Review Article

Primary Immunodeficiencies Inducing EBV-Associated Severe Illnesses

Toshio Miyawaki

Department of Pediatrics, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

ABSTRACT

Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus that infects about 95% of the adult population. The majority of primary infections occurs in early childhood and is generally subclinical; it can cause infectious mononucleosis (IM), which is usually a self-limiting lymphoproliferative disorder. However, infection of EBV occasionally results in severe, often lethal diseases, which include fatal IM, hemophagocytic syndrome, polyclonal lymphoproliferative disorders, and malignant lymphoma. These severe EBV-related illnesses occur secondary to some primary immunodeficiency diseases showing inefficient immune reaction to EBV. One example is X-linked lymphoproliferative disease (XLP), which is caused by mutations in the *SLAM-associated protein (SAP) gene*. The major clinical manifestations of XLP are fulminant IM, malignant lymphoma and dysgammaglobulinemia. Aplastic anemia, virus-associated hemophagocytic syndrome, and vasculitis have also been reported in XLP. We have developed a flow cytometric method using the anti-SAP monoclonal antibody to search for XLP. This clinically useful assay has successfully been used to identify XLP patients in Japan. In this review, clinical and mutational characteristics of XLP in Japan are mainly described. In addition, it is shown that the similar situations to XLP can occur in other primary immunodeficiencies involving T-cell killing function, such as autoimmune lymphoproliferative syndrome caused by *Fas gene* mutations or familial hemophagocytic lymphohistiocytosis caused by *perforin gene* mutations. Finally, the EBV-related terrible disease condition, namely chronic active EBV infection, which is common in Asian areas but its genetic background remains to be elucidated, will be touched on.

Keywords: Epstein-Barr virus, illness, infection, immunodeficiency

INTRODUCTION

Epstein-Barr virus (EBV) was discovered about 40 years ago by electron microscopy of cells cultured from Burkitt's lymphoma tissue by Epstein, Achong, and Barr.[1] Then, this virus was shown to be the etiologic agent for infectious mononucleosis (IM), and also was demonstrated to be associated with some malignant tumors, such as nasopharyngeal carcinoma or non-Hodgkin's lymphoma.

EBV is a member of the human herpes virus family.[2] EBV is infecting over 90 percent of humans, and persisting for the lifetime of a person. In the oropharynx, EB virus directly infects resting B cell or infects epithelial cells. The EBV-infected B cells undergo lytic infection with production of virus or express the full complement of the latent viral proteins, termed LMP-1 or LMP-2. These EBV-infected B-cell blasts should be checked by cytotoxic T cells or natural killer cells. Thereafter, EBV is present in the peripheral blood in latently infected memory B cells. These cells can undergo EBV reactivation, and they may be recognized and

Corresponding Author: Dr. Toshio Miyawaki, Department of Pediatrics, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama, Toyama 930-0194, Japan. Tel: (+81 76) 434 7310, Fax: (+8176) 434 5029, E-mail: toshio65@ms.toyama-mpu.ac.jp

destroyed by cytotoxic cells.

Primary EBV infection is usually asymptomatic in most people, and causes infectious mononucleosis (IM) in a minority of population. Unlike Western countries, primary infection in Asian areas occurs in infancy and early childhood.[3] Pediatricians occasionally see children with IM at the clinic. They can easily diagnose children suffering from IM. Patients with IM manifest the triad of fever, tonsillitis, and lymphadenopathy. Hepatosplenomegaly and liver dysfunction are present in most patients. We detect many atypical lymphocytes in the blood of IM patients, which are the *in vivo* activated T cells by EBV infected.[4] The important point is that IM is a self-limited and benign illness. However, it would be expected that the failure of cytotoxic T cells or natural killer cells to check EBV-infected B cells result in expansion of EBV-infected B-cells, presumably leading to clinically severe events in the host. These severe EBV-related illnesses are associated with primary immunodeficiency diseases as well as iatrogenic immunodeficiencies such as stem cell transplantation. The representative of the former is X-linked lymphoproliferative disease (XLP).[5] Similar situations to XLP can occur in some primary immunodeficiencies involving T-cell killing function, such as autoimmune lymphoproliferative syndrome (ALPS) caused by *Fas gene* mutations[6] or familial hemophagocytic lymphohistiocytosis (FHL) caused by *perforin gene* mutations.[7] Other primary immunodeficiencies, particularly with T-cell defect, may also cause EBV-related death in children, by causing malignant diseases or hematologic disturbance.[8-10] The unforgettable EBV-associated severe disorder is chronic active EBV infection (CAEBV), which is persistent IM-like illness, showing abnormally EBV antibody titers and hematologic abnormalities.[11] CAEBV has been occasionally found in Asian children.

X-linked Lymphoproliferative Disease (XLP)

In 1975, Purtilo et al. described an X-linked lymphoproliferative disease (XLP) as X-linked recessive progressive combined variable immunodeficiency, in which affected males present with a rapid fatal course following EBV infection.[5] In 1998, more than 20 years after the discovery of XLP, also known as Duncan's disease, its defective gene was identified by positional and functional cloning approaches, and was designated *SH2D1A/DHSP,* or the SLAM-associated protein (*SAP)* gene.[12-14] The gene contains 4 exons, and encodes a small intracytoplasmic protein of 128 amino acid residues, consisting of a single SH-2 domain and a unique short tail at the carboxyl-terminal. A variety of *SAP* gene mutations have been reported in XLP patients, and a disease-specific database has been organized (http://bioinf.uta.fi/SH2D1Abase). Exposure to EBV results in three clinical phenotypes in males with *SAP* mutations.[15] Fulminant and mostly fatal IM occurs in 58% of cases. The prognosis of fulminant IM is extremely poor. Lymphoproliferative disorders including malignant lymphoma develop in 30% of cases. Dysgammaglobulinemia is found in 31% of cases, some of whom occasionally exhibit various degrees of hypogammaglobulinemia. XLP patients presenting with hypogammaglobulinemia have a relatively favorable prognosis; particularly, if they are treated with an intravenous immunoglobulin replacement. Less frequent manifestations are aplastic anemia, vasculitis and lymphoid granulomatosis. Although XLP has long been considered rare in Japan, we surveyed for the possible presence of XLP patients in Japan by *SAP* mutation analysis.[16] We investigated 40 male patients with severe EBV-associated illnesses (fulminant IM, EBV-positive lymphoma, and CAEBV), including fatal cases. *SAP gene* mutations were detected in 10 patients from 9 families, but we

Table 1. Primary immunodeficiencies causing EBV-associated severe illnesses.

Diseases	Inheritance	Defective genes
X-linked lymphoproliferative disease (XLP)	X-linked	*SH2D1A/SAP*
Autoimmune lymphoproliferative syndrome (ALPS)	Autosomal	*Fas/TNFRSF6*
Familial hemophagocytic lymphohistiocytosis (FHL)	Autosomal	*Perforin*
Primary immunodeficiencies		
Wiskott-Aldrich syndrome	X-linked	*WASP*
Ataxia-telangiectasia	Autosomal	*ATM*
WHIM syndrome	Autosomal	*CXCR4*

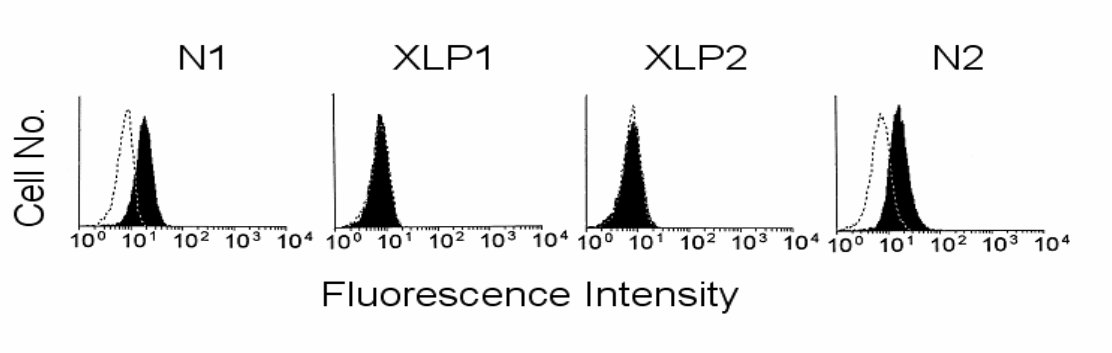

Figure 1. SAP expression in XLP patients with identified *SH2D1A* mutations. Peripheral mononuclear cells from XLP patients (XLP1 and XLP2) with identified *SH2D1A* mutations and normal donors (N1 and N2) were stimulated with PHA for 4 days. SAP expression in these PHA-activated cells was evaluated by a flow cytometric analysis using KST-3 monoclonal antibody.

could not identify mutations in patients with chronic active EBV infection.

Genetic analysis is often labor-intensive and time-consuming; and is usually carried out in specialized laboratories. To develop a simple and clinically useful method for the detection of XLP patients, we generated a monoclonal antibody, designated KST-3, against the XLP gene product, SAP.[17] We found that activated T cells substantially express SAP. To examine the possibility of detecting XLP patients by flow cytometric assay, we investigated SAP expression in activated T cells from two XLP patients with identified *SAP gene* mutations. Flow cytometric assay using the KST-3 monoclonal antibody demonstrated that there was much less expression of SAP in both patients compared with a normal control (Figure 1), indicating that the majority of *SAP* mutations in XLP could result in the defective expression of SAP. Therefore, we now are searching for additional XLP patients using this method.

Let me show one example, which we detected as XLP by a flow cytometric assay followed by genetic analysis. In this family, the proband manifested fatal infectious mononucleosis presenting after EBV infection. The proband was a 2-year-old boy, admitted to a hospital in February 2001 because of a 2-day history of high fever. He had edematous eyelids, cervical lymphadenopathy and hepatosplenomegaly. Laboratory tests documented elevated liver enzymes, thrombocytopenia, and absolute lymphocytosis with many atypical lymphocytes, inclining to a suspicion of acute EBV-induced infectious mononucleosis. Serologic studies indicated primary EBV infection. Family history revealed that his elder brother died at the age of 1 year and 11 months with a diagnosis of EBV-associated hemophagocytic lymphohistiocytosis. We suspected that this family might be a carrier of XLP.

As expected, a flow cytometric assay clearly demonstrated that the proband exhibited markedly deficient SAP expression in activated T cells (Figure 2). Repeated PCR reactions failed to generate products for the exons 3 and 4 of the *SH2D1A* gene in the patient, indicating the presence of genomic deletions containing the exons 3 and 4 of the gene. The examination of DNA extracted from the autopsy sample of his elder brother demonstrated the same deletion of the *SH2D1A* gene. Unfortunately, despite intensive supportive therapy, the proband died 53 days after admission. Subsequently, his mother gave birth to another boy, whose cord blood was immediately subjected to a flow cytometric analysis. This boy also showed SAP deficiency, indicating XLP (Figure 2). The same mutation in the *SH2D1A* gene to the proband was detected. The boy successfully received bone marrow transplantation, and is now healthy.

It is understood that SAP, the product of XLP gene, functions as an adaptor protein required for signalling through SLAM-related receptors, which are expressed on T cells or NK cells (Figure 3).[18] The interactions between SAP and SLAM-related receptors are necessary for cytokine production by T cells or cytotoxicity of T cells and NK cells. Although involvement of SAP dysfunction in the clinical

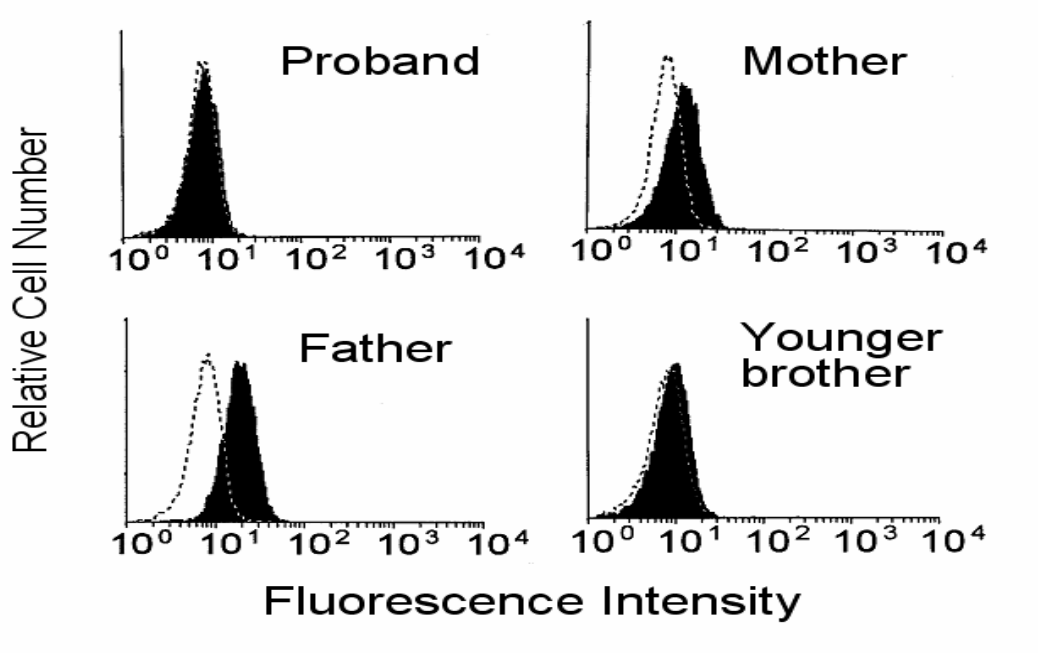

Figure 2. SAP expression in the members of an XLP family. SAP expression in PHA-activated T cells from each family member was analyzed by a flow cytometric analysis.

features of XLP is largely unknown, we understand that disturbed cytotoxicity of lymphocytes is related to uncontrolled expansion of EBV-infected B cells or activated T cells.

Autoimmune Lymphoproliferative Syndromes (ALPS)

There are some mechanisms of effector functions of cytotoxic T cells for target cells through apoptosis-mediated process.[19] One is the Fas-ligand and Fas system. This interaction can mediate intracytoplasmic caspase cascade to induce apoptosis in target cells. Another is an apoptosis-inducing system by granule-derived molecules (perforin and granzymes). Defects in the Fas-L and Fas system or perforin may result in reduced apoptosis in target cells. I hereby stress that mutations in apoptosis genes (Fas or perforin) could result in EBV-related clinical findings similar to XLP.

ALPS are the disease caused by genetic defect in the Fas-ligand/Fas system, and are clinically characterized by chronic, non-malignant lymphadenopathy, persistent splenomegaly, and recurrent auto-immune phenomena, that is, thrombocytopenia or hemolytic anemia.[20] The salient feature is an elevation in double negative T cells (TCR alfa-beta-positive CD4-negative CD8-negative lymphocytes). In 1995, it has been first reported that ALPS is caused by lymphocyte apoptotic defects and mutations in the *Fas gene*.[21] ALPS are categorized into three types. The common type is type Ia, which is caused by mutations in the Fas gene. We had taken care of siblings for years, who presented lymphadenopathy, splenomegaly and autoimmunities after primary EBV infection in early infancy. We examined the possibility that siblings might be ALPS having the Fas mutation. Both siblings had the significant elevation in double negative T cells, and we also found defect in Fas-mediated apoptosis of lymphocytes.[6] We further discovered that the Fas mutation was also found in patients diagnosed as chronic active EBV infection. Two candidates for ALPS had the elevation in double negative T cells. We confirmed that both showed a defective Fas-mediated apoptosis in lymphocytes. Using a genetic analysis, we identified *Fas gene* mutations in these 3 families. All mutations cause the deletion of the death domain in the intracytoplasmic region of the Fas molecule, which plays a key role in signal transduction of apoptosis.

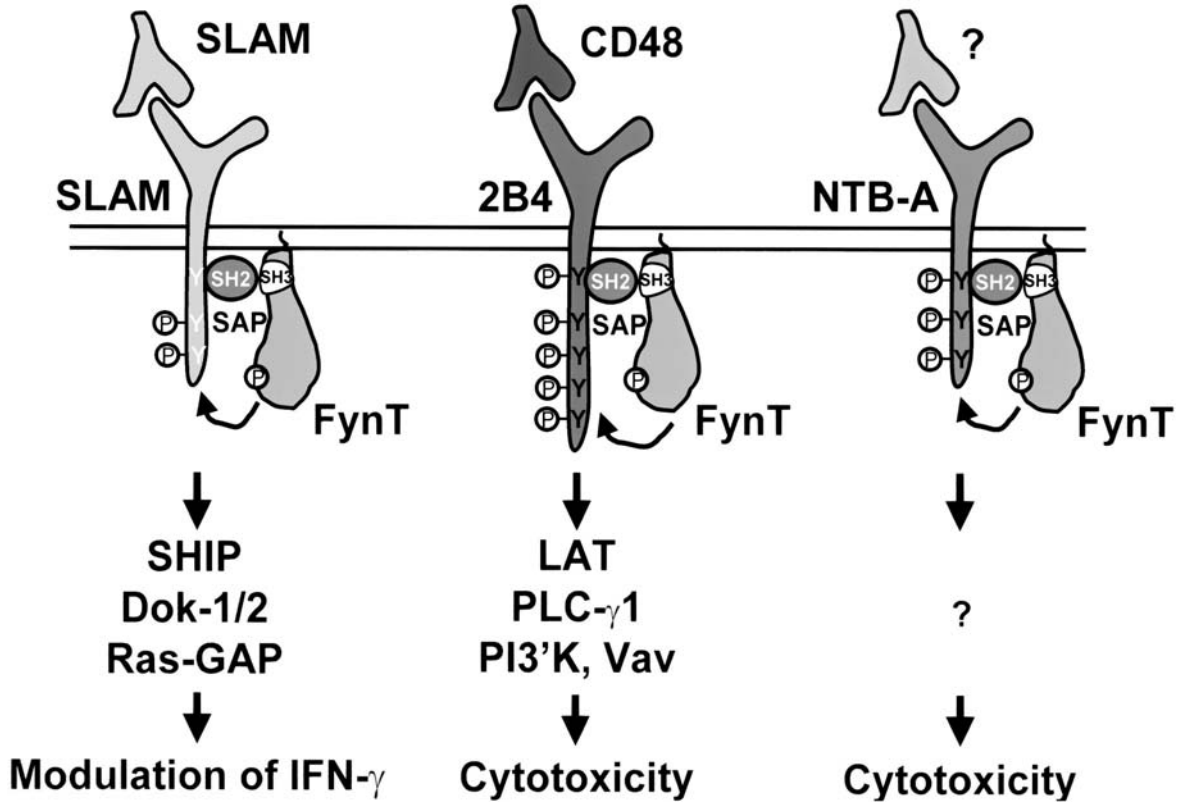

Figure 3. Model for differential impact of SLAM-related receptors on immune cell functions.[18] A model to explain the differential impact of three members of the SLAM family (SLAM, 2B4 or NTB4) on T cells or NK cells, respectively, is proposed.

Familial Hemophagocytic Lymphohistiocytosis (FHL)

It appears that mutations in the *perforin* gene can cause XLP-mimicking disease conditions. The mutations in the *perforin* gene have first been identified in patients with familial hemophagocytic lymphohistiocytosis (FHL).[22] This is a rare, fatal disorder presenting in early infancy and is characterized by fever, pancytopenia, hepatosplenomegaly, and disseminated intravascular coagulation. *Perforin gene* mutations were also identified in Japanese families with FHL.[23] Some FHL patients are associated with primary EBV infections. The phenotypes of FHL are closely similar to those seen in XLP, and it is proposed that FHL may be triggered by some kinds of viruses, including EBV.

Katano et al. have reported identification of *perforin gene* mutation in a patient diagnosed as having chronic active EBV infection.[7] This patient presented with IM followed by persistent lymphadenopathy and splenomegaly, and had later hemophagocytosis and T-cell lymphoma.

Other Primary Immunodeficiency Diseases

Although I have described XLP, ALPS and FHL as the examples of EBV-related severe illnesses of genetic origins, it is feasible to consider that some primary immunodeficiencies, particularly of T cell defect, can also lead to EBV-related lymphoproliferative diseases (Table 1). In fact, patients with Wiskott-Aldrich syndrome or ataxia-telangiectasia have been found to have EBV-related malignant lymphoma.[8,9] Recently, a patient with WHIM (wart, hypogammaglobulinemia, infections, and myelokathexis) syndrome, caused by mutations in the *CXCR4 gene*, has been reported which result to fatal EBV-associated T-lymphoproliferative disease with hemophagocytic syndrome.[10] Of course, there must be similar syndromes caused by the defect in other genes.

Chronic Active EBV Infection (CAEBV)

I would like to mention about another EBV-related severe and sometimes lethal illness, designated chronic active EBV infection (CAEBV), although the genetic background of CAEBV has remained elusive. CAEBV is the terrible EBV-associated disease condition, which is common in Eastern Asia.[11] CAEBV is characterized by severe, chronic or recurrent IM-like symptoms after a primary EBV infection, and, importantly, has a high morbidity and mortality from hepatic failure, lymphoma, sepsis, or hemophagocytic syndrome. CAEBV shows unusual patterns of anti-EBV antibodies, namely, high levels of IgG anti-VCA and EA, and absence of anti-EBNA. High EBV viral load is seen in peripheral blood of most patients with CAEBV. Now, we know that clonal expansion of EBV-infected T cells and NK cells, but not B cells, is responsible for the development of CAEBV.

In Conclusion

Many primary immunodeficiency diseases can induce EBV-related severe illnesses in the affected individuals. In future, the gene therapy will open the means to cure certain genetic diseases, but is at present far from its goal. Early diagnosis and rapid introduction of hematopoietic stem cell transplantation is very important when seeing children with EBV-related severe diseases, such as XLP, ALPS, FHL or CAEBV.

REFERENCES

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1964; 28,15:702-3.
2. Cohen JI. Epstein-Barr virus infection. N Engl J Med 2000; 343(7):481-92.
3. Kanegane H, Kanegane C, Yachie A, Miyawaki T, Tosato G. Infectious mononucleosis as a disease of early childhood in Japan caused by primary Epstein-Barr virus infection. Acta Paediatr Jpn 1997; 39(2):166-71.
4. Miyawaki T, Kasahara Y, Kanegane H, Ohta K, Yokoi T, Yachie A, et al. Expression of CD45R0 (UCHL1) by $CD4^+$ and $CD8^+$ T cells as a sign of in vivo activation in infectious mononucleosis. Clin Exp Immunol 1991; 83(3):447-51.
5. Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). Lancet 1975; 1(7913):935-40.
6. Kasahara Y, Wada T, Niida Y, Yachie A, Seki H, Ishida Y, et al. Novel Fas (CD95/APO-1) mutations in infants with a lymphoproliferative disorder. Int Immunol 1998; 10(2):195-202.
7. Katano H, Ali MA, Patera AC, Catalfamo M, Jaffe ES, Kimura H, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. Blood 2004; 103(4):1244-52.
8. Palenzuela G, Bernard F, Gardiner Q, Mondain M. Malignant B cell non-Hodgkin's lymphoma of the larynx in children with Wiskott Aldrich syndrome. Int J Pediatr Otorhinolaryngol 2003; 67(9):989-93.
9. Canioni D, Jabado N, MacIntyre E, Patey N, Emile JF, Brousse N. Lymphoproliferative disorders in children with primary immunodeficiencies: immunological status may be more predictive of the outcome than other criteria. Histopathology 2001; 38(2):146-59.
10. Imashuku S, Miyagawa A, Chiyonobu T, Ishida H, Yoshihara T, Teramura T, et al. Epstein-Barr virus-associated T-lymphoproliferative disease with hemophagocytic syndrome, followed by fatal intestinal B lymphoma in a young adult female with WHIM syndrome. Ann Hematol 2002; 81(8):470-3.
11. Kimura H, Hoshino Y, Kanegane H, Tsuge I, Okamura T, Kawa K, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. Blood 2001; 98(2):280-6.
12. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet 1998; 20(2):129-35.
13. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci U S A 1998; 95(23):13765-70.
14. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. Nature 1998; 395(6701):462-9.
15. Sumegi J, Seemayer TA, Huang D, Davis JR, Morra M, Gross TG, et al. A spectrum of mutations in SH2D1A that causes X-linked lymphoproliferative disease and other Epstein-Barr virus-associated illnesses. Leuk Lymphoma 2002; 43(6):1189-201.
16. Sumazaki R, Kanegane H, Osaki M, Fukushima T, Tsuchida M, Matsukura H, et al. SH2D1A mutations in Japanese males with severe Epstein-Barr virus--associated illnesses. Blood 2001; 98(4):1268-70.
17. Shinozaki K, Kanegane H, Matsukura H, Sumazaki R, Tsuchida M, Makita M, et al. Activation-dependent T cell expression of the X-linked lymphoproliferative disease gene product SLAM-associated protein and its assessment for patient detection. Int Immunol 2002; 14(10):1215-23.
18. Latour S, Veillette A. Molecular and immunological basis of X-linked lymphoproliferative disease. Immunol Rev 2003; 192:212-24.
19. Mullauer L, Gruber P, Sebinger D, Buch J, Wohlfart S, Chott A. Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutat Res 2001; 488(3):211-31.
20. Jackson CE, Puck JM. Autoimmune lymphoproliferative syndrome, a disorder of apoptosis. Curr Opin Pediatr

1999; 11(6):521-527.
21. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 1995; 81(6):935-46.
22. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 1999; 286(5446):1957-9.
23. Suga N, Takada H, Nomura A, Ohga S, Ishii E, Ihara K, et al. Perforin defects of primary haemophagocytic lymphohistiocytosis in Japan. Br J Haematol 2002; 116(2):346-9.