First, 2B4 can recruit the inhibitory kinase Csk, a process inhibited by SAP (151). Csk can phosphorylate 2B4, which, in the absence of SAP, may result in recruitment of the phosphatases SHP-1, SHP-2, and SHIP (96, 110, 151). Thus, 2B4 could provide a negative signal through a phosphatase-dependent mechanism (**Figure 4*b***). Alternatively, 2B4 may recruit EAT-2 (89, 92), which negatively regulates 2B4 function on murine NK cells (90) (**Figure 4*b***). EAT-2 binds constitutively to 2B4, and binding is reduced upon 2B4 phosphorylation, coincident with recruitment of SAP (92). Although not yet formally shown, SAP may displace EAT-2 from phosphorylated 2B4. Thus, in the absence of SAP, EAT-2 may remain associated with 2B4 and thus deliver an inhibitory signal.

2B4 functions as a ligand for CD48. Previous studies demonstrated that mAb-mediated ligation of CD48 enhanced activation of B and T cells (154, 155), raising the possibility that CD48 could act as a receptor to deliver activating signals to lymphocytes. This has recently been addressed. Mouse CD8$^+$ T or NK cells could augment proliferation of CD4$^+$ T cells, CD8$^+$ T cells, and NK cells, and cytotoxicity of CD8$^+$ T cells through a 2B4- and CD48-dependent mechanism

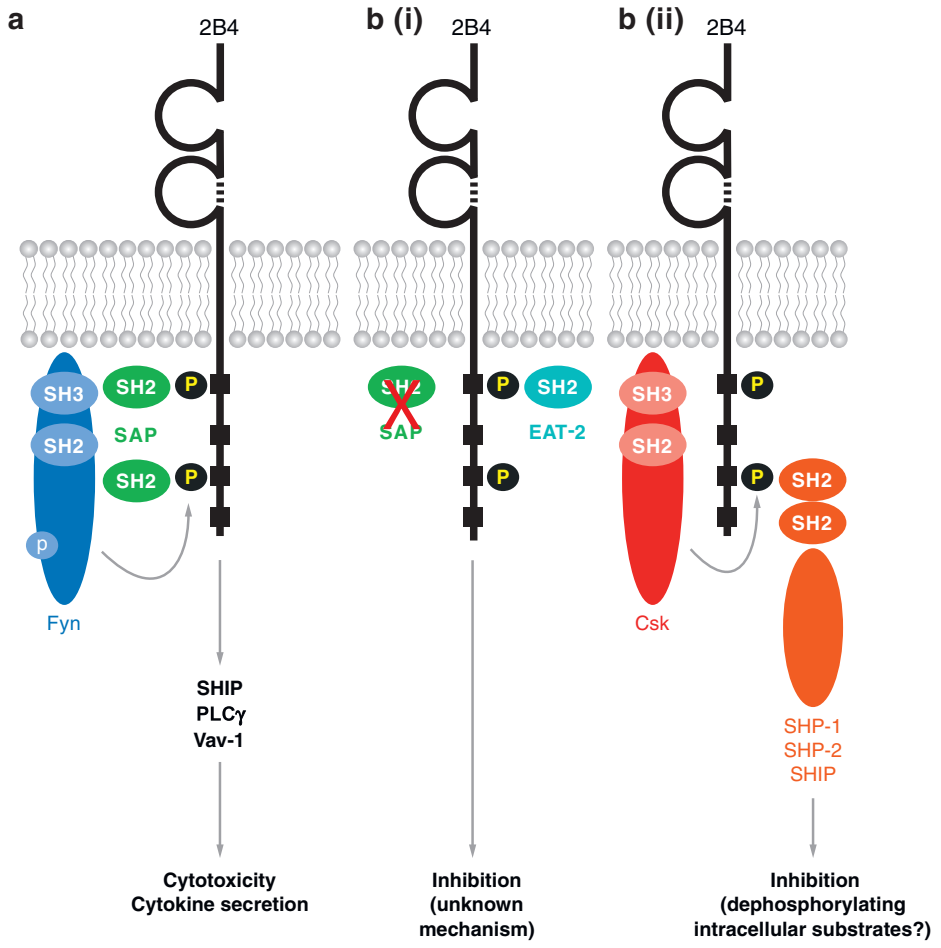


Figure 4

Positive and negative signaling pathways downstream of 2B4. (*a*) Following engagement with CD48, 2B4 becomes phosphorylated by Fyn, allowing recruitment of SAP, and the phosphorylation and activation of numerous intracellular substrates, such as Vav-1, PLCγ, SHIP, and c-Cbl. This initiates a positive signaling cascade, resulting in NK cell cytokine secretion and cytotoxicity. Binding of SAP to phosphorylated 2B4 prevents associations with Csk, EAT-2, and phosphatases. (*b*) In the absence of SAP, 2B4 may deliver negative signals to NK cells by at least two mechanisms: (*i*) EAT-2 can bind to 2B4, which negatively regulates NK cell function by a pathway that is yet to be characterized; (*ii*) Csk can bind and phosphorylate 2B4, resulting in the recruitment of inhibitory phosphatases such as SHP-1, SHP-2, and SHIP, which can attenuate signaling by dephosphorylating intracellular substrates.

(156–158). Consistent with these results, mAb to 2B4 or CD48 inhibited IL-2-induced proliferation of NK cells (131, 157), suggesting that cell-cell interactions contribute to NK cell expansion. It was anticipated that 2B4 would be responsible for delivering activating signals to the responding cells. However, because 2B4 is expressed on $CD8^+$, but not on $CD4^+$, T cells, and because proliferation of both cell subsets could be enhanced by co-cultured NK cells (156, 157), it appeared that 2B4 could act as a ligand for CD48. This was confirmed by demonstrating that when the P815 cell line was transduced to express 2B4, it substantially enhanced activation of anti-CD3 mAb-stimulated $CD8^+$ T cells (157).

A similar approach led to the conclusion that 2B4 can also stimulate cytokine secretion and cytotoxicity in human NK cells by interacting with CD48 (159). Although ligation of 2B4 does not augment cytotoxicity of activated human $CD8^+$ T cells (132, 134, 137), activated $2B4^+CD8^+$ T cells exhibit greater cytotoxicity than $2B4^-CD8^+$ T cells when tested against a variety of target cells (K562, P815, Jurkat) (131, 134). Although this finding could reflect differences in the maturation stage of these $CD8^+$ T cell subsets (135), it may result from 2B4 activating $2B4^+CD8^+$ T cells by delivering a signal through CD48.

These findings have several implications. First, 2B4 is expressed on monocytes. However, monocytes do not express SAP (43), and stimulation through 2B4 does not affect their cytokine production (132). Thus, rather than delivering signals to monocytes, 2B4 may act as a ligand to stimulate $CD48^+$ effector cells such as T cells and/or NK cells. However, it cannot be excluded that 2B4 activates monocytes via a SAP-independent mechanism or uses another intracellular adaptor protein such as EAT-2. Second, from a teleological perspective, the recognition that bidirectional signaling can occur through 2B4 and CD48 parallels the homotypic interactions exhibited by other members of the SLAM family, which can therefore also either deliver or receive stimulatory signals.

NTB-A

NTB-A was originally described in the mouse as Ly108 (100) and subsequently cloned in humans (101, 102). It is expressed on NK cells, T cells, B cells, and eosinophils, but not monocytes, macrophages, basophils, or neutrophils (48, 101, 102) (**Table 1**). In contrast to humans, mouse NK cells apparently do not express NTB-A (90). NTB-A is a self-ligand (160–162) and, similar to 2B4, becomes tyrosine phosphorylated following receptor ligation and recruits SAP to its cytoplasmic domain (101, 161). The ability of NTB-A to bind protein tyrosine phosphatases requires clarification because NTB-A could bind SHP-2, but not SHP-1, in primary human NK cells (101), but not in Jurkat T cells (161). These discrepancies may simply reflect the use of different cell lines for this analysis.

NTB-A appears to play a similar role as 2B4 on NK cells, in that cross-linking NTB-A results in enhanced cytotoxicity and cytokine secretion (101, 160, 162). Cytotoxicity of XLP NK cells was inhibited, rather than increased, when treated with anti-NTB-A mAb (101), whereas cytokine production was unaffected (162). These observations demonstrate a requirement for SAP in NTB-A-mediated activation of human NK cells. In contrast to 2B4, anti-NTB-A mAb enhanced proliferation and IFN-γ secretion of human and murine T cells stimulated with a suboptimal dose of anti-CD3 mAb (161). The role of SAP in this process as well as the consequences of SAP-deficiency on the function of NTB-A on human T cells remain to be determined.

Because administration of an NTB-A Fc fusion protein into mice decreased serum levels of IFN-γ-induced Ig isotypes (IgG2a, IgG3) and increased the IL-4-induced isotype IgG1, investigators proposed that NTB-A directs differentiation of $CD4^+$ T cells into Th1 effectors (161). Supporting this proposal was the finding that infusion of NTB-A Fc delayed the onset of EAE, a Th1-mediated autoimmune disorder, in susceptible mice (161). However, because NTB-A is a self-ligand, it cannot be excluded that the NTB-A Fc fusion protein blocked homotypic interactions or instead resulted in activation of $NTB\text{-}A^+$ cells, as has been demonstrated for this reagent in vitro (160, 162). Furthermore, it is difficult to reconcile these results with the phenotype of mice that lack exons 1 and 2 of *ly108* (*ly108*$^{\Delta E2+3}$). $CD4^+$ T cells from *ly108*$^{\Delta E2+3}$ mice produce normal amounts of IFN-γ but exhibit a partial reduction in IL-4 production (163). Thus, similar to SLAM, NTB-A may be primarily involved in polarizing $CD4^+$ T cells toward a Th2 response. Despite this discrepancy regarding the function of NTB-A on T cells, impaired signaling via NTB-A

likely affects the function of NK cells in XLP patients.

A striking feature of *ly108*$^{\Delta E2+3}$ mice was dysregulated neutrophil function, as evidenced by increased production of IL-12, TNF-α, and IL-6, but reduced production of reactive oxygen intermediates and reduced bactericidal activity (163). Consequently, *ly108*$^{\Delta E2+3}$ mice were highly susceptible to bacterial infections (163). Since SAP is not in neutrophils (48), the function of NTB-A on neutrophils is probably independent of SAP. It remains to be determined whether EAT-2 is expressed in granulocytes and whether EAT-2 mediates the effect of NTB-A on these cells. Interestingly, human neutrophils do not express NTB-A (48). Although human neutrophils may acquire NTB-A expression following activation or migration to different anatomical sites, it is unknown whether NTB-A plays an analogous regulatory role in the function of human neutrophils.

Ly9 (CD229)

Ly9 is unique among the SLAM family of receptors as it has four Ig domains in a duplicated V-C2 configuration in its extracellular region (**Figure 3**) (98, 99). Ly9 is expressed on ∼50% of CD4$^+$CD8$^+$ and CD4$^-$CD8$^-$ thymocytes, most CD4$^+$ and CD8$^+$ thymocytes, and peripheral mature B cells and T cells (98, 99, 114). It is expressed weakly on NK cells, but is absent from monocytes, granulocytes, erythrocytes, and platelets (99, 164) (**Table 1**). Ly9 is also a homotypic receptor (164) that can recruit SAP and EAT-2 (89, 108). Similar to its interaction with SLAM and 2B4, SAP is required for maximal tyrosine phosphorylation of Ly9 (108, 165). However, tyrosine phosphorylation of Ly9 was not completely absent in *sap*$^{-/-}$ thymocytes, indicating that other kinases can directly interact with Ly9 or that another adaptor molecule can mediate its phosphorylation (165). The cytoplasmic domain of Ly9, but not of the other SLAM family members, can also recruit Grb2 (166). This process is dependent on src kinase–mediated phosphorylation of a tyrosine-based motif (Y_{606}ENF) in the cytoplasmic domain of Ly9, which is distinct from the ITSM that recruits SAP ($TVY_{558}AQV/TIY_{581}CSI$) (108, 166). Beyond these biochemical studies, little is known about the function of Ly9 on lymphocytes. In vitro studies have shown that ligating Ly9 on primary human T cells rapidly induces its tyrosine phosphorylation and partially reduces ERK activation and IFN-γ production induced by anti-CD3 mAb (166). This suggests that Ly9 may have a negative role in T cell activation (166). Assessment of the function of Ly9 on XLP lymphocytes will be important to determine the significance of the proposed negative regulatory role of Ly9 on T cells.

To further address the role of Ly9 in vivo, Ly9-deficient mice have recently been described. Although Ly9 is expressed on a vast array of leukocytes, deletion of *ly9* induced only very mild lymphocyte defects (167). *ly9*$^{-/-}$ CD4$^+$ T cells had near-normal production of IL-4 and IFN-γ, but there was a subtle reduction in IL-2 production (167). On the other hand, NKT cell development and humoral responses to LCMV infection were unaffected. Thus, although Ly9 can bind to SAP and SAP deficiency severely compromises multiple arms of the host immune response, the specific contribution of Ly9 to cellular and humoral immunity remains unclear. Although gene targeting has identified unique functions of SLAM, 2B4, and NTB-A, functional redundancy among the SLAM family cannot be discounted, such that another SLAM-related receptor compensates for loss of Ly9 in *ly9*$^{-/-}$ mice.

CD84

CD84 is expressed on human CD4$^-$CD8$^-$, CD4$^+$, and CD8$^+$ thymocytes, but is absent from CD4$^+$CD8$^+$ thymocytes (114). CD84

is also expressed on human HSCs, mast cells, monocytes/macrophages, DCs, granulocytes, platelets, B cells, and T cells (48, 97, 109, 114, 168–170) (**Table 1**). Whereas human NK cells do not express CD84 (109, 164), murine NK cells do (90). Apart from NK cells, expression of CD84 on mouse leukocytes has not been reported. CD84 is also a self-ligand (169), and coengagement of CD84 and CD3 on activated T cells increased proliferation (91) and IFN-γ secretion (169). However, cross-linking CD84 by itself failed to enhance proliferation or IFN-γ production, indicating that it most likely acts as a costimulatory molecule (91, 169). Homotypic interactions between CD84 molecules also increased platelet spreading (46).

Ab-mediated cross-linking of CD84 on T cells or platelets results in phosphorylation of ITSMs and recruitment of SAP (46, 91, 108). This process is mediated by src kinases such as Lck (91). Tyrosine kinases such as Fyn, as well as those expressed in B cells, can probably also perform this function. Phosphorylation of CD84 does not require SAP expression because it can occur in SAP-deficient cell lines and T cells from XLP patients (91, 109). Although signaling downstream of CD84 has not been examined in detail, SAP is presumed also to facilitate the recruitment of Fyn to the CD84 and SAP complex, which will deliver signals to activated T cells. As CD84 is abundantly expressed on platelets (168), and platelets from *sap*$^{-/-}$ mice have impaired function (46), it will be important to assess signaling and functional consequences of CD84 ligation in platelets from XLP patients to determine whether compromised platelet function contributes to disease progression. CD84 also becomes rapidly tyrosine phosphorylated on B cells following ligation with anti-CD84 mAb (109). Because B cells do not express SAP (37, 43, 45), CD84 most likely uses an intracellular signaling pathway in B cells that is distinct from that in T cells. CD84-deficient mice have not been reported to date; thus, the cell-specific function of CD84 awaits further investigation.

CRACC

CRACC is expressed on all human NK cells, most CD8$^+$ T cells, and a small subset of CD4$^+$ T cells (103). It is also on mature DCs and B cells and is upregulated following CD40 stimulation, but is not expressed on monocytes or immature DCs (103) (**Table 1**). CRACC is also a self-ligand (171), and engaging CRACC on NK cells induces cytotoxicity (103, 171). Colonna and colleagues reported that CRACC associates with EAT-2, but not SAP (92, 103). In contrast, other investigators have found that human (but not murine) CRACC could associate with SAP, albeit with an apparently lower affinity than that of other SAP-associating receptors (104, 166). Irrespective of these findings, anti-CRACC mAbs could induce killing of FcR-expressing target cells by XLP NK cells (92, 103), suggesting CRACC-mediated NK cell cytotoxicity is independent of SAP. This is in stark contrast to the requirement of SAP for the lytic function of 2B4 and NTB-A on human NK cells.

Dissection of signal transduction pathways has shown that ligation of CRACC on human NK cell lines induces src kinase–mediated tyrosine phosphorylation of CRACC followed by the recruitment of EAT-2 and activation of PLCγ, PI3 kinase, Vav, and SHIP-1 (92). These pathways appear to be involved in the function of CRACC because pharmacological inhibition of src kinases or PLCγ abrogated CRACC-induced NK cell cytotoxicity (92). Because CRACC appears to recruit only EAT-2, it was proposed that EAT-2 positively regulates signaling through CRACC (92). This proposal has raised several important questions. First, the signaling pathways activated through CRACC in NK cells (i.e., PLCγ1/PLCγ2/PI3 kinase/Vav) were very similar to those induced by 2B4 (92, 148). Although it could be argued that 2B4 also elicits its effect by recruiting EAT-2 (89), binding of EAT-2 to 2B4 appears to be displaced by SAP following 2B4 phosphorylation (92). Thus, assuming that EAT-2 positively

regulates CRACC signaling, why can't EAT-2 compensate for the absence of functional SAP in XLP NK cells that fail to lyse target cells and activate Vav in response to 2B4 engagement (53, 110, 140, 148)? Possible answers are that (*a*) unidentified molecules are recruited to phosphorylated 2B4 in the presence of SAP, but not EAT-2; (*b*) SAP is required for localization of 2B4 to the immune synapse and this does not take place in cells that express only EAT-2; or (*c*) signaling in immortalized human NK cells differs from that in primary cells. To resolve this discrepancy, 2B4- and CRACC-mediated signaling events will need to be examined in parallel with those in normal human and XLP NK cells. Second, studies by Veillette and colleagues indicated that EAT-2 is a negative, rather than positive, regulator of NK cell function in mice (90). The inhibitory function of EAT-2 is abolished by mutating two tyrosine residues in its C-terminal domain. Whether one or both of these tyrosines are required for inhibitory function is unclear because an analysis of EAT-2 molecules bearing single mutations has not been reported. This is a relevant point because the C-terminal domain of human EAT-2 bears only one of these tyrosine residues (89, 109). Thus, human EAT-2 may indeed lack the inhibitory function of its mouse counterpart and, as proposed by Tassi et al. (92), act as a positive regulator of CRACC-induced lymphocyte activation.

INVOLVEMENT OF IMPAIRED SIGNALING THROUGH SLAM FAMILY RECEPTORS IN DEFECTS IN LYMPHOCYTE DEVELOPMENT AND BEHAVIOR IN XLP

By associating with up to six different SLAM family receptors, SAP may play a significant role in regulating the activation and effector function of multiple types of immune cells. Thus, the phenotype of XLP is likely due to defects in signaling through several SAP-associating receptors. Hence, it will be important to determine specific cell-cell interactions that involve different SAP-associating receptors to understand better how perturbed signaling results in key features of XLP, such as hypogammaglobulinemia, susceptibility to EBV infection, lymphoma, and NKT cell deficiency.

Hypogammaglobulinemia

The primary defect underlying hypogammaglobulinemia that develops in XLP patients and *sap*$^{-/-}$ mice is most likely related to the inability of CD4^{+} T cells to provide B cell help owing to reduced expression of important costimulatory molecules, such as ICOS, and diminished production of cytokines important for B cell differentiation (**Figure 5**) (35, 80). As a result, XLP patients fail to form GCs and have a paucity of memory B cells and long-lived PCs (35, 37, 79, 80). Despite these important findings, the SAP-associating receptors and the subset of CD4^{+} T cells that participate in TD B cell activation have not yet been identified. Furthermore, it is possible that NK and NKT cells further influence B cell differentiation, and that these cellular interactions are also impaired in XLP patients.

Role of CD4^{+} T cells in hypogammaglobulinemia in XLP. A candidate cell type that may be defective in XLP is the T follicular helper (T_{FH}) cell (83). T_{FH} cells localize to GCs by virtue of their expression of CXCR5, a lymphoid homing chemokine receptor (reviewed in 83). T_{FH} cells express CD40L and ICOS, produce IL-10, and support Ig secretion by B cells (reviewed in 83). Furthermore, they are the predominant source of IL-21 (83, 172), a cytokine capable of inducing proliferation and Ig production by human naive and memory B cells and mouse B cells (173, 173a). Lastly, T_{FH} cells express high levels of CD84, Ly9, and NTB-A, as well as SAP itself (83, 172; S.G. Tangye, unpublished results), and activated B cells have increased expression of several SAP-associating receptors (103, 109, 111, 112). Thus, T_{FH} cells

express an array of molecules, including SAP and SAP-associating receptors, that may directly contribute to their ability to promote B cell differentiation. This hypothesis is supported by the finding that T_{FH} cells are overrepresented in murine models of autoimmunity (83, 174). Therefore, compromised function of T_{FH} cells in the absence of SAP may cause impaired help to B cells for their differentiation to memory cells and PCs (**Figure 5**).

Role of NK and NKT cells in hypogammaglobulinemia in XLP. Although the above-listed features of T_{FH} cells highlight their likely involvement in humoral immune responses, NK and NKT cells can also induce human and murine B cells to secrete Ig via direct cell-cell interactions (41, 175, 176). 2B4-deficient NK cells are less capable of inducing isotype switching than are WT NK cells (176). Furthermore, B cells potently stimulate production of IL-13 by murine NK cells, a process dependent on expression of both 2B4 and SAP by the NK cell (138). Although this phenomenon has not been established for human NK cells, a subset of these cells produce high amounts of IL-13 and IL-10 (177, 178). Since NK cells can express CD40L, and IL-13 induces human B cells to secrete IgG and IgE (179), reduced production of "helper" cytokines (i.e., IL-10, IL-13) in response to stimulation through 2B4 on NK cells may also contribute to hypogammaglobulinemia in XLP.

The deficit of memory B cells in XLP patients contributes to hypogammaglobulinemia because these cells are the precursors of PCs (35). However, memory B cell deficiency may further contribute to defects in humoral immunity by a secondary, and complementary, mechanism. Murine NK cells localize to the splenic marginal zone (MZ) following viral infections (180), and MZ B cells primarily induce IL-13 production by NK cells (138). In human spleens, memory B cells localize to the MZ (181) and share many features with murine MZ B cells (39, 182). Thus, it is tempting to speculate that the paucity of memory B cells in the splenic MZ of XLP patients (37) would further compromise the ability of NK cells to provide "help" because NK cells would not receive the appropriate stimulatory signals from memory (i.e., MZ) B cells, which normally augment cytokine production.

Both XLP patients and *sap*$^{-/-}$ mice lack NKT cells (40). NKT cells express CD40L and can induce Ig secretion by human B cells (41). Furthermore, NKT cells secrete high amounts of effector cytokines such as IL-4, IL-10, IL-13 (42, 183, 184), and IL-21 (D. Godfrey, personal communication), all of which stimulate proliferation and Ig secretion by human B cells (39, 173, 173a). Thus, the selective absence in XLP patients of a discrete subset of lymphocytes potentially capable of regulating B cell behavior may also affect the ability of these patients to mount appropriate TD humoral immune responses.

Susceptibility to EBV

Mutations in *SH2D1A* render XLP patients exquisitely sensitive to infection with EBV. In contrast, responses of XLP patients to infections with other herpes viruses are apparently normal. This finding suggests that SAP and SAP-associating receptors are preferentially involved in anti-EBV immune responses. Indeed, several pieces of evidence support this proposal. First, CD48 is "superinduced" on B cells following EBV infection (185). Because the cytotoxic activity of NK cells is strictly regulated by signals transduced by stimulatory and inhibitory receptors (58), the increased density of CD48 on EBV-infected B cells may tip the balance in favor of 2B4-mediated NK cell–mediated cytotoxicity and subsequent target cell lysis. Second, 2B4 recruits SAP to its cytoplasmic domain (96), which is required for 2B4-dependent NK cell–mediated cytotoxicity (53, 110, 140). Third, 2B4 contributes to the cytotoxicity of lymphocytes by facilitating the polarization of the lytic machinery of these cells (143, 144). Thus, the combined inability of

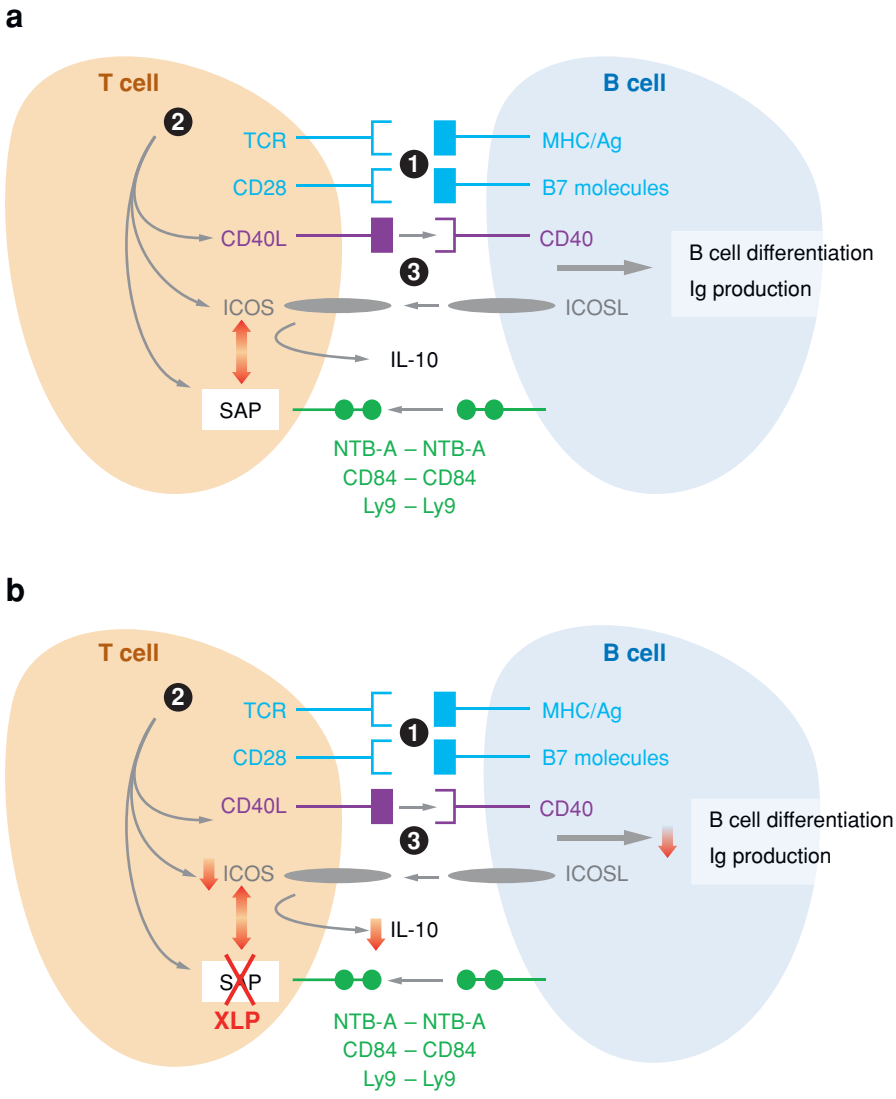


Figure 5

Impaired B cell differentiation in XLP may result from impaired ICOS expression and IL-10 production by $CD4^+$ T cells. (*a*) B cells present Ag in the context of MHC class II complexes to the TCR (1). In the presence of appropriate accessory signals (e.g., interaction between B7 molecules on B cells and CD28 on T cells), the T cells become activated (1). This results in the induction of expression of CD40L and ICOS, and an increase in SAP expression in the T cells (2). The interaction between CD40L on T cells and CD40 on B cells facilitates activation and Ig production by B cells (3). Signals delivered through ICOS by ICOSL (3) induce IL-10 secretion, which contributes to Ig production. Because the phenotype of ICOS deficiency resembles that of $sap^{-/-}$ mice and XLP patients, we hypothesize that interactions between SAP-associating SLAM family receptors on T_{FH} cells and B cells (e.g., CD84, Ly9, NTB-A) facilitate maximal expression of ICOS. (*b*) In the absence of SAP, ICOS expression on T cells is lower, resulting in insufficient signals for optimal B cell activation, causing impaired B cell differentiation, as evidenced in vivo by deficiencies in memory B cells and plasma cells and hypogammaglobulinemia.

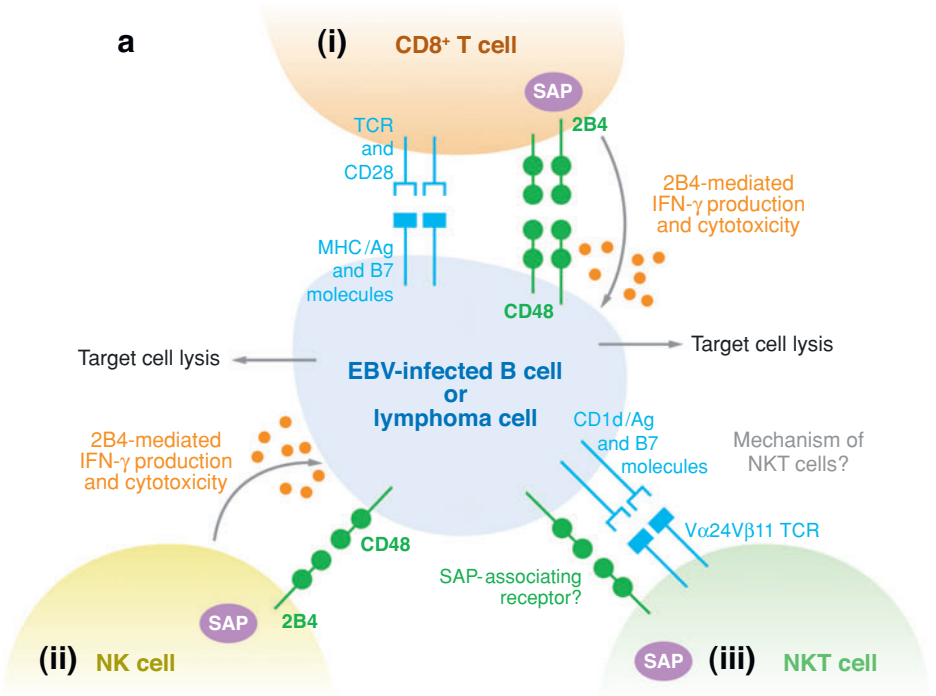
SAP-deficient NK cells to respond to 2B4 signals and of SAP-deficient $CD8^+$ T cells to form an appropriate cytotoxic synapse may lead to uncontrolled proliferation of EBV-infected B cells in XLP patients. The persistence of EBV-infected B cells could serve as a stimulus for continued T cell activation and Th1-type cytokine production, which would lead to the secondary activation of macrophages (**Figure 6**) (16). Lastly, there is a curious association between polymorphisms in the *IL-10* promoter and susceptibility to infection with EBV (186). Mononuclear cells from individuals with a −1082(G) polymorphism produced more IL-10 than did individuals with a −1082(A) polymorphism (186). The incidence of EBV infection was greater in individuals with the −1082(A) polymorphism, suggesting that the capacity to produce greater amounts of IL-10 provides a level of resistance to EBV infection (186). This is reminiscent of XLP $CD4^+$ T cells that produce less IL-10 than do $CD4^+$ T cells from normal donors (35).

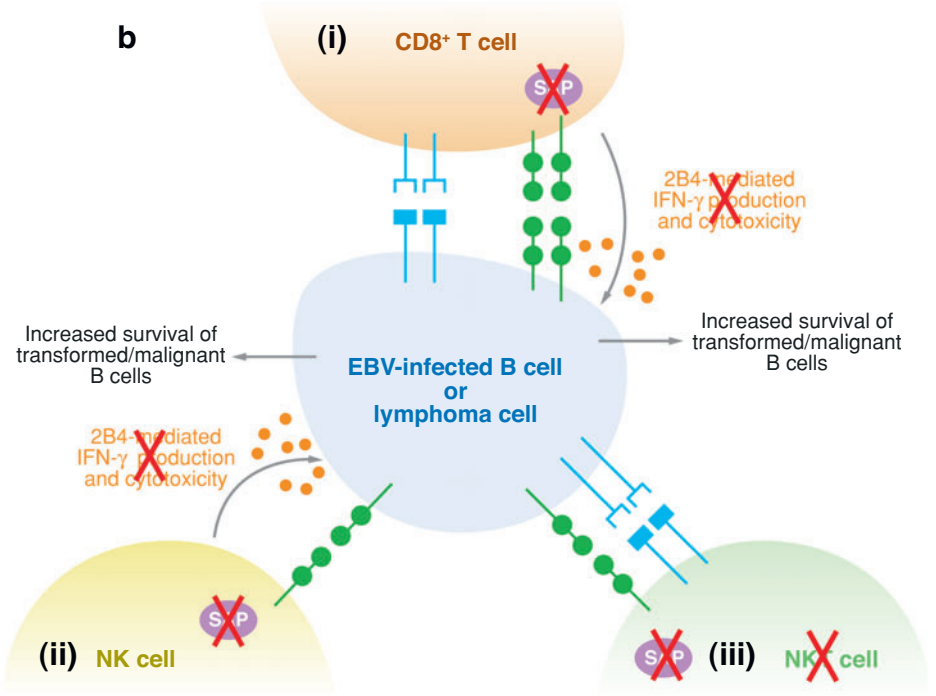
B Cell Lymphoma

One of the earliest cellular defects reported in XLP was impaired NK cell function (28, 30). XLP NK cells had reduced killing of the NK-sensitive cell line K562 in vitro, whereas Ab-dependent cell-mediated cytotoxicity (ADCC) was intact (28). This functional dichotomy of XLP NK cells led the authors to propose that separate populations of NK cells may be responsible for "natural killing" and ADCC (28). However, in light of more recent studies (53, 101, 110, 140), the defect in natural killing in XLP patients (28) is likely due to defects in activation through SAP-associating receptors, whereas ADCC, which signals through the CD16 FcR that does not involve SAP (58), is normal in these patients. Given the known role of NK cells in antitumor immunity (58), defects in NK cell activation through 2B4 and NTB-A may be permissive to the development of lymphoma in XLP patients. As mentioned above, CD48 expression is increased on EBV-infected B cells (185). Consequently, following EBV infection in normal individuals, the 2B4 pathway would be rapidly activated, resulting in NK cell–mediated lysis of EBV-infected B cells (53). However, in XLP, killing of EBV-infected B cells via this pathway may be severely compromised, especially if 2B4 can inhibit NK cell function in the absence of SAP (110). Thus, altered signaling or decreased activation through 2B4 on NK cells may facilitate the survival and persistence of transformed B cells, which could acquire additional genetic alterations and develop into true malignant B cells (16). Since $CD8^+$ T cells also have an important role in anti-EBV immunity (16), the defective function of 2B4 on these cells probably also manifests as increased host susceptibility to development of lymphoma (143). Lastly, based on the potential role of NKT cells in antitumor immunity in normal individuals (42), the NKT cell deficiency may also contribute to the development of lymphoma in XLP patients (**Figure 6**).

NKT Cell Development

The SLAM family receptor(s) required for NKT cell development is not known. Similarly, the pattern of expression of SLAM family receptors on developing and mature mouse NKT cells has not been reported. Investigations of gene-targeted mice have demonstrated that Ly9 is dispensable for NKT cell development (167). Although this has not been formally reported in mice deficient for SLAM, 2B4, or NTB-A (117, 145, 163), experimental evidence suggests that SLAM is not required for NKT cell development because *slam*$^{-/-}$ mice rapidly produce IL-4 following anti-CD3 mAb treatment in vivo (117), a response mediated by NKT cells (42). Interestingly, nonobese diabetic (NOD) mice are deficient in NKT cells (42). The defect maps to two loci, one of which (*Nkt1*) maps to the region of chromosome 1 that contains the *slam* and *ly108 (NTB-A)* genes (186a) and





can be partially corrected by introgression of C57BL/6-derived alleles in this region (A. Baxter, personal communication). Detailed analysis of NOD mice revealed that expression of SLAM is reduced on $CD4^+CD8^+$ thymocytes compared with those from C57BL/6 mice and this reduction in expression was corrected in NOD.*Nkt1* congenic mice. Furthermore, similar to the findings by Wakeland and colleagues (107, 187), NOD and C57BL/6 mice expressed different isoforms of *ly108* (A. Baxter, personal communication). Together, these findings suggest that SLAM and/or NTB-A/Ly108 may provide the instructive signal for progenitors to develop into NKT cells. However, confirmation of this proposal awaits detailed examination of mice deficient in these, or additional, SAP-associating receptors.

INVOLVEMENT OF SLAM FAMILY RECEPTORS IN AUTOIMMUNITY

A susceptibility locus, *Sle1*, for the development of murine lupus was recently mapped to a region on chromosome 1 (107) that includes genes encoding SLAM family receptors. Notably, the autoimmune haplotype contains several polymorphic variants of these genes (107). The polymorphisms are located in exons encoding the extracellular domains of 2B4, Ly9, CRACC, and CD84 (107). Although it is not understood how these polymorphisms contribute to the autoimmune phenotype, resulting amino acid differences may affect the affinities of these molecules for their ligands. Mice congenic for the Sle1 locus exhibited increased expression of CD84 and Ly108/NTB-A and had multiple copies of the *2b4* gene, further supporting a role for these molecules in the development of autoimmunity (107). However, perhaps the most significant finding was the differential expression of splice variants of *ly108* in the autoimmune (*Sle1b*) versus nonautoimmune (C57/BL6) haplotypes. *Sle1b* preferentially encodes a variant of *ly108 (ly108-1)* that contains three ITSMs, whereas the C57/BL6 locus encodes *ly108-2*, which has only two ITSMs (107). This resulted in up to fivefold higher levels of *ly108-1* expression in B and T cells from *Sle1b* mice (107, 187). Increased expression of *ly108-1* by B cells from *Sle1b* congenic mice appeared to be functionally significant because immature B cells from these mice exhibited reduced responsiveness to signaling through the B cell receptor (187). Consequently, there was increased survival and impaired deletion of autoreactive B cells in *Sle1b* congenic mice, a process apparently mediated by the *ly108-1* isoform (187). The molecular mechanism underlying this aberrant selection of autoreactive B cells is unknown. Since SAP does not appear to be expressed in B cells, it is unlikely to involve SAP. However, the

Figure 6

Defects in $CD8^+$ T cells and NK cells and the absence of NKT cells may contribute to impaired antiviral and antitumor immunity in XLP patients. (*a*) (*i*) $CD8^+$ T cells become activated by EBV-infected B cells presenting viral Ag bound to MHC class I and costimulatory molecules. Interactions between 2B4 on $CD8^+$ T cells and CD48 on target cells (EBV-infected B cells or lymphoma cells) activate SAP-dependent signaling pathways that result in synapse formation and the polarization of cytotoxic mediators such as perforin and death of the target cells. (*ii*) Similar to $CD8^+$ T cells, engagement of 2B4 on NK cells by CD48 on EBV-infected B cells and lymphoma cells induces IFN-γ production and target cell lysis. (*iii*) NKT cells are also believed to play a role in antiviral and antitumor immunity. Since NKT cells express SAP, interactions between SAP-associating receptors on NKT cells with their cognate ligands on target cells may initiate the effector functions of NKT cells. (*b*) In the absence of functional SAP in XLP, 2B4-mediated IFN-γ production and cytotoxicity by $CD8^+$ T cells and NK cells are impaired. In addition, NKT cells fail to develop. Collectively, defects in $CD8^+$ T cells and NK cells and a lack of NKT cells may compromise the ability of XLP patients to control EBV-infection and increase their susceptibility to lymphoma development.

fact that *ly108-1* contains an additional ITSM in its cytoplasmic domain suggests that active signaling through Ly108 on B and/or T cells may play a role in regulating B cell self-tolerance and T cell activation.

CONCLUSIONS AND FUTURE PERSPECTIVES

The identification of *SH2D1A* as the genetic lesion in XLP, coupled with the cloning and characterization of SAP-associating receptors and the generation of SAP and SLAM family receptor–deficient mice, highlighted the previously unappreciated and indispensable roles played by the SLAM family and SAP pathway in regulating multiple facets of lymphocyte development and function. Clearly, SAP is required in humans and mice for NKT cell ontogeny, as well as for induction of optimal TD B cell immune responses and aspects of NK and $CD8^+$ T cell activation. Despite recent advances in elucidating the function of the SLAM- and SAP-related families of molecules, several areas require further investigation. For example, future identification of the SAP-associating receptors that regulate NKT cell development and mature NKT cell activation will provide new insights into this population of regulatory lymphocytes. A more thorough dissection of the roles played by the SAP and SLAM families in different hematopoietic cell lineages may facilitate a clearer understanding of the molecular events guiding normal humoral, anti-EBV, and anti-tumor immune responses, as well as platelet function. These studies will also undoubtedly provide insights into the pathogenesis and management of XLP, as well as the development of autoimmune conditions. Recently, it was reported that SAP is expressed in neuronal cells and can associate with tropomyosin-related kinases (Trk), thereby attenuating signaling pathways downstream of Trk (52). This report raises the interesting possibility that the function of SAP is not restricted to the immune/hematopoietic systems, but may extend to the nervous system. Since XLP patients and $sap^{-/-}$ mice do not appear to have obvious neurological defects, the physiological relevance of these findings requires further investigation. Finally, ITSM sequences are present in the cytoplasmic domains of receptors belonging to the Ig and lectin superfamilies, such as signal regulatory proteins (SIRP), PD-1, CD31 (PECAM-1), and sialic-acid binding immunoglobulin-like lectins (Siglec-3, -5, -9, -10) (59). Thus, it will be important to determine whether the SAP family of adaptors contributes to intracellular signaling elicited through these receptors in cell types as diverse as lymphocytes, granulocytes, platelets, and neurons. Overall, we have learned a great deal about two complementary families of immunological molecules in a relatively short period of time. With numerous questions remaining unanswered, the next decade of investigation will undoubtedly provide further insight into the significance of the SLAM and SAP families in the development and function of the hematopoietic system, and how perturbations to these processes precipitate disease.

SUMMARY POINTS

1. The identification of *SH2D1A* as the genetic lesion in XLP revealed a novel signal transduction pathway utilized by the SLAM family of cell surface receptors and implicated this pathway in immune responses to EBV.
2. The SLAM family of cell surface receptors includes SLAM, 2B4, CD84, NTB-A, Ly9, and CRACC. These proteins are encoded by genes present on chromosome 1 that probably arose through gene duplication. Most of these molecules are self-ligands, with the exception of 2B4, which recognizes CD48. These receptors are broadly expressed on hematopoietic cells and have diverse functions following engagement

with their ligands. SAP can be recruited to all of these receptors (although there is some controversy regarding binding to CRACC), and the function of some of them is dependent on SAP expression.

3. SAP is a highly unconventional adaptor protein as its SH2 domain can (*a*) bind the cytoplasmic domain of SLAM independently of tyrosine phosphorylation and (*b*) recruit tyrosine kinases by binding a noncanonical motif in the SH3 domain of Fyn.
4. SAP has an important role, either directly or indirectly, in the development, differentiation, and effector function of B cells, $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and NKT cells.
5. EAT-2 and ERT (only in rodents) are structural homologs of SAP; however, based on the phenotype of *eat-2*$^{-/-}$ and *ert*$^{-/-}$ mice, they appear to be distinct from SAP, as they have a role in negatively regulating the function of NK cells.

FUTURE ISSUES

1. Identify the SAP-associating receptors that regulate NKT cell development and $CD4^+$ T cell function during humoral immune responses.
2. Establish the mechanism by which polymorphisms in molecules such as *ly108* underlie autoimmunity.
3. Determine the effect of SAP deficiency on the function of SLAM, CD84, and Ly9 on lymphocytes from XLP patients and the function and mechanism of action of these molecules on cells that do not normally express SAP (e.g., myeloid cells).
4. Assess the function of platelets and eosinophils in XLP patients, as these cells express SAP and several SAP-associating receptors.
5. Resolve the controversy regarding the expression and function of SAP in B cells.
6. Clarify why XLP patients, even though they have functional deficiencies in NK cells, B cells, and T cells, as well as a lack of NKT cells, are particularly susceptible to EBV infection. These global lymphocyte defects could be expected to render affected individuals susceptible to infection with other pathogens. However, in spite of this, XLP patients are reasonably healthy in the absence of EBV, suggesting that TI mechanisms of eliciting humoral immune responses may be sufficient for protection against many other infections.

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