SLAM Family Receptors and SAP Adaptors in Immunity

Jennifer L. Cannons,[1] Stuart G. Tangye,[2] and Pamela L. Schwartzberg[1]

[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892; email: jcannons@mail.nih.gov, pams@nhgri.nih.gov

[2]Immunology Program, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia; email: s.tangye@garvan.org.au

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Abstract

The signaling lymphocyte activation molecule (SLAM)-associated protein, SAP, was first identified as the protein affected in most cases of X-linked lymphoproliferative (XLP) syndrome, a rare genetic disorder characterized by abnormal responses to Epstein-Barr virus infection, lymphoproliferative syndromes, and dysgammaglobulinemia. SAP consists almost entirely of a single SH2 protein domain that interacts with the cytoplasmic tail of SLAM and related receptors, including 2B4, Ly108, CD84, Ly9, and potentially CRACC. SLAM family members are now recognized as important immunomodulatory receptors with roles in cytotoxicity, humoral immunity, autoimmunity, cell survival, lymphocyte development, and cell adhesion. In this review, we cover recent findings on the roles of SLAM family receptors and the SAP family of adaptors, with a focus on their regulation of the pathways involved in the pathogenesis of XLP and other immune disorders.

X-LINKED LYMPHOPROLIFERATIVE DISEASE

X-linked lymphoproliferative disease (XLP) is a rare genetic disorder that is generally characterized by a fatal response to infection with the Epstein-Barr virus (EBV) (1–7). EBV infects mature B lymphocytes, leading to a lymphocytosis characterized by a large expansion of lymphocytes that are primarily reactive CD8 T cells. Patients with XLP fail to clear EBV-infected B cells and have a massive lymphocytic expansion, which can lead to infiltration of the liver, bone marrow, and other organs. XLP patients also develop hemophagocytic lymphohistiocytosis syndrome (HLH) in which innate immune cells become hyperactivated and phagocytic; these complications are often fatal (8–11). Although EBV was initially identified as the catalyst for the presentation of XLP (1, 3, 4), it is now clear that several of the clinical features of XLP are EBV independent (11). This conclusion has been strengthened by the discovery of the gene responsible for most XLP cases, *SH2D1A*, which encodes the adaptor molecule SAP that binds to the SLAM family of cell surface receptors (12–14). SAP-deficient patients who survive EBV infection, or who have never been infected by EBV, still develop lymphoproliferative disorders, usually of B cell origin, and dysgammaglobulinemias that can progress to frank hypogammaglobulinemia with a similar incidence (8, 10, 15, 16). More recently, studies of gene-targeted mice and lymphocytes from XLP patients have revealed that XLP is associated with other immune abnormalities, including impaired germinal center (GC) formation and long-term humoral immunity (17–21); defects in the development of innate-type T lymphocyte subsets, including natural killer T (NKT) cells (22–24); and impaired T cell apoptosis (25–27). These findings underscore the diverse nature of this disorder and highlight its phenotypic overlap with other lymphoproliferative and immunodeficiency states, including common variable immunodeficiency (CVID), autoimmune lymphoproliferative syndrome (ALPS), and HLH (28–31). Indeed, several male patients initially diagnosed with CVID, primary HLH, or EBV^- non-Hodgkin's B cell lymphoma were subsequently found to have mutations in *SH2D1A* (28, 30–34). Thus, XLP is increasingly recognized as part of the differential diagnosis of a growing number of primary immunodeficiencies and immune disorders.

Despite the description of this disease over 35 years ago and the identification of the major genetic cause over 10 years ago, much about the broad nature of the pathogenesis of XLP remained unknown, including the profoundly abnormal responses to EBV, the predisposition to lymphoproliferative disorders, and the nature of the humoral defects. In this review, we examine recent findings on SAP and the SLAM family receptors that help provide insight into disease pathogenesis. However, it should be noted that SLAM family members have diverse cell functions, including cells that do not express SAP. Some of these are associated with functions of the related adaptor molecules EWS-Fli1-activated transcript-2 (EAT-2) and, in mice, EAT-2-related transducer (ERT), which are expressed in other hematopoietic cells, while others may be related to functions independent of these adaptors.

Although SAP has been implicated in most XLP cases, patients with fatal infectious mononucleosis and other XLP-like features have been recently found to have mutations that affect two additional proteins, XIAP (encoded by *BIRC4*) (7, 35–37) and ITK (IL-2-inducible T cell kinase) (38). XIAP deficiency is now designated XLP2 to distinguish it from SAP deficiency (which is termed XLP1) and is associated with a high incidence of HLH and a lack of lymphomas (36, 39). Mutations affecting *ITK*, a tyrosine kinase involved in T cell receptor (TCR) signaling, were reported in two sisters with fatal infectious mononucleosis and immunodeficiency (38). How mutations affecting *XIAP* and *ITK* lead to abnormal responses to EBV remains unclear, as does whether these mutations affect signaling from SLAM family receptors.

THE SAP FAMILY OF SH2 DOMAIN–CONTAINING PROTEINS

In 1998, two groups used positional cloning to identify a gene, *SH2D1A* (also called *DSHP*), which was mutated in most XLP1 cases (12, 13). At the same time, a third group identified SAP as a binding partner for the cytoplasmic domain of the costimulatory receptor SLAM and demonstrated that the gene encoding SAP was deleted or otherwise mutated in patients with XLP1 (14). Surprisingly, this gene encoded only a 128 amino acid protein (14 kDa), consisting almost entirely of a single Src homology 2 (SH2) interaction domain, which binds phosphorylated tyrosine residues. SAP is expressed primarily in lymphocytes, specifically T, NK, and NKT cells, as well as in eosinophils and platelets (12–14, 23, 40–47). Expression in transformed B cells and B cell lines has also been reported; however, expression in normal B cells has not been consistently observed (13, 20, 48–50).

The SAP SH2 domain has the greatest homology to the SH2 domains of EAT-2, SH2 domain–containing inositol-5-phosphatase (SHIP), and Abl (13, 14). Like SAP, EAT-2 (*Sh2d1b*) and ERT (*Sh2d1c*) are composed of a single SH2 domain and a short C terminus (51–53). EAT-2 has been detected in NK cells, dendritic cells (DCs), human CD8 T cells, macrophages, and platelets (44, 52, 54). Transcripts have also been detected in B cells and activated T cells (51, 56, 57). In humans, *SH2D1C* (*ERT*) is a pseudogene and not expressed (51), whereas murine ERT is exclusively found in NK cells (52).

SLAM FAMILY MEMBERS

Overview

It is now recognized that SLAM, the receptor to which SAP binds, is a member of a family of related proteins that broadly consists of SLAM (CD150, Slamf1), CD48 (Slamf2), 2B4 (CD244, Slamf4), Ly9 (CD229, Slamf3), CD84 (Slamf5), NK-T-B-antigen (NTB-A; also known as SF2000 in human or Ly108 in mouse, CD352, Slamf6), CD2-like receptor activating cytotoxic cells (CRACC; also known as CS1, CD319, Slamf7), B lymphocyte activator macrophage expressed (BLAME, Slamf8), and SF2001 (CD84H, Slamf9). These receptors are considered to be a subset of the greater CD2 superfamily of immunoglobulin (Ig) domain–containing molecules and are expressed on the surface of a wide variety of hematopoietic cells (**Table 1**). Cellular, biochemical, and genetic data suggest that these immunomodulatory receptors display diverse functions, including roles in regulating costimulation (SLAM) (18, 58, 59); T cell cytokine production (SLAM, Ly9, 2B4, CD84, Ly108) (58–68); NK cell– and CD8 T cell–mediated cytotoxicity (2B4, NTB-A/Ly108, and CRACC) (62, 69–81); adhesion between hematopoietic cells (CD84, Ly108, 2B4, and SLAM) (44, 82–85); T cell reactivation-induced cell death, RICD (NTB-A) (27); the development of innate T lymphocytes (SLAM and Ly108) (22–24, 86–91); as well as functions of neutrophils (Ly108) (64) and macrophages (SLAM) (67, 92) (**Table 2**).

The SLAM Receptor Gene Family

Most members of the SLAM family are encoded in a gene cluster on synteneic regions of mouse and human chromosome 1: The human and mouse genes encoding *BLAME* (93) and *SF2001* (94) as well as human *SH2D1B* (*EAT-2)* (53) and mouse *Sh2d1b* (*Eat-2*) and *Sh2d1c* (*Ert)* (52) are located close to the main SLAM locus (**Figure 1**). The genomic organization, location, and sequence suggest that the genes encoding the SLAM family arose from duplications of a common ancestral precursor (95–97). Multiple splice forms and polymorphisms have been identified for many of the genes encoding SLAM family members and in some cases are associated with altered biology, particularly predispositions to autoimmunity (see below).

The SLAM family members are type I glycoproteins characterized by an amino-terminal Ig variable (V)–like domain lacking canonical disulfide bonds and a membrane-proximal C2

Table 1 Expression pattern of SLAM receptor family members[a]

Receptor	Ligand	Expression pattern	References
SLAMF1 SLAM CD150	SLAM (CD150) Measles virus (H) Gram-negative OmpC and OmpF	HSCs, thymocytes (highest DP), B cells, DCs, activated T, macrophages, platelets, GC T_{FH}	14, 40, 41, 44, 58, 68, 83, 87, 92, 96, 114, 120, 183, 238, 284, 286
SLAMF2 CD48	CD2 CD244 (2B4) FimH	Widely expressed hematopoietic cells	105, 238, 288, 289
SLAMF3 Ly9 CD229	Ly9 (CD229)	Thymocytes, T, T_{FH}, NKT, B, NK (low), macrophages, DC	99, 100, 125, 140, 184, 234
SLAMF4 2B4 CD244	CD48	MPP, NK, γδ, activated CD8 T, CD8 iELs, monocytes, basophils, eosinophils	41, 74, 80, 81, 191, 201, 238, 290–294
SLAMF5 CD84	CD84	NK, NKT, B, T, monocytes, platelets, DC, eosinophils, neutrophils, T_{FH}	41, 44, 65, 83, 125, 182, 183, 234, 235, 295, 296
SLAMF6 (CD352) Human: NTB-A Mouse: Ly108	Human: NTB-A Mouse: Ly108	Thymocytes (highest DP), NK, T, B, DC (low), eosinophils, neutrophils, T_{FH}	41, 73, 83, 87, 94, 112, 297
SLAMF7 CRACC, CS1, CD319	CRACC (CD319)	NK, B, DC, plasma cells, activated CD4 and CD8 T	70, 71, 113, 125, 136, 298
SLAMF8 BLAME	No data		93
SLAMF9 SF2001 CD84-H1	No data		94, 108, 109

[a]Abbreviations: DC: dendritic cell; FimH: lectin expressed by (type 1 fimbriated) *Escherichia coli*; GC: germinal center; H: hemagglutinin; HSCs: hematopoietic stem cells; iELs, intraepithelial lymphocytes; MPP: multipotent hematopoietic progenitors; Omp: outer membrane protein; SLAM: signaling lymphocyte activation molecule; T_{FH}: T follicular helper cell.

domain containing two conserved disulfide bonds (98). Ly9 is the exception, in which the V-C2-like sequences are duplicated, resulting in an extracellular domain containing four Ig-like domains (99, 100). SLAM family members contain one or more immunoreceptor tyrosine-based switch motifs (ITSMs)—TxYxxI/V ("x" denotes any amino acid)—in their cytoplasmic domains (**Figure 2**) (14, 101), which have high affinity for SAP and/or EAT-2 (14, 56, 102, 103) (**Figure 2**). Although CD48 is encoded within the SLAM cluster and has homology in its extracellular domain, it is a glycosyl-phosphatidylinositol-anchored protein (104, 105); we refer readers to other reviews of its function (106). However, it is important to note that CD48 is the ligand for 2B4 and, although it is expressed on nearly all hematopoietic cells, it is highly upregulated on B cells following EBV infection (107). BLAME and SF2001 have short intracellular tails that lack any tyrosine motifs and may represent orphan ligands (93, 94, 108, 109). In this review, we focus on the six core SLAM family members that contain ITSMs: SLAM (SLAMF1), Ly108/NTB-A (SLAMF6), CD84 (SLAMF5), Ly9 (SLAMF3), 2B4 (SLAMF4), and CRACC (SLAMF7) (**Figure 2**).

Although SLAM family members share characteristics with CD2 and CD58 (human), the nucleotide and amino acid sequence identity between these receptor subgroups is low (<15%) (110). With the exception of 2B4, SLAM family members are homophilic

Table 2 Signal transduction pathways activated downstream of SLAM receptor family members and phenotype of mice deficient in SLAM family members[a]

Receptor	ITSM	Effectors	Phenotype of knockout mice	References
SLAMF1 SLAM CD150	Human: 2 Mouse: 2	Thymocytes: SAP, Fyn, SHIP, Dok1, Dok2, SHC, Ras-GAP T cells: SAP, Fyn, Akt, PKCθ, NF-κB, SHP-1, SHP-2 B cells: Fgr/Lyn, SHIP, SHP-2, Akt Platelets: SAP Macrophages: Vps34-Vps15-beclin complex	*Slam*$^{-/-}$ CD4 T cells: ↓ IL-4 and IL-13, ↑ IFN-γ production GC T_{FH} cells: ↓ IL-4 production Macrophages: ↓ NO, IL-12, TNF-α production, ↑IL-6 production, ↓ phagosome maturation Platelets: ↓ aggregation NKT development: in conjunction with Ly108	14, 18, 44, 59, 61, 67, 68, 87, 92, 101, 120, 140, 148, 150, 152, 154, 155, 161, 211, 299
SLAMF2 CD48	None		*Cd48*$^{-/-}$ T cells: ↓ proliferation and IL-2 production	300
SLAMF3 Ly9 CD229	Human: 2 Mouse: 2	T cells: SAP, Fyn, Grb2, μ2 AP-2, ERK, SHP-2	*Ly9*$^{-/-}$ T cells: ↓ proliferation, ↓ IL-4 and IL-2 production	63, 66, 116, 140, 153, 180
SLAMF4 2B4 CD244	Human: 4 Mouse: 4	NK cells: SAP, EAT-2/ERT, Fyn, LAT Vav1, CBL, PI3K, Ca^{2+} Flux, ERK1/2, 3BP2, CSK, SHP-1, SHP-2, SHIP CD8 T cells: SAP	*2b4*$^{-/-}$ NK cells: ↑ cytotoxicity	52, 62, 78, 80, 82, 85, 118, 139, 149, 160, 164, 165, 167, 169, 170, 172–174, 195
SLAMF5 CD84	Human: 2 Mouse: 2	T cells: SAP B cells: EAT-2, SHP-1, SHP-2	*Cd84*$^{-/-}$ T-B cell adhesion, impaired GC, ↓ T_{FH} cells, ↓ IL-21 production	57, 83, 140, 142, 182
SLAMF6 (CD352) Human: NTB-A Mouse: Ly108	Human: 2 Mouse: 2	Thymocytes: SAP, Fyn, Vav1, CBL T cells: Ca^{2+} flux, SAP, SHP-1 B cells: Ca^{2+} flux NK cells: SAP, SHP-1	*Ly108*$^{(\Delta 2+3)/(\Delta 2+3)}$ T cells: ↓ IL-4 production Neutrophils: ↓ ROS production, ↑ IL-12, IL-6, TNF-α production, ↓ bacterial killing NKT cells: ↓ cell number CD4 T: slight ↓ cell number	27, 64, 69, 87, 90, 123, 124, 127
SLAMF7 CRACC, CS1, CD319	Human: 1 Mouse: 1	NK cells: EAT-2, PLCγ1, PLCγ2, Ca^{2+} Flux Beads: SHP-1, SHP-2, Fyn, SHIP, CSK	*Cracc*$^{-/-}$ NK cells: ↓ killing targets, ↓ Ca^{2+} Flux CD4 T cells: ↓ IL-2, ↓ IFN-γ, ↓ proliferation	54, 70, 71, 301

[a]Abbreviations: 3BP2: Abl-SH3 binding protein 2; CBL: casitas *B*-lineage lymphoma; CD4 T: thymocyte-selected CD4 T cells; CSK: COOH-terminal Src kinase; Dok1/2: Docking protein 1/2; EAT-2: Ewing's sarcomas EWS/FLI1 activated transcript-2; ERT: EAT-2-related transducer; Grb-2: growth factor receptor–bound protein 2; LAT: linker for activated T cells; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: phosphoinositide 3-kinases; PLCγ: phospholipase Cγ; SAP: SLAM-associated protein; SHC: Src homology 2 containing; SHIP: SH2-containing inositol polyphosphate 5-phosphatase; SHP-1/2: SH2 domain–containing phosphatase 1/2; SLAM: signal lymphocyte activation molecule.

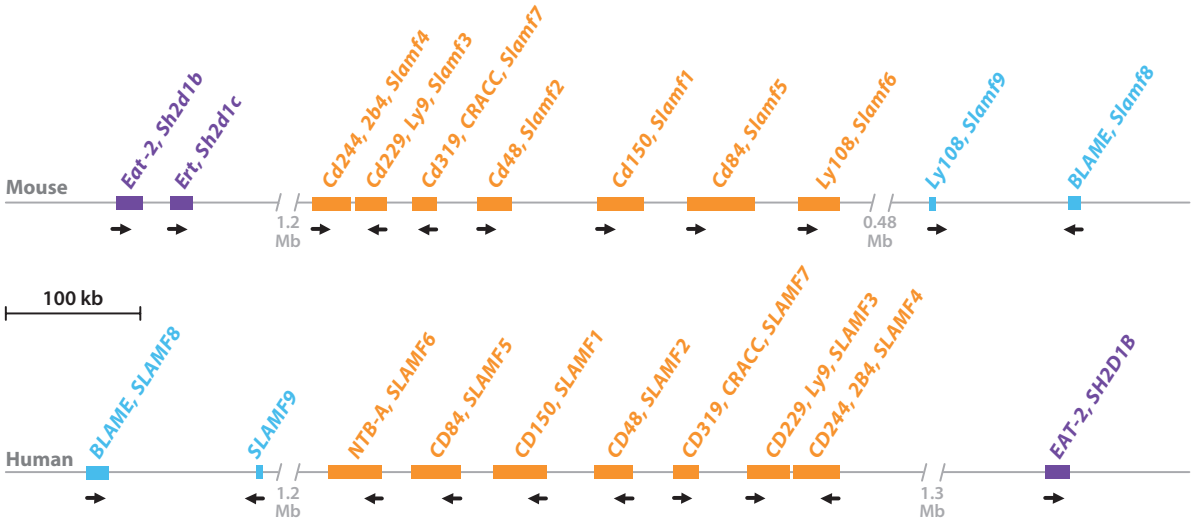


Figure 1

Genomic organization of the mouse and human *Slam* locus. The genes encoding members of the SLAM receptor family (SLAM locus) are located on human chromosome 1q23 and mouse chromosome 1H2. The genes encoding the SLAM family members are clustered in a genomic segment of 359 kb in humans and 392 kb in mice. Two genes (*SLAMF8* and *SLAMF9*) that belong to the SLAM family, which do not bind to SAP and/or EAT-2, are located in the same chromosome region, but outside of the SLAM locus. Human *SH2D1B* (*EAT2*) as well as mouse *Sh2d1b* (*Eat2a*) and *Sh2d1c* (*Eat2b*) are also located close to the *Slam* locus. The arrangement of the SLAM gene family is identical in mouse and human genomes with the exception of the gene orientation relative to the centromere. Black arrows signify the transcriptional direction of these genes.

receptors and thus are self-ligands (65, 73, 111–117). Data obtained from structural analysis have indicated that, except for Ly9, the homophilic and heterophilic interactions of these receptors span a distance consistent with localization within the T cell–antigen-presenting cell (APC) or NK cell–target cell interface (approximately 105 Å) (111, 114, 117–119). Despite the large binding interface of these receptors, changes in a single residue can significantly alter association (111, 116, 117, 119). Within the SLAM family, ligand affinities span three orders of magnitude (SLAM ~200 μM, 2B4-CD48 ~4 μM, NTB-A ~2 μM, CD84 sub-μM) (111, 114, 117, 119), which could contribute to functional differences between the various receptors.

Differential Isoform Usage of SLAM Family Members

Several different alternatively spliced isoforms of SLAM (58, 120), CD84 (121), CRACC (122), Ly108 (123, 124), Ly9 (125), and 2B4 (126) have been identified that differ in the length of the cytoplasmic domain. Notably, although Ly108 contains two ITSMs in its cytoplasmic domain, the Ly108.1 isoform has one additional unique tyrosine motif, whereas the Ly108.2 isoform contains two additional unique tyrosines (123, 127). Additional isoforms have also been reported (124). These isoforms are differentially expressed in mouse strains that have high antinuclear antibodies (ANAs) and can potentially elicit different downstream signaling pathways (see the sections below on Signal Transduction and on Autoimmunity) (123, 127). Furthermore, murine 2B4 has two isoforms: a short activating form (2B4-S) and a long inhibitory variant (2B4-L) differing in the number of ITSMs (126, 128). Because only 2B4-L is found in humans and is activating, the relevance of these features is not clear. Nonetheless, alterations in the number of ITSMs influence SAP and EAT-2 recruitment and thus affect signal transduction events. Two

splice variants of human 2B4 have been identified: 2B4-B has five additional amino acids between the V and C2 regions compared with 2B4-A (129, 130). However, whether this influences 2B4/CD48 receptor/ligand interactions remains to be determined.

In addition to splicing variants, multiple polymorphisms have been identified in the SLAM locus that segregate into two major haplotypes in inbred mouse strains. Murine Ly9 was initially identified as an alloantigen marker: The *Ly9.1* allele is expressed in most inbred mouse strains, whereas *Ly9.2* is expressed in C57Bl/6 and related strains (99). Sequence analysis of Balb/c and C57Bl/6 identified nine differences, four of which are located in the ligand-binding surface (131–133). 2B4 is also polymorphic: The 2B4 monoclonal antibody (mAb) recognizes 2B4 in C57Bl/6 and C58/J mice, whereas the C9.1 mAb recognizes 2B4 in most other strains including NZB, SJL, C3H, CBA, BALBc, DBA, A/J, and 129, which possess four copies of the gene (134). Moreover, Wakeland and colleagues (135) found that the ligand-binding domains of Ly9, CD48, and CD84 were highly polymorphic in natural mouse populations, suggesting that selection favored variability.

SIGNAL TRANSDUCTION BY SLAM FAMILY MEMBERS

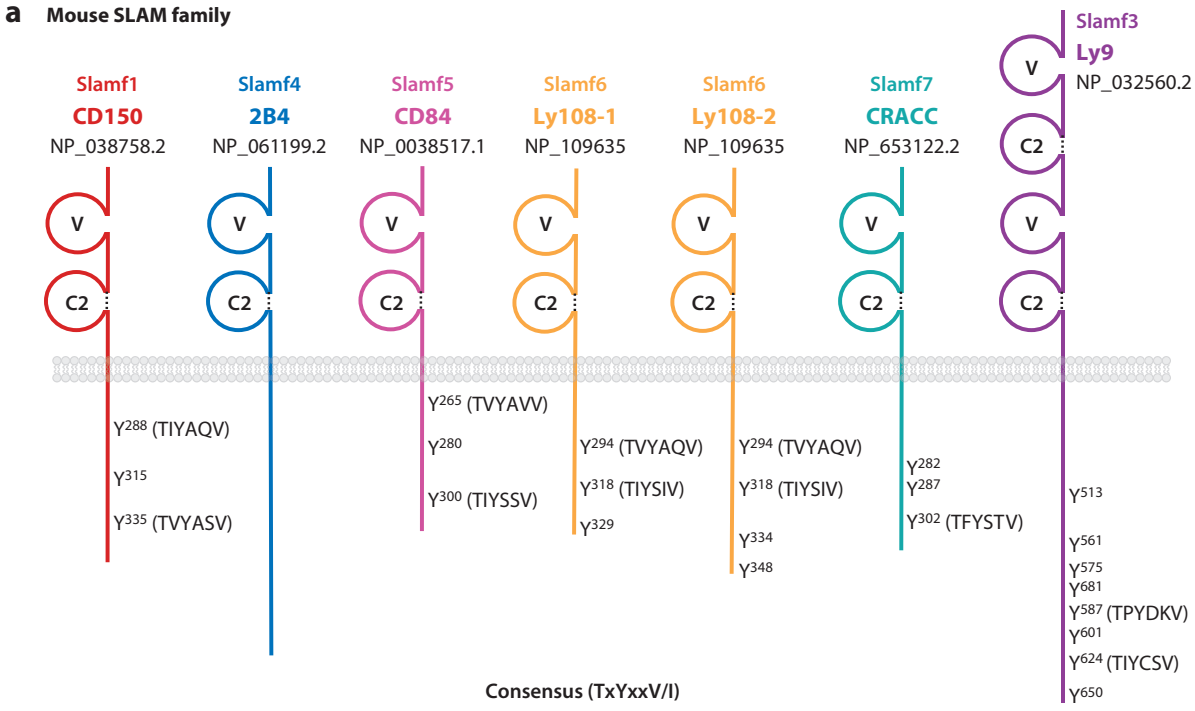
SAP Recruitment to SLAM Family Members

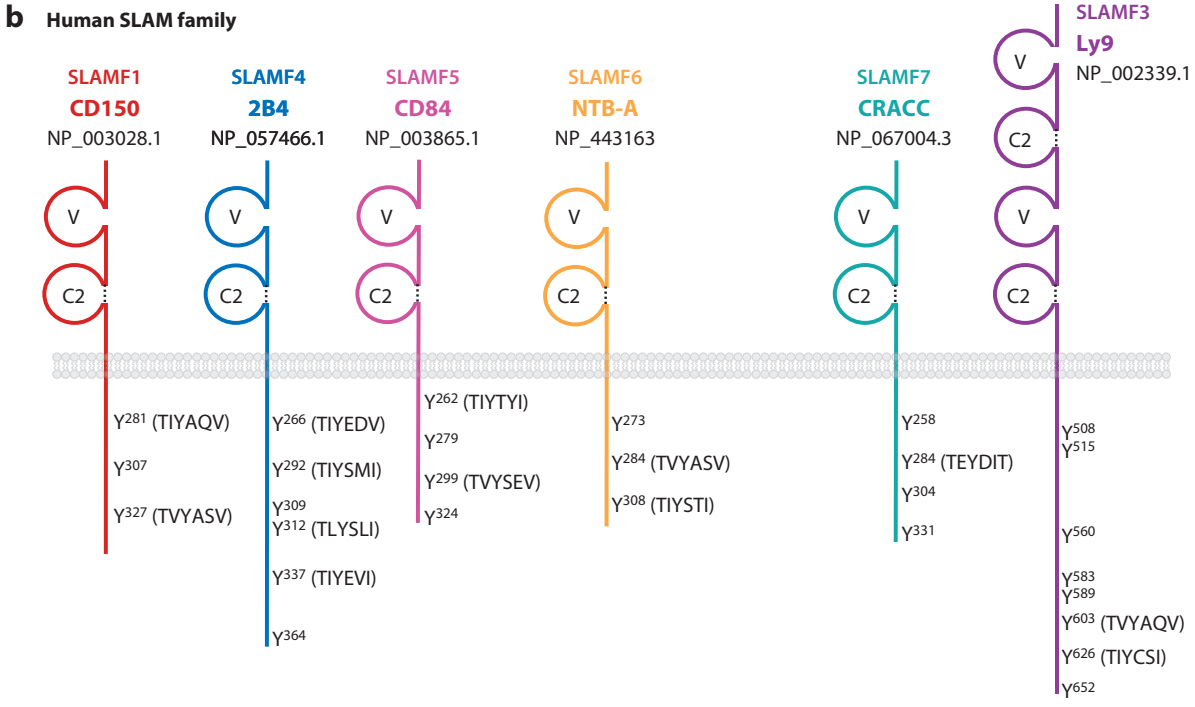
A key feature of the SLAM family of surface receptors is the presence of one or more SAP-binding ITSMs in their cytoplasmic domains (14, 101). The exception is CRACC: Although one study showed binding of SAP to human CRACC with low affinity (136), most studies indicate that human and murine CRACC only associate with EAT-2 (54, 70, 71) (see **Table 2** and **Figures 2** and **3**).

Although most SH2 domains require phosphorylated tyrosines for binding, SAP is unusual in that it can bind to the membrane proximal ITSM in the cytoplasmic tail of SLAM via a three-pronged binding mechanism. This structure stabilizes binding to the nonphosphorylated tyrosine, although binding is improved following phosphorylation (102, 103, 137, 138). In contrast, tyrosine phosphorylation of ITSMs is required for SAP binding to other SLAM family members, including 2B4 (69, 78, 139), NTB-A (69), Ly108 (124), Ly9 (140), and CD84 (57, 140).

When SAP was first identified, it was unclear how a single SH2 domain could participate in signal transduction and contribute to the diverse range of XLP1 phenotypes. SH2 protein interaction domains are usually part of larger proteins—which contain other interaction domains (adaptor proteins) or contain enzymatic activity—that are regulated by intra- or intermolecular interactions involving the SH2 domain. Initial studies, including the seminal cloning paper from the Terhorst group (14), suggested that SAP functioned to compete with binding of other SH2 domain–containing proteins to SLAM, hence the naming of the SAP-binding motif as a switch motif (ITSM) to distinguish it from immunoreceptor tyrosine activation motifs (ITAMs), which recruit Syk kinases, and immunoreceptor tyrosine inhibitory motifs (ITIMs), which recruit phosphatases (141). Consistent with this idea, SAP expression blocked the recruitment of the SH2 domain–containing protein tyrosine phosphatase (SHP)-2 to tyrosine phosphorylated SLAM residues (14), suggesting that SAP sterically hindered recruitment. Analyses of primary NK and CD8 cells, cell lines, and overexpression studies indicate that SAP also competes with SHP-1 and/or SHP-2 for recruitment to SLAM, CD84, 2B4, NTB-A, and Ly9 (14, 27, 69, 78, 101, 120, 139, 140, 142, 143).

Interestingly, both SAP and SLAM are recruited to the immunological synapse (the site of T cell–APC contact) (61, 144), and anti-CD3 stimulation was shown to increase SLAM phosphorylation (144). Similarly, 2B4 and NTB-A are recruited to the cytolytic synapse in NK and CD8 T cells (62, 145, 146). Moreover, TCR engagement on CD8 T cell blasts induces NTB-A recruitment to TCR/CD3 clusters





and increases NTB-A/SAP association while decreasing NTB-A/SHP-1 association (27). It is notable that a negative feedback pathway involving SHP-1 has been implicated in the discrimination between weak and strong ligands during TCR signaling and in activating and inhibitory signaling of NK cell receptors (147). Thus, SLAM/SAP signaling may prevent inhibitory signals by altering phosphatase recruitment, thereby enhancing TCR/NK cell–mediated signal transduction at the site of APC-target interaction and promoting cell activation.

However, an alternative view of SAP-mediated transduction arose from elegant studies from the groups of Veillette, Terhorst, and Eck, who demonstrated that SAP recruited the Src family tyrosine kinase Fyn to SLAM. Following ligation of SLAM, 2B4, or Ly108, SAP interacts with and recruits Fyn, leading to subsequent receptor tyrosine phosphorylation and the recruitment of downstream signaling intermediates (124, 148–150). Data suggest that an inducible SAP-Fyn interaction is initiated by a conformational change of SAP bound to SLAM (151). This interaction occurs between the SH3 domain of Fyn and an arginine-based motif in SAP that lies outside of the phosphotyrosine binding pocket, thus allowing SAP to simultaneously interact with a SLAM family member and Fyn (143, 148, 152). Mutation of a critical arginine (R78) of SAP dramatically reduces the ability of SAP to recruit Fyn and induce SLAM phosphorylation (143, 148, 152). This interaction appears specific for Fyn: Tyrosine phosphorylation of SLAM, Ly108, and 2B4 is markedly impaired in thymocytes and NK cells from either *Sh2d1a*$^{-/-}$ or *Fyn*$^{-/-}$ mice (124, 148–150, 152). However, data from a two-hybrid screen and glutathione S-transferase-pulldowns demonstrated a potential interaction of SAP with the kinase domain of Lck (153). In some of the original work implicating Src kinases in SLAM signal transduction, Fgr and Lyn were also associated with SLAM in B cell lines (154, 155). Interestingly, CD84 can be tyrosine phosphorylated in T cells from XLP1 patients (57), indicating that CD84 does not require SAP-mediated Fyn recruitment for receptor tyrosine phosphorylation, while still requiring SAP for downstream signal transduction. Thus, depending on the cell type, state of activation, and specific SLAM family member engaged, multiple mechanisms may exist for recruitment of Src family kinases. Notably, these two views of SAP-mediated signaling, in which SAP either acts as a competitor or acts as an adaptor to recruit Src kinases, may not be mutually exclusive and thus may contribute to varying effects of SLAM family signaling in the presence or absence of SAP (143).

EAT-2-Mediated Signal Transduction

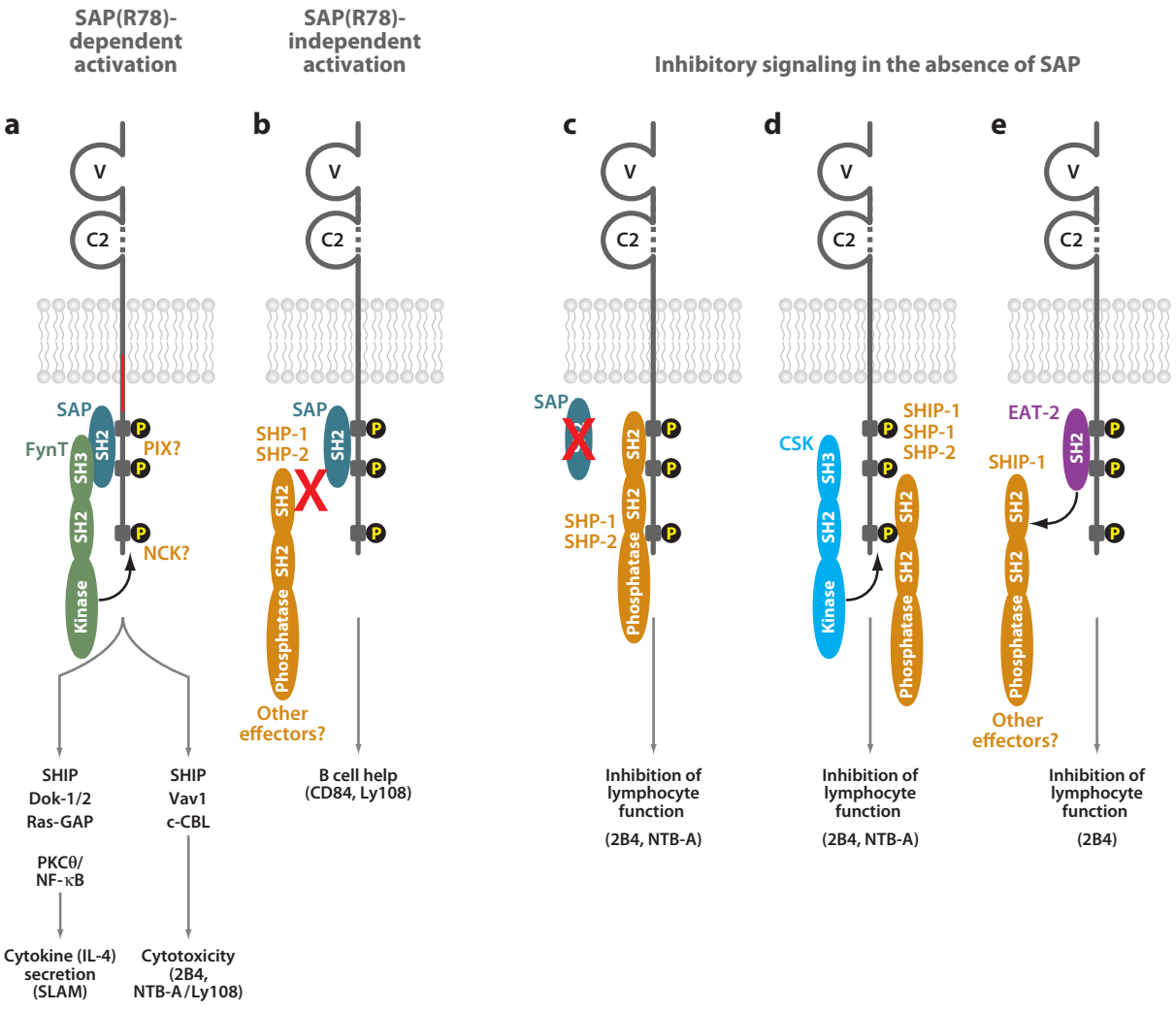
EAT-2 shares ~50% amino acid identity with SAP. Biochemical analysis of transfected cell lines demonstrated that EAT-2 is recruited to ITSMs of SLAM, Ly9 (56), CD84 (56, 57), 2B4 (54, 56), and CRACC (54). Despite their sequence homology, SAP and EAT-2 exhibit distinct features. Notably, the predominant ITSMs in the cytoplasmic domains of NTB-A,

Figure 2

SLAM receptor family members. SLAM family members are type I glycoproteins characterized by an N-terminal Ig V-like domain lacking canonical disulfide bonds and a membrane-proximal C2 domain containing two conserved disulfide bonds. The cytoplasmic tails of (*a*) mouse and (*b*) human SLAM, 2B4, CD84, Ly108/NTB-A, Ly9, and CRACC contain at least one ITSM, the binding site for SAP and EAT-2, as well as additional tyrosine residues. Only the mouse long 2B4 isoform is shown: The shorter variant contains only the membrane-proximal ITSM (128). Multiple isoforms of CD84, SLAM, CRACC, and Ly108/NTB-A have also been found. The numbers indicate the tyrosine position relative to the protein's N-terminal amino acid. Accession numbers are indicated below the protein name and correspond to Ensembl ID. Readers should note the difference between the NCBI and published sequence (71). (Abbreviations: CRACC, CD2-like receptor activating cytotoxic cells; EAT-2, EWS-Fli1-activated transcript-2; ITSM, immunoreceptor tyrosine-based switch motif; NTB-A, NK-T-B-antigen; SAP, SLAM-associated protein; SLAM, signaling lymphocyte activation molecule.)

2B4, and CD84 that bind SAP and EAT-2 are different (56, 57, 62). This likely explains the ability of NTB-A to associate simultaneously with SAP and EAT-2 (72). Moreover, although SAP can bind nonphosphorylated SLAM, association with EAT-2 is strictly phosphotyrosine dependent (56). Lastly, although recruitment of EAT-2 to CRACC increases upon receptor tyrosine phosphorylation, data suggest that its binding to 2B4 decreases (54). Thus, SLAM receptors interact with adaptor proteins with different affinities, and the quality of the interaction can be modified by changes in the phosphorylation state of SLAM receptors.

EAT-2 and ERT do not contain the arginine motif found in SAP(R78) that is responsible for Fyn binding. Rather, these molecules have tyrosine residues (two in murine EAT-2 and ERT, one in human EAT-2) in the C-terminal domain that are phosphorylated and required for mediating signaling (52). Overexpression studies and Biacore binding analyses provided evidence that Fyn can associate with the phosphorylated tyrosine residues in EAT-2 (51, 156), while yeast two-hybrid analysis indicated that EAT-2 directly binds to the catalytic domain of Src family kinases (51). These data suggest that EAT-2 and ERT



couple Src family kinases to SLAM family members via mechanisms distinct from SAP.

Signal Transduction Independent of SLAM Family Members

Although SAP was initially identified as a SLAM binding partner, evidence indicates that SAP-related adaptors influence signaling from other receptor families. SAP was shown to bind the neurotrophic receptor TrkB and to mediate signal transduction in the rat pheochromocytoma cell line PC12 (157). More recently, associations between SAP and CD22 as well as FcγRIIB were identified (158, 159). The physiological relevance of these interactions remains to be determined, as CD22 and FcγRIIB are predominantly expressed by B cells, which may not express SAP.

Interestingly, NK cells lacking EAT-2, ERT, or all three SAP-related proteins demonstrated enhanced activation in response to nonhematopoietic target cells that do not express SLAM family ligands (53, 160). Thus, EAT-2 and ERT may provide inhibitory signals downstream of other receptors. Alternatively, EAT-2 and ERT could signal via NK cell–NK cell interactions, endowing the cells with specific functions that influence other receptor pathways.

Signal Transduction Downstream of SLAM

SLAM family receptors have been shown to associate with distinct downstream signaling intermediates. SLAM ligation on thymocytes, or a T cell line, enhances SAP recruitment, SLAM tyrosine phosphorylation, and the recruitment and phosphorylation of SHIP, docking protein 1 (Dok1), Dok2, and Ras-GTPase-activating protein (Ras-GAP) (150) (**Figure 3**). This signaling cascade is dependent on the generation of a ternary SLAM-SAP-Fyn complex for SLAM phosphorylation and is defective in SAP-deficient, SAP(R78A)-expressing, and *Fyn*$^{-/-}$ thymocytes (148, 150, 152, 161).

However, SLAM-SAP interactions are likely to mediate other signal transduction pathways. SLAM cross-linking on B and T cells results in AKT activation (59, 155), which is activated downstream of phosphatidylinositol 3-kinase (PI3K). In CD4 T cells, SLAM engagement also potentiates and prolongs PKCθ recruitment to the site of APC contact in a SAP-dependent manner, as well as influences Bcl-10 phosphorylation and patterns of NF-κB activation (61). Although this interaction requires the R78 motif of SAP, SAP and PKCθ coimmunoprecipitate in both wild-type and *Fyn*$^{-/-}$ T cells (162). In overexpression studies, SAP

Figure 3

Signal transduction by SLAM family members. (*a*) SAP(R78)-dependent activation: SAP associates with the ITSM in the cytoplasmic domain of SLAM. The arginine 78 (R78) of SAP binds the SH3 domain of Fyn and recruits Fyn to the SLAM/SAP complex; Fyn subsequently phosphorylates tyrosine residues in the cytoplasmic domain of SLAM. These tyrosine-phosphorylated residues act as docking sites for SHIP, leading to tyrosine phosphorylation of the adaptor proteins Dok1 and Dok2 and Ras-GAP. SAP also contributes to signaling through the TCR by interacting with PKCθ and influencing Bcl-10 and patterns of NF-κB activation. SLAM-mediated pathways dependent on SAP(R78) influence CD4 T cell cytokine production. In addition, SAP is recruited to 2B4 and Ly108, leading to Vav1 and CBL phosphorylation, and is thought to affect NK cell– and CD8 T cell–mediated cytotoxicity. (*b*) SAP(R78)-independent activation: GC formation is rescued in *Sh2d1a*$^{-/-}$ mice by the transfer of retrovirally reconstituted SAP-deficient CD4 T cells expressing SAP or SAP(R78A). CD84 is tyrosine phosphorylated in the absence of SAP, and both CD84 and Ly108 participate in stable T-B cell conjugate pairing in vitro. (*c–e*) Inhibitory signaling in the absence of SAP. (*c*) SAP can block or sterically hinder the recruitment of phosphatases (SHP-1 and SHP-2) to SLAM family members including SLAM, CD84, 2B4, Ly9, and NTB-A. (*d*) 2B4 and NTB-A can recruit CSK, leading to receptor phosphorylation and the subsequent recruitment of SHIP, SHP-1, and SHP-2 phosphatases. (*e*) *Sh2d1b*$^{-/-}$ and/or *Sh2d1c*$^{-/-}$ mice revealed that EAT-2 and ERT can play either negative or positive roles in 2B4-mediated NK cell cytotoxicity. In addition, in the absence of SAP, EAT-2, and ERT, 2B4 engagement results in elevated SHIP phosphorylation. (Abbreviations: CSK, C-terminal Src kinase; Dok1/2, docking protein 1/2; EAT-2, EWS-Fli1-activated transcript-2; ERT, EAT-2-related transducer; ITSM, immunoreceptor tyrosine-based switch motif; NK cell, natural killer cell; NTB-A, NK-T-B-antigen; PKCθ, protein kinase Cθ; Ras-GAP, Ras-GTPase-activating protein; SAP, SLAM-associated protein; SHIP, SH2 domain–containing inositol-5-phosphatase; SHP-1/2, SH2 domain–containing protein tyrosine phosphatase 1/2; SLAM, signaling lymphocyte activation molecule; TCR, T cell receptor.)

also bound to PAK-interacting exchange factor (PIX), leading to synergistic NFAT (nuclear factor of activated T cells) activation in conjunction with ionomycin in Jurkat T cells (163) and to NCK1 (noncatalytic region of tyrosine kinase 1) (158) via the R78 motif. Thus, the R78 motif of SAP mediates several protein interactions, which may not all require SAP's recruitment of Fyn.

In B cells lines, SLAM associates with Fgr and/or Lyn (154, 155). B cell receptor (BCR) ligation induced tyrosine phosphorylation of SLAM, whereas anti-SLAM treatment resulted in reduced SLAM phosphorylation (154). SLAM ligation induced SHIP binding, but unlike T cells, this was not SAP dependent and resulted in reduced SHIP phosphorylation (101, 154). In light of more recent data on SLAM signal transduction, the antibody used may have been inhibitory. SLAM phosphorylation has also been observed in *Sh2d1a*$^{-/-}$ platelets (44). These data suggest that SLAM-mediated signal transduction pathways differ depending on the cell type examined.

Complexities of Signal Transduction Events in NK Cells: 2B4, Ly108/NTB-A, and CRACC

Engagement of 2B4 on murine NK cells results in phosphorylation of all ITSMs (149, 164) and induces a distinct SAP-dependent pathway. SAP can recruit Fyn to 2B4, leading to increased 2B4 phosphorylation and downstream phosphorylation of Vav1 and c-Casitas B-lineage lymphoma (c-CBL) (85, 149, 165). Synergy between NKG2D and 2B4 engagement on primary resting NK cells was required for a strong Vav1 signal that could overcome inhibition by c-CBL, resulting in Ca^{2+} flux, Erk activation, and NK cell cytotoxicity (82, 118, 165–167). 2B4-mediated activation is dependent on SAP-mediated recruitment of Fyn, as receptor phosphorylation in SAP-deficient and *Fyn*$^{-/-}$ murine NK cells and NK cells from XLP1 patients is impaired (149, 168). Interestingly, 2B4 associates with the adaptor protein LAT (linker for activated T cells) in membrane glycolipid–enriched microdomains (167, 169, 170). In addition, following 2B4 ligation on human NK cells, the adaptor 3BP2 can associate with the fourth phosphorylated ITSM (171). Phosphorylated 3BP2 can interact with Vav1, LAT, and phospholipase Cγ (PLCγ) (171–173) and potentially link 2B4 to downstream events regulating cytotoxicity.

In addition to SAP, 2B4 and NTB-A can recruit the inhibitory C-terminal Src kinase (CSK) and the phosphatases SHP-1, SHP-2, and SHIP-1; SAP prevents these interactions (69, 78, 139, 174). It is possible that, in the absence of SAP, phosphorylation of ITSMs by CSK results in the recruitment of these phosphatases (174). Interestingly, Vav1 is a primary substrate for dephosphorylation by SHP-1 during inhibitory receptor engagement on NK cells (175, 176). In addition, studies of SHIP-deficient mice suggest that alterations in the balance of phosphatases expressed in NK cells influence 2B4 isoform expression and signal transduction (177). The consequences of SAP, EAT-2, and phosphatase recruitment to 2B4 are discussed below, in the Cytotoxicity section.

Analysis of primary human NK cells established that NTB-A, 2B4, and CRACC associate with both endogenous SAP and/or EAT-2 (54, 72). Coexpression of EAT-2 increased the Src kinase–induced phosphorylation of 2B4 and CRACC, suggesting that EAT-2 is required for the generation of activation signals elicited by these receptors; recent data support a positive role for EAT-2 in both 2B4- and CD84-mediated cytolysis and phosphorylation of Vav1 (54, 55). Recently, the tyrosines in the C terminus of EAT-2 were also shown to bind PLCγ (178), which is activated following CRACC cross-linking (54). Thus, EAT-2 may mediate CRACC signaling in NK cells both through recruitment of Src kinases and through PLCγ-mediated pathways. Mutations that abolish the ability of EAT-2 to associate with NTB-A abrogated NK cell cytotoxicity (72). In contrast, siRNA-mediated downregulation of SAP or mutation of the SAP-binding ITSM in NTB-A had minimal effect on NTB-A-induced killing of target cells by transformed

human NK cell lines in vitro (72). However, this finding contradicts other studies using cells from XLP1 patients, which revealed a dependency on SAP for NTB-A-mediated NK cell cytotoxicity (69) (see the section below on Expression and Function of SLAM Family Receptors on Human and Murine NK Cells).

Ly108-Mediated Signal Transduction in Lymphocytes

Ligation of Ly108 on thymocytes requires SAP and Fyn for tyrosine phosphorylation and results in phosphorylation of Vav1 and CBL, similar to 2B4 (124). However, in human CD8 T cell blasts, NTB-A still associated with SAP when Fyn expression was markedly reduced by siRNA (27). Ly108 has multiple isoforms varying in the length of the cytoplasmic domain (see **Figure 2**): Engagement of Ly108.1 resulted in increased tyrosine phosphorylation of downstream proteins compared with Ly108.2. Altered expression of Ly108 isoforms including increased expression of Ly108.1 is associated with the lupus-prone *Sle.1b* haplotype (see the Autoimmunity section) (124). Anti-CD3 stimulation of peripheral *Sle.1b* T cells that predominantly express the Ly108.1 isoform revealed mildly elevated Ca^{2+} flux compared with cells expressing higher amounts of Ly108.2 (123). In contrast, immature B cells expressing Ly108.1 stimulated with α-IgM demonstrated reduced Ca^{2+} flux (127). This apparent discrepancy may reflect the developmental profile of the cells, the cell type examined, and the relative levels of SAP. Although it is not understood how Ly108 isoforms influence TCR-induced Ca^{2+} flux, Vav1 phosphorylation is induced by Ly108 engagement (124), and Vav1 affects TCR-induced Ca^{2+} mobilization (179).

Consequences of Ly9 Engagement

Similar to SLAM, Ly9 phosphorylation is reduced in SAP-deficient thymocytes (153). Ly9 ligation resulted in receptor phosphorylation and Grb2 recruitment to a tyrosine motif (YENF) distinct from the SAP-binding site (66, 140). Following TCR and Ly9 engagement, the μ2 chain of the clathrin-associated adaptor complex (AP-2) associates with Ly9, and the receptor is internalized (180). The consequences of Grb2 association or Ly9 internalization are not known. Ly9 ligation on human T cells reduces CD3-mediated ERK activation (66), suggesting that Ly9 could serve a negative regulatory function in T cell activation. However, to date, phenotypes of *Ly9*$^{-/-}$ mice have not revealed negative roles in T cell activation (63).

Summary

The SLAM receptor family has emerged as a complex series of immunomodulatory proteins that activate multiple downstream signaling molecules. The signals transduced downstream of this receptor family may depend on the mode of stimulation (antibody versus ligand), the receptor isoform expressed, and the activation status and cell type examined. Moreover, as discussed below, the relative expression of adaptor proteins SAP and EAT-2 and phosphatases SHP-1/2 and SHIP can influence the signals that are transduced and can profoundly affect cell function (**Figure 3**).

PHENOTYPES ASSOCIATED WITH DEFICIENCIES IN SAP-RELATED ADAPTORS AND SLAM FAMILY RECEPTORS

The cloning of *SH2D1A* and the recognition that its product associated with SLAM family receptors opened a new era in the understanding of XLP1 and the roles of SLAM and SAP in normal immune function. In particular, the generation of *Sh2d1a*$^{-/-}$ mice by several independent groups (18, 49, 50, 181) has provided critical insight into the immunological defects associated with XLP1 and revealed previously unappreciated phenotypes, including defects in the development of NKT and other cells with innate-like characteristics (22–24, 88, 90) as well as impaired T-B cell interactions and defective GC formation (83, 84). In the

next section, we review the roles of SAP and the SLAM family receptors in immune cell function and development in the context of these phenotypes.

CYTOTOXICITY

One of the salient features of XLP1 is the profoundly impaired response to EBV: NK and CD8 T cells from XLP1 patients exhibit defective cytolytic responses to EBV-infected B cell lines, whereas cytotoxicity against non-EBV-infected cells appears normal, highlighting the specificity of this phenotype (62, 69, 75, 78, 79). Impairment in cytolytic function is likely to lead to the accumulation of virus-infected B cells as well as the persistence of reactive inflammatory cells, both of which lead to the exaggerated responses seen in XLP1.

Expression and Function of SLAM Family Receptors on Human and Murine NK Cells

All human NK cells express 2B4, NTB-A, and CRACC (58, 69, 70, 182), and a subset expresses Ly9 (183). Murine NK cells express 2B4, CRACC, and Ly9 (52, 71, 74, 184). In contrast to human NK cells, murine NK cells constitutively express CD84 but not Ly108 (52, 124).

The effects of engaging 2B4 with anti-2B4 mAb or CD48-expressing targets on human NK cell effector function differ depending on the activation state of the responding cell (76–78, 80–82, 185). Although ligating 2B4 alone on activated NK cells or clones strongly induces granule exocytosis, cytotoxicity, and cytokine secretion (76–78, 80, 81, 185), resting NK cells are largely unresponsive to the stimulatory effects of anti-2B4 mAb or CD48 expressed on transfected target cells (82). It has since been established that 2B4 acts largely as a coreceptor on resting NK cells, such that its function depends on concomitant engagement of other activating receptors such as NKG2D, NKp44, NKp46, DNAM-1, and/or CD16 (82, 186). Thus, integration of diverse signaling pathways downstream of multiple stimulatory receptors is required for the induction of the stimulatory function of 2B4 on resting NK cells, a requirement that may safeguard against inappropriate activation of such cells. The mechanism underlying the switch in the ability of 2B4 to behave as a coreceptor in resting cells versus an activating receptor in stimulated cells remains incompletely understood but may reflect induced changes in expression levels of components of the 2B4 signaling pathways. Indeed, human resting NK cells express little SAP but abundant levels of EAT-2 (187). Furthermore, expression of SAP, but not EAT-2, markedly increases in human NK cells stimulated in vitro with IL-2, IL-12, IFN-α, or poly(I:C), and this correlates with the ability of such NK cells to be activated following 2B4 engagement in the absence of coengagement of additional stimulatory receptors (187). Thus, SAP levels in resting NK cells may be insufficient to endow 2B4 with an activating function, and increased expression of SAP following in vitro stimulation may convert 2B4 from a coreceptor to an activating receptor.

NTB-A/Ly108 and CRACC appear to play a similar role to 2B4 on human and murine NK cells: mAb- or homotypic ligand–mediated cross-linking enhances cytotoxicity and cytokine secretion in vitro (69–71, 73, 112). Furthermore, the cytotoxic function of NTB-A on human NK cells requires coengagement of complementary activating receptors, particularly NKp46 or NKG2D (69). It is yet to be determined whether CRACC acts predominantly as a coreceptor, as opposed to an activating receptor, on resting NK cells; in vitro studies that examined CRACC function utilized human NK cell clones (70) or populations of murine NK cells stimulated in vitro with IL-2 or in vivo with poly(I:C) (71). Nonetheless, CRACC may have a potentially important role in NK cell–mediated tumor surveillance because $CRACC^+$ tumor cells generated fewer lung metastases using the B16 melanoma model (71). Thus, 2B4, NTB-A, and CRACC have important roles in regulating the effector functions of human and murine NK cells.

The availability of SAP-deficient NK cells and polyclonal lines from XLP1 patients made it possible to investigate the requirement of SAP for 2B4, NTB-A, and CRACC function. Engagement of 2B4 or NTB-A on XLP1 NK cells failed to increase target cell lysis, thereby revealing a requisite role for SAP (69, 76, 78, 80, 188). Indeed, the profound defects in responses to EBV-infected B cells have been attributed to these defects; CD48, the ligand for 2B4, is highly induced on B cells upon EBV infection (107). Studies using *Sh2d1a*$^{-/-}$ mice also confirmed an obligatory requirement for SAP in 2B4-mediated activation of murine NK cells in vivo and in vitro (168); however, this finding is not universal (see below). Studies suggesting that EAT-2, but not SAP, is required for NTB-A-mediated cytotoxicity in an NK cell line (72) contrast with the inability of XLP1 NK cells to be activated through NTB-A (69). Such differences possibly reflect the use of primary NK cells versus transformed NK cell lines in which SAP expression is reduced by siRNA.

Interestingly, Moretta and colleagues found that mAb-mediated engagement of 2B4 or NTB-A on XLP1 NK cells actually inhibited the basal level of target cell lysis (69, 78, 189, 190). 2B4 also functioned as an inhibitory, rather than activating, receptor on immature NK cells derived from in vitro culture of CD34^{+} progenitors (189) and on decidual NK cells (190). The differential outcomes of 2B4 engagement partitioned with SAP expression; i.e., SAP was absent or expressed at low levels in those NK cells where 2B4 exhibited an inhibitory function (189, 190).

In contrast to the requirement of SAP in the activating function of 2B4 and NTB-A, CRACC remained functional on SAP-deficient human and murine NK cells, consistent with reports that CRACC does not associate with SAP (54, 70, 71).

EAT-2 Acts to Suppress the Activation of Murine NK Cells

Although EAT-2 was initially found to associate only with 2B4 in primary murine NK cells (52), a phosphotyrosine-dependent interaction between murine CRACC and EAT-2 was subsequently reported by the same group using stably transfected cell lines (71). NK cells lacking EAT-2 exhibited increased lysis of xenogeneic, but not allogeneic, target cells, and heightened production of IFN-γ following engagement of 2B4, CD16, and Ly49D (52). A weaker enhancement in the cytotoxicity and cytokine production was observed in ERT-deficient NK cells (52). EAT-2 and to a lesser extent ERT therefore act to suppress the effector function of activated murine NK cells. However, more recent data examining EAT-2-deficient mice on a C57Bl/6 background support a positive role for EAT-2 downstream of 2B4 and CD84, suggesting that EAT-2 signaling may vary, depending on the strain of mice or other factors, such as activation status of cells (55).

The inhibitory function of EAT-2 is dependent on two C-terminal tyrosine residues that become phosphorylated in activated NK cells (52). Interestingly, human EAT-2 contains only a single tyrosine residue in its C terminus (56, 182). Because human EAT-2 appears to mediate positive signaling through human CRACC (54) and potentially NTB-A (72), the additional tyrosine in murine EAT-2 could confer its negative signaling function.

Nonetheless, the cytotoxic activity of wild-type murine NK cells against CRACC^{+} targets was also strictly dependent on EAT-2 and independent of SAP (71). Similar to the inhibitory function of EAT-2 (presumably downstream of 2B4), the two C-terminal tyrosine residues of EAT-2 were also required for CRACC-mediated activation of murine NK cells (71). These features are consistent with studies showing intact CRACC function in NK cells derived from XLP1 patients (70) and with biochemistry, suggesting that EAT-2, via the recruitment and/or activation of PLCγ, regulates the activating function of CRACC (54, 178). EAT-2 may therefore regulate NK cell effector function downstream of different associating receptors by distinct mechanisms.

Interestingly, in cells lacking EAT-2, CRACC functioned as an inhibitory receptor

(71). This scenario resembles the findings that, depending on the presence or absence of SAP, 2B4 and NTB-A can function either as activating or inhibitory receptors, respectively, on human NK cells (69, 78, 189, 190). The cytoplasmic domain of murine CRACC contains three tyrosine residues: of these, Tyr281 within an ITSM is responsible for EAT-2 recruitment and the stimulatory function of CRACC, whereas Tyr261 is required for CRACC's inhibitory function (71). Thus, similar to 2B4, CRACC may switch from an activating to an inhibitory receptor by recruiting different signaling molecules to distinct binding sites within its cytoplasmic domain.

Gene Targeting Reveals that 2B4 Functions as an Inhibitory Receptor on Murine NK Cells

In contrast to data demonstrating that 2B4 functions as a stimulatory receptor, *2b4*$^{-/-}$ NK cells display increased cytotoxicity and exaggerated IFN-γ production toward CD48^{+} target cells (191, 192). In addition, wild-type NK cells lysed CD48^{+} target cells less efficiently than they lysed CD48^{-} target cells. Restoring 2B4 expression inhibited the ability of *2b4*$^{-/-}$ NK cells to kill CD48^{+} target cells. Similarly, CD48^{-} target cells transfected with CD48 were protected against NK cell–mediated cytotoxicity (191, 192). These observations suggest that signals delivered through murine 2B4 restrained NK cell effector functions. Paradoxically, homotypic NK cell interactions mediated by 2B4 and CD48 were necessary to license NK cells for their acquisition of cytotoxic effector function (193). Thus, although the general consensus is that 2B4 functions as an inhibitory receptor on murine NK cells, scenarios exist whereby it functions to activate cytolysis, consistent with data from human NK cells.

Studies using *2b4*$^{-/-}$, *Cd48*$^{-/-}$, and wild-type NK cells demonstrated that, depending on the context of activation, in the absence of 2B4-CD48 interactions, murine NK cells could lyse each other (194). Thus, 2B4 can inhibit NK-NK fratricide, providing a potential explanation for reduced cytotoxicity and proliferation in the absence of an activating signal. In addition, recent data indicate that *2b4*$^{-/-}$ NK cells can kill activated CD8 T cells in vitro and in vivo, suggesting that 2B4 plays a pivotal role in maintaining tolerance of activated NK cells in the early stages of persistent infections (195).

Although investigators have made attempts to explain the molecular mechanisms that underlie the inhibitory function of 2B4 on murine NK cells, these mechanisms remain unresolved. One potential explanation lies in the expression of two forms of 2B4 in murine cells—the short form has been proposed to exhibit activating function, whereas the long form was thought to be inhibitory (126, 128). The Schatzle group (126, 128) further found that the inhibitory function of 2B4 was independent of SAP (192). This would be consistent with the proposal that the function of murine 2B4 is regulated by the recruitment of distinct adaptor proteins, with SAP being required for the activating function of 2B4, while EAT-2 and/or ERT promote the inhibitory function of 2B4 (52, 71, 168, 196). In addition, murine NK cells overexpressing EAT-2 exhibited reduced lysis of susceptible target cells and impaired 2B4-induced tyrosine phosphorylation (52), suggesting that EAT-2 controls inhibitory signals from 2B4 in murine NK cells, in contrast to its role downstream of CRACC (54, 71).

Another group proposed that the switch in activating versus inhibitory function of 2B4 resulted from differences in levels of expression of 2B4 on the surface of effector cells, the degree of 2B4 engagement by a specific mAb or by CD48, and the relative availability of SAP within the responding cells. Thus, the inhibitory function of 2B4 was dominant under conditions of relatively high surface expression of 2B4 and low intracellular levels of SAP (197), consistent with data from SAP-deficient human NK cells (78, 189, 190) and SAP-deficient murine NK cells (168). However, data from another group indicate that the activating function of 2B4 on human NK cells positively correlates with its level of surface expression (198). Although differences

may result from the use of a T cell hybridoma engineered to express 2B4 in the Chlewicki et al. (197) study in which the consequences of 2B4 engagement on IL-2 production were assessed following TCR ligation, overall, the relative levels of SAP and 2B4 appear likely to contribute to 2B4 functional outcomes.

Additional explanations for the inhibitory activity of 2B4 and NTB-A can be gleaned from biochemical studies that examined interactions between these receptors and several SH2 domain–containing proteins. 2B4 and NTB-A can recruit the inhibitory kinase CSK and phosphatases SHP-1, SHP-2, and SHIP-1, and interactions with these mediators could be prevented by the presence of SAP (69, 78, 139, 160, 174). Thus, these receptors could provide a negative signal to NK cells in the absence of SAP via a phosphatase-dependent mechanism. Indeed, it has become increasingly evident that there may be alternative modes of signaling in the presence and absence of SAP (see **Figure 3*e***). Finally, recent data raise the interesting possibility that interpretation of 2B4 function in murine cells is complicated by the fact that CD48 can also bind CD2 in murine but not in human cells (178). Thus, competition between receptors may contribute to phenotypes elicited by engagement of 2B4.

CD8 T Cell–Mediated Cytotoxicity and Cytokine Production

Activated CD8 T cells express SLAM, 2B4, and Ly108, which have been associated with CD8 T cell effector function. Anti-SLAM stimulation of human CD8 T cells augmented TCR-mediated IFN-γ production, granule release, and cytotoxicity (199, 200). However, because SLAM mAbs can block homophilic interactions, it will be important to evaluate SLAM function in CD8 T cell–mediated cytotoxicity using targets expressing or lacking SLAM.

Initial experiments suggested that 2B4-CD48 interactions provide a costimulatory function augmenting TCR-mediated CD8 T cell proliferation and IL-2 production (201). In addition, 2B4-expressing CD8 T cells exhibit increased killing of both CD48-positive and CD48-negative targets, suggesting that 2B4-CD48 interactions occurred between CD8 T cells themselves as well as between CD8 T cells and target cells (81, 202). Interestingly, EBV-specific CD8 T cell lines from XLP1 patients exhibit decreased IFN-γ production and lytic activity in response to autologous EBV-transformed lymphoblastoid cell line stimulation, suggesting that defects in CD8 T cell cytotoxicity also contribute to defective responses to EBV in XLP1 (62, 79). This defect correlated with impaired 2B4 and perforin polarization at the contact site between the CTL and the CD48-expressing target (62). SAP also localizes to the NK cell–target cell contact site (146), suggesting that SAP expression is critical for facilitating 2B4 localization for efficient target lysis.

Recent data suggest that SAP specifically affects CD8 T cell cytolysis of B cell targets. XLP1-derived EBV-specific T cell clones killed EBV antigen–expressing fibroblasts yet exhibited reduced lysis of EBV antigen–expressing B lymphoblastic cell lines compared with control T cell clones. In addition, XLP1-derived EBV-specific CD8 T cell clones produced considerably more IFN-γ when incubated with EBV peptide–pulsed fibroblasts compared with B cell targets. The use of anti-NTB-A and anti-2B4 reagents synergistically increased IFN-γ production in response to antigen-expressing B cells (75). Although it is not clear whether these antibodies block receptor-ligand interactions or stimulate receptors, the impaired CD8 T cell effector function likely reflects a broader functional inability to effectively interact with and lyse B cell targets that may contribute to phenotypes of XLP1 (see below).

RESPONSES TO INFECTIONS

Although EBV does not infect murine cells, *Sh2d1a*$^{-/-}$ mice have been challenged with numerous pathogens. These studies have been useful in dissecting specific defects associated with SAP deficiency. Following lymphocytic choriomeningitis virus (LCMV) or *Toxoplasma*

gondii infection, *Sh2d1a*$^{-/-}$ mice developed increased numbers of antigen-specific CD8 T cells and a heightened effector response (18, 181). *Sh2d1a*$^{-/-}$ mice survive an acute LCMV infection yet fail to resolve a chronic infection and die (181, 203). Although the cause of death was not fully delineated, it is likely due to CD8 T cell–mediated immunopathology along with impaired antibody-mediated responses. In contrast, acute LCMV infection of *Slam*$^{-/-}$ mice generated normal numbers of antigen-specific CD8 T cells producing IFN-γ (204), although chronic LCMV infection was not examined.

Sh2d1a$^{-/-}$ mice have also been infected with murine gammaherpesvirus-68 (γHV-68) as a model for EBV infection in XLP1. γHV-68 establishes a lytic infection in the oropharynx and respiratory tract, followed by latency in B cells (205). γHV-68 infection established latency in *Sh2d1a*$^{-/-}$ mice, although there were reduced memory B cell numbers that translated into a reduced latent load (206). Interestingly, two reports demonstrated elevated percentages of splenic CD8 T cells as well as T cell infiltration of the lung and liver associated with increased tissue damage due to γHV-68 infection (49, 207). The accumulation of antigen-specific CD8 T cells postinfection correlated with a defect in RICD associated with reduced p73 expression (which can influence a mitochondrial cell death pathway) (208). These studies suggest that SAP-deficient mice recapitulate the CD8 T cell hyperproliferation and tissue infiltration–mediated immune pathology seen in XLP1 and propose a role for defective T cell apoptosis in this process. Recently, defective RICD, associated with decreased Bim expression, was observed in activated T cells from XLP1 patients, as well as in peripheral blood T cells in which SAP expression was reduced by RNAi (27). These data further suggested that SAP influenced RICD by affecting the strength of TCR signaling. Similar results were obtained by disruption of NTB-A expression, implicating NTB-A as an important SAP-associated receptor mediating cell death (27). In addition, studies using cell lines suggest that SAP inhibits the antiapoptotic function of valosin-containing protein (26). Although the mechanism of cell death differed between these reports, they support the premise that hyperactivation of CD8 T cells in XLP1 results from a failure of these cells to undergo RICD.

ALTERED T CELL CYTOKINE PRODUCTION

Engagement of SLAM Family Members on CD4 T Cells

One of the first recognized features of SLAM was its ability to affect T cell cytokine production. Stimulation with anti-SLAM mAbs mediated TCR-independent proliferation and IFN-γ production from previously activated human CD4 T cells (58) and further polarized human Th1 clones (60). Moreover, stimulation with antimurine SLAM mAb (59, 120), antihuman CD84 mAb (57), human CD84-Fc (65), anti-NTB-A mAb, and NTB-A Fc (209) all enhanced IFN-γ production in CD4 T cells in conjunction with TCR ligation. In contrast, homophilic SLAM interactions on a murine thymoma cell line (150) and homophilic SLAM associations between T cells and an artificial APC line that expressed SLAM reduced IFN-γ expression (61). These results suggest that the antibody(ies) used in these earlier studies blocked, rather than promoted, SLAM function. T cells from *Sh2d1a*$^{-/-}$ mice show increased IFN-γ production upon stimulation, supporting a negative role for SLAM in the regulation of IFN-γ (18, 61, 181).

However, perhaps more striking is the finding that SAP-deficient CD4 T cells exhibit a pronounced defect in IL-4 production, despite normal proliferation and IL-2 production (18, 61, 181). The defect in IL-4 expression was also shown in T cells from *Sh2d1a*$^{-/-}$*Ifn-γ*$^{-/-}$ mice, uncoupling the IL-4 defect from increased IFN-γ expression (61). Consistent with an intrinsic defect in Th2 cytokine expression, *Sh2d1a*$^{-/-}$ mice have low levels of serum IgE (18, 181) and are resistant to *Leishmania major* infection, a parasitic infection requiring Th2 cytokines for disease progression in the

Balb/c background (181). Nonetheless, when SAP-deficient CD4 T cells were activated in the presence of Th2 polarizing cytokines, production of IL-4, IL-13, and IL-10 was comparable to wild-type cells (18, 61, 181), indicating that responses to polarizing Th2 cytokines are intact. Supporting this, *Sh2d1a*$^{-/-}$ mice mounted a Th2 response to *Schistosoma mansoni* egg injection, a model in which IL-4 is initially produced by cells other than T cells (210).

Consistent with the idea that SLAM family members influence cytokine production, stimulation of wild-type CD4 T cells with APCs expressing SLAM leads to increased IL-4 (and decreased IFN-γ) production (162). Moreover, *Slam*$^{-/-}$ CD4 T cells exhibit reduced IL-4 production (67, 161) and impaired responses in an allergic asthma model (211). Recently, a newly described subpopulation of CD4 T follicular helper (T_{FH}) cells located in the GC (212) was found to require SLAM expression for IL-4 production, suggesting an important role for SLAM in the regulation of T_{FH} cytokine expression (68). In vitro, the IL-4 production defect observed in *Sh2d1a*$^{-/-}$ CD4 T cells is more pronounced than that in *Slam*$^{-/-}$ cells (67, 161), suggesting that other receptors are involved. Indeed, CD4 T cells from *Ly108*$^{\Delta exon2\text{-}3/\Delta exon2\text{-}3}$ mice and *Ly9*$^{-/-}$ also exhibit impaired IL-4 production, although not to the same extent as *Slam*$^{-/-}$ T cells (63, 64). However, although SLAM, Ly108, and Ly9 may all contribute to optimal CD4 T cell IL-4 production, the *Ly108*$^{\Delta exon2\text{-}3/\Delta exon2\text{-}3}$, *Ly9*$^{-/-}$, and *Slam*$^{-/-}$ mice were all generated on the 129 background and backcrossed to B6 and thus still have the 129 SLAM locus. Thus, these effects may need to be further evaluated on a pure genetic background (see the section on Autoimmunity).

Retroviral reconstitution of SAP-deficient CD4 T cells with the SAP(R78A) mutant or CD4 T cells from SAP(R78A) knock-in mice established that a SAP(R78)-dependent pathway is important for TCR-mediated IL-4 production (61, 161). Although this domain is critical for SAP-mediated Fyn binding and recruitment to SLAM (143, 148, 152), it can also mediate binding to PIX (163), NCK1 (158), and PKCθ; recent data support a role for PKCθ in SLAM-mediated IL-4 production (162). Indeed, even though Fyn has been implicated in SAP-mediated pathways leading to IL-4 expression, there are conflicting reports as to how Fyn affects Th2 cytokine production (213–215), and it is not entirely clear which SAP(R78)-dependent pathway(s) are instrumental in CD4 T cell IL-4 production.

HUMORAL RESPONSES

A cardinal feature of XLP1 is the development of dysgammaglobulinemias that can progress to frank hypogammaglobulinemia over time. Although the nature of the humoral defects in XLP1 has been enigmatic, the study of *Sh2d1a*$^{-/-}$ mice has recently provided insight into this phenotype.

Defects in Antibody-Mediated Immunity

Examination of serum immunoglobulins revealed that *Sh2d1a*$^{-/-}$ mice have low levels of IgE (18, 181) but either normal or variably reduced basal levels of IgM, IgG, and IgA (48, 49), perhaps reflecting the cleanliness of various animal facilities, the infectious history of the mice, and the age of the animals examined. Immunization with T-independent antigens also gave relatively normal results (210, 216). However, following immunization with T-dependent antigens (50, 210, 216, 217) or infection with various pathogens (17, 18, 49, 181, 203, 210, 217–219), *Sh2d1a*$^{-/-}$ mice exhibit striking defects in long-term antigen-specific antibody production. These results are in accordance with an early study showing defective responses to immunization with the bacteriophage PhiX178 (a T-dependent antigen) in XLP1 patients (220).

Sh2d1a$^{-/-}$ mice develop short-lived plasma cells, albeit at reduced levels (17, 18, 219) yet exhibit a striking defect in GC formation (17, 210, 216, 217, 219). As a consequence, *Sh2d1a*$^{-/-}$ mice have markedly reduced numbers of memory B cells and long-lived plasma cells

(17, 18, 210, 219). It is now appreciated that XLP1 patients also exhibit a paucity of GCs in their lymphoid organs and have an absence of IgG^+ and IgA^+ $CD27^+$ memory B cells (19–21).

Mechanisms for Humoral Immune Defects

Many lines of evidence indicate a T cell–intrinsic component to the humoral immune and GC defects in *Sh2d1a*$^{-/-}$ mice, including (*a*) analyses of T-dependent and -independent immunizations; (*b*) lymphocyte transfers into *Rag2*$^{-/-}$ (lymphocyte-deficient), SAP-deficient (204, 210, 217), or irradiated C57Bl/6 mice (17); (*c*) the generation of conditional gene-targeted mice in which *Sh2d1a* is selectively deleted in T or B cells (50); and (*d*) the demonstration of normal in vitro behavior of B cells from XLP1 patients (19, 20). Although several groups have reported that SAP is expressed in B cells, whether expression is limited to a particular subset is not apparent (48, 101, 159, 217). Some data suggest that SAP expression in B cells is required for humoral responses (48, 217), but this has been controversial. The nature of these differences is ambiguous but could reflect the variation in genetic background of the mice as well as the assay systems used. Interestingly, XLP1, some CVID, and hypogammaglobulinemia patients show an increase in transitional B cell populations (221). Although these findings are not observed in *Sh2d1a*$^{-/-}$ mice, they could be indicative of B cell–intrinsic defects. Alternatively, this phenotype may result from compensatory mechanisms in these primary immunodeficiencies, as reduced frequencies of memory B cells and increased frequencies of transitional B cells are also observed in immune-deficient conditions unrelated to XLP1, such as HIV infection (222) and STAT3 deficiency (223).

Considerable effort has been taken to ascertain the T cell–intrinsic defect in antibody-mediated responses in *Sh2d1a*$^{-/-}$ mice. The production of Th2 cytokines by CD4 T cells can influence humoral immunity, particularly the generation of IgG1- and IgE-producing cells in mice. However, in response to *Schistosoma mansoni* egg immunization, *Sh2d1a*$^{-/-}$ mice mounted a robust Th2 response yet failed to generate GC B cells and displayed reduced serum *S. mansoni*–specific antibody titers (210). Although the SAP(R78A) mutant failed to improve IL-4 production in vitro, GCs were rescued by the transfer of SAP-deficient antigen-specific CD4 T cells that were retrovirally reconstituted with either wild-type SAP or the SAP(R78A) mutant (204, 210). Thus, the SAP-dependent pathways required for GC formation appear independent of proteins that bind SAP(R78). Nonetheless, the humoral defects may not be completely distinct from the cytokine defects associated with SAP deficiency. T cells from XLP1 patients show decreased production of IL-10, which has been shown to affect antibody production (19). Similarly, in mice, IL-4 can influence GC formation in conjunction with IL-21 (224, 225): In vivo SAP-deficient CD4 T cells exhibit reduced IL-21 production (83, 226, 227) as well as an absence of IL-4-producing T_{FH} cells in the GC (68).

SAP Deficiency Affects CD4 T Cell Contacts with Cognate B Cells

The use of intravital imaging has recently provided insight into the nature of the GC defects associated with SAP deficiency. Postimmunization, SAP-deficient CD4 T cells were found to interact with antigen-presenting DCs, proliferate, upregulate markers of activation, and migrate toward the T-B cell border comparably to wild-type CD4 T cells (84). In striking contrast, whereas wild-type CD4 T cells formed long-lasting mobile conjugate pairs with antigen-presenting cognate B cells (84, 228), SAP-deficient CD4 T cells primarily formed short-lived conjugates (84). These data were confirmed using an in vitro conjugation system in which SAP-deficient T cells were unable to maintain adhesion to antigen-presenting B cells while sustaining conjugation to

antigen-presenting DCs (84). Thus, although SAP-deficient CD4 T cells become activated, they are unable to deliver signals for GC formation and maintenance. Consistent with these observations, SAP expression was crucial for later stages of T cell help for B cells (229), although the timing may be antigen dependent (218).

T_{FH} cells are activated, antigen-specific CD4 T cells that reside in the GC to help initiate and maintain a GC reaction. T_{FH} cells are characterized by the elevated expression of CXCR5, PD-1 (programmed death-1), ICOS (inducible costimulator), BTLA (B and T lymphocyte attenuator), SAP, CD84, Ly108, and the transcription factor Bcl-6 as well as high secretion of IL-21 (212, 230). SAP-deficient CD4 T cells initially upregulate T_{FH} markers postimmunization (83, 84); however, T_{FH} cell markers are not sustained, and IL-21 production is reduced (68, 83, 226, 227). Despite the detection of some SAP-deficient T cells in GCs (17, 229), intravital imaging provided evidence that SAP-deficient CD4 T cells are neither efficiently recruited nor retained within the GC (processes dependent on cognate T-B cell interactions) and fail to become functional T_{FH} cells in the GC (84). Data suggest that sustained B cell contact is required for efficient development of the T_{FH} effector cell lineage (84, 231–233). Impaired T-B cell adhesion may therefore prevent a final stage of T_{FH} cell maturation in SAP-deficient mice. However, a recent study has demonstrated that the nature of immunization can dictate the B cell requirement for T_{FH} cell development. Repeated delivery of peptide antigen permitted the generation of cells expressing T_{FH} markers not only in *Sh2d1a*$^{-/-}$ mice, but also in mice whose B cells were unable to present antigen (MHC class II deficient) or receive cognate T cell help (CD40 deficient) (226). Thus, final T_{FH} differentiation may require prolonged antigen stimulation rather than B cell–specific signals per se.

Transcriptional profiling and phenotypic analyses indicate that T_{FH} cells express elevated levels of SAP and SLAM family members including CD84, Ly108/NTB-A, and Ly9 (83, 226, 230, 234, 235). CD84 and Ly108 are also upregulated on GC B cells (83). Indeed, CD84 plays a key role in sustaining T-B cell contacts, T_{FH} cell function, as well as optimal GC formation, as these processes are defective in *Cd84*$^{-/-}$ mice, although not to the same extent as seen with SAP deficiency (83). Moreover, in vitro conjugation experiments revealed that both CD84 and Ly108 contribute to T-B cell adhesion (83).

Additional data suggest that although the integrins LFA-1 (lymphocyte function–associated antigen-1) and VLA-4 (very late antigen-4) are the primary adhesive receptors implicated in T cell–DC interactions and early T-B cell contacts, sustained T-B cell interactions also require SAP, CD84, and Ly108. CD4 T cells adhere to CD84 and Ly108 in a SAP-dependent manner, providing further support that these SLAM family members act as adhesion receptors (83). Intriguingly, CD84 and Ly108 are two SLAM family members with the strongest interactions (lowest Kds) (111, 117), perhaps contributing to their roles in the highly dynamic process of T-B cell interactions.

However, T-B cell adhesion may not be the only SAP-mediated contribution to GC formation. Recently, Crotty and colleagues (68) described a phenotypically distinct GC T_{FH} subset that was dependent on SLAM expression for IL-4 production during a viral infection. Whether SLAM family members contribute to other aspects of humoral immune responses beyond GC formation, and the potential contribution of B cells to such processes, will be of interest.

Nonetheless, data from other systems also support the role of SLAM family members as adhesive receptors. NK cells exhibit synergistic binding to target cells expressing intercellular adhesion molecule (ICAM)-1 and CD48 (82, 85). T cells overexpressing SLAM exhibited increased clumping (144), and T cell lines from XLP1 patients did not efficiently form aggregates upon PMA treatment (237). Interestingly, these XLP1 cells were maintained in

culture by stimulation with EBV-transformed B cells—whether this exacerbated this potential adhesion phenotype is not known. Finally, CD84 participates in platelet spreading, whereas SLAM contributes to stability of platelet aggregates in vitro and thrombus formation in vivo (44). Thus, platelet, NK/CD8 T cell–target cell, and T-B cell interactions provide examples in which cell-cell interactions mediated by integrins are associated with secondary contacts mediated by SLAM family members.

Notably, this selective defect in T cell interactions with B cells, but not with DCs, also provides intriguing insight into the phenotypes of XLP1: T cells from XLP1 patients become activated or even overactivated, but CD8 T cells fail to effectively kill EBV-infected B cells (75), and CD4 T cells fail to provide help to B cells for GC formation (19). Moreover, XLP1 patients have a high incidence of B cell lymphomas (34, 237), which could reflect a defect in immunosurveillance toward B cells (**Figure 4**). Thus, the phenotypes of XLP1 may be interpreted in the context of global defects associated with B cell interactions. Even the defect in NKT cell development has interesting parallels (see the Hematopoietic Development section), because NKT cells are selected through lymphocyte-lymphocyte interactions. Whether direct lymphocyte-lymphocyte interactions contribute to RICD is less clear.

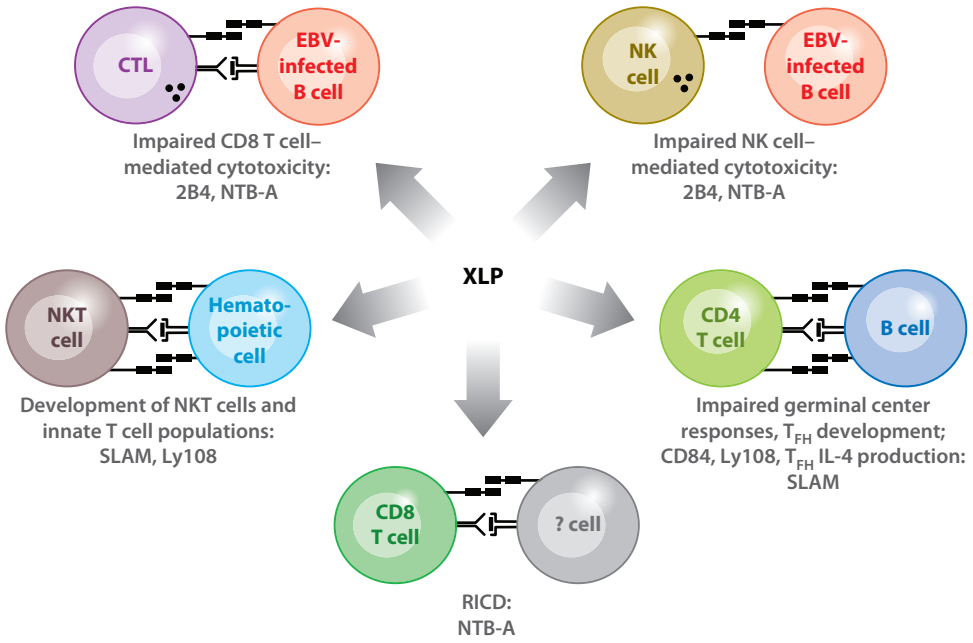


Figure 4

Cellular defects in XLP1 patients and the SLAM receptor family member implicated in the phenotype. The phenotypes of XLP1 are shown in the context of the lymphocyte-lymphocyte interactions affected. XLP1 patients exhibit impaired NK- and CD8-mediated killing of EBV-infected B cell targets, diminished humoral responses (reduced numbers of memory B cells) due to impaired T-B cell interactions, impaired development of NKT cells, and reduced sensitivity to reactivation-induced cell death (RICD). Whether RICD involves direct lymphocyte-lymphocyte interactions is unknown. SAP-deficient mice recapitulate several features of XLP1 and have revealed previously unappreciated XLP1 characteristics, providing insight into the B cell–centric phenotypes of this disease as well as a further understanding of normal immune physiology. (Abbreviations: EBV, Epstein-Barr virus; NK, natural killer; NKT, natural killer T; RICD, reactivation-induced cell death; SAP, SLAM-associated protein; SLAM, signaling lymphocyte activation molecule; T_{FH}, T follicular helper cell; XLP, X-linked lymphoproliferative syndrome.)

HEMATOPOIETIC DEVELOPMENT

Expression of SLAM Family Members on Hematopoietic Stem Cells

The selective expression of SLAM and 2B4 on long-term reconstituting hematopoietic stem cells, multipotent progenitors, and oligolineage-restricted progenitors in the bone marrow and fetal liver of mice has refined the isolation of specific progenitor populations (238–240). However, as hematopoiesis appears to be globally intact in the absence of SAP, SLAM, or 2B4, the relevance of this expression is not clear. Nonetheless, *Sh2d1a*$^{-/-}$ mice do show defects in the development of certain lymphocyte lineages.

Development of T Cells with Innate-Like Characteristics

Conventional thymocytes undergo positive selection by interacting with cortical epithelial cells. However, there are subsets of thymocytes with unconventional properties that are selected by hematopoietic cells, most likely other double-positive (DP) cells (241). Cells selected in this manner tend to traffic to nonlymphoid tissues and demonstrate phenotypic and functional characteristics similar to cells of the innate immune system, including expression of invariant antigen receptors, memory cell markers, and robust effector function. Perhaps the best recognized of these populations are NKT cells, which are selected by lipid antigens presented by CD1d on other DP thymocytes, express an invariant TCR (Vα24^{+}Vβ11^{+} in humans, Vα14^{+} in mice), and rapidly secrete high levels of cytokine following stimulation (241). Previous data demonstrated that *Fyn*$^{-/-}$ mice display reduced NKT cell numbers (242, 243). Strikingly, both *Sh2d1a*$^{-/-}$ mice and XLP1 patients exhibit an almost complete absence of these cells (22–24, 241). In *Sh2d1a*$^{-/-}$ mice, the block in NKT cell development occurs early following rearrangement of the canonical Vα14 TCR and initiation of TCR signaling for positive selection (86, 244, 245). SAP(R78A) knock-in mice, competitive bone marrow chimeras using SAP(R78A) bone marrow, and SAP(R78A) cells cultured on OP9-delta cells also generated reduced numbers of NKT cells. Nonetheless, the SAP(R78)-mutant NKT cells that developed were still able to produce cytokines rapidly (245), indicating that a SAP(R78) pathway is required for optimal NKT cell development but not for functional cytokine responses.

Evaluation of the development of these innate-like T cells in gene-targeted mice has provided some of the strongest evidence for redundancy between SLAM family members contributing to phenotypes associated with SAP deficiency. Although *Slam*$^{-/-}$ and *Ly108*$^{-/-}$ mice have either normal or only slightly reduced NKT cell numbers (64, 67, 87), mixed bone marrow chimeras using cells from *Slam*$^{-/-}$ mice and *Ly108*$^{-/-}$*Cd1d*$^{-/-}$ mice or from *Ly108*$^{-/-}$ and *Slam*$^{-/-}$*Cd1d*$^{-/-}$ mice provided evidence that the combined lack of SLAM and Ly108 caused a striking reduction of NKT cells. These "pseudo double knockouts" of SLAM and Ly108 forced selection so that neither receptor could be engaged in *trans* (87). Recently, investigators found that the transcription factor c-Myb plays a selective role in NKT cell development by regulating cell survival and expression of CD1d, SAP, SLAM, and Ly108 (246). Interestingly, polymorphisms in the SLAM locus, specifically SLAM and Ly108, in the NOD background correlate with reduced NKT cell numbers (247) and impaired cytokine secretion (248). Introgression of the B6 *Nk1* allele (SLAM locus) from B6 onto the NOD background improved NKT cell number and function, although it did not alter the course of spontaneous diabetes (249). More recent data have provided evidence that the *Slam* haplotype also influences liver NKT cell number and function (250).

SAP is also important for the differentiation of other innate T cells, including an unusual subpopulation of CD4 T cells (90). Human CD4 T cells can be selected on MHC class II^{+} hematopoietic cells in the thymus (251, 252). Although this does not occur in mice,

transgenic expression of MHC class II transactivator in T cells permits the generation of thymocyte-selected CD4 T cells that display innate-like cell characteristics, including rapid expression of cytokines. Like NKT cells (253, 254), these thymocyte-selected innate CD4 T cells express the transcription factor promyelocytic leukemia zinc finger (PLZF) (255). Notably, thymocyte-selected innate CD4 T cells are dependent on SAP and partially on Ly108 for their development (90, 256).

Reduced PLZF expression also severely affects NKT cell maturation and function (253, 254). Nonetheless, transgenic PLZF expression did not rescue NKT cell differentiation in *Sh2d1a*$^{-/-}$ and *Fyn*$^{-/-}$ mice. Furthermore, PLZF-mediated acquisition of effector/memory phenotypic changes in conventional T cells was not dependent on either SAP or Fyn (257), consistent with the notion that PLZF affects a different, later stage of NKT cell maturation involving acquisition of effector function.

Mice with TCR signaling defects due to a lack of the tyrosine kinase Itk or a mutation that affects the Itk-binding site of SLP-76, as well as mice deficient in *Inhibitor of differentiation gene 3* (*Id3*), develop a population of CD8 T cells that exhibit characteristics of innate-like cells including the expression of memory cell markers and rapid production of cytokines (258–261). SAP is also required for the development of these innate-like T cells in *Itk*$^{-/-}$ and *Id3*$^{-/-}$ mice, although the requirements for SLAM family members have not been evaluated (88, 262). Interestingly, recent data suggest that this CD8 T cell population found in *Itk*$^{-/-}$, *Kruppel-like factor2*$^{-/-}$, and *Id3*$^{-/-}$ mice develop by a non–cell autonomous mechanism that results from IL-4 produced by increased numbers of innate-type PLZF^{+} CD4 T cells that also arise in these mice (262, 263). These data argue that it is the CD4-innate T cell population that requires SAP for its selection or development, parallel to findings for invariant NKT cells.

Itk$^{-/-}$ and *Id3*$^{-/-}$ mice, as well as mice with SLP-76 tyrosine mutations, also have an increased percentage of Vγ1.1^{+}Vδ6.3^{+} $\gamma\delta$ T cells that display increased PLZF and IL-4 expression (86, 89, 91, 262, 264, 265). These cells are responsible for the increased IgE levels and GCs in *Itk*$^{-/-}$ mice (264). This subset is also reduced in *Sh2d1a*$^{-/-}$ and *Id3*$^{-/-}$*Sh2d1a*$^{-/-}$ mice (86, 89, 91). Whether these innate-type lymphocytes influence phenotypes observed in the absence of SAP is unknown.

B Cell Development

Within the bone marrow, when B cells expressing an autoreactive BCR encounter self-antigen, they either are deleted via apoptosis or undergo receptor editing to select for BCR specificities that no longer react strongly with self-antigen (266). Interestingly, expression of the NZW *Sle.1b* locus on the B6 background impaired normal tolerance mechanisms so that autoreactive B cells now entered the periphery (127). Given the link to altered Ly108 isoform usage, these findings suggest that Ly108 isoforms or other polymorphisms in genes encoding SLAM family members influence the B cell response to BCR ligation during development, thereby altering critical steps of tolerance induction. Whether SAP contributes to this process is unknown.

AUTOIMMUNITY

A less frequent manifestation of XLP1 is the development of autoimmune disorders, including vasculitis (16). Nonetheless, SAP deficiency has been shown to ameliorate autoimmune disease models associated with autoantibody production, including pristine-induced lupus (216), *Fas*^{lpr} mutation (267), and mutation of *Roquin* (227). In contrast, *Sh2d1a*$^{-/-}$ mice demonstrated enhanced susceptibility to a murine model of multiple sclerosis, EAE (experimental allergic encephalomyelitis), induced by immunization with myelin oligodendrocyte glycoprotein$_{38-50}$ peptide in complete Freund's adjuvant (216). Given the decreased interactions between T and B cells resulting from SAP deficiency, it is of interest

that a population of IL-10-producing "regulatory B cells" has recently been described that decreases EAE manifestations (268). In other studies, infusion of NTB-A Fc delayed onset of EAE in susceptible mice (209); however, it is unclear if this protein blocked homophilic interactions or resulted in Ly108 signaling.

Altered Expression of SLAM Family Members in Human Autoimmune Disorders

Human studies have demonstrated altered expression of SLAM family members in different autoimmune states, including elevated SLAM expression on monocytes and macrophages within the inflamed colon from Crohn's disease patients (269) and on synovial tissue lymphocytes (270) and increased CRACC expression on B cells from patients with systemic lupus erythematosus (SLE) (271). Although alteration in receptor expression could reflect the chronic activation status of the cells and the inflammatory environment, genome-wide association studies have also demonstrated association of certain SLAM haplotypes with increased susceptibility to autoimmune disorders (272). Recently, a LY9 variant [with a nonsynonymous change Val/Ile in the consensus ITSM, which may affect SAP and SHP-1 binding and stability (273)] has been proposed to contribute to SLE susceptibility (274). Variants in 2B4 have also been identified as genetic risk factors for SLE and rheumatoid arthritis (275).

Murine Models of Autoimmunity

Murine models have provided further evidence that polymorphic variations in the SLAM family of receptors contribute to the development of autoimmunity. Genetic studies of lupus susceptibility in crosses between the NZW and C57Bl/6 strains revealed that a locus that predisposes to the development of ANAs (*Sle.1b*) maps to the SLAM gene cluster: A C57Bl/6 congenic strain with NZW-derived SLAM locus (*Sle.1b*) was sufficient to mediate loss of tolerance and generate high ANAs (123, 276–278). Mouse genomic sequences indicate that two haplotypes exist in the region encompassing the *Slam* locus: One haplotype occurs in C57Bl/6 and the second in most other laboratory strains, including 129, NOD, and NZW (123, 276). Because many mouse gene-targeting experiments are performed in embryonic stem cells derived from the 129 strain and then backcrossed to C57Bl/6, this association is an important consideration for evaluating autoimmune phenotypes. Extensive polymorphisms in SLAM family members exist between lupus-susceptible and -nonsusceptible mouse strains, including the expansion of the *2b4* gene from one to four copies. Following antigen-receptor engagement, the Ly108.1 splice isoform that is preferentially expressed in lupus-susceptible strains demonstrates increased phosphorylation compared with Ly108.2 (**Figure 2**) (124). Thus, altered expression of Ly108 isoforms may contribute to both enhanced TCR-mediated responses and impaired B cell tolerance, predisposing the immune system for self-reactivity (123, 127). In addition, the *Sle.1b* haplotype exacerbates the autoimmune phenotype associated with either the *Yaa* translocation (TLR7 duplication) (279) or the Fas^{lpr} mutation (280). The lymphoproliferative disease in the B6.*Sle1b*.Fas^{lpr} mice was associated with an imbalance in the PI3K/PTEN signaling axis leading to elevated mTOR activation (280). Notably, SLAM cross-linking on B and T cells activated AKT (59, 155), a component of the PI3K-mTOR cascade. These data complement observations that SAP deficiency ameliorates disease in models associated with autoantibody production and decreases autoantibody production in *Sle.1b* mice (281).

Within the natural mouse population, SLAM family members CD48, CD229 (Ly9), and CD84 display extensive polymorphisms in the ligand-binding domain (135, 282) and thus could influence stability and duration of homophilic interactions. Polymorphisms in SLAM family members may have been selected as advantageous for responses to infectious agents, with the unfortunate consequence of increased susceptibility to autoimmunity.

Differences Between SAP and SLAM Receptor Deficiencies

The data reviewed here provide clear evidence that SAP not only is recruited to SLAM family members to mediate signal transduction but also prevents the coupling of receptors to inhibitory phosphatases. Therefore, the phenotypes associated with the SAP deficiency may be due to alternate (phosphatase and/or EAT-2/ERT) signals rather than to a loss of signal, as seen in deficiencies of individual SLAM family members. Indeed, loss of a single SLAM family member has not fully recapitulated a SAP-deficient phenotype. However, functional redundancy between family members has been documented in NKT cell development (87) and in T-B cell adhesion (83). Thus, multiple SLAM family members likely contribute to SAP-mediated and SAP-independent events within a given cell. Moreover, as discussed below, SLAM family members can signal in cells, such as in the myeloid lineages, that do not express SAP.

LINK BETWEEN INNATE AND ADAPTIVE IMMUNITY

SLAM Is a Receptor for Measles Virus

CD46 is expressed on all nucleated cells and was initially identified as a receptor for measles virus (MV), yet several strains can infect cells independently of CD46 expression (283). SLAM has been found to interact with MV hemagglutinin protein and to permit entry into the host cell (284). The generation of transgenic mice expressing SLAM under the control of the mouse Lck or CD11c promoter, as well as the creation of a SLAM knock-in mouse (by replacing the murine V with the human V domain), has established MV infection in mice and reproduced the lymphotropism and immunosuppression that is observed in human infection (285). Because some XLP1 patients have perturbed anti-MV immunity (9), it will be of interest to examine MV infection in SLAM knock-in mice on a SAP-deficient background.

Neutrophils

Although *Sh2d1a*$^{-/-}$ mice and XLP1 patients do not exhibit any defects in neutrophils, a striking feature of *Ly108*$^{\Delta exon2\text{-}3/\Delta exon2\text{-}3}$ mice is aberrant neutrophil function associated with elevated IL-12, TNF-α, and IL-6 production with reduced production of reactive oxygen species and bacterial killing (64). As a result, *Ly108*$^{\Delta exon2\text{-}3/\Delta exon2\text{-}3}$ mice are highly susceptible to *Salmonella typhimurium* infection (64), indicating that Ly108 is involved in the oxidative burst in neutrophils. It remains to be determined how Ly108 transmits signals within neutrophils. Although human neutrophils do not appear to express NTB-A, it will be interesting to ascertain if these cells alter receptor expression following activation.

Macrophages and Dendritic Cells

DCs are important APCs critical for T-dependent immune responses. DCs express EAT-2 but not SAP and also express Ly108/NTB-A, Ly9, and SLAM (40, 56, 183, 286). Stimulation of human CD40L-activated DCs with anti-SLAM mAb augmented secretion of IL-8 and IL-12 but had no effect on IL-10 (286). However, SLAM-SLAM engagement reduced IL-12 production from CD40L-stimulated DCs and impaired Th1 CD4 T cell differentiation (45), suggesting that the anti-SLAM reagent blocked homophilic association. Using a fibroblast cell line as the APC, researchers also found that SLAM expression decreased the amount of CD40L expressed on T cells (210).

Macrophages express Ly9, Ly108, CD84, and SLAM. Recent data suggest that CD84 engagement may influence cytokine production by LPS-activated bone marrow–derived macrophages (287). LPS, but not CpG, stimulation of *Slam*$^{-/-}$ macrophages resulted in reduced nitric oxide, IL-12, and TNF-α yet increased IL-6 production, whereas treatment with an antimurine SLAM antibody increased IL-12 production (67). *Slam*$^{-/-}$ mice also showed enhanced susceptibility to *Leishmania*

and impaired clearance associated with reduced IL-12 production by macrophages (67). Recently, SLAM was shown to function as a bacterial receptor recognizing the outer membrane proteins OmpC and OmpE from gram-negative bacteria (92). This study also provided evidence that SLAM regulates phagosome maturation and the production of free radicals by the NOX2 complex in macrophages, suggesting interesting parallels with Ly108 in neutrophils (92). CD48 is one of the receptors for FimH, a lectin on the pili of some Enterobacteriaceae (288). Thus, SLAM-related receptors may have dual functions as microbial sensors, providing further links between innate and adaptive immune responses.

CONCLUSIONS

The past several years have seen an explosion of data uncovering the roles of SLAM family members in immune cell function and their roles in the diverse phenotypes of XLP1. Many of these insights are derived from gene-targeted mice. In particular, the role of SLAM family members in lymphocyte-lymphocyte interactions has shed substantial light on the phenotypes of humoral immunodeficiency and the selective nature of the cytolytic defects against EBV-infected B cells. Studies of cells from XLP1 patients have also revealed new insights into the disease, particularly the effects of SAP on lymphocyte survival and on cytolysis of EBV-infected B cell targets. Together, these studies suggest that XLP1 is a disease mediated by defective interactions between lymphocytes.

In parallel, studies of XLP1 and mouse models have revealed new insight into basic immune cell function, contributing to knowledge regarding T_{FH} cells and their roles in GC formation, as well as delineating requirements for the development of subclasses of innate type T lymphocytes. The study of SAP and the related adaptor EAT-2 has furthered mechanistic analyses of NK cell cytolysis and the dual nature of signaling through SLAM family members in the presence or absence of these adaptors. Together, these studies highlight the importance of the SLAM family members and SAP-related adaptors and their profound influence on immune system development and function.

DISCLOSURE STATEMENT

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Errata

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