Table 1. *(continued)*

PID category	Hypopara-thyroidism	Diabetes mellitus T1D 1A	1B	T2D	Thyroid disease Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Non-autoimmune hypothyroidism	GHD	Hypogonadism	Isolated ACTH deficiency	Others	n	The number of PID patients 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	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.

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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.

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CASE REPORT

Delayed onset adenosine deaminase deficiency associated with acute disseminated encephalomyelitis

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Abstract Acute disseminated encephalomyelitis (ADEM) is a monophasic, immune-mediated demyelinating disorder that can appear after either immunizations or, more often, infections. Magnetic resonance imaging of patients shows inflammatory lesions in the brain and spinal cord. An immune-mediated mechanism may play a role in this disease, although its precise pathogenesis remains unclear. In this study, a 2-year-old boy presented with ADEM, and he showed improvement on treatment with high-dose intravenous corticosteroids. At the age of 3 years, the presence of recurrent bronchitis, bronchiectasia, and lymphopenia suggested that the patient was suffering from combined immunodeficiency. The patient was finally diagnosed with delayed onset adenosine deaminase deficiency. Delayed onset adenosine deaminase deficiency is frequently associated with autoimmune diseases, including thyroiditis and cytopenia, both of which were observed in the patient. The ADEM in this patient may be a presentation of delayed onset adenosine deaminase deficiency.

Keywords Acute disseminated encephalomyelitis · Adenosine deaminase · Bronchiectasia · Delayed onset · Lymphopenia

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Introduction

Adenosine deaminase (ADA) deficiency is a systemic purine metabolic disorder that primarily affects lymphocyte development and function [1, 2]. ADA deficiency has accounted for approximately 15 % of severe combined immunodeficiency (SCID) cases and 30–40 % of autosomal recessive SCID cases [3]. The *ADA* gene is encoded by a 32 kb region that contains 12 exons and is located in chromosome 20q13.11, and *ADA* mutations include missense, splicing, deletion and nonsense mutations. ADA is an enzyme of the purine salvage pathway that catalyzes the deamination of adenosine and deoxyadenosine, giving rise to inosine and deoxyinosine, respectively. The absence of ADA results in an accumulation of the substrate adenosine and deoxyadenosine. The latter is phosphorylated by nucleoside kinases, which results in the production of deoxynucleotide triphosphates (dATP). ADA deficiency may promote pro-apoptotic effects due to the accumulation of dATP, which may be responsible for the observed lymphopenia due to ADA deficiency. Thus, the clinical presentation of ADA-deficient patients is similar to that of SCID patients.

A majority of ADA-deficient patients have neonatal-onset disease and present with lymphopenia, an absence of cellular and humoral immunity, failure to thrive and a rapid disease course due to infections. However, approximately 15 % of ADA-deficient patients are diagnosed between 3 and 15 years of age or in adulthood, and their disorder has been referred to as "delayed onset type" and "late onset type" ADA deficiency. Patients with delayed onset ADA deficiency show variable clinical manifestations including recurrent sinopulmonary bacterial infections and septicemia. Laboratory data may show IgG2 deficiency, but a markedly elevated IgE titer and eosinophilia. Autoimmune diseases, including autoimmune hypothyroiditis, diabetes mellitus, hemolytic anemia and idiopathic thrombocytopenia, may be observed in conjunction with ADA deficiency. Here, we describe a Japanese child with delayed onset ADA deficiency.

Before the time of diagnosis, the patient had acute disseminated encephalomyelitis (ADEM), from which he recovered with minor residual disability. ADEM shows multiple inflammatory lesions in the brain and spinal cord, particularly in the white matter, suggesting that it may involve autoimmune demyelination [4]. Therefore, ADEM may be an early sign of autoimmune disease resulting from the onset of delayed onset ADA deficiency as autoimmune disease.

Case report

The patient had experienced recurrent bronchitis and mild leukopenia since 5 months of age, but the patient had no severe infections or adverse effects. At 2 years of age, he presented with a fever, gait disturbances and lethargy, and he was admitted to the hospital. Upon admission, he also had motor weakness and urinary retention. Physical examination revealed drowsiness and bilateral normal deep tendon reflexes, but no neck stiffness was observed. Laboratory investigations, which included a measure of anti-nuclear antibody titers, were normal. Cerebrospinal fluid (CSF) analysis revealed 51 white blood cells per microliter with 86 % lymphocytes and 14 % polymorphonuclear cells, and the total protein content was increased to 102 mg/dl. CSF culture was negative and the glucose level was normal. Myelin basic protein in CSF was increased to 5347 pg/ml (normal <102 pg/ml), suggesting the occurrence of demyelination. Magnetic resonance imaging (MRI) showed patch lesions of high signal intensity on the T2-weighted images and low signal on the T1-weighted images in the subcortical and central white matter regions, as well as the basal ganglia and spine (Fig. 1). These findings suggested demyelination and edema. Multiple asymmetric lesions were present.
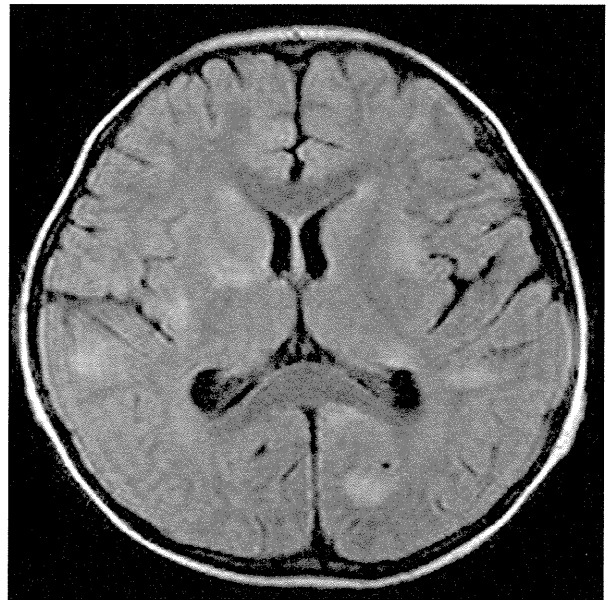

Fig. 1 Axial T2-weighted MRI of the patient's brain. Several high intensity signals are observed in the brain, as indicated

The clinical and MRI data led us to the diagnosis of ADEM, and the patient was immediately treated with high-dose intravenous methylprednisolone. The patient's level of consciousness and his neurological signs gradually improved over the next month. He retained only a residual hyperreflexia of the patella and Achilles tendons. Four months later, the brain MRI results were almost normal.

Frequent infectious episodes were again observed in the patient after he turned 3 years old, and he was finally admitted for investigation. A physical examination revealed that the boy's height was 100 cm (+0.5 SD), and his weight was 16.2 kg (+0.7 SD). Other clinical statistics were as follows: his temperature was 37.4 °C, heart rate was 135/min, respiratory rate was 25/min, blood pressure was 102/62 mmHg and SpO_2 was 95 % at room temperature. Occasional rales were heard over both lungs. Neither organomegaly nor enlarged lymph nodes were observed.

Laboratory tests revealed altered contributions of blood cells, but largely normal blood chemistry. The specific blood values recoded are as follows: hemoglobin, 10.2 g/dl; white blood cells, 2220/μl with 600/μl neutrophils and 580/μl lymphocytes; platelets, 276000/μl; and C-reactive protein, 4.43 mg/dl (normal range <0.29 mg/dl). Blood chemistry, which included liver enzymes and electrolytes, was normal. Chest X-rays appeared normal, although chest computed tomography revealed bronchiectasia. KL-6 was elevated to 3674 U/ml (normal <499 U/ml). Thyroid-stimulating hormone was elevated to 133 μIU/ml (normal 0.35–3.73 μIU/ml). However, free T3 and free T4 were roughly normal at 2.3 pg/ml (normal 2.2–4.1 pg/ml) and

0.6 ng/dl (normal 0.9–1.8 ng/dl), respectively. These data suggest that the patient had hypothyroidism. The presence of anti-thyroglobulin and anti-thyroid peroxidase antibodies indicated autoimmune thyroiditis, and the patient was treated with levothyroxine.

Unexpectedly, immunological studies showed hypergammaglobulinemia in the patient (Table 1). IgG2 levels were within the normal range, but a percentage of an IgG2 subclass (5.41 %) was lower than that in the normal controls (20–30 %). In addition, the patient tested positive for varicella zoster-specific IgG. Although the patient had recurrent pneumococcal infections, the level of pneumococcus-specific IgG2 was only 0.6 μg/ml (normal >3.0 μg/ml). Lymphocyte subpopulations revealed an extremely high frequency of activated (HLA-DR$^+$) CD3$^+$ T cells and memory (CD45RO$^+$) CD4$^+$ and CD8$^+$ T cells, and an extremely reduced number of CD20$^+$ B cells. An analysis

Table 1 Immunological studies in the patient

Test	Value	Unit	Normal value
Immunoglobulins			
IgG	1659	mg/dl	929 ± 228
IgA	51	mg/dl	56 ± 18
IgM	188	mg/dl	93 ± 27
IgE	62	IU/ml	0–170
IgG subclasses			
IgG1	1220	mg/dl	390.2–955.2
IgG2	72.9	mg/dl	58.5–292.1
IgG3	52.4	mg/dl	11.4–98.8
IgG4	3.0	mg/dl	1.2–76.7
Lymphocyte subpopulations			
CD3	70.3	%	71.4 ± 5.8
CD4	23.4	%	43.2 ± 11.5
CD8	44.2	%	22.3 ± 6.6
HLA-DR/CD3	76.5	%	<1.0
CD45RO/CD4	74.8	%	21.9 ± 4.4
CD45RO/CD8	39.6	%	14.9 ± 5.6
CD20	0.2	%	12.5 ± 6.7
Lymphoproliferative response to mitogen			
Phytohemagglutinin	7438	cpm	20500–56800
Natural killer cell activity	6	%	18–40
TRECs quantification			
At birth	1.011 × 10³	copies/μg DNA	6.2 ± 3.2 × 10³
Present	Undetectable		
Autoantibodies			
Anti-thyroglobulin	538	IU/ml	<27.9
Anti-thyroid peroxidase	185	U/ml	<0.29
Anti-nuclear	Positive		Negative
Anti-neutrophil	Positive		Negative

of the T-cell receptor Vβ repertoire revealed a strongly skewed pattern in CD8$^+$ T cells but not in the repertoire of CD4$^+$ T cells (data not shown). Lymphocyte proliferation was impaired in response to phytohemagglutinin, and natural killer cell activity was low. T-cell receptor excision circles (TRECs) were quantified by real time-PCR, as previously described [5]. When measured with the patient's neonatal Guthrie card, the copy number of TRECs was lower than normal, but they were well detectable. However, TRECs were undetectable in this patient at the age of 3 years. The delayed-type hypersensitivity skin test, which uses purified protein from tuberculosis, was negative despite the fact that the patient had been immunized with the bacille Calmette–Guérin vaccine. Furthermore, the patient was positive for various autoantibodies, including anti-thyroglobulin and anti-nuclear antibodies.

Although the patient showed hypergammaglobulinemia, the presence of humoral and cellular immune defects in addition to various autoimmune features suggested a diagnosis of delayed onset ADA deficiency. Therefore, ADA enzyme activity was assayed by the radiochemical thin-layer chromatography method, as previously described [6, 7]. The levels of adenosine nucleotide (AXP) and deoxyadenosine nucleotides (dAXP) in erythrocytes were determined, as previously described [8]. The patient's ADA activity in mononuclear cells was detectable at 8.6 nmol/min/10⁸ cells, but this value is approximately one-tenth of activity found in normal controls (102.6 nmol/min/10⁸ cells) (Fig. 2). Consistent with this observation, the patient's ADA level in red blood cells (RBC) was 0 nmol/h/mg (normal 26.4 ± 10.0 nmol/h/mg), and the toxic metabolite dAXP levels in RBC were increased to 9.4 % (normal <1 %). These data indicated that the ADA activity observed in the patient might be mild. The parents' ADA levels in RBC were intermediate between that of the patient's level and that of a normal control (mother
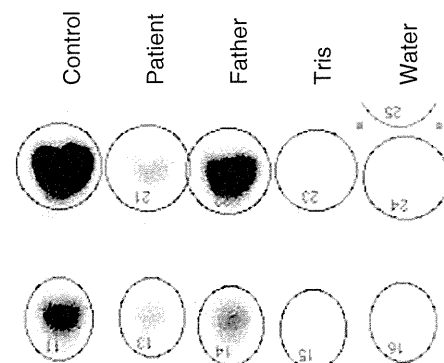

Fig. 2 ADA enzymatic activity. Each lane corresponds to a different sample as follow: control individual (102.6 U), the patient (8.6 U), the patient's father (39.6 U), Tris (1.0 U), and water (1.0 U). U denotes nmol/min/10⁸ cells

12.2 nmol/h/mg, father 14.1 nmol/h/mg), which suggests that they are carriers of the ADA deficiency. Gene analysis of *ADA* revealed compound heterozygous mutations in the patient (R156C and V177M), each contributed by one parent: the mother had contributed the R156C mutation and the father passed on a V177M mutation, respectively, thereby confirming the parents' carrier status.

After the diagnosis of delayed onset ADA deficiency, the patient was treated with intravenous immunoglobulin, and he received oral administration of trimethoprim–sulfamethoxazole, and acyclovir. Following the prophylactic treatment, the patient was nearly free from infections. However, serum immunoglobulin levels were decreased (IgG 1069 mg/dl, IgA 21 mg/dl, and IgM 33 mg/dl) at the age of 4 years. Therefore, we searched for a human leukocyte antigen-identical donor and identified his healthy sister as a suitable donor. At the age of 4 years, he underwent a bone marrow transplant preceded by a reduced-intensity conditioning regimen. This regimen included reduced dose intravenous busulfan (8.8 mg/kg total) and fludarabine (total dose: 180 mg/m^2) with standard cyclosporine A prophylaxis. Total nucleated cell and CD34$^+$ cell counts were 6.9×10^8 and 3.1×10^6 cells/kg, respectively. Thus, the patient's condition was good and he exhibited immune reconstitution with nearly complete chimerism.

Discussion

The recurrent infectious episodes in the patient presented herein suggested that he harbored a primary immunodeficiency, and bronchiectasis demonstrated by computed tomography strongly suggested that it was specially a humoral immunodeficiency. Although he had hypergammaglobulinemia, the relatively low frequency of IgG2 subclasses, low levels of pneumococcus-specific IgG2 and the decreased number of B cells demonstrated that the patient had humoral immune defects. Nonetheless, the absolute number of T cells was decreased, and naïve T cells were profoundly diminished in the patient. Impaired lymphocyte proliferation in response to mitogen and the lack of TRECs also indicated that a cellular immune deficiency was present. Hyperproduction of immunoglobulins by scanty B cells suggests that the patient's B cells may be oligoclonal. Furthermore, it remains to be determined whether a specific autoantibody target could be associated with the development of ADEM in this patient, particularly because he also presented with autoimmune disease, such as autoimmune thyroiditis. The combined presence of delayed onset combined immune deficiency and autoimmunity suggested a diagnosis of either delayed onset ADA deficiency or RAG deficiency. Our patient was finally diagnosed with delayed onset ADA deficiency.

The various phenotypes observed in ADA deficiency exhibit a strong correlation with their respective genotypes. For instance, alleles are grouped according to the resulting levels of ADA activity: deletion and nonsense alleles formed Group 0, which assumes no activity, whereas the amino acid substitutions are placed in Groups I–IV with increasing ADA activity [9]. The levels of soluble ADA activity and immunoreactive ADA protein expressed by mutant *ADA* cDNA were measured. ADA proteins bearing the patient's R156C or V177M mutations were included in Groups I and II, respectively. Patients with Group 0 or I alleles might show SCID, whereas patients with Group II might present with delayed onset phenotypes. The phenotype resulting from a combination of R156C and V177M mutations is compatible with that of delayed onset ADA deficiency.

ADEM is defined as a first episode of inflammatory demyelination with polyfocal neurological deficits (altered behavior or consciousness) [4]. MRI features of diffuse, bilateral lesions support ADEM. While the pathophysiology of ADEM remains undefined, it is believed to include autoimmune responses mounted de novo or following an infection. There is no report of an association between ADEM and ADA deficiency, although ADEM is rarely associated with immune deficient individuals. For instance, in one report, a child with common variable immune deficiency was associated with ADEM and Lennox–Gastaut syndrome [10], and ADEM has been observed in several patients with primary HIV infections [11–14]. Abnormal T cells and/or B cells that may be present under conditions of immune deficiency may promote an autoimmune process that results in ADEM. In support of this notion, patients with delayed onset ADA deficiency are frequently associated with autoimmune diseases, including autoimmune thyroiditis. The combination of an oligoclonal T-cell repertoire and a specific autoantibody produced by B cells may contribute to the development of ADEM in the patient. This study suggested that the patient presented ADEM as an autoimmune disease associated with delayed onset ADA deficiency.

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Successful bone marrow transplantation with reduced intensity conditioning in a patient with delayed-onset adenosine deaminase deficiency

Kanegane H, Taneichi H, Nomura K, Wada T, Yachie A, Imai K, Ariga T, Santisteban I, Hershfield MS, Miyawaki T. Successful bone marrow transplantation with reduced intensity conditioning in a patient with delayed-onset adenosine deaminase deficiency.

Abstract: In this case report, we describe successful BMT with RIC in a patient with delayed-onset ADA deficiency. A three-yr-old Japanese boy was diagnosed with delayed-onset ADA deficiency because of recurrent bronchitis, bronchiectasia, and lymphopenia. In addition, autoimmune thyroiditis and neutropenia were present. At four yr of age, he underwent BMT with a RIC regimen, including busulfan and fludarabine, from an HLA-identical healthy sister. Engraftment after BMT was uneventful without GVHD. Decreased ADA levels in blood immediately increased following BMT, and the patient was disease-free 13 months after BMT. These results suggest that BMT with RIC may sufficiently restore immune regulation in delayed-onset ADA deficiency. A longer follow-up period is needed to confirm these observations.

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ADA deficiency is a disorder of purine metabolism, which results in abnormalities in immune system development and function (1, 2). A majority of ADA deficiency cases indicate SCID

Abbreviation: ADA, adenosine deaminase; BMT, bone marrow transplantation; dAXP, deoxyadenosine nucleotides; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; PEG, polyethylene-glycosylated; PEG-ADA, polyethylene-glycosylated bovine ADA; RIC, reduced intensity conditioning; SCID, severe combined immunodeficiency; sjKRECs, signal joint κ-deleting recombination excision circles; TCR, T-cell receptor; TRECs, T-cell receptor excision circles.

during infancy; however, approximately 15% of ADA-deficient patients present with symptoms after infancy, which is referred to as a delayed- or late-onset type. Patients with delayed-onset ADA deficiency exhibit variable clinical symptoms, including bacterial infections and autoimmune manifestations. Allogeneic hematopoietic stem cell transplantation has long been a gold standard for the treatment of ADA-SCID; however, two other second-line options are available for ADA-SCID: Enzyme replacement therapy with PEG-ADA and hematopoietic stem cell gene therapy (3). The treatment of choice for delayed-onset ADA deficiency remains unclear because of

the clinical variety. We report on a four-yr-old Japanese boy with delayed-onset ADA deficiency who underwent BMT with RIC from a HLA-identical healthy sister.

Case report

The patient was previously described (4). He is a boy who was admitted to our hospital at three yr of age for the investigation of recurrent infectious episodes. The patient did not have a neurological deficit. Laboratory data revealed neutropenia $(600/\mu L)$, lymphocytopenia $(580/\mu L)$, elevated C-reactive protein (7.43 mg/dL; normal, <0.29 mg/dL) and elevated thyroid-stimulating hormone (133 $\mu IU/mL$; normal, 0.35–3.73 $\mu IU/mL$). Anti-neutrophil, anti-nuclear, anti-thyroglobulin, and anti-thyroid peroxidase antibodies were positive, indicating that autoimmune neutropenia and thyroiditis were present. Chest computed tomography disclosed bronchiectasia. An immunological study indicated hypergammaglobulinemia, but a low percentage of IgG2 subclass antibodies (5.41%; normal, 20–30%) was obtained. The lymphocyte subsets revealed an expansion of the CD45RO$^+$ (memory) populations of CD4$^+$ and CD8$^+$ T cells (74.8% and 39.6%, respectively) and an extremely reduced number of CD20$^+$ B cells (0.2%). TRECs and signal joint κ-deleting recombination excision circles (sjKRECs) were quantified by real-time PCR as previously described (5, 6) and were undetectable. Flow cytometry analysis of the TCR Vβ repertoire was performed as described previously (7), and the analysis revealed an extremely skewed pattern in CD8$^+$ T cells but not in CD4$^+$ T cells (Fig. 1). Therefore, the patient was clinically presumed to have a combined immunodeficiency with autoimmune manifestations, possibly indicating delayed-onset ADA deficiency. The ADA and dAXP levels in whole blood were measured using the extracts of dried blood spots (8). ADA was found to be decreased (1.0 $\mu mol/h/mg$ protein; normal, 26.4 ± 10.0 $\mu mol/h/mg$ protein), and %dAXP increased to 10.8% (normal < 1%). These data led to a diagnosis of ADA deficiency. An analysis of the *ADA* gene disclosed that the patient had compound heterozygous mutations (R156C and V177M). This genotype is compatible with delayed-onset ADA deficiency (9, 10).

The patient was treated with intravenous immunoglobulin replacement therapy, and oral administration of trimethoprim–sulfamethoxazole, acyclovir, and levothyroxine. He was nearly free from infections; however, his serum immunoglobulin levels gradually decreased. We iden-
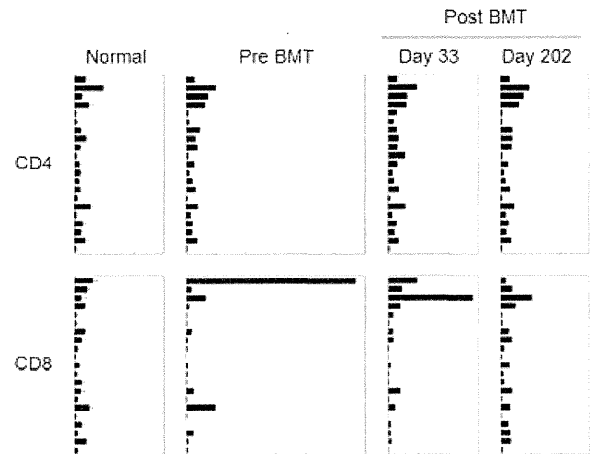

Fig. 1. TCR Vβ repertoire in the CD4$^+$ and CD8$^+$ T cells that were analyzed pre-BMT and post-BMT on days 23 and 202. The TCR Vβ repertoire was analyzed by flow cytometry as previously described (7).

tified his healthy sister as an HLA-identical donor with no mutation in the *ADA* gene. At the age of four yr, the patient underwent BMT. He was treated with conditioning, which consisted of fludarabine (30 mg/m^2/day × six days, days −7 to −2) and intravenous reduced dose busulfan (4.4 mg/kg/day × two days, days −3 to −2). The patient received 6.9 × 10^8 nucleated cells/kg containing 3.1 × 10^6 CD34$^+$ cells/kg to achieve rapid engraftment. Cyclosporin A was used as GVHD prophylaxis. The post-transplant clinical course was without major complications, and no signs of acute GVHD were observed. The patient did not receive blood transfusion, and engraftments of neutrophils ($>500/\mu L$) and thrombocytes ($>50\ 000/\mu L$) were achieved at days 18 and 35, respectively. Cyclosporin A was stopped at day 46. The patient is currently well and has not suffered from any major infectious episodes. The patient received levothyroxine at a low dose; however, anti-thyroglobulin and anti-thyroid peroxidase antibodies became negative. The patient went off immunoglobulin replacement therapy 11 months after BMT.

Donor engraftment was evaluated by PCR amplification of the microsatellite marker D8S1179. Donor engraftment in granulocytes and B cells was observed at days 33 and 83, respectively. Complete donor engraftment in whole cells was achieved at day 323 (Table 1). Consistent with high chimerism, the patient exhibited a rapid increase in ADA activity and fast metabolic detoxification by day 83 (Table 2). In addition, immunological studies indicated rapid reconstitution of the lymphocyte subpopulation, and B cells increased to a normal level $(305/\mu L$;

Table 1. Engraftment of donor cells in different cell lineages

| Post-BMT | Donor cell engraftment (%) | | | | |
	Whole blood	Lymphocytes	T cells	B cells	Granulocytes
Day 12	8.5	NA	14.2	NA	0
Day 33	50.7	33.0	NA	NA	100.0
Day 83	80.5	NA	16.5	100.0	100.0
Day 323	>95.0	NA	>95.0	100.0	100.0

NA, not applicable.
Analyses of donor cell engraftment according to a chimerism assay in the peripheral blood of the patient at different time points after BMT.

Table 2. ADA activity in the whole blood of the patient

Samples	ADA (μmol/h/mg protein)	%dAXP
Pre-BMT	1.0	10.8
Day 25	8.7	1.1
Day 83	33.7	0.0
ADA-SCID	0.38 ± 0.5	50.3 ± 18.0
Normal levels	26.4 ± 10.0	<1

The data are from the analyses of the extracts of dried blood spots.

Table 3. TREC and sjKREC levels as measured by quantitative PCR in the peripheral blood at different time points

	Post-BMT Day 25	Day 83	Day 202	Normal values
TRECs	0	8.1×10^3	1.9×10^3	$3.5 \pm 2.8 \times 10^3$
sjKRECs	0	2.8×10^2	6.4×10^3	$4.8 \pm 0.6 \times 10^3$
RNaseP	4.5×10^4	1.6×10^5	2.0×10^5	

All of the units are copies/μg DNA. The RNaseP gene was amplified as an internal control. The normal values indicate the copy numbers of the age-matched controls (2-6 yr of age).

age-matched control, 278–922/μL) at day 97 (Fig. 2). TREC and sjKREC levels reached normal levels at days 83 and 202, respectively (Table 3). The CD45RO$^+$ (memory) populations of CD4$^+$ T cells decreased to a normal range (21.9 ± 4.4%) soon after BMT. Sequential TCR Vβ repertoire analyses revealed that the polyclonal patterns in CD4$^+$ T cells were consistent after BMT, and the extremely skewed pattern in CD8$^+$ T cells had improved by day 202 (Fig. 1).

Discussion

ADA-SCID is a complex immune and metabolic disorder that results from a lack of ADA, which is a key enzyme in purine metabolism. Patients with ADA-SCID have recurrent and severe infections, growth retardation and organ failure. The first treatment of choice is BMT from an HLA-identical sibling donor, if available, fol-
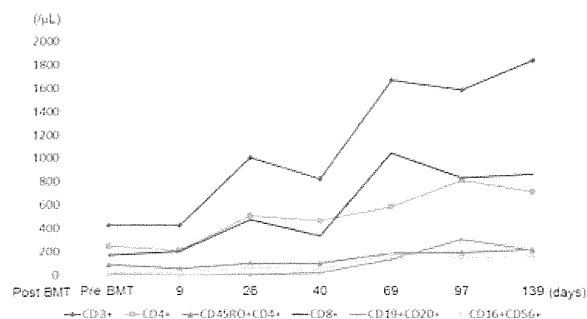

Fig. 2. Kinetics of the lymphocyte subpopulations in the patient.

lowed by treatments for other forms of SCID. Second-line treatments for patients without an HLA-identical donor include enzyme replacement therapy with PEG-ADA, matched unrelated donor hematopoietic stem cell transplantation and hematopoietic stem cell gene therapy (3). Although the treatment strategy for ADA-SCID is well-established, treatment for delayed-onset ADA deficiency is not standardized because of the various clinical conditions. In this study, an HLA-identical healthy sibling donor was available, and we selected BMT from this donor to treat the patient. In cases of ADA-SCID, BMT from an HLA-identical donor is undertaken without a preparative conditioning regimen. The largest series of SCID patients from the European SCE-TIDE database included 475 patients (11). Of these patients, 51 patients with ADA-SCID had a three-yr survival rate of 81% for HLA-matched transplantation, but 29% for HLA-mismatched transplantation. A recently published cohort study demonstrated that hematopoietic stem cell transplantation in patients with SCID, including ADA deficiency, resulted in engraftment and long-term survival for the majority of patients with or without conditioning (12). However, transplantation without conditioning may result in partial donor engraftment, causing reduced immune reconstitution. Alternatively, hematopoietic stem cell gene therapy is effective for ADA-SCID patients who lack an HLA-identical sibling donor (13). Autologous CD34$^+$ bone marrow cells were transduced with a retroviral vector containing the ADA gene and infused into 10 patients with ADA-SCID after non-myeloablative conditioning. However, two patients have required enzyme replacement after gene therapy (14). ADA gene therapy has been performed in total 31 patients in Italy, the United Kingdom, and the United States. Twenty-one patients have been successful, whereas 10 patients have received enzyme replacement therapy (15). Recently, Cancrini et al. (16) described two ADA-SCID patients from the same family who both underwent BMT. One patient underwent BMT without conditioning, whereas

the other patient was administered a RIC regimen (busulfan and fludarabine) following the failure of cord blood transplantation. Engraftment and immune reconstitution were compared in these patients. The patient who received conditioning exhibited stable mixed chimerism in all of the cell lineages, whereas the patient who underwent BMT without conditioning exhibited slow immune reconstitution, especially in B and myeloid cells. This observation indicated that the use of conditioning resulted in faster immunologic and metabolic reconstitution. In these patients, the immune reconstitution of B and myeloid cells was slower than that of T and NK cells. Interestingly, the reconstitution of myeloid and B cells appeared earlier than that of T cells in our patient. The patient with delayed-onset ADA deficiency had a substantial number of T cells but no B cells before BMT, and the generation of new T cells may take longer than B cells.

Patients with delayed-onset ADA deficiency often have chronic pulmonary insufficiency and autoimmune phenomena, including cytopenia and anti-thyroid antibodies, as observed in our patient. Patients with a delayed- or late-onset type may survive undiagnosed in the first decade of life or beyond; however, the longer the disease goes undiagnosed, the more the immune function deteriorates. The serum immunoglobulin levels of our patient gradually decreased from the point of diagnosis. Our patient had a substantial number of T cells; however, TRECs were undetectable in his peripheral blood. Therefore, we decided that the patient would receive BMT preceded by a RIC regimen, including busulfan and fludarabine. The use of RIC in BMT from an HLA-identical donor in this patient resulted in rapid and complete immune and metabolic reconstitution, and there was no treatment-related toxicity. However, a longer follow-up period is required to confirm these observations. If patients have an HLA-identical sibling donor, BMT with a RIC regimen may be the treatment of choice in patients with delayed-onset ADA deficiency.

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ORIGINAL ARTICLE

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

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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

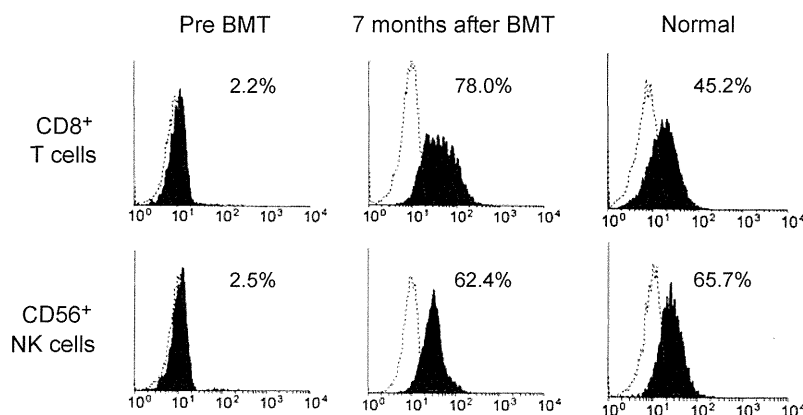Pre BMT   7 months after BMT   Normal

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

Phenotype	Present study (33 cases)		Seemayer (272 cases) (2)		Booth (91 cases) (17)	
	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.

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CASE REPORT

Two cases of partial dominant interferon-γ receptor 1 deficiency that presented with different clinical courses of bacille Calmette–Guérin multiple osteomyelitis

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Abstract We experienced two cases of unrelated Japanese children with bacille Calmette–Guérin (BCG) multiple osteomyelitis with partial interferon (IFN)-γ receptor 1 (IFNGR1) deficiency. Heterozygous small deletions with frame shift (811 del4 and 818 del4) were detected, which were consistent with the diagnosis of partial dominant IFNGR1 deficiency. Case 1: a 2-year-old boy visited us because of limb and neck pain. He had been vaccinated with BCG at 17 months of age. Multiple destructive lesions were observed in the skull, ribs, femur, and vertebral bones. *Mycobacterium bovis* (BCG Tokyo 172 strain by RFLP technique) was detected in the bone specimen. The BCG multiple osteomyelitis was treated successfully without recurrence. Case 2: an 18-month-old girl developed multiple osteomyelitis 9 months after BCG inoculation. Radiologic images showed multiple osteolytic lesions in the skull, ribs, femur, and vertebrae. *M. bovis* (BCG Tokyo 172 strain) was detected in the cultures from a bone biopsy. Her clinical course showed recurrent osteomyelitis and lymphadenitis with no pulmonary involvement. The effects of high-dose antimycobacterial drugs and IFN-γ administration were transient, and complete remission has since been achieved by combination antimycobacterial therapy, including levofloxacin. Partial dominant IFNGR1 deficiency is a rare disorder, but it should be considered when a patient presents with multiple osteomyelitis after BCG vaccination. The cases that are resistant to conventional regimens require additional second-line antituberculous drugs, such as levofloxacin.

Keywords Interferon-γ receptor 1 deficiency · Multiple osteomyelitis · Bacille Calmette–Guérin · Mycobacterial infection · Levofloxacin

Introduction

Interleukin-12 (IL-12)- and IFN-γ (IFNG)-mediated immunity plays an important role in host defense against intracellular pathogens [1]. Mendelian susceptibility to mycobacterial disease (MSMD) is a rare disorder and sometimes lethal disease that occurs in response to poorly virulent mycobacteria, such as bacille Calmette–Guérin (BCG) and environmental nontuberculous mycobacteria (NTM). In patients with MSMD, different types of mutations in six genes—IFNGR1, IFNGR2, IL12RB1, IL12B, STAT-1, and NEMO—have been revealed [2].

Sasaki et al. [3] previously reported a partial IFNGR1 mutation in three Japanese children with BCG osteomyelitis and in the father of one of the patients. We have followed the two unrelated cases over 10 years since their onset in the same department (Koshigaya Municipal Hospital). Based on our longitudinal experience, we intend to provide important clinical information for the diagnosis and treatment of IFN-γR1 deficiency in Japan.

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