Published in final edited form as:

J Immunol. 2017 September 01; 199(5): 1531–1533. doi:10.4049/jimmunol.1701007.

SAP and Lessons Learned from a Primary Immunodeficiency

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Approximately 19 years ago, Terhorst and colleagues reported the cloning of the gene encoding a small adaptor protein SAP (SLAM Associated Protein), named by virtue of its binding to the intracellular tail of a cell surface receptor called SLAM (Signaling Lymphocyte Activation Molecule) (1). Genetic mapping, along with 2 independent papers using positional cloning approaches (2, 3), revealed that this gene, now known as *SH2D1a*, was mutated in X-linked Lymphoproliferative (XLP1) syndrome, a rare primary immunodeficiency characterized by fulminant mononucleosis triggered by Epstein-Barr Virus (EBV). The study of SAP and the associated SLAM family members has led to multiple discoveries about the genetic immunodeficiency XLP1, as well as insight into the biology of lymphocyte:lymphocyte interactions, regulation of germinal center (GC) formation, requirements for cytolysis of B cells, development of invariant (i) NKT cells, and induction of T cell restimulation-induced cell death (RICD) (4–6). Moreover, recent studies of SAP-independent functions of the SLAM family members have identified novel roles for these receptors in immune cell homeostasis and pattern recognition (7–9).

XLP1 was first described in the 1970s as a fatal immunodeficiency characterized by lymphoproliferation, hemophagocytosis, and abnormal Ab levels (10). Although such patients initially appear healthy, they are unable to clear EBV, with fatal infectious mononucleosis resulting in the majority of cases. XLP1 is a noteworthy example of a primary immunodeficiency characterized by susceptibility mainly to one infectious agent; however, the findings of lymphoproliferation, lymphomas, and altered serum immunoglobulins even in the absence of EBV infection argued that these patients have a broader immune dysfunction (4, 5, 11). The cloning of *SH2D1a* allowed identification of other family members with XLP1 and generation of mouse models, thereby providing further insight into this disease and the role of SAP and SLAM family members in immune cell function and homeostasis.

SLAM was first cloned as a T cell costimulatory receptor that influenced patterns of T cell IFNγ production (12). SLAM is now recognized as a member of a larger family of receptors including 2B4 (SLAMF4, CD244), LY9 (SLAMF3, CD229), CD84 (SLAMF5), NTB-A/Ly108 (SLAMF6, CD352), and CRACC (SLAMF7, CD319). With the exception of 2B4, the SLAM family members are homophilic (i.e., self-ligands), many of which function as cytolytic receptors on NK and $CD8^+$ T cells. A larger family of receptors has homology to this superfamily and includes CD2 and CD48 (SLAMF2, the ligand for 2B4), although not

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all of these receptors bind SAP, which is expressed mainly in T and NK cells (5, 6); some B cell expression has also been reported (13).

One of the most striking features of SAP is that it consists primarily of one Src homology 2 (SH2) domain: SH2 domains are conserved domains that bind to phosphotyrosine-based motifs and are usually part of larger proteins, including multi-modular domain adaptors and enzymes (14). Surprisingly, SAP binds to a tyrosine-based motif on SLAM in the absence of phosphorylation, although binding improves upon phosphorylation and binding to other SLAM family receptors does require tyrosine phosphorylation (15, 16). So how does SAP help transmit signals from these receptors? In this landmark paper (1), Terhorst and colleagues hypothesized that SAP functions by blocking the recruitment of phosphatases, in part due to the similarity of its binding site to immunotyrosine inhibitory motifs. They further showed using overexpression in heterologous cells that SAP can compete with the phosphatase SHP2 (1). However, subsequent data from Veillette and colleagues, as well as from Terhorst and Eck, provided contrasting evidence that SAP functions as an adaptor molecule, binding to the Fyn SH3 domain (17, 18). Other evidence demonstrated that SAP also binds Lck (19, 20). Together, these data supported a model in which SAP functions as an adaptor molecule required for recruiting Src family kinases, leading to phosphorylation and transmission of positive signals downstream of SLAM family receptors. Nonetheless, an increasing body of data now supports both pathways, demonstrating that SAP can also compete for binding of inhibitory molecules such as SHP1, SHP2, and SHIP to multiple SLAM family members (21–24). Thus, SAP appears to function as a switch that determines whether SLAM family members transmit positive signals (if SAP is present) or function as inhibitory receptors (in the absence of SAP). This model was put forth by Siderenko, Clark, and colleagues when they proposed the SAP binding motif be called an Immunotyrosine Switch motif (25, 26) and was supported by early work on 2B4 (21).

So how does SAP affect immune cell function? Even prior to the cloning of SAP, NK cells from XLP1 patients were found to exhibit impaired killing (27). Subsequent data demonstrated that $CD8^+$ T cells from XLP patients were unable to kill EBV-infected B cell targets (28–30), suggesting a reason why XLP1 patients fail to clear EBV. Yet other data suggested that the SLAM family member 2B4 functioned as an inhibitory receptor in the absence of SAP, supporting the idea of SLAM family members as inhibitory receptors (31–33). More recently, SAP-deficient T cells have been found to exhibit defective RICD, again as a result of inhibitory activity of a SLAM family member, NTB-A; this may contribute to the lymphoproliferation seen in XLP1 (34).

Evaluation of SAP-deficient mice has highlighted several additional phenotypes, and these have helped provide insight into the mechanistic underpinnings of XLP1. First, based on the connection with Fyn, which affects iNKT cell development, SAP-deficient mice were shown to have a severe block in iNKT cell development (35–37). A dearth of NKT cells, even in the absence of EBV infection, is now recognized as a feature of XLP1. Second, challenge with infectious agents or immunization revealed that SAP deficiency leads to profound defects in long-term humoral immunity and the generation of memory IgG B cells (38) that are associated with impaired GC formation (39). Additional data from mice demonstrated that the humoral immune defect is primarily T cell-intrinsic (39, 40), although in some genetic

backgrounds, i.e., Balb/c, B cell contributions have also been shown (13). Accordingly, SAP-deficient mice have been used in myriad studies that have helped uncover requirements for T cell help in B cell GC formation, including many studies of follicular T helper cells (5, 41). Interestingly, a lack of GCs was noted in some of the earliest XLP1 studies (10); however, the detailed mechanistic findings in mice, as well as subsequent evaluation in larger numbers of XLP1 patients (11) clarified that humoral defects are a key feature of this disorder.

Intravital microscopy in mice, complemented by in vitro assays, together have provided further insight into these phenotypes, revealing that SAP-deficient T cells exhibit a defect in stable conjugation to B cells, despite relatively normal interactions with dendritic cells (42). This defect likely accounts for the inability of SAP-deficient T cells to deliver contact-dependent cognate help for GC formation. Importantly, these observations helped crystallize an appreciation that defective lymphocyte:lymphocyte interactions provide a common pathophysiological mechanism for the phenotypes associated with SAP deficiency (43). Thus, in XLP1, $CD8^+$ T cells and NK cells are activated but fail to effectively kill EBV-infected B cells, $CD4^+$ T cells are activated but fail to provide cognate help for B cells for GC formation, and NKT cells (which are selected by interactions with double positive thymocytes) fail to develop. Indeed, the occurrence of B cell lymphomas, which can be EBV-negative in XLP1, suggests a basic defect in immunosurveillance of B cell malignancies.

So why are these defects specific for B cells (and other hematopoietic cells)? Clues came from expression of SLAM family members, which are expressed primarily on hematopoietic cells, and at very high levels on activated B cells. Indeed, CD48, the ligand for 2B4, was first described as a marker that was highly induced on B cells by EBV infection (44). Thus, in the absence of SAP, strong inhibitory signals from SLAM family members (particularly 2B4 and Ly108/NTB-A) are triggered by EBV-infected B cells, thereby preventing effective T cell activation and killing of B cell targets. Other data suggest that SAP is important for regulating killing of hematopoietic targets in general (6). It is notable that many of the phenotypes associated with SAP deficiency require the presence of a SLAM family member to transmit an inhibitory signal, a fundamentally different mechanism of signaling than when SAP acts as an adaptor. The strongest data supporting this interpretation include those showing that blocking or mutation of SLAM family members improved phenotypes associated with SAP deficiency, such as killing of B cell targets (24, 29, 30), and the demonstration that deficiency in Ly108 markedly improved the GC and iNKT cell developmental defects in SAP-deficient mice (45). The strong inhibitory effects of these receptors in the absence of SAP, combined with their roles in transmitting both positive and negative signals, may account for why deficiencies of SLAM receptors do not recapitulate many features associated with SAP deficiency.

Nonetheless, studies of the SLAM family have also been revealing about lymphocyte biology. The SLAM family receptors are encoded in a polymorphic gene cluster on mouse and human chromosome 1 that has been linked to autoimmunity in both species. Interestingly, polymorphisms in specific SLAM family members have been implicated in the development of autoantibodies in the lupus-prone mouse strains, supporting the roles of

these receptors in regulating humoral immunity (46). Similarly, variation in NKT cell numbers in various strains of mice has also been linked to the *SLAM* family locus (47). But also fascinating is growing evidence for SAP-independent functions of SLAM family members as pattern recognition receptors, particularly in myeloid cells, where SLAM has been found to bind to the outer membrane protein of *E. coli* and other gram-negative bacteria (7). SLAM also serves as a receptor for measles virus (48). More recent data have implicated CRACC (SLAMF7) as a CD47-independent "Eat-me" signal that helps clear dying cells (9). Thus, the SLAM family members are now recognized as regulators of multiple aspects of immune homeostasis.

These studies of SAP and SLAM family members have helped uncover many new insights into lymphocyte interactions and immune cell biology, most of which were triggered by findings in this featured paper from the Terhorst laboratory (1). Together, this work provides an excellent example of how the identification of genes implicated in primary immunodeficiencies has spurred not only knowledge of the disease, but also helped uncover new insights into the workings of the immune system.

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