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X-linked lymphoproliferative disease

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X-linked lymphoproliferative disease (XLP) is an immunodeficiency disorder characterized by an increased susceptibility to severe infection with Epstein-Barr virus (EBV), which causes infectious mononucleosis [1]. Unlike immunocompetent individuals in whom EBV infection induces transient lymphocyte proliferation, boys with XLP experience uncontrolled EBV-associated lymphocyte expansions and often develop lymphoma and, paradoxically, hypogammaglobulinemia [2]. Although the clinical features of XLP initially were believed to be solely attributable to EBV infection, investigations have revealed that a subset of patients develop manifestations of the disease without evidence of previous EBV exposure [3–5]. The immune defect underlying XLP is broader than originally described and likely involves more general disruptions of lymphocyte function and homeostasis. In 1998, three groups of investigators identified *SH2D1A* (Src homology 2 [SH2] domain containing gene 1A; previously known as Duncan syndrome human protein [DSHP], SAP [signaling lymphocytic activation molecule (SLAM)-associated protein]), the gene that is mutated in most patients with XLP [6–8]. Characterization of the SH2D1A protein is elucidating novel biochemical pathways that regulate lymphocyte activation and antiviral immune responses. This article describes the clinical features of XLP and discusses the scientific advances that are providing insight into the pathogenesis underlying this immunodeficiency disorder.

Clinical features

In 1975, Purtilo and investigators reported XLP after identifying a large kindred in which several male members died from the development of an

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uncontrolled immune response following primary EBV infection [1]. Initially called “Duncan syndrome” after the original affected family, the disorder also was named “X-linked recessive progressive combined variable immunodeficiency” based on its X-linked pattern of inheritance and its evolving features of hypogammaglobulinemia and malignant lymphoma. In 1978, an International XLP Registry was created to aid in the identification of patients, characterization of disease manifestations, and coordination of clinical and basic science research projects. As of December 2000, 89 families are entered into this registry, with over 309 affected boys and men represented [5].

Evaluation of registry data has revealed important epidemiologic, genetic, and clinical features of XLP. XLP is a rare disorder that affects approximately one out of every million males [5]. No ethnic or racial predilection toward developing the disease seems to exist, with patients from around the world entered into the registry. There is no standard XLP phenotype; rather, three clinical manifestations characterize most patients with XLP: (1) fulminant infectious mononucleosis (FIM), (2) malignant lymphoma, and (3) dysgammaglobulinemia (Table 1) [2]. Less commonly reported manifestations include vasculitis, pulmonary lymphomatoid granulomatosis, and hematological cytopenias such as aplastic anemia [2,9]. Manifestations of XLP can occur as isolated events, or two or more manifestations can develop sequentially in susceptible individuals. The clinical features of XLP vary between kindreds and between members of the same family, suggesting that environmental or genetic factors influence their expression. Although FIM is by definition caused by EBV infection, other XLP manifestations, including lymphoma and hypogammaglobulinemia, occasionally develop in EBV seronegative patients [5]. The disease is generally fatal, with most patients dying by age 10 years [10]. Death most often is attributed to organ dysfunction following EBV-induced hemophagocytic syndrome or to infectious complications resulting from lymphoma treatment or hypogammaglobulinemia.

Females with XLP display no overt clinical manifestations. Because only 50% of their lymphocytes express *SH2D1A* due to random X chromosome inactivation [11], normal lymphocytes appear to compensate for those in which the gene is defective. Despite the absence of obvious clinical abnormalities, many carriers

Table 1
Clinical Features of XLP

Disease phenotype	Patients with feature (%) ^a	Median age (y) (range)	Surviving patients (%) ^b	Patients with EBV+ (%)
Fulminant infectious mononucleosis	63	3 (0.5–40)	7	100
Dysgammaglobulinemia	30	9 (0.1–34)	51	56
Lymphoma, lymphoproliferation	28	6 (2–33)	26	49
Aplastic anemia	6	8 (2–24)	18	71
Vasculitis	1	6.5	0	55

Data from the International XLP Registry, supplied by T.G. Gross.

^a Percentage of patients diagnosed with each feature at presentation with XLP. The total adds up to more than 100% because patients can present with more than one feature.

^b Percentage of surviving patients was determined at the end of 1999.

develop persistent elevations in anti-EBV antibody titers, indicating chronically active viral infection [12]. A few female carriers have had baseline perturbations in serum immunoglobulin levels [13]; however, no carriers with increased susceptibility to lymphoma or clinically significant hypogammaglobulinemia have been described.

FIM

Reflecting the ubiquitous nature of EBV infection, FIM is the most common XLP phenotype and affects approximately 60% of patients [10,14]. FIM is also the most aggressive XLP feature; only 7% of patients have been documented as having survived FIM [10,15]. The median age at FIM onset is 3 years (range, 0.5 to 40), and average survival is 2 months [5,10]. Pathologically, the bone marrow, liver, spleen, thymus, lymph nodes, intestinal tract, and other affected organs show extensive infiltration by polyclonal populations of EBV-infected B cells and reactive CD4⁺ and CD8⁺ T lymphocytes [10,16–18]. The excessive secretion of interleukin-2 (IL-2) and interferon- γ (IFN- γ) by activated T lymphocytes contributes to uncontrolled macrophage activation and culminates in erythrophagocytosis, tissue destruction, and depletion of T and B lymphocytes [10,18]. Despite increased susceptibility to EBV, boys with XLP exhibit normal immune responses to other herpesviruses and to most other viral and bacterial pathogens [2]. The mechanisms underlying the dysregulated proliferation of T and B lymphocytes, heightened secretion of T_H1 cytokines, and apparently ineffective immune response against EBV are beginning to be understood. These immunologic features suggest that the XLP gene product regulates critical steps during the development of EBV-specific immunity. Re-evaluation of registry data has shown that 44 of 119 (37%) XLP patients with a history of EBV infection never experienced FIM. Although it is not known why these patients did not develop EBV-associated hemophagocytosis, it is possible that specific *SH2D1A* mutations, or other genetic or environmental factors, influenced susceptibility to EBV [5].

Dysgammaglobulinemia

Affecting 20% to 30% of patients, dysgammaglobulinemia is the second most common XLP phenotype [2,14]. Most patients with this manifestation have global decreases of serum immunoglobulin levels; however, some patients present with increased immunoglobulin M (IgM) or IgA levels and variable deficiencies in the IgG1 and IgG3 subclasses [19]. The median age at the time of hypogammaglobulinemia diagnosis is 7 to 9 years [2,13,14]. Patients with hypogammaglobulinemia usually have a more favorable prognosis than do patients with FIM or lymphoma, especially when treated with monthly immunoglobulin infusions to protect against infection. Although hypogammaglobulinemia most often occurs after EBV infection, some patients with XLP develop hypogammaglobulinemia without any previous evidence of EBV [5], which indicates that the immune defects associated with XLP are not limited to the aberrant EBV response.

Lymphoma

Lymphomas develop in 20% to 30% of patients with XLP and most commonly occur following EBV infection [10]. Most lymphomas are of B-cell immunophenotype and are characterized by a small, noncleaved (Burkitt's) histology or diffuse, large-cell histology [20]. Karyotype analysis has not been extensively performed; however, in a limited series of cases, the t(8;14) associated with Burkitt's lymphoma has been identified [21]. In rare instances, patients develop Hodgkin's disease or T-cell lymphoproliferative disorders, including lymphoblastic lymphoma and lymphomatoid granulomatosis [10]. Most lymphomas are extranodal in origin and involve the terminal ileum, liver, kidney, central nervous system, thymus, and tonsils [22]. Studies revealed that, like hypogammaglobulinemia, lymphomas can develop in the absence of detectable EBV infection [3,5]. The median age at onset of lymphomas is either 5 years (range, 2 to 19) or 8 years (range, 3 to 33) in patients with or without EBV infection, respectively [5,23]. Median survival depends on EBV status, with a poorer prognosis in patients with EBV. Although lymphomas respond to initial treatment, they often recur over time. Because lymphomas arise in EBV-negative patients and can be of differing clonal origin [3,5], SH2D1A might participate in antitumor immune surveillance.

Diagnosis

Before the identification of *SH2D1A* as the genetic basis of XLP, it was difficult to make a diagnosis based solely on clinical or laboratory features. Laboratory investigations of lymphocyte number, immunophenotype, and function generally were not helpful, because results were normal [24–26] or hard to interpret because of immune dysfunction following EBV infection or lymphoma treatment. Evaluation of humoral responses displayed wide variation, with some patients who had normal values [25] and other patients who had increases in IgA or IgM levels and variable deficiencies in total IgG, IgG1, and IgG3 levels [19]. The only consistent finding was an inability to switch from IgM to IgG following secondary challenge with ϕ X174 bacteriophage [27], an observation that also can be seen with other inherited T-cell deficiencies. Investigations of EBV-specific immunity also have not been helpful in making a definitive diagnosis. Initial studies suggested that patients with XLP could not mount an effective anti-EBV immune response, as demonstrated by deficient production of EBV-specific antibodies [28–30] and by defective killing of EBV-infected B cells by autologous T or natural killer (NK) cells [26,31–35]. More recent studies, however, have demonstrated that some patients with XLP generate anti-EBV antibodies [36] and normal T-cell responses during acute and latent infection [34,36]. Because of these issues, establishing an XLP diagnosis formerly relied on the identification of two or more maternally related males who demonstrated a characteristic phenotype (such as FIM, lymphoma, or hypogammaglobulinemia) following EBV infection [37]. The diagnosis can be made more easily now based on identification of abnormal

SH2D1A gene sequence. Not all patients with XLP, however, harbor detectable *SH2D1A* mutations. For patients exhibiting typical features, but lacking detectable gene mutations, a diagnosis may be entertained based on absent SH2D1A protein expression [38].

The differential diagnosis for XLP is broad and includes sporadic fatal infectious mononucleosis (also known as EBV-associated hemophagocytic lymphohistiocytosis), X-linked hyper-IgM syndrome, X-linked agammaglobulinemia, common variable immunodeficiency, chronic active EBV infection, and familial hemophagocytic lymphohistiocytosis (Table 2). When one of these diagnoses is suspected, male patients should be considered for *SH2D1A* gene testing. Establishing the diagnosis of XLP facilitates heightened surveillance for primary EBV infection or lymphoma and preventive treatment with monthly immunoglobulin replacement or bone marrow transplantation. Knowledge that a

Table 2
Diseases included in the differential diagnosis of XLP

Disease	Age at diagnosis	Gene defect	Features
Sporadic fatal infectious mononucleosis ^a	Peaks between 6–13 years	None known	Severe EBV, symptoms similar to FIM No lymphoma, dysgammaglobulinemia
X-linked agammaglobulinemia	Peaks between 1–2 years	<i>BTK</i>	Low or absent B cells Hypogammaglobulinemia Recurrent bacterial infections No lymphoma or severe EBV infections
X-linked hyper-IgM syndrome	Peaks between 1–2 years	<i>CD40L</i>	Normal or increased IgM Decreased IgG, IgE, IgA Bacterial and opportunistic infections Lymphoma may occur Severe EBV infections are rare
Common variable immunodeficiency	Peaks between 20–30 years	None known	Heterogenous disorder Variable hypogammaglobulinemia Increased autoimmunity Increased lymphoma later in life Severe EBV infections are rare
Familial hemophagocytic lymphohistiocytosis	Peaks between 1–3 years	<i>PRF1</i> , others	Severe EBV, symptoms similar to FIM No lymphoma, dysgammaglobulinemia
Chronic active EBV infection	Variable	None known	Prolonged or recurrent IM-like features Abnormal EBV serology Dysgammaglobulinemia T-cell or NK-cell malignancies occur

Abbreviations: EBV, Epstein-Barr virus; FIM, fulminant infectious mononucleosis; IM, infectious mononucleosis; NK, natural killer.

^a Also known as EBV-associated hemophagocytic lymphohistiocytosis.

patient has XLP expedites prompt initiation of treatment for primary EBV infection before progression to a full-blown hemophagocytic syndrome. A genetic diagnosis also allows counseling and prenatal testing for affected individuals and other family members.

Prognosis and treatment

Without treatment, XLP, in most cases, is a fatal disorder [10]. Definitive treatment involves allogeneic hematopoietic stem-cell transplantation (HSCT) to replace the defective immune system before development of typical XLP manifestations [23,39–43]. Fifteen patients with XLP have undergone conventional stem-cell transplantation. Although HSCT corrected the abnormal immune response in 11 patients, 4 (27%) died from infectious or therapy-related causes. Nonmyeloablative transplantation offers a potential advantage for patients with XLP or other immunodeficiencies [44]. Because lower doses of radiation and chemotherapy are used, this approach may result in less transplant-related toxicity. To date, only 1 patient with XLP has been reported as having undergone nonmyeloablative transplantation [44]. Although this patient's disease recurred following transplantation, the efficacy of nonmyeloablative therapy requires further investigation.

The treatment of XLP component illnesses varies and depends on the phenotype affecting a given patient. Antiviral therapies, including IFN- α , have been used to treat primary EBV infection [33]; however, none have proven particularly effective. A promising treatment for EBV-associated hemophagocytic syndrome includes the chemotherapy agent etoposide, a topoisomerase II inhibitor that is cytotoxic against activated macrophages. Glucocorticoids and cyclosporine often are used concomitantly to suppress reactive T lymphocytes (K.E. Nichols, MD, T.G. Gross, MD, PhD, personal communication, 2002). Patients who develop hypogammaglobulinemia require regular immunoglobulin infusions to prevent bacterial and viral infections. Immunoglobulin infusions, however, do not guarantee protection from primary EBV infection in EBV seronegative patients [2,33]. For patients with lymphoma or cytopenias, standard medical approaches can be used. Although medical treatments can induce disease remissions, relapses and other XLP phenotypes often occur [14].

Genetics

In 1998, the authors and one other group of investigators cloned the XLP gene based on its previous genetic linkage to the Xq25 chromosomal locus [6,45]. Following identification of patients harboring constitutional deletions at Xq25, the affected gene *SH2D1A* (also known as *DSHP*) was isolated through large-scale sequencing of this region and mutational evaluation of isolated candidate genes. A third group of investigators concurrently identified the murine homologue *SAP*

through studies of the interacting protein SLAM (or CD150), a putative T-cell costimulatory molecule [8].

SH2D1A is comprised of 4 exons that span 25 kilobases of genomic DNA. The gene encodes a 128 amino acid protein consisting of a single SH2 domain and a short carboxyl terminal tail (Fig. 1). Although the carboxyl terminal tail has no homology with other known protein domains, the SH2 domain shares structural similarity with the SH2 domains contained within important lymphocyte regulatory proteins, including the SH2 domain-containing inositol polyphosphate 5' phosphatase (SHIP) and the Abl tyrosine kinase (cABL). The SH2D1A protein is highly homologous to EWS/Flt1 activated transcript 2 [46], a 132 amino acid protein of unknown function that also is comprised exclusively of an SH2 domain and short carboxyl-terminal tail. SH2D1A is highly conserved across species, with 87% amino acid identity to the murine protein.

SH2 domains are modular protein structures that mediate protein–protein interactions by binding to phosphorylated tyrosine residues. Because SH2D1A contains an SH2 domain, it has been postulated to function by participating in tyrosine phosphorylation-dependent signaling pathways [6,8,45]. However, because SH2D1A contains only a single SH2 domain, without other signature motifs, the authors and other investigators have speculated that it may act as an inhibitor of signaling proteins that contains a similar SH2 domain [8,47–49]. By

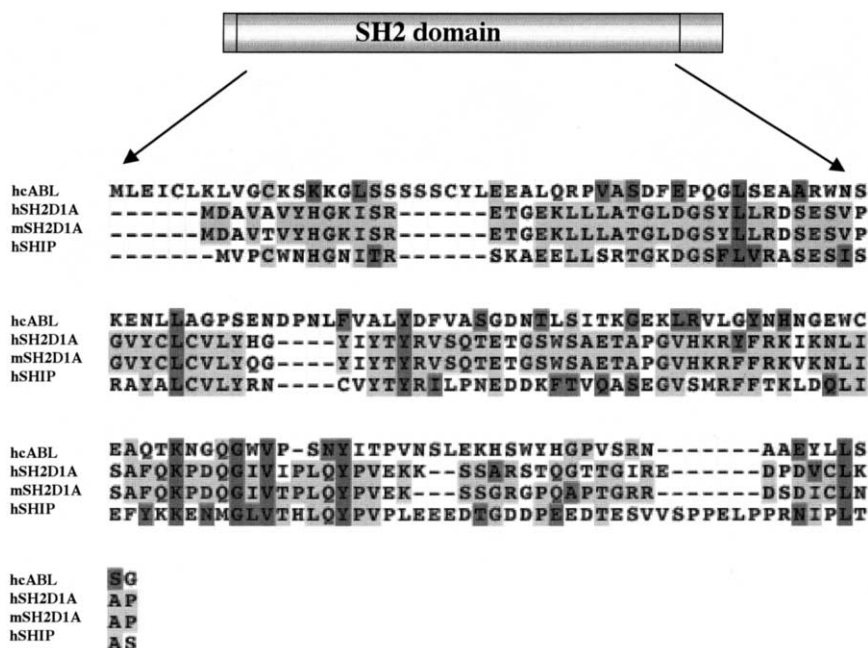


Fig. 1. SH2D1A protein structure and amino acid alignment between human and murine SH2D1A, and the SH2 domains of the human cABL (hcABL) tyrosine kinase, and human SHIP (hSHIP).

binding to phosphotyrosine residues within interacting proteins, SH2D1A might compete with the binding of these other SH2 domain-containing molecules. In so doing, SH2D1A could regulate the signals generated by these multiprotein complexes (see later in article; Fig. 2A). Other investigators have suggested a model in which SH2D1A functions by recruiting additional molecules or by stabilizing signaling complexes (Fig. 2B) [50].

Genetic analyses of patients with XLP identified over 50 unique mutations spanning the length of the *SH2D1A* gene [3,5,6,8,45,47,51–54]. The incidence of mutations varies, depending on whether patients come from classic XLP pedigrees that demonstrate an X-linked pattern of inheritance. In this regard, constitutional *SH2D1A* mutations have been identified in up to 97% of well-defined XLP kindreds [5]. In contrast, *SH2D1A* mutations are absent or rare in boys with features of XLP who lack a positive family history (so-called “sporadic” XLP) [5,51–54]. No mutations have been identified in a limited number of male patients with chronic active EBV infection [5,53]. Several possibilities might explain the

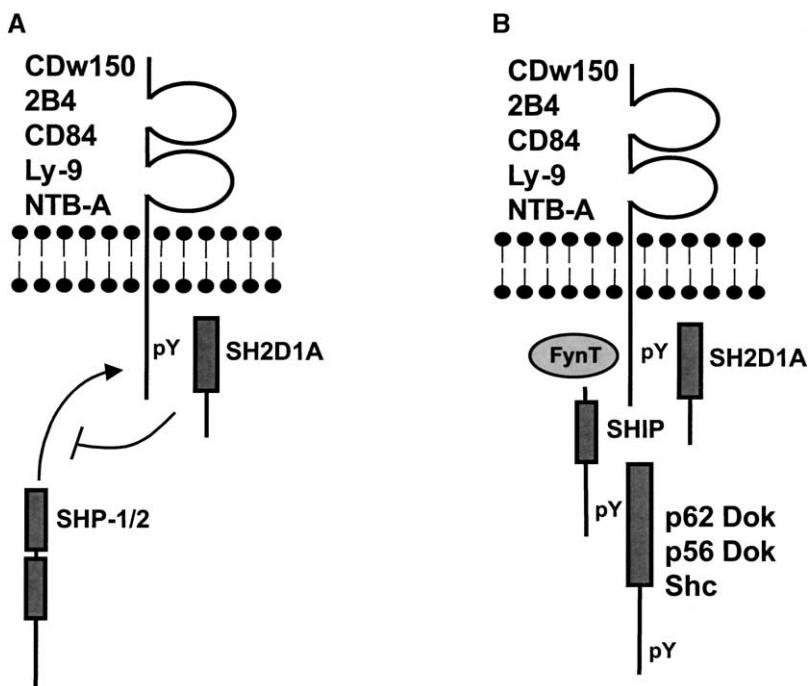


Fig. 2. Possible models of SH2D1A function. (A) SH2D1A functions as a physiologic inhibitor of protein interactions. By binding phosphotyrosine residues on the cytoplasmic tail of SLAM, 2B4, CD84 and Ly-9, SH2D1A can block the binding of the SHP-1 and SHP-2 tyrosine phosphatases. (B) Alternatively, SH2D1A might recruit the tyrosine kinase Fyn, thereby facilitating phosphorylation of SLAM and possibly other SH2D1A-interacting receptors. This leads to phosphorylation of downstream signaling intermediates, including SHIP, p62 Dok, p56 Dok, and Shc. Using these modes of action, SH2D1A can modulate NK cell cytotoxicity, T-cell proliferation, cytokine secretion, and possibly B-cell survival and antibody production.

absence of mutations in certain patients with XLP, including: mutations residing outside of the *SH2D1A* coding region that might influence RNA expression, splicing, or stability; misdiagnosis of XLP based on its similarity to other genetically unrelated immunodeficiencies; or existence of other unidentified X-linked genes that, when mutated, cause features that resemble XLP.

The spectrum of XLP-associated *SH2D1A* mutations is broad and includes large and small deletions and insertions, single nucleotide substitutions, and splice-site abnormalities. Most mutations are believed to affect normal function by deleting all or part of the *SH2D1A* gene, truncating the protein within its SH2 domain, or altering amino acid residues that are required for phosphotyrosine binding. These observations argue that a genetic loss of function underlies the abnormal immune response in patients with XLP. Some missense alterations, however, lead to amino acid substitutions outside the region of the critical SH2 domain phosphotyrosine-binding pocket. Although these mutations have been shown to alter function and protein stability in *in vitro* biochemical assays [47,49], their *in vivo* effects on *SH2D1A* function and expression remain uncharacterized. An extensive genetic analysis of 35 families with histories of XLP did not reveal a correlation between *SH2D1A* genotype and the type or severity of clinical manifestations of XLP [5].

Expression of *SH2D1A*

Because of the use of different polyadenylation sequences, *SH2D1A* is expressed as two RNA species measuring 2.5 and 0.9 kilobases [8,45]. These transcripts are found exclusively in lymphoid tissues, including the spleen, thymus, tonsils, and peripheral blood [6,8,45], with moderate levels of expression in most primary T and NK cells and in transformed T and NK cell lines [8,45,55,56]. *SH2D1A* is expressed throughout thymocyte development and in CD4⁺ and CD8⁺ peripheral T cells [55]. In contrast to T lymphocytes, *SH2D1A* has a more limited expression in B cells. The transcript has been reported in human tonsillar lymphocytes of the memory phenotype (K.E. Nichols, unpublished results), rare B lymphoma or leukemia cell lines [8,57], and in certain human B-cell malignancies [45]. Consistent with investigations of RNA expression, the *SH2D1A* protein is present in activated T and NK cells [8,45,55,56] and possibly in some B lymphocytes [8,57,60]. Western blot analyses using Jurkat T cells demonstrate *SH2D1A* expression in cytoplasmic and membrane fractions, with little change in intracellular redistribution following activation using agonistic anti-T-cell receptor antibodies (K.E. Nichols, unpublished results).

As anticipated for a molecule involved in antiviral immune responses, *SH2D1A* RNA levels vary depending on activation status and cellular context. In this regard, *SH2D1A* message steadily increases following *in vitro* stimulation of human or murine NK cells with IL-2 [55,56]. In contrast, studies of *SH2D1A* expression have shown differing patterns in activated T lymphocytes. In the authors' studies, expression gradually increases over 1 to 5 days following T-cell activation using the mitogens phytohemagglutinin, concanavalin A, and the

agonistic anti-CD3 antibody OKT3 [45]. Sayos and investigators, however, reported an initial decrease in *SH2D1A* expression, followed by a rise in RNA levels [8]. This bimodal pattern might indicate that the relative abundance of SH2D1A differentially influences T cells early or late in the activation process. Such alterations in the levels of SH2D1A relative to its various partner proteins might influence formation of SH2D1A signaling complexes.

To date, there are few published reports of the expression of mutant SH2D1A protein in tissues derived from patients with XLP. To investigate this question, the authors performed immunohistochemistry using tissue from a patient with XLP who harbored an A→G nucleotide substitution at position 319 of the *SH2D1A* cDNA sequence, a mutation that results in the substitution of cysteine for tyrosine at codon 7. In contrast to normal control lymphoid tissues, the lymphocytes from this affected patient did not have detectable SH2D1A protein, suggesting that this *SH2D1A* mutation might lead to disease by decreasing protein expression levels (Fig. 3). The authors are completing a more comprehensive study of *SH2D1A*

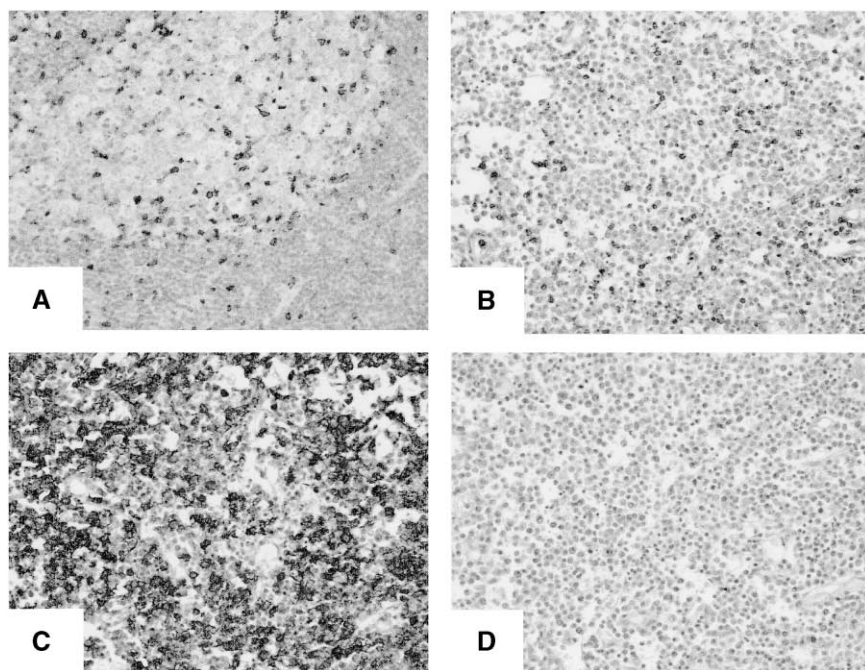


Fig. 3. Immunohistochemical analysis of the expression of SH2D1A. (A) Staining of a normal control lymph node obtained during infectious mononucleosis using anti-SH2D1A polyclonal antibody. (B–D) Sections from lymphoproliferative disease tissue obtained from a patient with XLP who harbored an A319G substitution are stained with the T cell marker anti-CD3 (B), the B cell marker anti-CD20 (C), or anti-SH2D1A (D). (D) Lack of brown staining indicates the absence of a detectable SH2D1A protein. Stain, avidin-biotin complex (ABC) technique followed by diaminobenzidine (DAB) stain. Original magnification, 400 $\times$. (Courtesy of S. Pittaluga, MD, PhD and E. Jaffe, MD, Bethesda, MD.).

expression in paraffin-embedded tissues obtained from patients with XLP and known genetic mutations.

Structural modeling of SH2D1A

The murine homologue of SH2D1A first was identified as a protein that could interact with the cellular receptor SLAM using a yeast two hybrid screen [8]. Because tyrosine phosphorylation does not occur in yeast, this observation suggested that SH2D1A might function differently from more conventional SH2 domain-containing molecules. Typically, the protein interactions mediated by SH2 domains are stabilized by contacts between the phosphotyrosine-binding pocket with a phosphorylated tyrosine residue and several amino acids carboxyl-terminal to this tyrosine [58]. Crystallography and nuclear magnetic resonance (NMR) investigations of the interaction between SH2D1A and SLAM revealed that, in contrast to this characteristic configuration, the amino acid residues both carboxyl- and amino-terminal to SLAM tyrosine 281 (Y281) stabilize this intermolecular interaction [58,59]. The energy contributed by interaction with amino acids on both sides of Y281 might explain how SH2D1A binds to nonphosphorylated SLAM. The implications of the unusual interaction between SH2D1A and phosphorylated and nonphosphorylated SLAM remain unclear. It has been speculated that by interacting with nonphosphorylated SLAM, SH2D1A might block the binding of tyrosine kinases, preventing the generation of more conventional SH2 domain binding sites [58,59]. Alternatively, by interacting with phosphorylated SLAM, SH2D1A might prevent the delivery of downstream signals mediated by recruitment of other SH2 domain-containing molecules.

Proteins interacting with SH2D1A

Identification of interacting proteins is expanding the understanding of how SH2D1A regulates lymphocyte function. SH2D1A has been reported to bind to six partner proteins, including the CD2 family members SLAM (CD150) [8,47,50,55,60], 2B4 (CD244) [55,56,61–63], CD84 [47,64], Ly-9 (CD229) [64], NK-T-B-antigen (NTB-A) [7], and the cytoplasmic adaptor p62 downstream of kinase (Dok) (also known as Dok1) [66]. This discussion focuses on the association of SH2D1A with SLAM, 2B4, and NTB-A because data exist to support the potential biologic relevance of these protein interactions.

Signalling lymphocytic activation molecule

The signaling lymphocytic activation molecule is a glycosylated type-1 trans-membrane protein that is present on memory T cells, immature thymocytes, dendritic cells, and a subset of B cells [67,68]. Expression of SLAM is upregulated rapidly on T and B cells following activation [67–69]. Agonistic anti-SLAM monoclonal antibodies induce proliferation and IFN- γ production by

activated T cells [67,70,71] and stimulate proliferation and immunoglobulin synthesis by activated B cells [72]. Based on these properties, SLAM is speculated to mediate the interactions that occur between activated T and B lymphocytes during antiviral and other immune responses. In addition to its role as a coreceptor for cellular activation, SLAM has been found to function as a cellular receptor for measles [73]. It not yet is known whether measles infection of SLAM-expressing lymphocytes results in cellular activation or cytolysis. Some investigators have suggested, however, that SLAM-mediated entry of virus into lymphocytes might contribute to the profound immune suppression accompanying measles infection [73].

Different models have been proposed to explain how SH2D1A might regulate signaling by SLAM. Using *in vitro* model systems, several investigators have shown that overexpression of SH2D1A prevents the interaction between phosphorylated SLAM and SH2 domain containing phosphatase 2 (SHP-2), a protein tyrosine phosphatase that is associated with inhibitory function (see Fig. 2A) [8,47,55]. These data support the model that SH2D1A functions as an inhibitor of protein interactions, and it remains possible that by displacing SHP-2, SH2D1A affects downstream signaling by changing the phosphorylation status of SLAM. Alternatively, phosphorylated SLAM might sequester SHP-2 away from the T-cell, and possibly B-cell, receptors [74]. The loss of functional SH2D1A, which occurs in XLP, might prolong T- and B-cell receptor signaling and result in enhanced antigen-driven lymphocyte expansion and function. Latour and investigators have proposed that, instead of inhibiting protein interactions, SH2D1A might facilitate the generation of multiprotein complexes involving SLAM (see Fig. 2B) [50]. Specifically, these investigators have shown that following SLAM ligation, SH2D1A recruits the Fyn tyrosine kinase to the cytoplasmic tail of SLAM. By recruiting Fyn to SLAM, SH2D1A promotes the phosphorylation of SLAM itself and promotes the recruitment and phosphorylation of the inositol phosphatase SHIP and several downstream signaling intermediates. In B cells, both models may apply. Shlapatska and investigators have shown that, in certain B-cell lines, SH2D1A serves as a molecular switch that controls the interaction between SLAM and either SHP-2 or SHIP [60]. These investigators speculate that, depending on SH2D1A expression levels, SLAM might transmit a SHP-2- or SHIP-dependent signal.

2B4

2B4 is a receptor expressed on NK cells, most CD8⁺ T cells, thymocytes, $\gamma\delta$ T cells, monocytes, and basophils [63,75–77]. Although its role during T-cell activation is not clear [78], the ligation of 2B4 on NK cells results in increased activation, IFN- γ secretion, and cytolytic function [75–80]. Because its amino acid sequence is similar to that of SLAM, several investigators evaluated 2B4 as a molecule that is capable of interacting with SH2D1A. These studies revealed that in human NK cells and NK cell lines [55,61,62], or in transfected heterologous cell lines [47,55,63], SH2D1A inducibly binds to the cytoplasmic

tail of 2B4. Unlike the interaction between 2B4 and SLAM, the interaction between 2B4 and SH2D1A requires tyrosine phosphorylation [47,51,55,62,63], and little or no association is seen in the absence of cellular stimulation. In a manner similar to SLAM, phosphorylated 2B4 has been found to interact with SHP-2 [55,63] and with the related tyrosine phosphatase SHP-1 [47,62]. Whether SH2D1A interferes with the recruitment of these phosphatases in NK cells has not been reported yet.

Four different groups of investigators simultaneously analyzed the expression and function of 2B4 on NK cells derived from patients with XLP [56,61,62,81]. Using flow cytometry, expression of 2B4 was found to be identical on control NK cells and XLP NK cells, indicating that SH2D1A is not required for the expression of this receptor [56,61,62]. Ligation of 2B4 with a specific monoclonal antibody or its natural ligand CD48 revealed decreased cytotoxic activity of XLP peripheral blood mononuclear cells, lymphokine-activated killer cells, NK cell clones, and polyclonal cell lines against a variety of target cells, including EBV-infected B cells [56,61,62,81]. In contrast, SH2D1A-deficient NK cells were capable of killing when activated by way of receptors that do not associate with SH2D1A, such as CD2, CD16 [56,61,62], and the natural cytotoxicity receptors NKp46, NKp44 and NKp30 [61,62]. Therefore, SH2D1A seems to be required for the initiation of activating or costimulatory signals delivered by way of 2B4. Parolini and investigators also have shown that in XLP NK cells, ligation of 2B4 actively inhibits killing mediated by other NK cell receptors [62]. The inability to specifically activate NK cells, or to abnormally suppress cytotoxic function via other NK cell receptors, might contribute to the abnormal immune responses that follow EBV or other viral infections. It is not known whether similar defects lead to functional impairments of normal immune surveillance and increased lymphoma susceptibility.

NTB-A

Bottino and investigators described a novel member of the immunoglobulin superfamily NTB-A, a receptor expressed on all human NK, T, and B cells [65]. Like 2B4, antibody-mediated cross-linking of NTB-A induces NK cytotoxicity against EBV⁺ and EBV⁻ target cells. NTB-A cooperates with 2B4 and NKp46 to induce maximal NK cell lytic activity. NTB-A contains two tyrosine-based motifs that conform to the SH2D1A consensus (TxYxxV/I) [58,59]. Following phosphorylation, NTB-A associates with SH2D1A, SHP-1, and SHP-2. As noted for 2B4, XLP NK cells demonstrate defects in NTB-A-mediated function, including deficiencies in cytotoxic activity and inappropriate suppression of killing by way of other NK cell receptors [65].

SH2D1A and its role during control of EBV infection

A model explaining how SH2D1A might regulate EBV-specific responses can be inferred from data demonstrating interactions between SH2D1A and its other

partners, including SLAM and possibly 2B4, NTB-A, CD84, and Ly-9. Because these proteins, and their cognate ligands, are expressed broadly during lymphocyte activation, they might regulate the interactions that take place between EBV-infected B cells and reactive cytotoxic and helper T cells. If SH2D1A modulates T-cell responsiveness by binding to these receptors, absence of functional protein could lead to excessive T-cell proliferation and aberrant cytokine production. In addition to T-cell defects, XLP NK cells seem to be unable to kill EBV-infected target cells using the 2B4 or NTB-A receptors. This action could lead to further accumulation of EBV-infected B cells, thereby increasing T-cell activation and proliferation. It is not yet clear whether aberrant signaling through one or more SH2D1A-associated receptors contributes to progressive hypogammaglobulinemia and increased lymphoma development.

Animal models of XLP

To clarify how SH2D1A regulates lymphocyte function *in vivo*, two groups of investigators generated mice that are deficient in the expression of *SAP* because of targeted gene disruption [74,82]. Consistent with humans with XLP, mutant animals are viable and fertile, transmit the gene defect in an X-linked, Mendelian fashion, and display no significant defects in the number and phenotype of developing thymocytes or circulating T, B, and NK cells [74,82]. Following infection, *SAP*-deficient mice also recapitulate important aspects of the human disease. These mice exhibit abnormal lymphocyte activation after infection with the lymphocytic choriomeningitis virus (LCMV), which, like EBV, elicits strong CD8⁺ cytotoxic T-cell responses. When injected with LCMV Armstrong, a strain that causes acute infection, mutant animals develop increased numbers of total and LCMV-specific CD4⁺ and CD8⁺ T cells [74,82]. T lymphocytes that are reactive to LCMV demonstrate heightened cytotoxicity [82] and increased secretion of IFN- γ following *in vitro* stimulation [74,82] or secondary LCMV infection [82]. Morbidity and mortality increase following injection with the virulent strain LCMV-clone 13 [74] or the hepatotropic strain LCMV-we [82], suggesting that the increased T-cell responsiveness of *SAP*-deficient animals worsens tissue inflammation that results from chronic infection. In comparison with T-cell hyperactivation, B-cell responses seem to be blunted, with a dramatic decrease in the numbers of total and LCMV-specific antibody-secreting cells over time [74]. In addition to their heightened sensitivity to LCMV, *SAP* knockout mice develop abnormal cellular and humoral responses following infection with other pathogenic agents, including the intracellular protozoa *Toxoplasma gondii* [74] and *Leishmania major* [82]. These data indicate that *SAP* in mice regulates immune responses that extend beyond acute or chronic LCMV infection.

Previous investigations of patients with XLP demonstrated elevated levels of IFN- γ during infectious mononucleosis [10], suggesting that a bias toward production of T_H1 cytokines might contribute to the overwhelming macrophage

activation that follows EBV infection. This clinical observation supports the finding that *SAP*-deficient mice produce more IFN- γ and less IL-4 at baseline and following in vitro and in vivo stimulation [74,82]. Defects are not caused by a general inability to generate a T_H2 response, because *SAP*-deficient T cells produce normal levels of IL-4 following incubation under T_H2 polarizing conditions [74,82]. The increased T-cell activation, bias toward T_H1 cytokine secretion, and defects in antibody production that occur in *SAP*-deficient mice suggest that these mice are useful in improving the understanding of fulminant infectious mononucleosis and how SH2D1A regulates in vivo immune responses to viral and other infectious agents.

Summary

Studies of the genetics and function of SH2D1A have brought about several advances in the field of XLP research. Investigations of genetically affected patients and *SAP*-deficient mice revealed that the immune defect that typifies XLP is not limited to EBV infection and extends to a variety of known and unknown pathogenic agents. Crystallographic studies demonstrated that SH2D1A might function differently from other, more conventional SH2 domain-containing molecules. By binding with varying affinity to nonphosphorylated or phosphorylated tyrosine residues, SH2D1A differentially might regulate the formation of larger protein complexes. The identification of SLAM and other SH2D1A-associated proteins clarifies new functional and biochemical pathways that regulate antiviral and more general immune responses.

Despite these advances, several important questions remain unanswered. Why is EBV the dominant clinical manifestation typifying XLP? Does this observation relate to the fact that EBV is a ubiquitous virus that delivers an extremely potent antigenic stimulus? Or are certain SH2D1A-dependent pathways specifically required for controlling infection with this virus? What explains the development of progressive hypogammaglobulinemia in patients with XLP and *SAP*-deficient mice? Could SH2D1A have a role in regulating B-cell activation or survival, or are defects in immunoglobulin production solely caused by skewed profiles of T-cell cytokine secretion? How does one explain the development of lymphoma in patients with XLP who have no history of previous EBV infection? Do defects in the normal immune surveillance mediated by T, NK, or NKT cells have roles in lymphoma susceptibility? What are the mechanisms by which SH2D1A functions? Does this protein act as an inhibitor or as a facilitator of protein interactions? What are the critical partner proteins of SH2D1A, and how do they function in concert with SH2D1A to control lymphocyte responsiveness following infectious or other immunologic challenges? Future clinical and scientific investigations will continue to improve the understanding of XLP and highlight the importance of SH2D1A during the regulation of EBV-specific and other immune responses.

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