Alterations of the X-linked lymphoproliferative disease gene *SH2D1A* in common variable immunodeficiency syndrome

Massimo Morra, Olin Silander, Silvia Calpe, Michelle Choi, Hans Oettgen, Laurie Myers, Amos Etzioni, Rebecca Buckley, and Cox Terhorst

X-linked lymphoproliferative (XLP) disease is a primary immunodeficiency caused by a defect in the *SH2D1A* gene. At least 3 major manifestations characterize its clinical presentation: fatal infectious mononucleosis (FIM), lymphomas, and immunoglobulin deficiencies. Common variable immunodeficiency (CVID) is a syndrome characterized by immunoglobulin deficiency leading to susceptibility to infection. In some patients with CVID, a defective *btk* or *CD40-L* gene has been found, but most often there is no clearly identified etiology. Here, 2 unrelated families in whom male members were affected by CVID were examined for a defect in the *XLP* gene. In one family previously reported in the literature as having progressive immunoglobulin deficiencies, 3 brothers were examined for recurrent respiratory infections, whereas female family members showed only elevated serum immunoglobulin A levels. A grandson of one of the brothers died of a severe *Aspergillus* infection secondary to progressive immunoglobulin deficiency, FIM, aplastic anemia, and B-cell lymphoma. In the second family, 2 brothers had B lymphocytopenia and immunoglobulin deficiencies. X-linked agammaglobulinemia syndrome was excluded genetically, and they were classified as having CVID. The occurrence of FIM in a male cousin of the brothers led to the XLP diagnosis. Because the *SH2D1A* gene was found altered in both families, these findings indicate that XLP must be considered when more than one male patient with CVID is encountered in the same family, and *SH2D1A* must be analyzed in all male patients with CVID. Moreover, these data link defects in the *SH2D1A* gene to abnormal B-lymphocyte development and to dysgammaglobulinemia in female members of families with XLP disease. (Blood. 2001;98:1321-1325)

Introduction

X-linked lymphoproliferative (XLP) disease is characterized by extreme complications of Epstein-Barr virus (EBV) infection.[1-5] Its identification was first reported more than 25 years ago.[1] XLP has 3 major phenotypes: fulminant infectious mononucleosis (FIM) (50%), B-cell lymphoma (20%), and dysgammaglobulinemia (30%).[6,7] Aplastic anemia, vasculitis, and pulmonary lymphomatoid granulomatosis are also often associated with the syndrome.

The gene responsible for the disease has been cloned and named *SAP* (for SLAM-associated protein) or *SH2D1A*.[8-10] The human and mouse *SH2D1A* genes consist of 4 exons and 3 introns spanning approximately 25 kilobase (kb).[8,11] In the mouse, *SH2D1A* is highly expressed in thymocytes and peripheral T cells with a prevalent expression on Th1 cells.[11] Although *SH2D1A* is also expressed by natural killer cells,[12,13] its presence in B lymphocytes is unclear.

The SH2D1A protein consists of 128 amino acids comprising an SH2 domain and a 24–amino acid tail.[8,10] It has been shown to bind a family of surface immune receptors, the SLAM family, which belongs to the immunoglobulin family of receptors.[10,13,14] SLAM (CD150), 2B4 (CD244), CD84, and Ly-9 are the molecules that bind SH2D1A.[10,13-15] An SH2D1A-like molecule named *EAT-2*[16] interacts with the same SLAM family members as SH2D1A in non-T hematopoietic cells (M.M. et al, manuscript submitted, 2001).[3,5]

Among the different XLP phenotypes, FIM is the only one clearly linked to EBV infection. However, immunoglobulin deficiencies and non-Hodgkin B-cell lymphomas have been observed in patients with XLP who were seronegative or polymerase chain reaction (PCR)–negative for EBV.[17,18] Immunoglobulin deficiency and chronic respiratory infections associated with XLP clinically resemble common variable immunodeficiency (CVID).[19,20] CVID is a primary immunodeficiency syndrome characterized by decreased, often fluctuating serum immunoglobulins and clinical features of recurrent bacterial infections.[19,20] Atypical inflammatory gastrointestinal diseases and autoimmune diseases, including autoimmune hemolytic anemia, thrombocytopenia, rheumatoid arthritis, and pernicious anemia, develop in some patients. Patients with CVID also have an increased risk for cancer, particularly lymphoma.[20]

Our working hypothesis is that a subset of male patients with CVID that lacks a clearly defined etiologic factor may have alterations in the *SH2D1A* gene. In the past, X-linked agammaglobulinemia (XLA) and X-linked and autosomal hyper-immunoglobulin (Ig)M syndrome (X-HIM and HIM) disease genes have been

From the Division of Immunology, Beth Israel Deaconess Medical Center, and the Division of Immunology, Children's Hospital, Harvard Medical School, Boston, MA; the Division of Allergy and Immunology, Department of Pediatrics, Duke University School of Medicine, Durham, NC; and the Division of Pediatrics, Rambam Medical Center, B Rappaport School of Medicine, Technion, Haifa, Israel.

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Reprints: Cox Terhorst and Massimo Morra, Division of Immunology, RE-204, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215; e-mail: cterhors@caregroup.harvard.edu; mmorra@caregroup.harvard.edu.

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found mutated in some CVID patients, whereas most patients remain without a clearly identified etiologic factor.[19,21] Here, 10 males of 2 families in whom CVID had been previously diagnosed were found to have alterations in the XLP gene *SH2D1A*. Our results indicate that mutations in the *SH2D1A* gene must be studied in all male patients with CVID.

Materials and methods

Detection of mutations in the *SH2D1A* gene

Genomic polymerase chain reaction. Peripheral blood lymphocytes from families were collected in EDTA-containing test tubes. When lymphoblasts were available, they were grown in RPMI 1640 supplemented with 10% fetal bovine serum under standard culture conditions. DNA was isolated using standard techniques.[22] Coding sequences, 5′ regulatory region (300 nucleotides from the transcription initiation site), and intronic splice-site sequences were amplified by PCR (GeneAmp/XL PCR kit; PerkinElmer, Branchburg, NJ). From each family, at least 2 affected members, 2 carriers, and 2 healthy members were analyzed for mutations in the *SH2D1A* gene. PCR was performed in 50 μL with a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA), under the following conditions: 94°C for 3 minutes, 94°C for 1 minute, 60°C for 1.5 minutes, 72°C for 1 minute for 35 cycles; 72°C for 10 minutes; 4°C (∞). Primer combinations were: exon 1 F5′-GCC CTA CGT AGT GGG TCC ACA TAC CAA CAG-3′; exon 1 R5′-GCA GGA GGC CCA GGG AAT GAA ATC CCC AGC-3′; exon 2 F5′-GGA AAC TGT GGT TGG GCA GAT ACA ATA TGG-3′; exon 2 R5′-GGC TAA ACA GGA CTG GGA CCA AAA TTC TC-3′; exon 3 F5′-GCT CCT CTT GCA GGG AAA TTC AGC CAA CC-3′; exon 3 R5′-GCT ACC TCT CAT TTG ACT TGC TGG CTA CAT C-3′; exon 4 F5′-GAC AGG GAC CTA GGC TCA GGC ATA AAC TGA C-3′; exon 4 R5′-ATG TAC AAA AGT CCA TTT CAG CTT TGA C-3′. Genomic DNA from the Raji human cell line (American Type Culture Collection, Manassas, VA) was used as a positive control, and distilled water was used as a negative control. PCR products were visualized on a 1.5% agarose gel and subjected to direct sequencing procedure or to subcloning followed by sequencing (samples from female donors).

For direct nucleotide sequencing, PCR products were purified using Microcon-PCR centrifugal filters (Amicon-Millipore, Danvers, MA) and were sequenced with appropriate end-labeled primers. For subcloning, 1μL PCR product was ligated in a TA-cloning vector (TA cloning kit; Invitrogen, Carlsbad, CA). After insertion, the vector was transformed in INV-α bacteria (Invitrogen), and selection was performed through blue-white screening on Luria Bertani (LB) plates containing ampicillin (50 mg/mL) and X-Gal (40 μL of a 100mg/mL solution on each plate; Sigma, St Louis, MO). White colonies were grown overnight in LB-Amp liquid media. Plasmid was isolated using a Miniprep kit (Qiagen, Valencia, CA). For each exon, at least 10 colonies were subjected to sequencing. Sequencing data were analyzed using the programs EditSeq and MegAlign (Dnastar Software, Madison, WI).

Reverse transcription–PCR. Total RNA was isolated from peripheral blood lymphocytes of patients, carriers, and healthy persons by TRIzol Reagent (BRL, Gaithersburg, MA). One microgram total RNA was reverse-transcribed using a one-step reverse transcription (RT)–PCR system (Access RT-PCR kit; Invitrogen). The primer combinations used were F5′-GCC TGG CTG CAG TAG CAG CGG CAT CTC CC-3′ and R5′-ATG TAC AAA AGT CCA TTT CAG CTT TGA C-3′. Annealing temperature for both primer pairs was 60°C.

Measurement of serum immunoglobulin levels

Immunoglobulin isotypes G, A, and M were determined using standard laboratory procedures.[23] Part of the data reported in Table 1 were obtained from Buckley and Sidbury.[24] Measured values were considered normal, above average, or below average relative to the standards of the laboratory in which measurements were taken. Patient studies were conducted in accordance with the Helsinki protocol. Arbitrary initials were given to protect the patients' identities.

Table 1. Immunoglobulin levels in members of family 1 and family 2A

Subject	Age (y)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)
C.L.	$0^{6/12}$	391	74 ↑	75
	$1^{2/12}$	779	25	35
	$1^{5/12}$	373	0 ↓	37
	$1^{6/12}$	119 ↓	8 ↓	31
	$1^{7/12}$	248	11 ↓	72
	$1^{8/12}$	264	10 ↓	97
C.G.*	$6^{6/12}$	760	690 ↑	114 ↑
	$8^{4/12}$	410 ↓	67 ↓	44
	$9^{4/12}$	340 ↓	59 ↓	12 ↓
	10	265 ↓	53 ↓	12 ↓
C.E.*	$14^{4/12}$	55 ↓	0 ↓	10 ↓
C.F.*	$11^{9/12}$	423 ↓	253	59
	$13^{8/12}$	363 ↓	167	36
	$14^{8/12}$	290 ↓	117	18 ↓
	$15^{6/12}$	223 ↓	122	25 ↓
C.B.* (mother)	39	1263	896 ↑	33 ↓
	41	1200	1274 ↑	30 ↓
	42	950	1000 ↑	42
	43	900	1000 ↑	39
C.A.* (father)	43	690	450	71
	45	900	315	46
	46	870	340	64
A.C.	3	120 ↓	3 ↓	5 ↓
A.B.	$0^{10/12}$	90 ↓	0 ↓	4 ↓

↑ indicates value above normal for age; ↓, value below normal for age. For normal ranges, see "Materials and methods."

*Data reprinted with permission from Pediatric Research.[24]

Results

Clinical and immunologic presentation of the patients

Family 1. Patient C.L. (Figure 1A) was born in 1996. After the first 6 months of life, the patient was examined for recurrent infections of the upper and lower respiratory tracts (bronchitis, pneumonia, and otitis media) and of the gastrointestinal tract. Determination of serum immunoglobulin levels indicated only elevated IgA at 6 months and slightly low IgM at 14 months (Table 1). Because of the family history of immunodeficiency, he did not receive live vaccines. At the age of 17 months, serum concentrations of IgG and IgA declined, and he had almost no detectable antibody titer against tetanus and diphtheria toxoids despite repeated immunizations. B- and T-cell numbers were normal. Lymphocyte proliferation tests at the age of 18 months indicated normal responses to phytohemagglutinin and concanavalin A, with a low response to pokeweed mitogen. At the age of 19 months, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase liver enzyme levels were elevated. Abdominal ultrasonography results were normal, and findings were negative for hepatitis A, B, and C, cytomegalovirus, and human immunodeficiency virus. Two months later, the patient was admitted to the hospital because of fever, pneumonia, a diffuse morbilliform rash, and an enlarged liver. Absolute neutropenia and thrombocytopenia then developed, and the patient was found to be EBV positive by PCR testing of his blood and cerebrospinal fluid. He was treated with acyclovir, granulocyte transfusions, and intravenous immunoglobulin (IVIG), but he died 5 weeks after admission because of an overwhelming *Aspergillus* infection

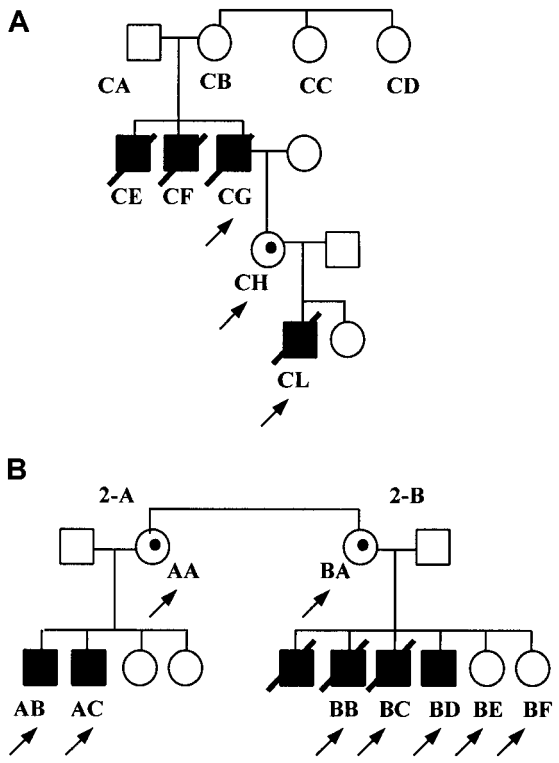

Figure 1. Pedigree of the 2 CVID families investigated. Genetic trees of family 1 and family 2 are shown in panels A and B, respectively. Patients are labeled with numbers and letters as indicated in the text. An arrow indicates patients analyzed for the presence of *SH2D1A* gene mutations. (box) Males; (circle) females; (line through) deceased; (filled) affected male; (dotted) patient carrier; (open) unaffected subjects.

secondary to aplastic anemia. Autopsy showed disseminated aspergillosis (involving lungs, kidney, esophagus, large and small intestines, pericardium, and diaphragm); acute and organizing bronchopneumonia; hypocellular bone marrow; lymphodepletion of thoracic and abdominal lymph nodes (necrosis with rare B cells and moderate number of T cells) positive for the EBV antigens LMP and EBNA-2); severe thymic atrophy with no evidence of thymopoiesis; acute and organizing splenic infarcts; centrilobular liver congestion, cholestasis, and peritoneal serous effusion; and large B-cell lymphoma involving peripancreatic lymph nodes only (LMP and EBNA-2 positive).

C.L.'s family is a well-studied sibship, previously reported by Buckley and Sidbury[24] (Figure 1A), affected by a variety of progressive immunoglobulin abnormalities in male and female members. C.L.'s grandfather (C.G.), together with his 2 brothers C.E. and C.F., were first seen at Duke University Medical Center in 1963 because all 3 were affected with frequent respiratory infections that were particularly severe in C.E. C.G. had only a late onset of mild infections. When he was first seen at the age of 6.5 years, C.G.'s findings were reported to be normal, but by the time he was 8.5 years of age, splenomegaly and a low lymphocyte count were noted. Immunologic studies over a period of 4 years showed a progressive deficiency of all 3 immunoglobulin isotypes (Table 1). He had a normal number of B cells initially, but this declined with time. He was treated with IVIG, but a B-cell lymphoma of the small intestine developed. This was successfully treated, but he had chronic severe diarrhea and died of bacterial pneumonia at the age of 38 years. The oldest brother, C.E., was first seen at the age of 14 years. He was affected by severe repeated respiratory infections, and examination of his serum revealed marked deficiencies of IgA, IgM, and IgG. C.E. failed to respond to blood group A and blood group B substances and to diphtheria, tetanus, and polio vaccines. He had 4 episodes of acute pneumonia before the age of 11 and died at 15 years because of acute respiratory failure. Another brother, C.F., remained well until late childhood. After the age of 10 years, he had repeated episodes of acute pneumonia. Analysis of serum immunoglobulin (Table 1) showed progressive reduction of his IgM and IgG levels over a 4-year period beginning in 1963. He died at the age of 25 years from acute pulmonary infiltrates and carcinomatous meningitis. The mother of the 3 boys (C.B.) had no history of severe infections. Her immunologic study findings (Table 1) demonstrated marked polyclonal IgA hyperglobulinemia, selective unresponsiveness to blood group B substance injections, and poor responses to immunization with diphtheria and polio vaccines. Two maternal aunts (C.C. and C.D.) also had IgA hyperglobulinemia and low isohemagglutinin titers. The father of the 3 boys (C.A.) was healthy and had normal levels of serum immunoglobulins (Table 1). There was no history of conditions similar to those of the boys on either side of the family.

Family 2. A 2.5-year-old boy (A.C.) (Figure 1B, 2-A) was brought to the Rambam Medical Center (Haifa, Israel) in 1988 after several episodes of pneumonia and *Escherichia coli* sepsis starting when he was 1 year old. His B-lymphocyte count was very low (1%-2%), as were his serum IgG and IgM levels, and serum IgA was undetectable (Table 1). A presumptive diagnosis of XLA was made, and he was started on IVIG therapy. B cell levels rose to 7% to 8% over years. He had no major medical problems until the present; he is now 12 years of age. In 1993, his brother (A.B.) (Figure 1B, 2-A) was born and was found to have a normal number of B cells. Nevertheless at age 10 months, pneumonia and hypogammaglobulinemia developed (Table 1). IVIG treatment was begun, and, like his brother, he is now doing well. A mutation in *btk* was ruled out (courtesy of Dr M. E. Conley), and, thus, CVID was diagnosed.

In 1999, their cousin B.C. (age 2 years) (Figure 1B, 2-B) was admitted to the hospital with clinical signs and symptoms compatible with FIM. He had marked hepatosplenomegaly and rapid deterioration of liver function. Anti–viral caspid antigen (VCA) IgM was positive, and liver biopsy showed typical features of FIM. He was treated with high-dose methylprednisolone and VP-16, but, unfortunately, he died before bone marrow transplantation could be performed. Family history (Figure 1B, 2-B) revealed that 2 other brothers died of FIM at approximately the same age range. In one of them, EBV was detected by biopsy of the liver. His 2 sisters were healthy.

Analysis of the *SH2D1A* gene in the 2 families

Family 1. Clinical and autopsy findings of C.L. led us to consider the possibility that he and his ancestors could have had XLP. Genomic DNA of C.G. and C.L. was extracted from B lymphocytes immortalized with EBV virus. DNA sequencing results (Figure 2A) indicated the presence of a single nucleotide substitution, C→T, in position 462 of the *SH2D1A* gene. This substitution alters the triplet CGA, which codes for amino acid R55, into the stop codon TGA. Because of the premature stop signal, only a 54–amino acids hypothetical SH2D1A protein (R55X) could be generated (Figure 2C). This abnormal protein has previously been described in other patients with XLP.[25]

Family 2. The clinical presentation of B.C. (November 1999), compatible with XLP, prompted us to examine his DNA and that of his cousins (A.C. and A.B.) for mutations in the *XLP* gene. Genomic DNA was extracted from peripheral blood lymphocytes of members of the 2 families (2-A and 2-B) to be tested for

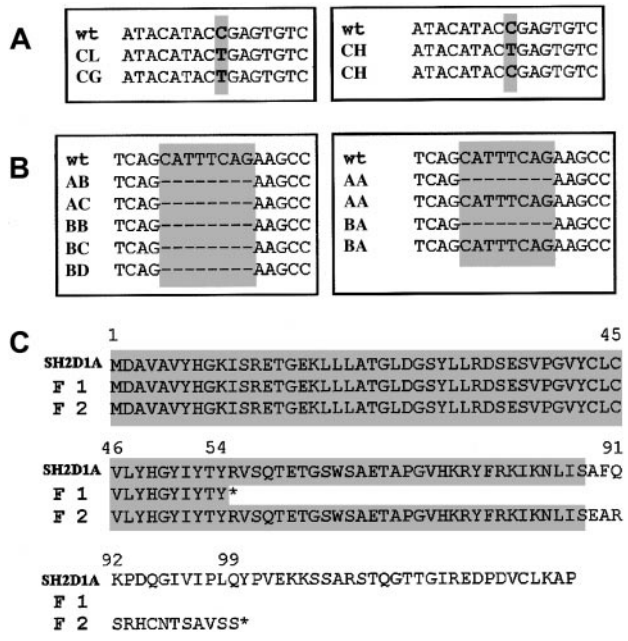

Figure 2. Alterations in the *SH2D1A* gene and the SH2D1A protein. (A) Alignment of the wild-type SH2D1A complementary DNA (cDNA) sequence with the nucleotide sequences obtained from patients C.L. and C.G. (left) and C.H. sequences (right). Panels depict the gene segment of interest. Nucleotide differences are indicated in gray. A single nucleotide substitution was detected at position 462 of the SH2D1A cDNA coding region of patients C.L. and C.G. (C462T) (left). After subcloning, approximately half the exon 2 PCR products of C.H. (mother of C.L. and daughter of C.G.) contained the C462T nucleotide substitution (right panel). (B) The SH2D1A cDNA nucleotide sequence was aligned with the sequences obtained from patients A.C., A.B., B.B., B.C., and B.D. (left) and A.A. and B.A. sequences (right). Panels depict the gene segment of interest. Nucleotide differences are indicated in gray. An 8-nucleotide deletion was detected between positions 548 and 555 of the SH2D1A cDNA coding region of patients A.C., A.B., B.B., B.C., and B.D. (left). After subcloning, approximately half the exon 3 PCR products of A.A. (mother of A.B. and A.C.) and B.A. (mother of B.B., B.C., and B.D.) contained the 8-nucleotide deletion detected in their sons (right). (C) Comparison of the 2 mutant protein sequences with wild-type SH2D1A. The single nucleotide C462T substitution detected in family 1 resulted in a change of the triplet CGA that coded for R55 to the stop codon triplet TGA. This generated a shorter SH2D1A protein of 54 amino acids (R55X) (indicated in the figure as F1). The 8-nucleotide deletion in the third exon of family 2 resulted in a change of the protein reading frame, generating a premature ending signal at a position corresponding to Y100 in SH2D1A. The shorter SH2D1A protein of 99 amino acids (Y100X) is indicated in the figure as F2. The gray area indicates the identity of residues among wild-type SH2D1A and the 2 mutant proteins. Asterisks mark the premature stop codon signals.

alterations in the *SH2D1A* gene. DNA sequencing (Figure 2B) indicated that the male family members B.B., B.C., A.B., and A.C. had an 8–base pair (bp) deletion located in the third exon (nucleotides 548 to 555). This alteration in the *SH2D1A* gene was previously unreported. Curiously, the sequence deleted in these patients (GCATTTCA) is repeated twice in the third exon, and this deletion is situated adjacent to an internal splice acceptor site located in the third exon. This low-frequency splice acceptor site generates a physiologically shorter form of the SH2D1A protein, named SAPΔ55, which is found in all healthy persons. Because of a shift in the reading frame, this deletion leads to a premature stop codon (at a position corresponding to residue 100). This premature stop codon generates a short, altered SH2D1A protein of 99 amino acids (Y100X) (Figure 2C). The same *SH2D1A* gene microdeletion was also found in the 2 mothers (B.A. and A.A.), identifying them as genetic carriers.

Another brother, an asymptomatic 11-month-old (B.D.) (Figure 1B, 2-B) tested positive for the same *SH2D1A* gene deletion. In December 1999 the patient underwent bone marrow transplantation (BMT) from a completely matched donor (a 9-year-old sister). No complications occurred during or after BMT.

Discussion

CVID is a heterogeneous syndrome both clinically and immunologically.[19,20] A precise clinical and laboratory definition of the disease has been difficult because of the heterogeneity in phenotypes. In a large study of 248 patients with CVID,[20] 40% had impaired T-cell proliferation to mitogens. Based on B-lymphocyte responses to plate-bound α-IgM, patients with CVID were divided into 4 subgroups.[19] Numerous studies have attempted to establish diagnostic criteria for the disease and to determine molecular etiologies. Recently, guidelines for the evaluation of CVID have been published.[26] A much stricter definition of the disease must now include the genetic exclusion of mutations in *btk*, *CD40-L*, *AID*, and *SH2D1A* genes.[19,21,27]

Cellular immunologic alterations in patients with XLP are not well understood. T and B lymphocytes undergo sustained proliferation in XLP. Extensive tissue infiltration and multi-organ failure are the primary causes of death in these patients.[6] The failure to eliminate EBV-transformed B cells in XLP does not seem to be caused by a defect in the B cell.[28] SH2D1A expression in B lymphocytes is probably limited only to certain subpopulations.[12] Moreover, no major B-lymphocyte defects have been found in SH2D1A null mice (C. Gullo, C. Terhorst, personal communication). On the contrary, variable defects in T cells and natural killer cells of patients with XLP have been reported. SH2D1A-deficient natural killer cells are unable to lyse appropriate target cells.[29-33] B-lymphocyte developmental abnormalities were detected in one member of the 2 families. Such a defect in B cells has been described in the past.[34] Whether these B-lymphocyte abnormalities and abnormal immunoglobulin levels result from a SH2D1A deficiency in B cells or from abnormal T–B lymphocyte interactions among SLAM-family members is unknown at this time. The SH2D1A-interacting molecules SLAM and CD84 and the 2B4-ligand CD48 are highly expressed in B cells,[35] and their expression increases after cell activation or EBV infection. In particular, SLAM has been demonstrated to play a role in B-lymphocyte proliferation and immunoglobulin synthesis after ligation by its soluble form (sSLAM).[36] The complex network of interactions among SH2D1A, EAT-2, and their ligands SLAM, 2B4, CD84, and Ly-9 may account for the clinical variability of manifestations in XLP. Recent data (M.M. et al, manuscript submitted, 2001) indicate that EAT-2 is probably the SH2D1A-like molecule functional in B lymphocytes. One could predict that mutations of EAT-2 might give rise to CVID.

Decreases in serum immunoglobulin levels with time in patients C.L., C.E., C.G., and C.F. (family 1) suggest that a cumulative effect of sequential environmental factors must play a strong role in determining the expression of the SH2D1A mutations. Because SLAM has been recently identified as another receptor for the measles virus,[37] a role for measles virus as a potential precipitant of disease expression in SH2D1A-deficient patients can be presumed. Dysgammaglobulinemia complicated by disseminated measles has been described in the past.[38,39]

Of particular interest is the fact that female members of family 1 had abnormal immunoglobulin levels. Female carriers of XLP have been reported to have abnormal antibody responses to EBV.[40] In male patients with XLP, IgG_1 and IgG_3 serum levels are often low with elevated IgA and IgM classes.[17] Therefore, in females with 1 of 2 altered SH2D1A alleles, a modest reduction in SH2D1A protein levels could result in mild laboratory alterations, such as the hyper-IgA reported in family 1. Decreased cellular levels of the SH2D1A protein could lead to immunoglobulin dysregulation through alterations in the T–B lymphocyte network. Patients with XLP who have reduced SH2D1A protein levels have been described. One patient had a critically reduced SH2D1A wild-type

protein level because of a regulatory mutation in the 5′ splicing acceptor site of the second exon.[10]

Family 1 is of further interest because all 3 major phenotypes developed in C.L. in only few months, and he died before the age of 2 years. The other affected male relatives had hypogammaglobulinemia or hypogammaglobulinemia and malignant lymphoma and lived until 15 years and 38 years. This clinical variation in patients with the same SH2D1A mutation indicates that other host or environmental factors are important in determining disease expression. Environmental factors are not limited to EBV infection because XLP phenotypes may develop in its absence. Age may be a critical factor in determining disease severity—hypogammaglobulinemia developed in C.L. at 17 months of age, and he succumbed of FIM and aplastic anemia at 20 months of age.

In conclusion, the work reported here indicates the presence of *SH2D1A* mutations in patients diagnosed with CVID. Therefore, together with *btk*, *CD40-L*, and *AID* genes, we suggest that *SH2D1A* must be included in the molecular diagnosis of CVID. Because of the high rate of new mutations occurring in other human X-linked immunodeficiencies, such as XLA,[41] the *SH2D1A* gene should be studied in all male patients with CVID. Clinically polarized XLP presentations must be considered when patients with CVID are encountered. A similar conclusion could be drawn from data published elsewhere.[42] This is particularly true when more than one male member of a family is affected. Besides allowing genetic counseling, a correct diagnosis of XLP will allow for the selection of more aggressive therapy (such as BMT) because the prognosis for XLP is much worse than for CVID syndrome in general.

References

1. Purtilo D-T, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). Lancet. 1975;1:935-940.
2. Seemayer T-A, Gross T-G, Egeler R-M, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. Pediatr Res. 1995;38:471-478.
3. Howie D, Sayos J, Terhorst C, Morra M. The gene defective in X-linked lymphoproliferative disease controls T cell dependent immune surveillance against Epstein-Barr virus. Curr Opin Immunol. 2000;12:474-478.
4. Sullivan J-L. The abnormal gene in X-linked lymphoproliferative syndrome. Curr Opin Immunol. 1999;11:431-434.
5. Morra M, Howie D, Simarro Grande M, et al. X-linked lymphoproliferative disease, a progressive immunodeficiency. Annu Rev Immunol. 2001;19:657-682.
6. Hamilton JK, Paquin LA, Sullivan JL, et al. X-linked lymphoproliferative syndrome registry report. J Pediatr. 1980;96:669-673.
7. Sullivan J-L, Byron K-S, Brewster F-E, Baker S-M, Ochs H-D. X-linked lymphoproliferative syndrome: natural history of the immunodeficiency. J Clin Invest. 1983;71:1765-1778.
8. Coffey A-J, Brooksbank R-A, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet. 1998;20:129-135.
9. Nichols K-E, Harkin D-P, Levitz S, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci U S A. 1998;95:13765-13770.
10. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. Nature. 1998;395:462-469.
11. Wu C, Sayos J, Wang N, Howie D, Coyle A, Terhorst C. Genomic organization and characterization of murine SAP: the gene coding for X-linked lymphoproliferative disease. Immunogenetics. 2000;51:805-815.
12. Nagy N, Cerboni C, Mattsson K, et al. SH2D1A and SLAM expression in human lymphocytes and derived cell lines. Int J Cancer. 2000;88:439-447.
13. Sayos J, Nguyen K-B, Wu C, et al. Potential pathways for regulation of NK and T cell responses: differential X-linked lymphoproliferative syndrome gene product SAP interactions with SLAM and 2B4. Int Immunol. 2000;12:1749-1757.
14. Tangye S-G, Lazetic S, Woollatt E, Sutherland G-R, Lanier L-L, Phillips J-H. Human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. J Immunol. 1999;162:6981-6985.
15. Sayos J, Martin M, Chen A, et al. The cell surface receptors Ly-9 and CD84 recruit the XLP gene product SAP. Blood. 2001;97:3867-3879.
16. Thompson A-D, Braun B-S, Arvand A, et al. EAT-2 is a novel SH2 domain containing protein that is up regulated by Ewing's sarcoma EWS/FLI1 fusion gene. Oncogene. 1996;13:2649-2658.
17. Grierson H-L, Skare J, Hawk J, Pauza M, Purtilo D-T. Immunoglobulin class and subclass deficiencies prior to Epstein-Barr virus infection in males with X-linked lymphoproliferative disease. Am J Med Genet. 1991;40:294-297.
18. Strahm B, Rittweiler K, Duffner U, et al. Recurrent B-cell non-Hodgkin's lymphoma in two brothers with X-linked lymphoproliferative disease without evidence for Epstein-Barr virus infection. Br J Haematol. 2000;108:377-382.
19. Spickett G-P, Farrant J, North M-E, Zhang J-G, Morgan L, Webster D-B. Common variable immunodeficiency: how many diseases? Immunol Today. 1997;18:325-328.
20. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34-48.
21. Farrington M, Grosmaire L-S, Nonoyama S, et al. CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. Proc Natl Acad Sci U S A. 1994;91:1099-1103.
22. Wang B, Biron C, She J, et al. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3E gene. Proc Natl Acad Sci U S A. 1994;91:9402-9406.
23. Jacobs D-S, Demott W-R, Grady H-J, Horvat R-T, Huestis D-W, Kasten B-L. Laboratory Test Handbook. Hudson, OH: Lexi-Comp; 1996.
24. Buckley R-H, Sidbury J-B. Hereditary alterations in the immune response: coexistence of 'agammaglobulinemia,' acquired hypogammaglobulinemia and selective immunoglobulin deficiency in a sibship. Pediatr Res. 1968;2:72-84.
25. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the *SH2D1A* gene and Epstein-Barr virus (EBV) infection with clinical phenotype and outcome in X-linked lymphoproliferative disease (XLP). Blood. 2000;96:3118-3125.
26. Conley M-E, Notarangelo G-L, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Clin Immunol. 1999;93:190-197.
27. Minegish Y, Lavoie A, Cunningham-Rundles C, et al. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. Clin Immunol. 2000;3:203-210.
28. Jager M, Benninger-Doring G, Prang N, et al. Epstein-Barr virus-infected B cells of males with the X-linked lymphoproliferative syndrome stimulate and are susceptible to T-cell–mediated lysis. Int J Cancer. 1998;76:694-701.
29. Sullivan J-L, Byron K-S, Brewster F-E, Purtilo D-T. Deficient natural killer cell activity in X-linked lymphoproliferative syndrome. Science. 1980; 210:543-545.
30. Rousset F, Souillet G, Roncarolo M-G, Lamelin J-P: Studies of EBV-lymphoid cell interactions in two patients with the X-linked lymphoproliferative syndrome: normal EBV-specific HLA-restricted cytotoxicity. Clin Exp Immunol. 1986;63:280-289.
31. Parolini S, Bottino C, Falco M, et al. X-linked lymphoproliferative disease: 2B4 molecules displaying inhibitory rather than activating function killer cells to kill Epstein-Barr virus-infected cells. J Exp Med. 2000;3:337-346.
32. Benoit L, Wang X, Dutz J, Tan R. Defective natural killer cell activation in x-linked lymphoproliferative disease. J Immunol. 2000;165:3549-3553.
33. Tangye S-G, Phillips J-H, Lanier L-L, Nichols KE. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. J Immunol. 2000;165:2932-2936.
34. Purtilo D, Zelkowitz L, Harada S, et al. Delayed onset of infectious mononucleosis associated with acquired agammaglobulinemia and red cell aplasia. Ann Intern Med 1984;101:180-186.
35. Tangye S-G, Phillips J-H, Lanier L-L. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK and other immune cells. Semin Immunol. 2000;12:149-157.
36. Punnonen J, Cocks B-G, Carballido J-M, et al. Soluble and membrane-bound forms of signaling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. J Exp Med. 1997;185:555-561.
37. Tatsuo H, Ono N, Tanaka K, Yanagi Y. SLAM (CDw150) is a cellular receptor for measles virus. Nature. 2000;406:893-897.
38. Mawhinney H, Allen I-V, Beare J-M, et al. Dysgammaglobulinaemia complicated by disseminated measles. Br Med J. 1971;2:380-381.
39. Lipsey A-I, Kahn M-J, Bolande R-P. Pathologic variants of congenital hypogamma-globulinemia: an analysis of 3 patients dying of measles. Pediatrics. 1967;39:659-674.
40. Sakamoto K, Seeley J-K, Lindsten T, et al. Abnormal anti-Epstein Barr virus antibodies in carriers of the X-linked lymphoproliferative syndrome and in females at risk. J Immunol. 1982;128:904-907.
41. Conley M-E, Mathias D, Treadaway J, et al. Mutations in btk in patients with presumed X-linked agammaglobulinemia. Am J Hum Genet. 1998; 62:1034-1043.
42. Gilmour K-C, Cranston T, Jones A, et al. Diagnosis of X-linked lymphoproliferative disease by analysis of SLAM-associated protein expression. Eur J Immunol. 2000;30:1691-1697.