106. Gordon TP, Kowanko IC, James M, Roberts-Thomson PJ: **Monosodium urate crystal-induced prostaglandin synthesis in the rat subcutaneous air pouch.** *Clin Exp Rheumatol* 1985, **3**:291-296.

107. Terkeltaub R: **Pathogenesis and treatment of crystal-induced inflammation.** In *Arthritis and Allied Conditions,* edn 15th. Edited by Koopman W, Moreland LW.Lippincott Williams and Wilkins; 2005:2357.

108. Shimada M, Johnson RJ, May WS Jr, Lingegowda V, Sood P, Nakagawa T, Van QC, Dass B, Ejaz AA: **A novel role for uric acid in acute kidney injury associated with tumour lysis syndrome.** *Nephrol Dial Transplant* 2009, **24**:2960-2964.

109. Denoble AE, Huffman KM, Stabler TV, Kelly SJ, Hershfield MS, McDaniel GE, Coleman RE, Kraus VB: **Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation.** *Proc Natl Acad Sci U S A* 2011, **108**:2088-2093.

110. Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT: **Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury.** *J Immunol* 2011, **187**:2626-2631.

111. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A: **Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors.** *Nature* 2002, **416**:603-607.

112. Boule MW, Broughton C, Mackay F, Akira S, Marshak-Rothstein A, Rifkin IR: **Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes.** *J Exp Med* 2004, **199**:1631-1640.

113. Sirois CM, Jin T, Miller AL, Bertheloot D, Nakamura H, Horvath GL, Mian A, Jiang J, Schrum J, Bossaller L *et al.*: **RAGE is a nucleic acid receptor that promotes inflammatory responses to DNA.** *J Exp Med* 2013, **210**:2447-2463.

114. Hornung V, Latz E: **Intracellular DNA recognition.** *Nat Rev Immunol* 2010, **10**:123-130.

115. Bernard JJ, Cowing-Zitron C, Nakatsuji T, Muehleisen B, Muto J, Borkowski AW, Martinez L, Greidinger EL, Yu BD, Gallo RL: **Ultraviolet radiation damages self noncoding RNA and is detected by TLR3.** *Nat Med* 2012, **18**:1286-1290.

116. Hefeneider SH, Cornell KA, Brown LE, Bakke AC, McCoy SL, Bennett RM: **Nucleosomes and DNA bind to specific cell-surface molecules on murine cells and induce cytokine production.** *Clin Immunol Immunopathol* 1992, **63**:245-251.

117. Ishii KJ, Suzuki K, Coban C, Takeshita F, Itoh Y, Matoba H, Kohn LD, Klinman DM: **Genomic DNA released by dying cells induces the maturation of APCs.** *J Immunol* 2001, **167**:2602-2607.

118. Decker P, Singh-Jasuja H, Haager S, Kötter I, Rammensee HG: **Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation.** *J Immunol* 2005, **174**:3326-3334.

119. Ronnefarth VM, Erbacher AI, Lamkemeyer T, Madlung J, Nordheim A, Rammensee HG, Decker P: **TLR2/TLR4-independent neutrophil activation and recruitment upon endocytosis of nucleosomes reveals a new pathway of innate immunity in systemic lupus erythematosus.** *J Immunol* 2006, **177**:7740-7749.

120. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT: **Extracellular histones are major mediators of death in sepsis.** *Nat Med* 2009, **15**:1318-1321.

121. Kaczorowski DJ, Scott MJ, Pibris JP, Afrazi A, Nakao A, Edmonds RD, Kim S, Kwak JH, Liu Y, Fan J *et al.*: **Mammalian DNA is an endogenous danger signal that stimulates local synthesis and release of complement factor B.** *Mol Med* 2012, **18**:851-860.

122. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H *et al.*: **Suppression of inflammation by a synthetic histone mim** *Nature* 2010, **468**:1119-1123.

123. Burnstock G, Knight GE: **Cellular distribution and functio P2 receptor subtypes in different systems.** *Int Rev Cytol* **240**:31-304.

124. Mariathasan S, Monack DM: **Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation.** *Nat Rev Immunol* 2007, **7**:31-40.

125. Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams **The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that pror chemotaxis and inhibit O2 generation, respectively.** *J Cl Invest* 1990, **85**:1150-1157.

126. Zhang M, Alicot EM, Chiu I, Li J, Verna N, Vorup-Jensen T, Kessler B, Shimaoka M, Chan R, Friend D *et al.*: **Identificati the target self-antigens in reperfusion injury.** *J Exp Med* **203**:141-152.

127. Zhang M, Takahashi K, Alicot EM, Vorup-Jensen T, Kessler Thiel S, Jensenius JC, Ezekowitz RA, Moore FD, Carroll MC **Activation of the lectin pathway by natural IgM in a mod ischemia/reperfusion injury.** *J Immunol* 2006, **177**:4727-47

128. Weiser MR, Williams JP, Moore FD Jr, Kobzik L, Ma M, Hechtman HB, Carroll MC: **Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement.** *J Exp Med* 1996, **183**:2343-2348.

129. Zhang M, Austen WG Jr, Chiu I, Alicot EM, Hung R, Ma M, Ver Xu M, Hechtman HB, Moore FD Jr *et al.*: **Identification of specific self-reactive IgM antibody that initiates intestin ischemia/reperfusion injury.** *Proc Natl Acad Sci U S A* 20 **101**:3886-3891.

130. Fu H, Karlsson J, Bylund J, Movitz C, Karlsson A, Dahlgren **Ligand recognition and activation of formyl peptide recep in neutrophils.** *J Leukoc Biol* 2006, **79**:247-256.

131. Carp H: **Mitochondrial N-formylmethionyl proteins as chemoattractants for neutrophils.** *J Exp Med* 1982, **155**: 264-275.

132. Hauser CJ, Sursal T, Rodriguez EK, Appleton PT, Zhang Q, Itagaki K: **Mitochondrial damage associated molecular patterns from femoral reamings activate neutrophils thr formyl peptide receptors and P44/42 MAP kinase.** *J Orth Trauma* 2010, **24**:534-538.

133. Gawlowski DM, Benoit JN, Granger HJ: **Microvascular pres and albumin extravasation after leukocyte activation in hamster cheek pouch.** *Am J Physiol* 1993, **264**:H541-H54

134 • Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brc Itagaki K, Hauser CJ: **Circulating mitochondrial DAMPs c inflammatory responses to injury.** *Nature* 2010, **464**:104-1
Mitochondria are evolutionary endosymbionts that were derive bacteria. This paper identified that mitochondria as a source for signals including mitochondria DNA and N-formyl peptides.

135. Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM Lima BH, Lopes GA, Russo RC, Avila TV, Melgaco JG *et al.* **Chemokines and mitochondrial products activate neutro to amplify organ injury during mouse acute liver failure.** *Hepatology* 2012, **56**:1971-1982.

136. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tam Oyabu J, Murakawa T, Nakayama H, Nishida K *et al.*: **Mitochondrial DNA that escapes from autophagy cause inflammation and heart failure.** *Nature* 2012, **485**:251-255

137. Julian MW, Shao G, Bao S, Knoell DL, Papenfuss TL, VanGundy ZC, Crouser ED: **Mitochondrial transcription fac serves as a danger signal by augmenting plasmacytoid dendritic cell responses to DNA.** *J Immunol* 2012, **189**:433

138. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Naml Abe T, Kiyonari H, Matsumoto K *et al.*: **IL-33 is a crucial amp of innate rather than acquired immunity.** *Proc Natl Acad Sc A* 2010, **107**:18581-18586.

Clinical Endocrinology (2012) 77, 628–634 doi: 10.1111/j.1365-2265.2012.04390.x

ORIGINAL ARTICLE

Endocrine complications in primary immunodeficiency diseases in Japan

Takafumi Nozaki*, Hidetoshi Takada*, Masataka Ishimura*, Kenji Ihara*, Kohsuke Imai†, Tomohiro Morio†, Masao Kobayashi‡, Shigeaki Nonoyama§ and Toshiro Hara*

*Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, †Department of Pediatrics and Developmental Biology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, ‡Department of Pediatrics, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, and §Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan

Summary

Background In spite of the accumulating evidence on the interaction between the immune and endocrine systems based on the recent progress in molecular genetics, there have been few epidemiological studies focused on the endocrine complications associated with primary immunodeficiency diseases (PID).

Objective To investigate the prevalence and clinical features of endocrine complications in patients with PID in a large-scale study.

Design and participants This survey was conducted on patients with PID who were alive on 1 December 2008 and those who were newly diagnosed and died between 1 December 2007 and 30 November 2008 in Japan. We investigated the prevalence and the clinical data of the endocrine complications in 923 patients with PID registered in the secondary survey.

Results Among 923 PID patients, 49 (5·3%) had endocrine disorders. The prevalence of the endocrine diseases was much higher in patients with PID than in the general population in the young age group, even after excluding patients with immune dysregulation.

Conclusions Endocrine disorders are important complications of PID. Analysis of the endocrine manifestations in patients with PID in a large-scale study may provide further insights into the relationship between the immune and endocrine systems.

(Received 15 November 2011; returned for revision 23 December 2011; finally revised 19 January 2012; accepted 13 March 2012)

Introduction

A wide variety of clinical complications have been described in primary immunodeficiency diseases (PID).[1,2] PID have been reported to be associated with an increased risk of cancer, in particular non-Hodgkin lymphoma,[2] and the contribution of immune dysfunction in PID to cancer risk is receiving much attention. It is also well known that patients with PID often have complications such as autoimmune and allergic disorders.[1,3] Recently, the interaction between the immune and endocrine systems has been getting increasing attention.[4,5] However, there have so far been no reports focusing on the endocrine complications associated with PID in a large-scale survey.

Many endocrine disorders in patients with PID are thought to be due to the development of the autoimmunity, which is closely related to the pathophysiology of PID.[6] However, it is not known how the immunological and molecular defects in individual PID contribute to the development of various autoimmune endocrine disorders. In addition, the genetic defects in some PID can lead to these complications directly or indirectly via nonimmunological mechanisms.[6]

We analysed the endocrine complications in PID from the information obtained from the nationwide PID survey in Japan conducted in 2008. This is the first large-scale survey focusing on the endocrine complications in PID.

Materials and methods

This survey was performed according to the nationwide epidemiological survey manual of patients with intractable diseases (2nd edition 2006, Ministry of Health, Labour and Welfare of Japan) as described previously.[7] PID classification was based on the criteria of the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee in 2007.[8] The survey was conducted on patients with PID who were alive on 1 December 2008 and those who were newly diagnosed and died between 1 December 2007 and 30 November 2008 in Japan. The initial survey covered 1224 paediatric departments and 1670 internal medicine departments, which were randomly selected according to the number of beds among the 2291 paediatric departments and 8026 internal medicine departments in Japan. Primary questionnaires regarding the number of patients and the disease names based on the PID classification

Correspondence: Takafumi Nozaki, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5421; Fax: +81 92 642 5435; E-mail: t-nozaki@pediatr.med.kyushu-u.ac.jp

were sent to the selected hospitals. The initial survey was conducted to investigate the prevalence of the respective PID. The secondary survey was performed to study the detailed clinical features of individual patients with PID. Secondary questionnaires regarding age, gender, clinical manifestations and complications other than those related to haematopoietic stem cell transplantation of individual patients with PID were sent to the respondents who answered that they observed at least one PID patient with characteristics listed in the primary questionnaires. The details of the methods of the questionnaire investigation, the response rates and the breakdown of the number of patients in both paediatric and internal medicine departments were described elsewhere.[9] The questionnaires were designed to elucidate the clinical characteristics including the manifestations and laboratory data of the patients. In this study, all endocrine manifestations in patients with PID were included as complications of PID, even if they were well known major symptoms of PID.

Results

Detailed clinical information was available from 923 (secondary survey) out of 1240 patients with PID (initial survey).[9] Among the 923 patients with PID, 49 (5·3%) had endocrine disorders. As shown in Table 1, more than two thirds of the patients with PID were <20 years old and the prevalence of endocrine diseases was much higher in the young population of patients with PID than that in the general young population,[7,10–14] even after excluding patients with immune dysregulation (PID category IV). As expected, hypoparathyroidism was the most common endocrine disorder, because it is very frequently observed in patients with DiGeorge syndrome. Endocrine manifestations were also common in patients with diseases of immune dysregulation, such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Although the number of patients with defects in innate immunity was small, endocrine complications seemed to be more common than expected. Interestingly, endocrine disorders were not observed in patients with complement deficiencies. In addition, Graves' disease and Addison's disease were not observed in any of the patients with PID in this study.

Type 1 diabetes mellitus (T1D) was observed in six patients with PID (Tables 1 and 2) including four with type 1A (autoimmune) and two with type 1B (autoantibody-negative, idiopathic). Type 1A diabetes mellitus occurred frequently in patients with IPEX or IPEX-like syndrome (two of six patients, 33·3%) (Table 1). One patient of unknown aetiology in PID category IV showed type 1A diabetes and Hashimoto's thyroiditis along with recurrent viral infections (Tables 1, 2 and S1). In the cases of type 1A diabetes mellitus, anti-glutamic acid decarboxylase (GAD) autoantibodies and anti-insulin autoantibodies (IAA) were positive in all patients and in two of four patients, respectively (Table 2). The patients with IPEX and IPEX-like syndrome had a history of diabetic ketoacidosis with poor glycaemic control, and they developed T1D at a younger age than the other patients with PID. The first case of warts, hypogammaglobulinaemia, infections, and myelokathexis (WHIM) syndrome with T1D and hypothyroidism was included (Tables 2 and S2).[15] With regard to type 1B diabetes mellitus, the patient with hypogammaglobulinaemia of unknown aetiology had diabetic ketoacidosis (Table 2). On the other hand, type 2 diabetes mellitus (T2D) was observed in two patients with PID (Table 1).

Hashimoto's thyroiditis was observed in five patients with PID (Tables 1 and S1). The onset was very early in the patient with IPEX syndrome (at birth). All patients had at least 1 autoantibody among the anti-thyroid peroxidase (TPO), anti-thyroglobulin (Tg) and thyroid stimulating hormone receptor autoantibodies (TRAb).

Nonautoimmune hypothyroidism was reported in seven patients with PID (Tables 1 and S2). Anti-thyroid autoantibodies were all negative when measured. Among these, three patients with X-linked agammaglobulinaemia (XLA), IgG subclass deficiency or WHIM syndrome had primary (congenital) hypothyroidism detected by newborn mass screening. Hypothyroidism in the other four patients with normal TSH levels was considered to be due to central hypothyroidism, a disorder of the pituitary, hypothalamus or hypothalamic-pituitary portal circulation. Two patients with severe combined immunodeficiency (SCID) developed hypothyroidism before they received haematopoietic stem cell transplantation.

Growth hormone deficiency (GHD) was observed in six patients with PID (Tables 1 and S3), whose heights at the diagnosis of GHD ranged from −11·3 SD to −2·5 SD. Five patients were treated with growth hormone. One patient with SCID received cord blood transplantation when she was 20 months old, without conditioning chemotherapy or radiation.

Hypogonadism was observed in three patients with PID (Tables 1 and S4). Among them, two had hypergonadotrophic (primary) hypogonadism, whereas the other had hypogonadotrophic (central) hypogonadism. None of the patients received haematopoietic stem cell transplantation.

One common variable immunodeficiency disease (CVID) patient had isolated ACTH deficiency (Table 1). The other endocrine complications included hypophosphataemia, pseudohypoaldosteronism, adrenal crisis, hypoglycaemia and hypophosphataemic rickets as shown in Table 1.

Discussion

This is the first nationwide survey focusing on the endocrine complications of PID. Among these, hypoparathyroidism was the most common, observed in patients with DiGeorge syndrome and APECED.[16,17] In APECED, the calcium-sensing receptor has been reported to be the autoantigen responsible for hypoparathyroidism.[18] Although it has been reported that 79% of patients with APECED have hypocalcaemia due to hypoparathyroidism,[17] only 1 (25%) among four patients with APECED developed hypoparathyroidism in this study, which might be one of the clinical characteristics of patients with APECED in Japan.

The prevalence (33·3%) of T1D in patients with IPEX syndrome in this study seemed to be lower than that (>70%) of the previous reports.[19,20] The low prevalence of T1D might be due to

Table 1. Endocrine complications in PID patients

PID category	Hypopara-thyroidism	Diabetes mellitus			Thyroid disease		GHD	Hypogonadism	Isolated ACTH deficiency	Others	The number of PID patients			
		T1D		T2D	Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Non-autoimmune hypothyroidism					*n*	0–19 years	Total	Percent in total
		1A	1B											
I. Combined T and B cell immunodeficiencies											**4**	**67**	**75**	**5·3**
RAG1 deficiency						1					1	6	6	16·7
CD4 deficiency					1						1	2	2	50·0
Undetermined											2	10	10	20·0
T-B-SCID						1	1				2	4	4	50·0
II. Predominantly antibody deficiencies											**13**	**231**	**378**	**3·4**
X-linked agammaglobulinaemia						1				2*	3	93	138	2·2
Common variable immunodeficiency disorders			1		1††		1		1	2†	6	29	93	6·5
IgG subclass deficiency						2					2	45	50	4·0
Undetermined			1				1**	1**			2	9	9	22·2
III. Other well-defined immunodeficiency syndromes											**20**	**126**	**165**	**12·1**
Hyper-IgE syndrome							1	1		1‡	3	31	46	6·5
DiGeorge syndrome	14										14	29	32	43·8
Ataxia telangiectasia				1							1	8	13	7·7
Chronic mucocutaneous candidiasis					1††						1	9	13	7·7
ICF syndrome								1			1	0	1	100·0
IV. Diseases of immune dysregulation											**6**	**31**	**38**	**15·8**
IPEX syndrome		2			1					1§	4	5	6	66·7
APECED	1										1	3	4	25·0
Undetermined		1**			1**						1	2	2	50·0
V. Congenital defects of phagocyte number, function or both											**3**	**106**	**153**	**2·0**
Chronic granulomatous disease						1	1				2	54	87	2·3

Table 1. *(continued)*

PID category	Hypopara-thyroidism	Diabetes mellitus: T1D 1A	T1D 1B	Diabetes mellitus: T2D	Thyroid disease: Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Thyroid disease: Non-autoimmune hypothyroidism	GHD	Hypogonadism	Isolated ACTH deficiency	Others	The number of PID patients: *n*	0–19 years	Total	Percent in total
Shwachman–Diamond syndrome							1				1	2	2	50·0
VI. Defects in innate immunity											**2**	**9**	**12**	**16·7**
NEMO deficiency										1[¶]	1	7	7	14·3
WHIM syndrome		1[**]				1[**]					1	2	3	33·3
VII. Autoinflammatory disorders											**1**	**54**	**74**	**1·4**
Familial Mediterranean fever				1[††]							1	23	36	2·8
VIII. Complement deficiencies											**0**	**18**	**23**	**0**
IX. Undetermined											**0**	**3**	**5**	**0**
Total	15	6		2	5	7	6	3	1	7	49	645	923	5·3
Estimated prevalence per 10 000 in the young population (0–19 years) of PID patients (95% CI)	232·6 (141·4–380·1)	93·0 (42·7–201·5)		15·5 (2·7–87·3)	46·5 (15·8–135·9)	108·5 (52·7–222·3)	93·0 (42·7–201·5)	46·5 (15·8–135·9)	15·5 (2·7–87·3)					
Prevalence per 10 000 in the general young Japanese population	0·072[‡‡]	1·19		0·461[§§]	30·0[§§]	13·5[¶¶]	1·47	ND	0·035					
References	[7]	[10]		[10]	[11]	[12]	[13]	ND	[14]					

SCID, severe combined immunodeficiency; ICF, immunodeficiency with centromeric instability and facial anomalies; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; APECED, autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy; NEMO, NF-κB essential modulator; WHIM, warts, hypogammaglobulinaemia, infections, and myelokathexis; T1D, type 1 diabetes; T2D, type 2 diabetes; GHD, growth hormone deficiency.

*Hypophosphatemia 1, Obesity 1.

†Obesity 2.

‡Pseudohypoaldosteronism 1.

§Adrenal crisis, Hypoglycaemia 1.

¶Hypophosphatemic rickets 1.

**Two endocrine disorders were observed in the same patient.

††the case whose onset age of a endocrine complication is 20 years or older, *n*: number of PID patients who had endocrine disorders, CI: confidence interval.

‡‡prevalence in all age groups.

§§incidence data.

¶¶prevalence in the United States, ND: no data available.

Table 2. Clinical data of T1D patients

Case		1	2	3	4	5	6
Disease		IPEX syndrome	IPEX-like syndrome	Immune dysregulation (undetermined)	WHIM syndrome	CVID	Hypogammaglobulinaemia (unknown aetiology)
Genetic mutations (gene name)		+ (*FOXP3*)	Unknown	Unknown	+ (*CXCR4*)	Unknown	NT
HSCT		–	–	–	–	–	–
Sex		M	M	F	F	F	M
Present age		8 years 5 months	14 years 5 months	21 years 8 months	18 years 9 months	19 years 1 month	25 years 3 months
Onset age of T1D		3 months	10 months	7 years 9 months	5 years 7 months	7 years 9 months	6 years 5 months
Type of T1D		1A	1A	1A	1A	1B	1B
Clinical symptoms		Polydipsia, polyuria	Polydipsia, weight loss	ND	Polydipsia, polyuria	None	None
Diabetic ketoacidosis		+ (pH 7·112)	+ (pH 7·012)	–	–	–	+ (urine ketone body (4+))
Laboratory data	Normal range						
Fasting blood glucose (mmol/l)	3·9–6·1	31·7	29·1	6·1*	7·6	8·3	7·7
HbA1c (%)	4·3–5·8	7·9	8·3	8·7*	8·9	5·6	9·1
Plasma CPR (nmol/l)	0·33–0·93	ND	0·27	0·10*	ND	0·27	ND
Urinary CPR (μg/day)	20–100	ND	ND	2·5*	15	NT	ND
Anti-GAD Ab							
Result		+	+	+*	+	None	None
Value (U/ml)	<1·5	69·1	4860	9·3*	92	ND	ND
Anti-IAA Ab							
Result		–	ND	+*	+	ND	ND
Value (nIU/ml)	<125	2·8		ND	ND		
Treatment							
Age at the start		3 months	10 months	7 years 9 months	5 years 7 months	8 years 1 month	6 years 5 months
Content		Insulin	Insulin	Insulin	Insulin	Insulin	Insulin

NT, not tested; ND, no data available; FOXP3, forkhead box P3; CXCR4, CXC chemokine receptor 4; HSCT, haematopoietic stem cell transplantation; CPR, C-peptide immunoreactivity; GAD, glutamic acid decarboxylase; IAA, insulin autoantibody.
*Post-treatment data.

some genetic factor, because the Japanese have been have reported to be one of the races with the lowest incidence of T1D.[21] With regard to the patient with WHIM, Takaya *et al.*[15] have reported that mutations of *CXCR4*, the gene responsible for WHIM syndrome, might be closely related to the development of T1D, because recent findings have suggested that impaired CXCR4 signalling is involved in the pathogenesis of T1D. The prevalence of T1D in patients with CVID was 1·1% (one in 93 patients) in our study, which was almost equal to that in the previous report.[3]

The development of T2D was observed in only one of 13 patients with ataxia telangiectasia (AT) (7·7%) in contrast to the high prevalence of T2D in the previous report (five of eight patients),[22] suggesting the unique clinical characteristics of patients with AT in Japan.

Hashimoto's thyroiditis is a relatively common endocrine manifestation in patients with IPEX syndrome.[19,20] The prevalence of Hashimoto's thyroiditis in patients with CVID in our study was 1·1% (one in 93 patients), which was similar to that of the previous report.[23] There have been only a few reports of Hashimoto's thyroiditis in patients with (S) CID.[24,25] Interestingly, this was the first report of Hashimoto's thyroiditis in a patient with CD4 deficiency, while autoimmune cytopenia is frequently associated with this disease (19%).[26] The patient with a patient with CD4 deficiency and Hashimoto's thyroiditis did not receive stem cell transplantation, suggesting that this complication was caused by autoimmunity based on the combined immunodeficiency. Nagpala *et al.*[25] reported an infant with autoimmune thyroiditis and hypothyroidism with SCID due to adenosine deaminase deficiency despite an extremely low number of T cells and a low level of IgG, which suggested that the leaky SCID phenotype permitted the survival of a few T cells with autoimmune potential.[27]

Central hypothyroidism (no TSH elevation) was observed in two patients with SCID before they received haematopoietic stem cell transplantation (Table S2), also suggesting the possibility that this complication was related to the combined immunodeficiency itself. In addition, this was the first report of primary hypothyroidism (elevated TSH levels at birth) in patients with

XLA or IgG subclass deficiency, although the aetiologies remain to be determined.

Of note, the prevalence of GHD in patients with PID seemed much higher than that in the general population (Table 1). Until now, GHD has been reported in patients with several diseases in PID including SCID, CVID and Shwachman–Diamond syndrome, as shown in our study.[28–30] However, to the best of our knowledge, this was the first report of GHD in patients with hyper-IgE syndrome (HIES) and chronic granulomatous disease (CGD). Some SCID patients with GHD have been reported to have *STAT5b* gene mutations.[31] However, the gene was not investigated in our patient with SCID. With respect to the mechanism underlying the development of GHD in patients with CVID, common impairment in the IGF-1 and IgG pathways has been suggested as a cause of the growth retardation in some patients with CVID.[32] In addition, anti-pituitary antibodies have been detected in some of these patients.[33] The patient with congenital agammaglobulinaemia had various other complications in addition to GHD (Table S3), suggesting that this patient might have had a novel primary immunodeficiency.

Hypogonadism in patients with immunodeficiency with centromeric instability and facial anomalies (ICF) syndrome has been reported previously[34], although the mechanism is unclear. On the other hand, this was the first report of hypogonadism in patients with congenital agammaglobulinaemia and HIES. It is possible that hypogonadism has not been a major concern in PID for clinicians.

Isolated ACTH deficiency usually occurs during adult life, and only a few cases have been reported in childhood.[35] However, the development of isolated ACTH deficiency in a 14-year-old girl with CVID has been reported[35], in addition to the present case (Table 1). Therefore, a common pathological background is suspected in some of the patients with CVID.

Several limitations of this study should be considered. First, there were only a small number of adult patients with PID reported in this study, from which we could not estimate the accurate prevalence of endocrine manifestations in adults. Second, not all of the patients with PID were given sufficient examinations by endocrinologists and different examination methods were used at the respective hospitals.

There has been growing evidence of the interaction between the immune and endocrine systems.[4,5] In this study, we have found an increased prevalence of endocrine complications in patients with PID, which appear to be caused by immune dysregulation or by the underlying genetic disorders of the respective PID. Although various endocrine abnormalities have been reported to occur after stem cell transplantation,[36] therapy-related endocrine abnormalities were not included in the present study. A large-scale study such as a nationwide survey, focusing on the endocrine diseases, may have the potential to provide further insights into the mechanisms or pathophysiology of endocrine disorders in non-PID as well as patients with PID.

Conflicts of interest/financial disclosure

We declare that we have no conflicts of interest.

Acknowledgements

We appreciate the support and contributions of the numerous doctors who cared for and provided information on patients with PID in Japan and would also like to thank the support of the Japanese Research Group on Primary Immunodeficiency Diseases, which is supported by Japan's Ministry of Health, Labour and Welfare.

References

1 Hayakawa, H., Iwata, T. & Yata, J. *et al.* (1981) Primary immunodeficiency syndrome in Japan. I. Overview of a nationwide survey on primary immunodeficiency syndrome. *Journal of Clinical Immunology*, **1**, 31–39.
2 Vajdic, C.M., Mao, L., van Leeuwen, M.T., *et al.* (2010) Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? *Blood*, **116**, 1228–1234.
3 Cunningham-Rundles, C. & Bodian, C. (1999) Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clinical Immunology*, **92**, 34–48.
4 Imura, H., Fukata, J. & Mori, T. (1991) Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. *Clinical Endocrinology*, **35**, 107–115.
5 Fabris, N., Mocchegiani, E. & Provinciali, M. (1995) Pituitary-thyroid axis and immune system: a reciprocal neuroendocrine-immune interaction. *Hormone Research*, **43**, 29–38.
6 Brandt, D. & Gershwin, M.E. (2006) Common variable immune deficiency and autoimmunity. *Autoimmunity Reviews*, **5**, 465–470.
7 Nakamura, Y., Matsumoto, T., Tamakoshi, A. *et al.* (2000) Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. *Journal of Epidemiology*, **10**, 29–33.
8 Geha, R.S., Notarangelo, L.D., Casanova, J.L. *et al.* (2007) Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *The Journal of Allergy and Clinical Immunology*, **120**, 776–794.
9 Ishimura, M., Takada, H. & Doi, T. *et al.* (2011) Nationwide survey of patients with primary immunodeficiency diseases in Japan. *Journal of Clinical Immunology*, **31**, 968–976.
10 Kitagawa, T., Owada, M., Urakami, T. *et al.* (1994) Epidemiology of type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in Japanese children. *Diabetes Research and Clinical Practice*, **24**(Suppl), S7–S13.
11 Inoue, M., Taketani, N., Sato, T. *et al.* (1975) High incidence of chronic lymphocytic thyroiditis in apparently healthy school children: epidemiological and clinical study. *Endocrinologia Japonica*, **22**, 483–488.
12 Rallison, M.L., Dobyns, B.M., Meikle, A.W. *et al.* (1991) Natural history of thyroid abnormalities: prevalence, incidence, and regression of thyroid diseases in adolescents and young adults. *American Journal of Medicine*, **91**, 363–370.
13 Tani, N. (1985) Epidemiological study of dwarfism in Niigata Prefecture. *Folia Endocrinologica Japonica*, **61**, 1295–1309.
14 Kitakami, H., Ishikawa, E., Hidaka, H. *et al.* (2007) Clinical feature, incidence, and prevalence of isolated ACTH deficiency (IAD). *ACTH Related Peptides*, **18**, 29–32.
15 Takaya, J., Fujii, Y., Higashino, H. *et al.* (2009) A case of WHIM syndrome associated with diabetes and hypothyroidism. *Pediatr Diabetes*, **10**, 484–486.

16 Driscoll, D.A. & Sullivan, K.E. (2007) DiGeorge Syndrome: A chromosome 22q11.2 deletioin syndrome. In: H.D. Ochs, C.I. Smith, J.M. Puck eds. Primary Immunodeficiency Diseases. Oxford University Press, NewYork, 485–495.
17 Peltonen-Palotie, L., Halonen, M. & Perheentupa, J. (2007) Autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy. In: H.D. Ochs, C.I. Smith & J.M. Puck eds. Primary Immunodeficiency Diseases. Oxford University Press, New York, 342–354.
18 Li, Y., Song, Y.H., Rais, N. *et al.* (1996) Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *The Journal of Clinical Investigation*, **97**, 910–914.
19 Torgerson, T.R., Gambineri, E. & Ziegler, S.F. *et al.* (2007) Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance. In: H.D. Ochs, C.I. Smith & J.M. Puck eds. Primary Immunodeficiency Diseases. Oxford University Press, New York, 355–366.
20 Gambineri, E., Perroni, L., Passerini, L. *et al.* (2008) Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: Inconsistent correlation between forkhead box protein 3 expression and disease severity. *The Journal of Allergy and Clinical Immunology*, **122**, 1105–1112.
21 DIAMOND Project Group (2006) Incidence and trends of childhood Type 1 diabetes worldwide 1990–1999. *Diabetic Medicine*, **23**, 857–866.
22 Schalch, D.S., McFarlin, D.E. & Barlow, M.H. (1970) An unusual form of diabetes mellitus in ataxia telangiectasia. *New England Journal of Medicine*, **282**, 1396–1402.
23 Quinti, I., Soresina, A., Spadaro, G. *et al.* (2007) Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. *Journal of Clinical Immunology*, **27**, 308–316.
24 Geffner, M.E., Stiehm, E.R., Stephure, D. *et al.* (1986) Probable autoimmune thyroid disease and combined immunodeficiency disease. *American Journal of Diseases of Children*, **140**, 1194–1196.
25 Nagpala, P., Newfield, R., Bastian, J. *et al.* (2007) Autoimmune thyroiditis and acquired hypothyroidism in an infant with severe combined immunodeficiency due to adenosine deaminase deficiency. *Thyroid*, **17**, 585–587.
26 Griscelli, C., Lisowska-Grospierre, B. & Mach, B. (1989) Combined immunodeficiency with defective expression in MHC class II genes. *Immunodeficiency Reviews*, **1**, 135–153.
27 Cunningham-Rundles, C. (2011) Autoimmunity in primary immune deficiency: taking lessons from our patients. *Clinical and Experimental Immunology*, **164**(Suppl. 2), 6–11.
28 Tang, M.L. & Kemp, A.S. (1993) Growth hormone deficiency and combined immunodeficiency. *Archives of Disease in Childhood*, **68**, 231–232.
29 Ogershok, P.R., Hogan, M.B., Welch, J.E. *et al.* (2006) Spectrum of illness in pediatric common variable immunodeficiency. *Annals of Allergy, Asthma & Immunology*, **97**, 653–656.
30 Kornfeld, S.J., Kratz, J., Diamond, F. *et al.* (1995) Shwachman-Diamond syndrome associated with hypogammaglobulinemia and growth hormone deficiency. *The Journal of Allergy and Clinical Immunology*, **96**, 247–250.
31 Bernasconi, A., Marino, R., Ribas, A. *et al.* (2006) Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics*, **118**, e1584–e1592.
32 van Bilsen, K., Driessen, G.J., de Paus, R.A. *et al.* (2008) Low level IGF-1 and common variable immune deficiency: an unusual combination. *Netherlands Journal of Medicine*, **66**, 368–372.
33 Delvecchio, M., De Bellis, A., De Mattia, D. *et al.* (2009) Growth hormone deficiency and antipituitary antibodies in a patient with common variable immunodeficiency. *Journal of Endocrinological Investigation*, **32**, 637–640.
34 Shirohzu, H., Kubota, T., Kumazawa, A. *et al.* (2002) Three novel DNMT3B mutations in Japanese patients with ICF syndrome. *American Journal of Medical Genetics*, **112**, 31–37.
35 Tovo, P.A., Lala, R., Martino, S. *et al.* (1991) Isolated adrenocorticotropic hormone deficiency associated with common variable immunodeficiency. *European Journal of Pediatrics*, **150**, 400–402.
36 Mazzolari, E., Forino, C., Guerci, S. *et al.* (2007) Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency. *The Journal of Allergy and Clinical Immunology*, **120**, 892–899.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical data of patients with Hashimoto's thyroiditis.

Table S2. Clinical data of patients with nonautoimmune hypothyroidism.

Table S3. Clinical data of patients with GHD.

Table S4. Clinical data of patients with hypogonadism.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

ORIGINAL ARTICLE CLINICAL IMMUNOLOGY

Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis

Hirokazu Kanegane[1], Xi Yang[1], Meina Zhao[1], Kazumi Yamato[2], Masami Inoue[3], Kazuko Hamamoto[4], Chie Kobayashi[5,6], Ako Hosono[7], Yoshikiyo Ito[8], Yozo Nakazawa[9], Kiminori Terui[10], Kazuhiro Kogawa[11], Eiichi Ishii[12], Ryo Sumazaki[6] & Toshio Miyawaki[1]

[1]Department of Pediatrics, Graduate School of Medicine, University of Toyama, Toyama, Japan; [2]Department of Pediatrics, Graduate School of Medicine, Osaka City University, Osaka, Japan; [3]Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; [4]Department of Pediatrics, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan; [5]Department of Pediatrics, Ibaraki Children's Hospital, Mito, Japan; [6]Department of Child Health, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan; [7]Department of Pediatrics, National Cancer Center Hospital, Tokyo, Japan; [8]Department of Hematology, Harasanshin Hospital, Fukuoka, Japan; [9]Department of Pediatrics, Shinshu University Graduate School of Medicine, Matsumoto, Japan; [10]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [11]Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan; [12]Department of Pediatrics, Ehime University Graduate School of Medicine, Toon, Japan

To cite this article: Kanegane H, Yang Xi, Zhao M, Yamato K, Inoue M, Hamamoto K, Kobayashi C, Hosono A, Ito Y, Nakazawa Y, Terui K, Kogawa K, Ishii E, Sumazaki R, Miyawaki T. Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis. *Pediatric Allergy Immunology* 2012: **23**: 488–493.

Keywords
flow cytometry; genetic analysis; hematopoietic stem cell transplantation; SLAM-associated protein; X-linked lymphoproliferative syndrome

Correspondence
Hirokazu Kanegane, Department of Pediatrics, Graduate School of Medicine, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan
Tel.: 81 76 434 7313
Fax: 81 76 434 5029
E-mail: kanegane@med.u-toyama.ac.jp

Accepted for publication 18 January 2012

DOI:10.1111/j.1399-3038.2012.01282.x

Abstract

Objective: X-linked lymphoproliferative syndrome (XLP) type 1 is a rare immunodeficiency, which is caused by mutations in *SH2D1A* gene. The prognosis of XLP is very poor, and hematopoietic stem cell transplantation (HSCT) is the only curative therapy. We characterized the clinical features and outcome of Japanese patients with XLP-1.

Methods: We used a combination of flow cytometric analysis and genetic analysis to identify XLP-1 and reviewed the patient characteristics and survival with HSCT.

Results: We identified 33 patients from 21 families with XLP-1 in Japan. Twenty-one of the patients (65%) who did not undergo a transplant died of the disease and complications. Twelve patients underwent HSCT, and 11 of these (92%) survived.

Conclusion: We described the clinical characteristics and outcomes of Japanese patients with XLP-1, and HSCT was the only curative therapy for XLP-1. The rapid and accurate diagnosis of XLP with the combination of flow cytometric assay and genetic analysis is important.

X-linked lymphoproliferative syndrome (XLP) is a rare inherited immunodeficiency estimated to affect approximately one in one million males, although it may be under-diagnosed (1). XLP is characterized by extreme vulnerability to Epstein–Barr virus (EBV) infection, and the major clinical phenotypes of XLP include fulminant infectious mononucleosis (FIM) or EBV-associated hemophagocytic lymphohistiocytosis (HLH) (60%), lymphoproliferative disorder (30%), and dysgammaglobulinemia (30%) (2). In addition, XLP is associated with a variety of other clinical manifestations including vasculitis, aplastic anemia, and pulmonary lymphoid granulomatosis. Patients with XLP often develop more than one phenotype over time.

The responsible gene was first identified as *SH2D1A/SLAM-associated protein (SAP)* located in the region of Xq25 (3–5). However, some of the presumed patients with

XLP do not harbor *SH2D1A* mutations, although they are clinically and even histologically similar to XLP patients with *SH2D1A* mutations. A second causative gene that encodes X-linked inhibitor of apoptosis protein (XIAP), namely *XIAP* or *BIRC4* gene, has been identified (6). Patients with XLP-2 (XIAP deficiency) sometimes present with splenomegaly and hemorrhagic colitis, but no lymphoma. The *SH2D1A* and *XIAP* genes are close together at Xq25, but the molecular pathogenesis and clinical features of these diseases seem to be distinct (7, 8).

The vast majority of patients with XLP die in childhood; the survival rate is very poor, even with treatment (2). Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for XLP (9, 10). Therefore, rapid definitive diagnosis and immediate treatment are extremely significant for better prognosis and survival of patients with XLP. We previously established the anti-SAP monoclonal antibody (mAb) and applied it to flow cytometric diagnosis of patients with XLP-1 (11). We performed a nationwide survey for XLP-1 with the flow cytometric assay and genetic analysis and identified a total of 33 patients from 21 families with XLP-1 in Japan (11–15). In this study, we elucidated the clinical and genetic characteristics of these patients. Twelve patients with XLP-1 underwent HSCT, and 11 of these (92%) survived. We also describe the outcomes of HSCT in Japan.

Materials and methods

Study subjects

The subjects in this study were largely male patients with FIM or EBV–HLH treated until the end of 2011. In addition, a few male patients with lymphoma or hypogammaglobulinemia with unknown genetic origin were suspected of having XLP. After written informed consent was obtained, 5–10 ml of venous blood was collected into heparin-containing syringes and delivered to the laboratory. Patients and families provided informed consent for genetic analyses in accordance with the 1975 Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the University of Toyama. Several patients were described in our previous reports (11–15).

Flow cytometric analysis of SAP

Flow cytometric analysis of SAP was performed as previously described (11, 12). The peripheral blood mononuclear cells (PBMC) were isolated by Ficoll–Hypaque density gradient centrifugation and immediately fixed in 1% paraformaldehyde for 30 min at room temperature and then permeabilized in 0.5% saponin for 15 min on ice. To test the expression of SAP in lymphocytes, these cells were incubated with 2 μg/ml anti-SAP mAb, termed KST-3 (rat IgG1) or irreverent rat IgG1, for 20 min on ice and further stained with a 1:1000 dilution of FITC-labeled goat anti-rat IgG antibody (Zymed, South San Francisco, CA, USA) or Alexa Fluor 488-conjugated goat anti-rat IgG antibody (Molecular Probes, Eugene, OR, USA) for 20 min on ice. To evaluate SAP expression in $CD8^+$ T and NK cells, PBMC were stained with phycoerythrin (PE)-conjugated anti-CD8 and anti-CD56 mAbs (DAKO Japan, Kyoto, Japan), respectively, before cellular fixation and permeabilization. The stained cells were analyzed using a flow cytometer (EPICS XL-MCL; Beckman Coulter KK, Tokyo, Japan).

SH2D1A mutation detection

The *SH2D1A* mutations were detected by direct sequencing as described previously (5, 14). Genomic DNA was purified from PBMC with a QIAamp Blood Kit (Qiagen, Hilden, Germany) and amplified using primers encompassing each exon–intron boundary of the *SH2D1A* genes. The sequencing reaction was carried out using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with an automated ABI PRISM 310 DNA sequencer (Applied Biosystems).

Results

SAP expression in patients with XLP-1

Fresh blood cells were available in 19 patients with XLP-1. All the examined patients demonstrated markedly deficient SAP expression in lymphocytes, especially in $CD8^+$ T cells and NK cells (Fig. 1 and Table 1).

SH2D1A mutations

All the mutations including unpublished data are summarized with the clinical data (Table 1). There were three gross deletions (the whole gene and two exons 3 and 4), four nonsense mutations (all Arg55stop), eight missense mutations (Ala3Ser, Tyr7Cys, two His8Asp, Gly27Ser, Asp33Tyr, Ser34Gly and Gly49Val), two small deletions (584delA and 1021delAA), two small insertions (312insG and 545insA), and two splicing anomalies (416C > T and IVS2 + 1G > A). The substitution of 416C with T revealed an aberrantly spliced cDNA with deletion of the last 22 bases of exon 1, and IVS2 + 1G > A resulted in skipping of exon 2.

Clinical characteristics of Japanese patients with XLP-1

Eighteen of the 33 patients (55%) had FIM or EBV–HLH, 12 patients (36%) had hypogammaglobulinemia, seven patients (21%) had malignant lymphoma or lymphoproliferative disease, and two patients (P4.2 and P7.2) had lymphocytic vasculitis. One patient (P7.1) had aplastic anemia. Twenty-seven patients (82%) were associated with EBV infection at the disease onset. Two patients (P16.1 and P19.3) presented with non-EBV–HLH. Interestingly, malignant lymphoma and lymphocytic vasculitis in P4.2 were not associated with EBV infection, but the patient later developed EBV–HLH at the age of 14 yr and died of HLH. Two patients (P17.2 and P21.1) had encephalitis: and P17.2 developed acute disseminated encephalomyelitis caused by human

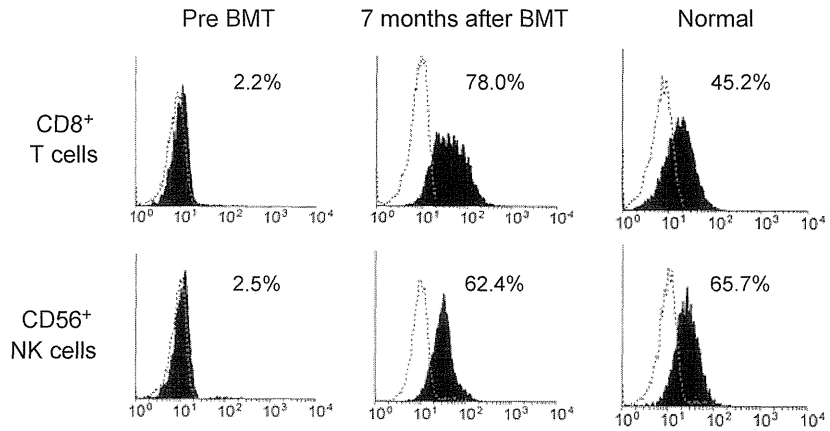

Figure 1 The SAP expression in $CD8^+$ T cells and NK cells from the patient (P16.1) and a normal adult donor. Dotted lines and shaded areas indicate staining by the control antibody and anti-SAP mAb (KST-3), respectively. A flow cytometric analysis demonstrated that deficient SAP expression in $CD8^+$ T cells and NK cells from the patient increased after he had undergone hematopoietic stem cell transplantation.

herpes virus 6 infection and P21.1 developed EBV encephalitis. Approximately 70% of the patients (23 of 33) were diagnosed by the time they were 5 yr of age, but two patients (P13.1 and P20.1) were diagnosed in adulthood. Eleven families (52%) had X-linked family histories. Ten patients (30%) presented with more than one clinical manifestation over time. Ten sibling cases were observed in this study, and seven families manifested different phenotypes. Fifteen patients (45%) were treated with intravenous immunoglobulin replacement therapy. In this study, the mortality rate was 21 of 32 patients (66%), and all the living patients were post-transplanted. Clinical characteristics of this study are summarized in comparison with those of previous study (Table 2).

Hematopoietic stem cell transplantation for patients with XLP-1

Twelve patients with XLP underwent HSCT in Japan (Table 3), and one patient (P9.2) died of *Pseudomonas* sepsis and multiple organ failure 14 days after HSCT. Two patients (P1.2 and P7.2) were transplanted from matched sibling donors, but the other patients were transplanted from matched or one-locus-mismatched unrelated donors, or mismatched familial donors. Various types of conditioning regimen were performed. Five patients (P1.2, P7.2, P9.1, P10.1, and P14.1) underwent HSCT following myeloablative conditioning, but the other patients did so following reduced intensity conditioning (RIC). Acute graft versus host disease (GVHD) was observed in 6 of 11 patients (Grade I, two patients; Grade II, three patients; Grade III, one patient). Chronic GVHD was observed in five patients, among whom 4 (P1.2, P7.2, P10.1, and P18.1) had extensive types and one (P14.1) had a limited type. Eleven patients (92%) have survived and had complete chimerism with a median follow-up of 7 yr and 9 months. A flow cytometric assay could be conducted to evaluate SAP expression in $CD8^+$ T cells and NK cells after HSCT in five patients (P7.2, P10.2, P16.1, P17.2, and P18.1). All the patients demonstrated an increase in SAP expression in $CD8^+$ T cells and NK cells after undergoing HSCT (Fig. 1).

Discussion

X-linked lymphoproliferative syndrome is a rare but life-threatening disease. A large cohort showed that most patients with XLP died by the age of 40 yr and more than 70% of the patients died before the age of 10 yr (2). Early diagnosis in non-familial cases may be difficult because XLP is heterogeneous in its clinical presentation. The ability to screen rapidly and make an accurate diagnosis of patients with XLP facilitates the initiation of life-saving treatment and preparation for HSCT. In a previous study, we generated an anti-SAP mAb, termed KST-3, which was applied to the flow cytometric evaluation of SAP deficiency (XLP-1) (11). All the patients evaluated in this study showed deficient SAP expression, although some patients with missense mutations might demonstrate normal expression of SAP, as shown in Western blotting (16).

Various types of *SH2D1A* mutation have been identified in Japan (11–15). The *SH2D1A*base (http://bioinf.uta.fi/SH2D1Abase) discloses that 133 unrelated patients were identified to have *SH2D1A* mutations. Missense and nonsense mutations appear in one-quarter each, and other types of mutation appear in half of the patients in this database. In the present study, Arg55stop mutations were most frequently found, in keeping with the *SH2D1A*base. No genotype and phenotype correlation was evident in this study, as well as in previous studies (1, 17).

Large cohort studies have shown that the major clinical phenotypes of XLP include FIM (60%), dysgammaglobulinemia (30%), and malignant lymphoma (30%) (1, 2). Aplastic anemia, lymphoid granulomatosis, and systemic vasculitis are minor clinical presentations at frequencies of approximately 3%. Although the present study included a limited number of patients with XLP-1, the distribution of the clinical manifestations seems to be similar to that in previous large studies

Table 1 Clinical and genetic data of patients with X-linked lymphoproliferative syndrome

Patient ID	Age at diagnosis	Family history	Clinical presentation	Epstein–Barr virus status	IVIG	Outcome	Cause of death	Age at death or presence	*SH2D1A* mutation	SAP expression
1.1	12 yr	+	Hypo-γ, LPD	+	+	Dead	GVHD	12 yr	NE	NE
1.2	7 yr	+	Hypo-γ, LPD	+	+	Alive*		21 yr	Asp33Tyr	NE
2.1	3 yr	−	FIM	+	−	Dead	FIM	3 yr	Arg55stop	NE
3.1	2 yr	+	FIM	+	−	Dead	FIM	2 yr	Arg55stop	NE
3.2	2 yr	+	FIM	+	−	Dead	FIM	2 yr	Arg55stop	NE
4.1	2 yr	+	FIM	+	−	Dead	FIM	2 yr	416C>T, fs	NE
4.2	4 yr	+	ML, vasculitis, HLH	−	−	Dead	HLH (MOF)	14 yr	416C>T, fs	Deficient
5.1	1 yr	+	FIM	+	+	Dead	FIM	1 yr	del of whole gene	NE
6.1	1 yr	−	FIM	+	−	Dead	FIM	1 yr	Gly27Ser	NE
7.1	1 yr	+	Hypo-γ, aplastic anemia	+	+	Dead	Sepsis	1 yr	NE	NE
7.2	3 yr	+	Hypo-γ, vasculitis	−	+	Alive*		30 yr	His8Asp	Deficient
8.1	1 yr	−	FIM	+	+	Dead	FIM	1 yr	584delA, fs	NE
9.1	6 yr	+	Hypo-γ	+	+	Alive*		18 yr	Arg55stop	Deficient
9.2	6 months	+	FIM	+	+	Dead*	Sepsis	6 yr	Arg55stop	Deficient
10.1	4 yr	+	ML	+	−	Alive*		15 yr	Gly49Val	Deficient
10.2	0 months	+	Healthy	−	−	Alive*		4 yr	Gly49Val	Deficient
11.1	1 yr	+	FIM	+	+	Dead	FIM (MOF)	1 yr	del of exons 3, 4	NE
11.2.	2 yr	+	FIM	+	+	Dead	FIM (MOF)	2 yr	del of exons 3, 4	Deficient
11.3	0 month	+	Healthy	−	+	Alive*		9 yr	del of exons 3, 4	Deficient
12.1	12 yr	+	Hypo-γ, ML	+	−	Dead	ML	12 yr	Ser34Gly	Deficient
12.2	10 yr	+	Hypo-γ	+	−	Unknown	Unknown	Unknown	Ser34Gly	Deficient
13.1	23 yr	−	FIM	+	−	Dead	FIM	23 yr	Tyr7Cys	Deficient
14.1	8 yr	−	Hypo-γ, ML	+	−	Alive*		16 yr	Arg55stop	Deficient
15.1	2 yr	−	FIM	+	−	Dead	FIM	2 yr	His8Asp	NE
16.1	10 yr	−	Hypo-γ, HLH	−	+	Alive*		17 yr	545insA, fs	Deficient
17.1	2 yr	+	FIM	+	−	Dead	FIM	2 yr	IVS2+1G>A	Deficient
17.2	2 yr	+	ADEM	−	−	Alive*		8 yr	IVS2+1G>A	Deficient
18.1	6 yr	−	Hypo-γ	+	+	Alive*		12 yr	312insG, fs	Deficient
19.1	10 months	+	Hypo-γ	+	+	Dead	DIC	10 months	NE	NE
19.2	1 yr	+	FIM	+	−	Dead		1 yr	NE	NE
19.3	3 yr	+	Hypo-γ, HLH, ML	+	+	Alive*		18 yr	del of exons 3, 4	Deficient
20.1	41 yr	−	FIM	+	−	Dead	FIM	42 yr	Ala3Ser	Deficient
21.1	3 yr	−	Encephalitis, LPD	+	−	Dead	Encephalitis	3 yr	538insA, fs	Deficient

Hypo-γ, hypogammaglobulinemia; LPD, lymphoproliferative disease; GVHD, graft versus host disease; FIM; fulminant infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis; MOF, multiple organ failure; ML, malignant lymphoma; ADEM, acute disseminated encephalomyelitis; DIC, disseminated intravascular coagulation; NE, not examined; fs, frameshift; del, deletion; ins, insertion.
P17.1 and 17.2 are monozygotic twins. Asterisk indicates the patients who underwent hematopoietic stem cell transplantation. P1.2, P2.1, P3.1, P3.2, P4.1, P5.1, P6.1, P7.2, P8.1, and P10.1 were described by Sumazaki et al. (14) P5.1 was described by Honda et al. (13) P9.1, P9.2, P11.1, P11.2, P11.3, P12.1, and P12.2 were described by Shinozaki et al. (11) P13.1 was described by Hoshino et al. (15) P16.1, P17.1, P17.2, P18.1, P19.3, and P20.1 were described by Zhao et al. (12). [Correction added on 10 April 2012, after first online publication: the *SH2D1A* mutation of P21.1 has been corrected.]

(Table 2) (2, 17). Lymphoid granulomatosis was not found in Japanese patients, but two patients have presented with systemic vasculitis (18). The vasculitis in these patients mainly affected the brain and was associated with encephalopathy. The mortality was different among clinical phenotypes, and the mortality of each phenotype in our study decreased from that in the XLP registry (2). However, in a recent worldwide study, the mortality associated with HLH decreased to 65%, lymphoproliferative disease to 8%, and dysgammaglobulinemia to 5% (16).

Hematopoietic stem cell transplantation is the only curative treatment for XLP-1. Twenty-one patients with XLP-1 did not undergo HSCT, and these patients died of the disease and complications. The outcome of one patient (P12.2) was unknown. Twelve patients underwent HSCT in Japan, and 11 patients survived. Most of the transplants were performed in different institutions, but the outcomes are similar to previously published data (9, 10, 17). This study revealed that unrelated donors could be used as donors as well as sibling donors. Although various types of conditioning regimen were performed, more than half included RIC regimen, and the result of RIC regimen is similar to that of myeloablative regimen. The RIC regimen should be performed for patients with XLP-1 to avoid regimen-related toxicity or morbidity (17). In

Table 2 Clinical phenotypes of patients with X-linked lymphoproliferative syndrome

	Present study (33 cases)		Seemayer (272 cases) (2)		Booth (91 cases) (17)	
Phenotype	Incidence	Mortality	Incidence	Mortality	Incidence	Mortality
FIM or HLH	18 (55%)	16/18 (89%)	157 (58%)	127/132 (96%)	35.2%	65.6%
ML or LPD	7 (21%)	3/7 (43%)	82 (30%)	46/71 (65%)	24.2%	9.0%
Hypogammaglobulinemia	12 (36%)	4/11 (36%)	84 (31%)	34/75 (45%)	50.5%	13.0%

FIM, fulminant infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis.

Table 3 Characteristics of HSCTs

Patient ID	Age at HSCT	Donor	Sources	Conditioning regimen	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Outcome
1.2	7 yr	MSD (6/6)	PBSC	TBI/CY	CsA/sMTX	Grade I	Extensive	Alive (14 yr 8 months)
7.2	24 yr	MSD (6/6)	BM	BU/CY/ATG	CsA/sMTX	Grade II	Extensive	Alive (6 yr 6 months)
9.1	8 yr	MUD (6/6)	BM	BU/VP/CY	FK/sMTX	None	None	Alive (10 yr 6 months)
9.2	6 yr	mMFD (3/6)	BM	TBI 6Gy/BU 4 mg/kg	MMF/sMTX/mPSL	NE	NE	Dead (14 days)
10.1	4 yr	mMUD (5/6)	BM	BU/CY/AraC	FK/sMTX	Grade II	Extensive	Alive (11 yr 2 months)
10.2	1 yr	MUD (6/6)	BM	BU/TAI 3Gy/Flu/CY/ATG	FK/sMTX	None	None	Alive (3 yr 3 months)
11.3	8 months	mMUD (5/6)	PBSC	Flu/Mel/ATG/TAI 6Gy	FK/sMTX/mPSL	Grade II	None	Alive (9 yr 2 months)
14.1	10 yr	MUD (6/6)	BM	BU/CY	CsA/sMTX	Grade III	Limited	Alive (8 yr 2 months)
16.1	11 yr	mMUD (5/6)	BM	BU/TAI 3Gy/Flu/CY/ATG	FK/sMTX	None	None	Alive (5 yr 6 months)
17.2	3 yr	mMFD (4/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	Grade I	None	Alive (8 yr 10 months)
18.1	7 yr	MUD (6/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	None	Extensive	Alive (4 yr 7 months)
19.3	15 yr	MUD (6/6)	BM	Flu/Mel/TBI 3 Gy	FK/sMTX	None	None	Alive (3 yr 7 months)

MSD, matched sibling donor; MUD, matched unrelated donor; mMFD, mismatched familial donor; mMUD, mismatched unrelated donor; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; CY, cyclophosphamide; BU, busulfan; ATG, anti-thymoglobulin; VP, etoposide; Gy, gray; AraC, cytosine arabinoside; TAI, total abdominal irradiation; Flu, fludarabine; Mel, melphalan; GVHD, graft versus host disease; CsA, cyclosporine A; sMTX, short methotraxate; FK, tacrolimus; MMF, mycophenolate mofetil; mPSL, methylprednisolone; NE, not evaluated; HSCT, hematopoietic stem cell transplantation.

this study, two patients (P10.2 and P11.3) were diagnosed because of a family history and presented no clinical features of XLP. Their parents wanted them to undergo HSCT because of the poor prognosis of the disease. Although the decision to transplant a relatively well child has been more challenging, these patients underwent transplant and were free from chronic GVHD.

In conclusion, this study verified the clinical usefulness of a flow cytometric assessment of SAP to search for XLP-1 (SAP deficiency). Flow cytometric analysis of XIAP is also useful to detect patients with XLP-2 (7, 19, 20). A male with any of the clinical phenotypes of XLP with or without EBV infection should be initially examined with a flow cytometric assay to evaluate both SAP and XIAP (21). We also identified nine Japanese patients with XIAP deficiency with a combination of flow cytometry and genetic analysis (22). Needless to say, a mutation analysis is the gold standard for confirming a definite diagnosis. The outcome of patients with XLP-1 seemed to be poor in Japan, and HSCT is the only curative treatment for patients with XLP-1.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a grant from the Ministry of Health, Labour, and Welfare of Japan. The authors would like to thank Ms. Chikako Sakai and Mr. Hitoshi Moriuchi for their excellent technical assistance and Dr. Rebecca A. Marsh for critical discussion. We would also like to thank many doctors for providing blood samples and medical records of the patients: Sadao Suga, Akihiro Yachie, Takeshi Shichijo, Tadashi Matsubayashi, Takeshi Taketani, Hiroyuki Moriuchi, Tatsuo Kondo, Takumi Hoshio, Yo Umeda, Mariko Fujimatsu, Junichi Kiyasu, and Takeo Mukai.

References

1. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood* 2000: **96**: 3118–25.
2. Seemayer TA, Gross TG, Egeler RM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res* 1995: **38**: 471–8.
3. Coffey AJ, Brooksbank RA, Brandau O, et al. (1998) Host response to EBV

infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* 1998: **20**: 129–35.
4. Nichols KE, Harkin DP, Levitz S, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci USA* 1998: **95**: 13765–70.
5. Sayos J, Wu C, Morra M, et al. (1998) The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998: **395**: 462–9.
6. Rigaud S, Fondanèche MC, Lambert N, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* 2006: **444**: 110–4.
7. Filipovich AH, Zhang K, Snow AL, Marsh RA. X-linked lymphoproliferative syndromes: brothers or distant cousins?. *Blood* 2010: **116**: 3398–408.
8. Razaei N, Mahmoudi E, Aghamohammadi A, Das R, Nichols KE. X-linked lymphoproliferative syndrome: a genetic condition typified by the triad of infection, immunodeficiency and lymphoma. *Br J Haematol* 2011: **152**: 13–30.
9. Gross TG, Filipovich AH, Conley ME, et al. Cure of X-linked lymphoproliferative disease (XLP) with allogeneic hematopoietic stem cell transplantation (HSCT): report from the XLP registry. *Bone Marrow Transplant* 1996: **17**: 741–4.
10. Lankester AC, Visser LF, Harwig NG, et al. Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature. *Bone Marrow Transplant* 2005: **36**: 99–105.
11. Shinozaki K, Kanegane H, Matsukura H, et al. (2002) 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**: 1215–23.
12. Zhao M, Kaneganea H, Kobayashi C, et al. An early and rapid detection of X-linked lymphoproliferative syndrome with *SH2D1A* mutations by flow cytometry. *Cytometry B Clin Cytom* 2011: **80**: 8–13.
13. Honda K, Kanegane H, Eguchi M, et al. Large deletion of the X-linked lymphoproliferative disease gene detected by fluorescence in situ hybridization. *Am J Hematol* 2000: **64**: 128–32.
14. Sumazaki R, Kanegane H, Osaki M, et al. SH2D1A mutations in Japanese males with severe Epstein-Barr virus-associated illnesses. *Blood* 2001: **98**: 1268–70.
15. Hoshino T, Kanegane H, Doki N, et al. X-linked lymphoproliferative disease in an adult. *Int J Hematol* 2005: **82**: 55–8.
16. Nistala K, Gilmour KC, Cranston T, et al. X-linked lymphoproliferative disease: three atypical cases. *Clin Exp Immunol* 2000: **126**: 126–30.
17. Booth C, Gilmour KC, Veys P, et al. X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, managements and outcome of the disease. *Blood* 2011: **117**: 53–62.
18. Kanegane H, Ito Y, Ohshima K, et al. X-linked lymphoproliferative syndrome presenting with systemic lymphocytic vasculitis. *Am J Hematol* 2005: **78**: 130–3.
19. Marsh RA, Villanueva J, Zhang K, et al. A rapid flow cytometric screening test for X-linked lymphoproliferative disease due to XIAP deficiency. *Cytometry B Clin Cytom* 2009: **76**: 334–44.
20. Zhao M, Kanegane H, Ouchi K, Imamura T, Latour S, Miyawaki T. A novel *XIAP* mutation in a Japanese boy with recurrent pancytopenia and splenomegaly. *Haematologica* 2010: **95**: 688–9.
21. Marsh RA, Bleesing JJ, Filipovich AH. Using flow cytometry to screen patients for X-linked lymphoproliferative disease due to SAP deficiency and XIAP deficiency. *J Immunol Methods* 2010: **362**: 1–9.
22. Yang Xi, Kanegane H, Nishida N, et al. Clinical and genetic characteristics of XIAP deficiency in Japan. *J Clin Immunol* 2012 Jan 8. [Epub ahead of print].

J Clin Immunol (2012) 32:411–420
DOI 10.1007/s10875-011-9638-z

Clinical and Genetic Characteristics of XIAP Deficiency in Japan

Xi Yang · Hirokazu Kanegane · Naonori Nishida · Toshihiko Imamura · Kazuko Hamamoto · Ritsuko Miyashita · Kohsuke Imai · Shigeaki Nonoyama · Kazunori Sanayama · Akiko Yamaide · Fumiyo Kato · Kozo Nagai · Eiichi Ishii · Menno C. van Zelm · Sylvain Latour · Xiao-Dong Zhao · Toshio Miyawaki

Received: 26 July 2011 / Accepted: 14 December 2011 / Published online: 8 January 2012

Abstract Deficiency of X-linked inhibitor of apoptosis (XIAP) caused by *XIAP/BIRC4* gene mutations is an inherited immune defect recognized as X-linked lymphoproliferative syndrome type 2. This disease is mainly observed in patients with hemophagocytic lymphohistiocytosis (HLH) often associated with Epstein–Barr virus infection. We described nine Japanese patients from six unrelated families with XIAP deficiency and studied XIAP protein expression, *XIAP* gene analysis, invariant natural killer T (iNKT) cell counts, and the cytotoxic activity of $CD8^+$ alloantigen-specific cytotoxic T lymphocytes. Of the nine patients, eight patients presented with symptoms in infancy or early childhood. Five patients presented with recurrent HLH, one of whom had severe HLH and died after cord blood transplantation. One patient presented with colitis, as did another patient's maternal uncle, who died of colitis at

X. Yang · H. Kanegane (✉) · N. Nishida · T. Miyawaki
Department of Pediatrics, Graduate School of Medicine,
University of Toyama,
2630 Sugitani,
Toyama 930-0194, Japan
e-mail: kanegane@med.u-toyama.ac.jp

X. Yang · X.-D. Zhao
Division of Immunology,
Children's Hospital of Chongqing Medical University,
Chongqing, China

T. Imamura
Department of Pediatrics, Graduate School of Medical Sciences,
Kyoto Prefectural University of Medicine,
Kyoto, Japan

K. Hamamoto
Department of Pediatrics, Hiroshima Red Cross Hospital,
Hiroshima, Japan

R. Miyashita
Department of Pediatrics, Izumiotsu Municipal Hospital,
Izumiotsu, Japan

K. Imai · S. Nonoyama
Department of Pediatrics, National Defense Medical College,
Tokorozawa, Japan

K. Sanayama
Department of Pediatrics, Japanese Red Cross Narita Hospital,
Narita, Japan

A. Yamaide
Department of Allergy and Rheumatology,
Chiba Children's Hospital,
Chiba, Japan

F. Kato
Department of Pediatrics,
Tokyo Women's Medical University Medical Center East,
Tokyo, Japan

K. Nagai · E. Ishii
Department of Pediatrics,
Ehime University Graduate School of Medicine,
Toon, Japan

M. C. van Zelm
Department of Immunology, Erasmus MC,
Rotterdam, The Netherlands

S. Latour
INSERM U768, Hôpital Necker-Enfants Malades,
Paris, France

4 years of age prior to diagnosis with XIAP deficiency. Interestingly, a 17-year-old patient was asymptomatic, while his younger brother suffered from recurrent HLH and EBV infection. Seven out of eight patients showed decreased XIAP protein expression. iNKT cells from patients with XIAP deficiency were significantly decreased as compared with age-matched healthy controls. These results in our Japanese cohort are compatible with previous studies, confirming the clinical characteristics of XIAP deficiency.

Keywords X-linked lymphoproliferative syndrome · X-linked inhibitor of apoptosis · Epstein–Barr virus · hemophagocytic lymphohistiocytosis · invariant natural killer T cell

Abbreviations

BIR	Baculovirus IAP repeat
CTL	Cytotoxic T lymphocyte
HSCT	Hematopoietic stem cell transplantation
HLH	Hemophagocytic lymphohistiocytosis
IAP	Inhibitor of apoptosis
LCL	Lymphoblastoid cell line
MMC	Mitomycin C
mAb	Monoclonal antibody
MFI	Mean fluorescence intensity
iNKT	Invariant natural killer T
PCR	Polymerase chain reaction
PBMC	Peripheral blood mononuclear cells
TCR	T cell receptor
XIAP	X-linked inhibitor of apoptosis
XLP	X-linked lymphoproliferative syndrome

Introduction

X-linked lymphoproliferative syndrome (XLP) is a rare inherited immunodeficiency estimated to affect approximately one in one million males, although it may be underdiagnosed [1]. XLP is characterized by extreme vulnerability to Epstein–Barr virus (EBV) infection, and the major clinical phenotypes of XLP include fulminant infectious mononucleosis (60%), lymphoproliferative disorder (30%), and dysgammaglobulinemia (30%) [2]. In addition, XLP is associated with a variety of additional clinical phenotypes such as vasculitis, aplastic anemia, and pulmonary lymphoid granulomatosis. Patients with XLP often develop more than one of these phenotypes. The gene responsible for XLP was identified as *SH2D1A*, located on Xq25 and encoding the SLAM-associated protein (SAP) [3–5]. However, gene analysis revealed *SH2D1A* mutations in only 50–60% of presumed XLP patients [6]. Importantly, a mutation in the gene that encodes the X-linked inhibitor of apoptosis (XIAP) called *XIAP* or *BIRC4* was identified as a second causative gene for XLP [7]. *XIAP* is located close to the *SH2D1A* gene on the X chromosome and consists of six coding exons [8–10]. XIAP produces an anti-apoptotic molecule that belongs to the inhibitor of apoptosis (IAP) family proteins. It contains three baculovirus IAP repeat (BIR) domains that, together with flanking residues, bind to caspases 3, 7, and 9, thereby inhibiting their proteolytic activity [11].

The clinical presentations of XIAP-deficient patients have been frequently reported [7,12,13]. More than 90% of patients with XIAP deficiency develop hemophagocytic lymphohistiocytosis (HLH) which is often recurrent. Therefore, it was recently suggested that the phenotype of XIAP deficiency fits better with the definition of familial HLH than with XLP disease [12]. However, familial HLH is characterized by defects in $CD8^+$ T and NK cell cytotoxicity responses, while these responses are normal in XIAP deficiency [7,12]. Other symptoms of XLP, such as splenomegaly, hypogammaglobulinema, and hemorrhagic colitis, have been reported in patients with XIAP deficiency, but lymphoma has never been noted [7,12–15].

We searched for patients with XIAP deficiency in Japan by detection of *XIAP* gene mutations and flow cytometric assessment of lymphoid XIAP expression. We previously reported the first case of XIAP deficiency in Japan [14]. Thereafter, we identified eight additional cases from five families with XIAP deficiency in our country. In this study, we describe the clinical and laboratory findings from nine patients from six unrelated families with XIAP deficiency, including previous cases, to help further the understanding of the pathogenetic features of this disease.

Materials and Methods

Patient and Family Member Samples

Patients without indentified *SH2D1A* mutations but with presumed XLP phenotypes were screened for *XIAP* mutations. Their family members were also screened for the same mutation. Upon identification of *XIAP* mutations, the patients were enrolled in this study. Patient 2.2 passed away before a genetic diagnosis of XIAP deficiency was made, but he was the maternal uncle of patient 2.1 and had presented with a XLP phenotype (Table I). In the end, nine patients from six different families were found to have XIAP deficiencies, three of whom had been reported previously [13,14]. Upon the approval of the Ethics Committee of the University of Toyama and after obtaining informed consent, 5–10 mL heparinized venous blood was collected from the patients, their mothers, and 25 age-matched healthy children (1–13 years of age). All of the samples were

transferred to our laboratory at room temperature within 24 h for analysis.

Mutation Analysis of the *XIAP* Gene

DNA was extracted from peripheral blood using the QuickGene-Mini 80 nucleic acid extraction system (FUJIFILM Co., Tokyo, Japan). The coding regions and the exon–intron boundaries of the *XIAP* gene were amplified by polymerase chain reaction (PCR) using primers flanking each of the six exons by standard methods. PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplification. Sequencing analysis was performed on an Applied Biosystems Prism 310 Capillary Sequencer (Applied Biosystems).

Flow Cytometric Analysis of XIAP Protein Expression in Lymphocytes

XIAP protein expression was studied by flow cytometric techniques as previously described [16,17]. Peripheral blood mononuclear cells (PBMC) from patients 1, 2.1, 3.1, 3.2, 4, 5, 6.1, 6.2, and 25 age-matched healthy children were prepared by density gradient centrifugation over Histopaque-1077 (Sigma-Aldrich, Inc., St. Louis, MO, USA). The cells were first fixed in 1% paraformaldehyde in PBS for 30 min at room temperature and then permeabilized in 0.5% saponin in washing buffer. The fixed and permeabilized cells were then incubated with an anti-XIAP monoclonal antibody (mAb) (clone 48 (BD Biosciences, Franklin Lakes, NJ, USA) or clone 2 F1 (Abcam, Cambridge, UK)) for 20 min on ice, washed, and then incubated with a FITC-labeled anti-mouse IgG1 antibody (SouthernBiotech, Birmingham, AL, USA) for 20 min on ice. The stained cells were analyzed on the FC500 flow cytometer (Beckman Coulter, Tokyo, Japan).

Western Blot Analysis of XIAP Protein Expression in Lymphocytes

PBMC from normal controls and patients 3.1, 5, and 6.2 were washed and pelleted. The cells were then lysed in 10 μL of lysing solution (1% Triton-X 100; 150 mmol/L NaCl; 10 mmol/L Tris–HCl, pH 7.6; 5 mmol/L EDTA-Na; 2 mmol/L phenylmethylsulfonyl fluoride) per 10^6 cells for 30 min on ice. The lysed cells were centrifuged for 10 min at 15,000*g* to remove nuclei, and the supernatants were diluted in the same volume of Laemmli's sample buffer. Samples were then electrophoresed in sodium dodecyl sulfate–polyarylamide 10% to 20% gradient gel and blotted on nitrocellulose filters. Blots were blocked in 5% skim milk in PBS for 1 h, treated with anti-XIAP mAb (clone 28 or clone 2F1) for 2 h, and then incubated with peroxidase-conjugated anti-mouse IgG antibody (Invitrogen, Grand Island, NY, USA) for 1 h. Immunoblots were developed by the ECL Western blotting detection system (GE Healthcare UK Ltd., Buckinghamshire, England).

Flow Cytometric Identification of Invariant Natural Killer T Cells

PBMC from eight patients (1, 2.1, 3.1, 3.2, 4, 5, 6.1, and 6.2) and 25 controls were incubated with fluorochrome-conjugated anti-CD3 (Dako Japan KK, Kyoto, Japan), anti-TCRVα24, and anti-TCRVβ11 mAbs (Beckman Coulter) to identify invariant natural killer T (iNKT) cells by flow cytometry. After the electronic gating of 100,000 $CD3^+$ T cells, iNKT cell populations were defined by the co-expression of TCRVα24 and TCRVβ11. The iNKT cell counts were evaluated at the diagnosis of XIAP deficiency.

Establishment of Alloantigen-Specific Cytotoxic T Lymphocyte Lines and Analysis of Cytotoxic T Lymphocyte-Mediated Cytotoxity

Alloantigen-specific $CD8^+$ cytotoxic T lymphocyte (CTL) lines were generated as described previously [18,19]. Briefly, PBMC were obtained from patients 1, 2.1, 3.1, and unrelated healthy individuals. These cells were co-cultured with a mitomycin C (MMC)-treated B lymphoblastoid cell line (LCL) established from an HLA-mismatched individual (KI-LCL). Using cell isolation immunomagnetic beads (MACS beads; Miltenyi Biotec, Auburn, CA, USA), $CD8^+$ T lymphocytes were isolated from PBMC that had been stimulated with KI-LCL for 6 days. $CD8^+$ T lymphocytes were cultured in RPMI 1640 medium supplemented with 10% human serum and 10 IU/mL interleukin-2 (Roche, Mannheim, Germany) and stimulated with MMC-treated KI-LCL three times at 1-week intervals. These lymphocytes were then used as $CD8^+$ alloantigen-specific CTL lines. The cytotoxic activity of CTLs was measured by a standard ^{51}Cr-release assay as described previously [20]. Briefly, alloantigen-specific CTLs were incubated with ^{51}Cr-labeled allogeneic KI-LCL or TA-LCL, which did not share HLA antigens with KI-LCL, for 5 h at effector/target cell ratios (E/T) of 2.5:1, 5:1, and 10:1. Target cells were also added to a well containing only medium and to a well containing 0.2% Triton X-100 to determine the spontaneous and maximum levels of ^{51}Cr release, respectively. After 5 h, 0.1 mL of supernatant was collected from each well. The percentage of specific ^{51}Cr release was calculated as follows: (cpm experimental release − cpm spontaneous release) / (cpm maximal release − cpm spontaneous release)×100, where cpm indicates counts per minute.

Table I Summary of our data

	Patient 1 [13]	Patient 2.1 [12]	Patient 2.2 [12]	Patient 3.1	Patient 3.2	Patient 4	Patient 5	Patient 6.1	Patient 6.2
Age at initial presentation	20 months	7 months	3 months	2 months	Asymptomatic	2 months	6 months	17 months	15 months
Current age	4 years	Deceased	Died of colitis	12 years	17 years	15 years	2 years	1 year	12 years
Family history	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes
HLH	+	+	−	+	−	−	+	+	+
Recurrent HLH	+	+	−	+	−	−	+	−	+
Fever	+	+	+	+	−	−	+	+	+
Splenomegaly	+	+	ND	−	−	−	−	+	+
Cytopenia	+	+	ND	+	−	−	+	+	+
EBV	+	−	ND	+	−	−	−	+	+
Hypogammaglobulinemia	−	+	ND	−	−	+	−	−	−
Colitis	−	−	+	−	−	−	+	−	−
Treatment	PSL	PSL		PSL			PSL, Dex		
	CsA	CsA	ND	CsA	−	IVIG	CsA, IVIG	IVIG, Dex	PSL
	Dex	Dex					Infliximab		
Allogeneic HSCT	−	+	−	−	−	−	−	−	−
Mutation	R238X	R381X	ND	W217CfsX27	W217CfsX27	E349del	Del of exons 1-2	N341YfsX7	N341YfsX7
XIAP protein expression	±	−	ND	−	−	+	±	±	±

HLH hemophagocytic lymphohistiocytosis, *ND* no data, *EBV* Epstein–Barr virus, *PSL* prednisolone, *CsA* cyclosporin A, *Dex* dexamethasone, *IVIG* intravenous immuglobulin, *HSCT* hematopoietic stem cell transplantation, + yes or positive, − no or negative, ± residual expression

Statistical Analysis

Student's *t*-test was used for statistics, with *P*-values <0.05 considered to be statistically significant.

Results

Clinical Manifestations of the Patients

Most of our patients presented with disease symptoms at very early ages; five patients presented in infancy and three patients presented in childhood (Table I). Three of the six families had family history records. Five of the nine patients had recurrent HLH, fever, splenomegaly, and cytopenia. EBV infection and hypogammaglobulinemia were also observed in multiple patients. Most patients with HLH were treated with corticosteroids with or without cyclosporin A to prevent an otherwise rapidly fatal disease course. Patients 2.2 and 5 presented with colitis, whereas patient 2.2 died; patient 5 improved with anti-TNF alpha mAb (infliximab®) treatment. Patient 2.1 underwent cord blood transplantation but died of complications. Patient 4 had a history of recurrent otitis media and pneumonia since 2 months of age, and he was found to have hypogammaglobulinemia. The patient was treated with intravenous immunoglobulin replacement therapy alone, and he is currently doing well. No patient developed lymphoma.

Detection of *XIAP* Mutations

We identified *XIAP* mutations in patients from all six unrelated families (Fig. 1) and analyzed all of the data using the US National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/SNP) to check for single-nucleotide polymorphism in the *XIAP* gene. As previously reported, patient 1 possessed a nonsense mutation, 712 C > T, resulting in an early stop codon R238X [14]. Patient 2.1 had a nonsense mutation in exon 5, 1141 C > T, resulting in R381X [13]. Patient 2.2 might have the same mutation as patient 2.1 because patient 2.2 was the maternal uncle of patient 2.1 [13]. Patients 3.1 and 3.2 were siblings and were found to have a one base pair deletion (650delG) in exon 1, resulting in a frameshift and premature stop codon (W217CfsX27). Patient 4 was found to have one amino acid deletion (1045_1047delGAG; E349del) in exon 3. Patient 5 has a large deletion, spanning exons 1 and 2. Patients 6.1 and 6.2 were brothers and had a two-nucleotide deletion (1021_1022delAA), which resulted in a frameshift and premature stop codon (N341YfsX7). All of the mothers of the patients from families 1–5 were heterozygote carriers of the mutations. Interestingly, we could not find any *XIAP* mutation in the mother of patients 6.1 and 6.2. We identified deleterious *XIAP* mutations in nine patients from six unrelated Japanese families that are likely to underlie their XLP phenotypes.

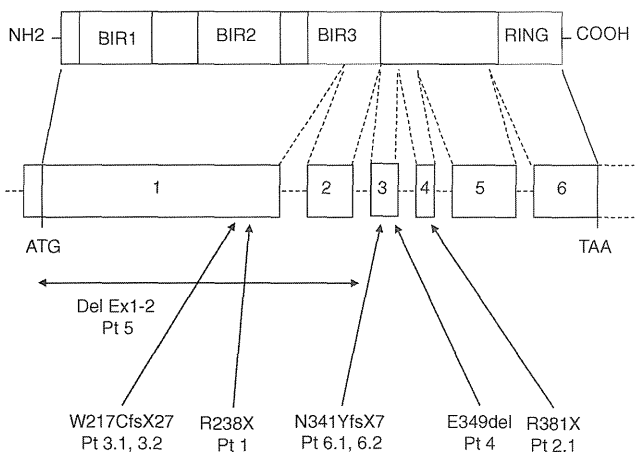

Fig. 1 *XIAP* gene mutations and their consequences for XIAP protein. *XIAP* comprises six exons and encodes the XIAP protein, which consists of 497 amino acids. XIAP contains three BIR domains and one RING domain. Mutations identified in our patients are indicated

XIAP Expression in Lymphocytes from the Patients and Carriers by Flow Cytometry

XIAP expression levels were analyzed in the lymphocytes of patients from all six families (Fig. 2). The lymphocytes of

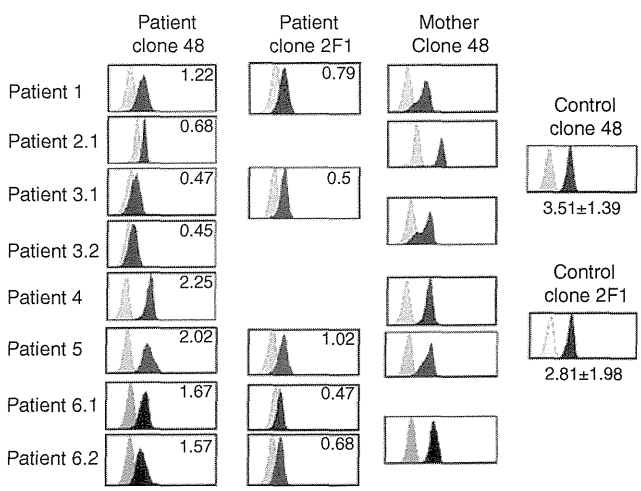

Fig. 2 XIAP protein expression in lymphocytes from the patients and their carriers. Flow cytometric detection of intracellular XIAP in lymphocytes from patients and their maternal carriers. The *gray* and *black areas* indicate the negative control and anti-XIAP staining, respectively. Anti-XIAP staining was performed using the clones 48 and 2 F1 antibodies where indicated. The *number in the box* indicates the log scale difference between the mean fluorescence intensity (ΔMFI) stained by the isotype antibody and that by the anti-XIAP antibodies. XIAP expression in 25 normal controls was also analyzed by the clone 48 and 2 F1 antibodies. The data of mean ± standard deviation of ΔMFI and each representative profile were shown

patients 1, 3.1, 5, 6.1, and 6.2 were examined by two different anti-XIAP mAbs. Using clone 48 antibody, patients 1, 2.1, 3.1, 3.2, 6.1, and 6.2 showed reduced XIAP expression, whereas XIAP was normally expressed in the lymphocytes of patients 4 and 5. In contrast to clone 48, clone 2F1 antibody showed reduced XIAP expression in patient 5. The effects of heterozygous *XIAP* mutations were studied in the lymphocytes of the patients' mothers by anti-XIAP mAb clone 48. The mothers of patients 1, 3.1, and 3.2 showed a bimodal pattern of XIAP protein (Fig. 2). The mothers of patients 2.1, 6.1, and 6.2 did not show a clear mosaic pattern, but all of these patients had reduced XIAP expression levels. Similarly to patients 4 and 5, the mothers of patients 4 and 5 demonstrated a normal XIAP expression pattern.

XIAP Expression in Lymphocytes from the Patients by Western Blot

Western blot analysis was used to evaluate the expression level of XIAP to determine the impact of patient *XIAP* mutations on protein expression and to compare this to the flow cytometric analysis. PBMCs from patients 3.1, 5.1, and 6.2 were available for Western blotting. All of these patients showed a reduction in XIAP protein expression (Fig. 3), fitting with the results obtained by flow cytometric analysis.

iNKT Cell Counts in the Patients

SAP-deficient patients had reduced numbers of NKT cells that expressed an invariantly rearranged T-cell receptor (TCR) consisting of TCRVα24 and TCRVβ11 chains [21,22]. The rare subset of iNKT cells was originally reported to be reduced in XIAP-deficient patients as well [7] but seemed to be present in normal numbers in a later study involving a larger patient cohort [23]. We analyzed the iNKT cell frequencies in 100,000 $CD3^+$ T cells in our XIAP-deficient patients and compared these with healthy controls (Fig. 4). The average frequency of iNKT cells within the $CD3^+$ T cell compartment of our XIAP patients was significantly reduced by twofold when compared with healthy

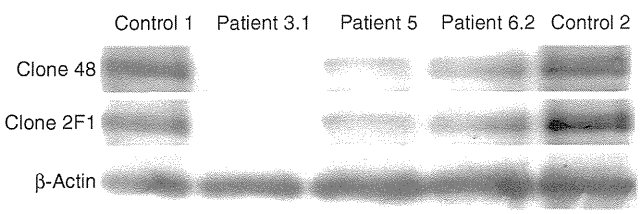

Fig. 3 XIAP expression in lymphocytes from the patients by Western blot. Analysis of XIAP expression in PBMC generated from patients with XIAP deficiency and normal controls using the antibody clone 48 (*upper panel*), the antibody clone 2 F1 (*middle panel*), and the β-actin antibody as an internal control (*lower panel*)

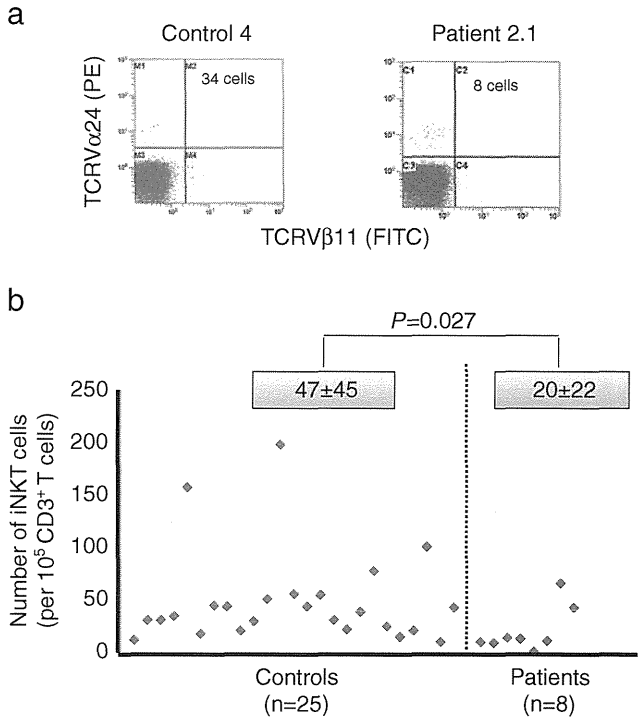

Fig. 4 iNKT cell counts in the patients and healthy controls. **a** Representative flow cytometric analysis of iNKT cells in $CD3^+$ lymphocytes from one XIAP-deficient patient and one healthy control. **b** Comparison of the number of iNKT cells in 100,000 $CD3^+$ lymphocytes between XIAP-deficient patients and control individuals. Statistical significance between patients and controls was determined with the Student's *t*-test (*p*-value=0.027)

controls (20 vs. 47 per 10^5 $CD3^+$ T cells). Therefore, we concluded that the number of iNKT cells was reduced in our patients with XIAP deficiency.

Functional Analysis of CTL Lines Established from the Patients

To test whether our XIAP-deficient patients have similar defects in $CD8^+$ T cell cytotoxicity as described in other subtypes of familial HLH [20,38], we generated $CD8^+$ alloantigen-specific CTL from patients 1, 2.1, 3.1, and three healthy controls (Fig. 5). The cytotoxic activity of the CTL of these patients was similar to that of the healthy controls, indicating that XIAP patients clearly differ from other familial HLH patients in this aspect of the disease.

Discussion

XIAP deficiency is a rare but severe and life-threatening inherited immune deficiency [12,13]. Early diagnosis and life-saving treatment such as hematopoietic stem cell transplantation is especially important. The causative gene for