

vaccination 3 months after birth. This indicates normal functioning of tissue macrophages, because protective immunity to mycobacteria is dependent upon the interleukin (IL)-12/IL-23-interferon (IFN)- γ axis, possibly mediated by intracellular killing of phagocytes following the production of IFN- γ by CD4 T lymphocytes in response to IL-12/IL-23 secreted by infected macrophages [3]. Patients with MonoMAC/DCML deficiency show very low numbers of circulating monocytes and no detectable myeloid or plasmacytoid dendritic cells in the peripheral blood, but relatively normal numbers of Langerhans cells and tissue macrophages accompanied by prominent hemophagocytosis in the bone marrow [1, 2]. This supports the idea that tissue and marrow macrophages, in addition to Langerhans cells, may be maintained by a distinct precursor from circulating monocytes or dendritic cells [1].

Mansour et al. [8] reported that the age of onset of MDS/AML in Emberger syndrome is 9–14 (median 11) years of age. This appears to be earlier than that of MDS/AML in MonoMAC syndrome (7–52 years, median 32 years) [2]. Moreover, the level of WT1-mRNA in the peripheral blood increases significantly as MDS progresses and is a strong predictor of rapid AML transformation in adult patients with de novo MDS [11]. The level of WT1-mRNA in the peripheral blood of the current patient was as high as that in patients that show worse survival than those with a low level WT1 mRNA (10^2 – 10^4 vs. $<10^2$ copies/ μ g) [12]. However, it is unclear whether phenotypic variation and increased WT1 mRNA level are related to hematological disease progression. In any case, neutropenic patients who suffer recurrent infections and/or MDS are likely to need a transplant in the near future. Therefore, for such cases, we perform hematopoietic stem cell transplantation with a reduced intensity conditioning regimen before the disease has progressed [4]. Table 1 summarizes the clinical features of MonoMAC, Emberger syndrome, and the present case.

Our observations suggest that children with recurrent or prolonged common infections that respond to antibiotics and recover well may suffer from unknown primary immunodeficiencies. Although the relationship between *GATA-2* and lymphedema or deafness requires further investigation, tissue-specific lesions such as lymphedema provide important clues to primary immunodeficiencies that also affect non-hematopoietic cells.

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ORIGINAL ARTICLE

Endocrine complications in primary immunodeficiency diseases in Japan

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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.

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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

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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, pseudo-hypoadosteronism, 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	Hypoparathyroidism	Diabetes mellitus			Thyroid disease			Hypogonadism	Isolated ACTH deficiency	Others	The number of PID patients			
		T1D			Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Non-autoimmune hypothyroidism	GHD				n	0-19 years	Total	Percent in total
		1A	1B	T2D										
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														
Common variable immunodeficiency disorders										2*	3	93	138	2.2
IgG subclass deficiency											6	29	93	6.5
Undetermined														
III. Other well-defined immunodeficiency syndromes											20	126	165	12.1
Hyper-IgE syndrome														
DiGeorge syndrome	14						1	1		1†	3	31	46	6.5
Ataxia telangiectasia				1							14	29	32	43.8
Chronic mucocutaneous candidiasis						1††					1	8	13	7.7
ICF syndrome								1			1	9	13	7.7
IV. Diseases of immune dysregulation											6	31	38	15.8
IPEX syndrome						1								
APECED	1										1§	4	5	6
Undetermined											1	3	4	25.0
V. Congenital defects of phagocyte number, function or both											3	106	153	2.0
Chronic granulomatous disease							1	1			1	2	2	50.0
											2	54	87	2.3

Table 1. (continued)

PID category	Hypoparathyroidism	Diabetes mellitus			Thyroid disease			GHD	Hypogonadism	Isolated ACTH deficiency	Others	The number of PID patients		
		T1D	T1D	T2D	Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Non-autoimmune hypothyroidism	Others					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†	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.

‡Pseudohypoadosteronism 1.

§Adrenal crisis, Hypoglycaemia 1.

¶Hypophosphatemic rickets 1.

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

††the case whose onset age of an 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	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 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 previously³⁴, 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 reported³⁵, 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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Multiple Reversions of an IL2RG Mutation Restore T cell Function in an X-linked Severe Combined Immunodeficiency Patient

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Abstract Reversion mosaicism is increasingly being reported in primary immunodeficiency diseases, but there have been few cases with clinically improved immune function. Here, a case is reported of X-linked severe combined immunodeficiency (SCID-X1) with multiple somatic rever-

sions in T cells, which restored sufficient cell-mediated immunity to overcome viral infection. Lineage-specific analysis revealed multiple reversions in T cell receptor (TCR) $\alpha\beta^+$ and TCR $\gamma\delta^+$ T cells. Diversity of the TCRV β repertoire was comparable to normal and, furthermore, mitogen-induced

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proliferation of the patient's T cells was minimally impaired compared to healthy controls. *In vivo* and *in vitro* varicella antigen-specific T cell responses were comparable to those of healthy controls, although a reduced level of T cell receptor excision circles suggested that recent thymic output was low. During long-term evaluation of the patient's immunologic status, both the number of CD4+ and CD8+ T cells and T cell proliferation responses were stable and the patient remained healthy. This case demonstrates that multiple but restricted somatic reversions in T cell progenitors can improve the clinical phenotype of SCID-X1.

Keywords Severe combined immunodeficiency · reversion · multiple

Introduction

X-linked severe combined immunodeficiency (SCID-X1) is a recessive hereditary disease characterized by a lack of T cells and natural killer (NK) cells. Without stem cell transplantation, persistent infections with opportunistic organisms uniformly lead to death in the first 2 years of life, except in those with atypically attenuated phenotypes [1–3]. Recently, spontaneous genetic reversion has been reported in primary immunodeficiency disorders. Somatic reversion mosaicism is considered to be 'natural gene therapy'; however, few cases are reported with reversions that restore functional immunity [4–9]. Here, an atypical case of SCID-X1 with somatic mosaicism due to multiple reversions in T cells, which restored sufficient T cell immunity, is described.

Materials and Methods

Patient

A male infant was born prematurely at 34 weeks and 4 days of gestation with a birth weight of 1,660 g to healthy

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parents. There was no family history of consanguinity or immunodeficiency. He was well until 14 months of age, when he started to have recurrent bacterial respiratory tract infections. At the age of 21 months, laboratory tests were performed. Patient results were compared to age-matched normal controls (controls). Examination of serum Ig revealed a decreased level of IgG (IgG, 1.93 g/L [range of controls: 7.15–9.07 g/L]), and normal levels of IgA (IgA, 0.33 g/L [range of controls: 0.22–1.44 g/L]) and IgM (IgM, 0.72 g/L [range of controls: 0.34–1.28 g/L]). His serum IgG was constantly under 2.0 g/L. In addition, he had a reduced number of CD4+ cells (358/ μ l, [mean of controls: 1,683 \pm 874]) and CD56+ cells (39/ μ l [mean of controls: 306 \pm 207]), while CD3+ cells (1,803/ μ l [mean of controls: 2,997 \pm 1,751]), CD8+ cells (1,067/ μ l [mean of controls: 1,683 \pm 874]) and CD19+ cells (1,850/ μ l [mean of controls: 1,114 \pm 976]) were within the normal limits. The patient's T cell proliferative response to phytohemagglutinin (PHA) (stimulation index (S.I.) of 172 [range of controls: 105–225]) and to concanavalin-A (Con-A) (S.I. of 140 [range of controls: 68–154]) was within the normal ranges for his age. From these data, he was diagnosed with common variable immunodeficiency (CVID) at that time. Intravenous immunoglobulin therapy was started and he remained in good health thereafter. Without receiving vaccination, varicella infection at 5 years of age did not cause fever, and he was successfully treated with oral acyclovir at an outpatient clinic. At 9 years of age, warts developed and spread over his body, and he was referred to our hospital for assessment of his immunological status. Physical examination revealed neither detectable lymph nodes nor tonsils, and his thymus appeared hypoplastic on CT scan. Before the laboratory studies were performed, informed consent was obtained from the patient and his parents, in accordance with the institutional review board of Kyoto University Hospital and the Declaration of Helsinki.

Flow Cytometry

Flow cytometric analysis was performed according to standard protocols with a FACSCalibur flow cytometer (Becton Dickinson, USA). The following fluorochrome-conjugated antibodies (Abs) were used for flow cytometric analysis: CD3 (clone SK7), CD4 (clone CK3), CD8 (clone SK1), CD14 (clone M5E2), CD19 (clone SJ25C1), CD56 (clone B159), CD45RA (clone HI100), CD45RO (clone UCHL1) (BD Biosciences Pharmingen, USA), TCR $\alpha\beta$ (clone IP26A), TCR $\gamma\delta$ (clone IMMU 510) (Beckman Coulter, Inc., USA), CCR7 (clone 150503, R&D Systems Inc., USA), CD27 (clone O323, eBioscience, Inc., USA), CD132 (clone TUGh4, BD Biosciences Pharmingen), and rabbit anti-Human IgD polyclonal Ab (DAKO Japan Co., Japan).

Sequencing of Genomic DNA and cDNA, and Subcloning Analysis

Peripheral blood mononuclear cells (PBMCs) were obtained from the patient and his parents and various cell lineages were sorted using a FACSVantage (Becton Dickinson). The genomic DNA was isolated from the sorted samples and the cDNA was obtained using reverse transcriptase Super Script II (Invitrogen, USA) with Oligo (dT)₂₀ primer. Genomic DNA and cDNA were amplified with the proofreading PCR enzyme, KOD -Plus- (Toyobo, Japan). Direct sequencing analysis of all exons of the *IL2RG* gene, including introns at least 50 bases adjacent, were performed on an ABI 3700 (Applied Biosystems, USA). For analysing revertant subclones in each PBMC lineage, the genomic DNA and the cDNA isolated from sorted cell fractions were amplified by PCR with primer pairs 5'-TCCCAGAGGTT CAGTGT TTTG-3' and 5'-TTGCAACTGACAGCCA GAAG-3', and 5'-CGCCATGTTGAAGCCATC-3' and 5'-TTGCAACTGACAGCCAGAAG-3', for the region spanning exons 2 and 3 of *IL2RG*, respectively. These PCR products were subcloned using a TOPO TA Cloning Kit (Invitrogen) and sequenced.

T cell Functional Assays

To obtain PHA-induced T cell blasts, PBMCs were stimulated with PHA (Invitrogen) at 1:100 dilution and cultured in RPMI 1640 (RPMI) supplemented with 5 % fetal calf serum (FCS) with recombinant human IL-2 (50 IU/ml, kindly provided by Takeda Pharmaceutical Company, Japan) at 37 °C for 7 days. After being rested in RPMI with 5 % FCS overnight, the T cell blasts were stimulated with various concentrations of IL-2 for 48 h, and [³H]-thymidine uptake assays were performed as previously described [8]. T cell receptor (TCR) V β repertoire analysis and CDR3 spectratyping were performed as described [10, 11]. *In vitro* cytokine production against varicella zoster virus (VZV) antigen was performed as previously described [12]. Spots were enumerated automatically using the KS ELISPOT system (Carl Zeiss). The *in vivo* delayed-type hypersensitivity (DTH) reaction to subcutaneous purified VZV antigen (BIKEN, Japan) was performed as previously described [13]. The T cell receptor excision circles (TRECs) from the patient PBMCs were measured as previously described [14].

Tyrosine Phosphorylation of STAT5 by IL-2

PBMCs (1×10^6) were cultured in RPMI with 5 % FCS at 37 °C for 2 h and then treated with or without IL-2 (10,000 U/ml) for 10 min. The cells were fixed and permeabilized with BD Cytofix Buffer and Phosflow Perm Buffer

III (BD Biosciences Pharmingen) according to the manufacturer's instructions. After washing with PBS containing 1 % FCS, the cells were stained with mouse anti-pSTAT5 (pY694) (clone 47, BD biosciences), anti-CD4 and anti-CD8 mAbs and analyzed by flow cytometry.

Results and Discussion

At the age of 9 years, the patient presented with generalized warts and no detectable lymph nodes and tonsils. This, coupled with his prior hypogammaglobulinemia, prompted a re-evaluation of his immunological status. He showed a decreased level of IgA and a normal level of IgM but no isohemagglutinin. Mitogen-induced proliferation assays showed a slightly reduced response to PHA and Con A (Table I). Surface marker analysis of PBMCs revealed slightly decreased levels of CD3+ and CD4+ T cells, and a normal level of CD8+ T cells (Table II). Naïve CD4+ T cells

Table I Laboratory investigations (patient aged 9 years)

	Patient (IVIG)	Healthy controls
Blood counts		
White blood cells (count/ μ l)	7,400	3,600–9,800
Neutrophil (count/ μ l)	4,773	3,000–5,000
Lymphocyte (count/ μ l)	2,028	2,500–4,500
Monocyte (count/ μ l)	340	200–950
Eosinophil (count/ μ l)	252	0–700
Basophil (count/ μ l)	7	0–150
Red blood cells ($\times 10^6$ count/ μ l)	5.15	4.08–5.07
Hemoglobin (g/dl)	12.5	11.6–14.1
Platelet ($\times 10^3$ count/ μ l)	275	201–409
Serum Immunoglobulin levels		
IgG (g/L)	7.69	10.79 \pm 2.63
IgA (g/L)	0.26	2.46 \pm 0.91
IgM (g/L)	1.08	0.83 \pm 0.21
IgD (mg/L)	<6	55 \pm 16
IgE (IU/mL)	<5	<170
isohemagglutinin	Undetectable	
T cell proliferation		
None (cpm)	163	127–456
Phytohemagglutinin (cpm)	16,800	20,500–56,800
Concanavalin A (cpm)	16,600	20,300–65,700
DTH reaction to subcutaneous varicella virus antigen		
Erythematous change (mm in diameter)	18	\geq 5.0

Control values of blood counts are shown as the range from 95 % of healthy children aged 9 to 12 years. Control values of serum immunoglobulin levels are based on children aged 8 to 10 years and are shown as the mean \pm SD. IVIG indicates monthly intravenous infusion of 2.5 g immunoglobulin

Table II Surface marker analysis of peripheral blood mononuclear cells (patient aged 9 years)

	Patient (count/ μ l)	Healthy controls (count/ μ l)
CD3+	1,080	2,813 \pm 1,197
CD4+	357	1,699 \pm 850
CD8+	582	972 \pm 457
TCR $\alpha\beta$ +	890	2,154 \pm 1,004
TCR $\gamma\delta$ +	190	324 \pm 182
CD4+CD45RA+CCR7+	8	1,290 \pm 756
CD8+CD45RA+CCR7+	25	655 \pm 503
CD8+CD45RA+CCR7-	114	221 \pm 95.3
CD8+CD45RA-CCR7+	33	30.1 \pm 27.6
CD8+CD45RA-CCR7-	410	132 \pm 87.4
CD19+	894	1,238 \pm 605
CD19+CD27+smIgD-	0.4	86.6 \pm 61.3
CD19+CD27+smIgD+	14.3	172 \pm 123
CD3-CD56+	Undetectable	271 \pm 186

Absolute numbers of cells expressing surface markers are shown. Healthy control values are from children aged 2 to 9 years and are shown as mean \pm SD

(CD4+/CD45RA+/CCR7+), naïve CD8+ T cells (CD8+/CD45RA+/CCR7+), and both switched memory B cells (CD19+/CD27+/smIgD-) and unswitched memory B cells (CD19+/CD27-/smIgD-) were scarce. In addition, natural killer (NK) cells (CD3-/CD56+) were absent. This suggested the existence of a genetic defect causing lack of NK cells, such as an *IL2RG* deficiency and *JAK3* deficiency, and therefore the expression of IL2RG (also known as the common gamma chain or CD132) was examined by flow cytometry. Reduced expression was found on B cells and monocytes, although T cells expressed normal levels of CD132 (Fig. 1a). To determine whether CD132-dependent signal transduction was functioning, STAT5 phosphorylation was analyzed on patient CD4+ and CD8+ T cells in response to IL-2. It was found to be comparable with that of normal controls (Fig. 1b). In addition, a proliferation assay of PHA-induced T cell blasts in response to exogenous IL-2 was performed (Fig. 1c). This confirmed that the patient T cells, which were expressing normal levels of CD132, also had intact IL-2 signaling.

To elucidate the genetic cause of the lineage-dependent CD132 expression abnormalities, *IL2RG* genomic sequencing was performed in various cell lineages. Genomic sequencing of *IL2RG* in B cells, monocytes and buccal mucosa revealed a point mutation, c.284-15A>G, in intron 2 of *IL2RG*. This has been reported as a causative mutation of SCID-X1 [15], producing aberrant mRNA with an insertion of 14 bases spanning nucleotide -14 to -1 of exon 3 (Fig. 2a, b). Genomic sequencing of *IL2RG* in T cells showed overlapping bases at and around the mutation sites, while the cDNA of *IL2RG* from

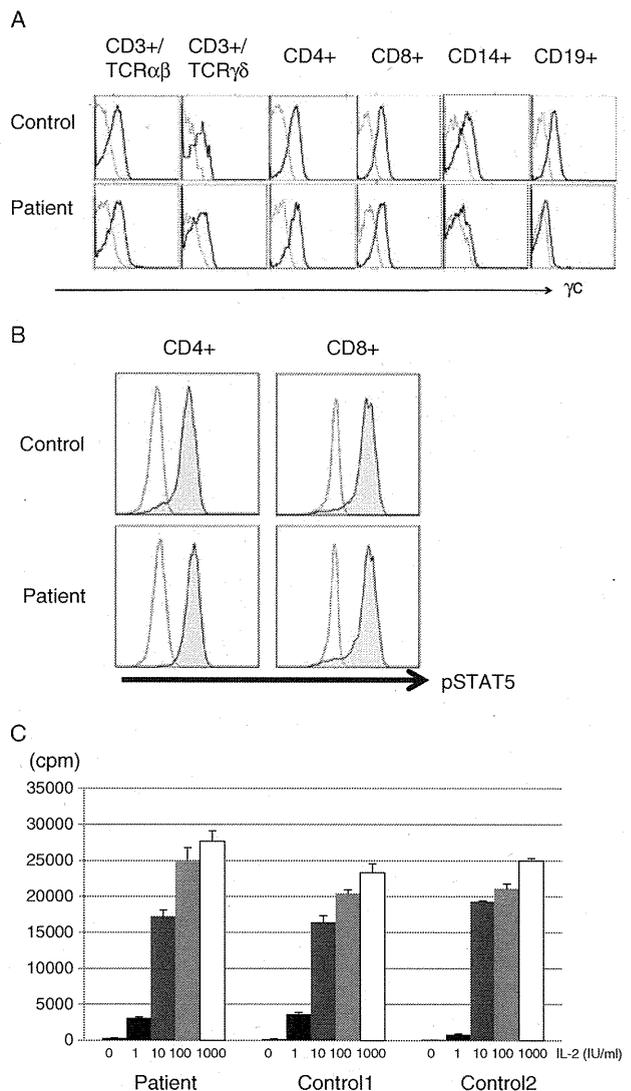
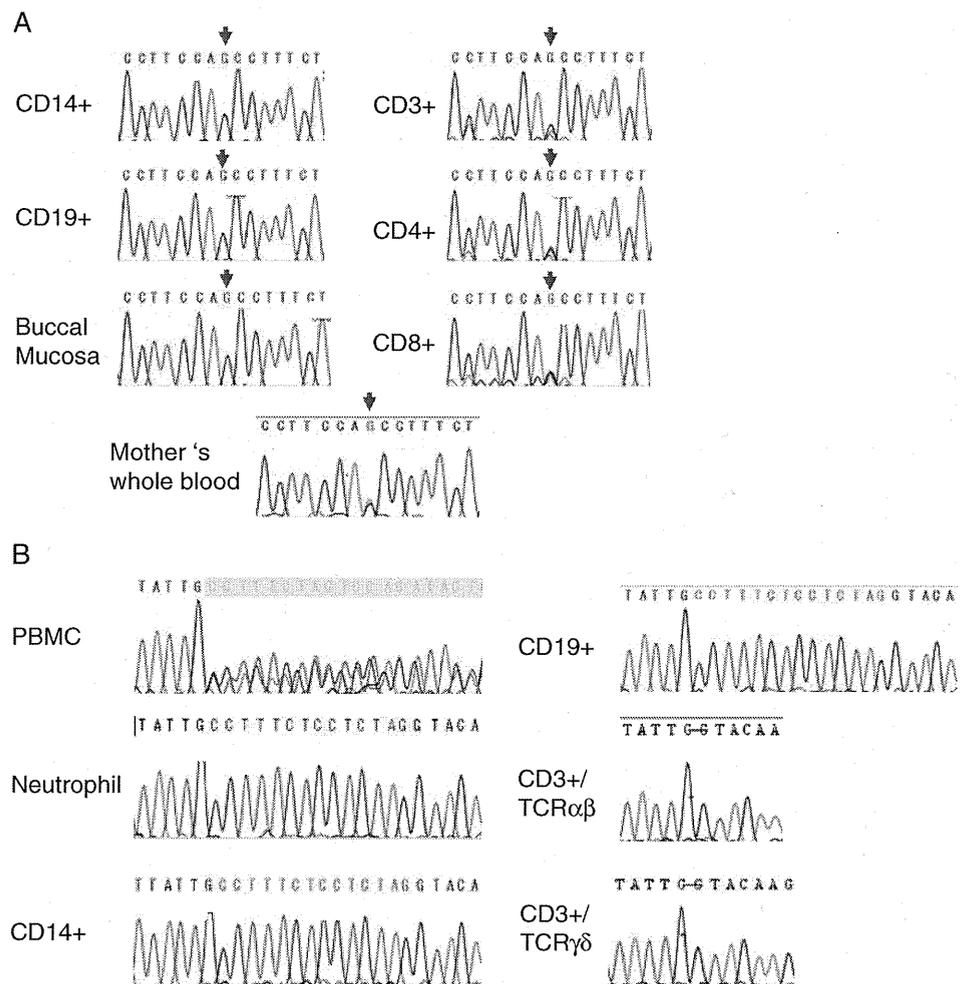


Fig. 1 IL2RG expression and T cell function at 9 years old. **a** Surface expression of IL2RG on PBMCs from the patient and healthy control gated according to the expression of the indicated lineage surface markers. *Black lines* indicate staining for IL2RG (with anti-CD132 Ab) and *gray lines* indicate staining with the isotype control. Data represent one of three independent experiments. **b** STAT5 tyrosine phosphorylation in patient and control CD4+ and CD8+ cells after incubation with (*shaded histograms*) or without IL-2 (*open histograms*). **c** Proliferation of PHA-induced T cell blasts in response to IL-2 stimulation from the patient and two controls. Data are shown as means \pm SD

the T cells was normal (Fig. 2a, b). Genomic sequencing of PBMCs from the patient’s mother confirmed her as a carrier of the mutation. The possibility of maternal engraftment was excluded by FISH analysis of sex chromosomes (data not shown), and it was concluded that the patient inherited the mutation from his mother and that reversion occurred in the patient’s T cells, which led to somatic mosaicism.

To explore the reversions that could have occurred to restore normal *IL2RG* expression in the patient’s T cells,

Fig. 2 Genetic analysis of various cell lineages at 9 years old. **a** Sequencing chromatograms of the patient's DNA from various immune cell lineages and buccal mucosa. Red arrows indicate the mutated base position c. 284–15. PBMCs from the patient's mother carried the same mutation. The patient's T cells show overlapping base changes at or around the mutated site. Data represent one of three independent experiments. **b** Sequencing chromatograms of the patient's cDNA from various cell types. Red characters indicate the inserted 14 bases spanning nucleotide -14 to nucleotide -1 of exon 3



subcloning and sequencing analysis of genomic DNA and cDNA was performed in various cell lineages. In B cells and monocytes, no reversion was detected and all of the cDNA clones had aberrant splicing (Table III). Analysis of TCR $\alpha\beta$ + cells revealed seven reversions, a true-back reversion, two fully compensating same-site reversions and four second-site reversions, all of which favored a functional reversion according to the splicing analysis software NNSPLICE0.9 [16] (Table IV). None of these base changes were detected in 200 clones from four healthy controls, indicating that the identified intron changes were unlikely to be due to PCR errors. The multiple reversions seen in this

case differed from the single reversions seen in other reported cases of reversion mosaicism of SCID-X1 [2, 3]. One possible reason for this is that, compared with the previously reported exonic mutations, an intronic mutation is more likely to acquire additional reversions on top of a true-back mutation. Additionally, the nine-year lifespan of the patient may have provided increased opportunities for extra reversions to occur. TCR β V-to-DJ rearrangement is reported to be impaired in some SCID-X1 patients, suggesting that differentiation arrest occurs at the CD4 immature single positive (ISP) stage at which TCR β V-to-DJ recombination is completed in normal T cells [17]. Therefore, the

Table III Clonal analysis of *IL2RG* cDNA in various cell lineages

	CD3+	CD4+	CD8+	CD14+	CD19+
Wild-type cDNA	100 % (25/25)	100 % (31/31)	100 % (30/30)	0 % (0/45)	0 % (0/34)
Aberrant cDNA	0 % (0/25)	0 % (0/31)	0 % (0/30)	100 % (45/45)	100 % (34/34)

Data represent the percentages of wild-type or aberrant spliced cDNA subclones in each lineage. The ratio indicates the number of each clone as compared to the total number of clones analyzed, based on subcloning and sequencing analysis

Table IV Multiple additional mutations detected in subclones of the *IL2RG* gene

	Subclones	Mutations
Wild type	TT CCTCT T CCT T CCAACC	Wild type
Inherited mutation	TT CCTCT T CCT T CCAGCC	c.284-15A>G
No.1	TT CCTCT T CCT T CCATCC	c.284-15A>T
No.2	TT CCTCT T CCT T CCAACC	c.284-15A>C
No.3	TT CCTCT T CAT T CCAACC	c.284-15A>G, c.284-21C>A
No.4	TT <u>AGAGTGG</u> CCTCT T CCT T CCAACC	c.284-15A>G, c.284-29_284-28insAGAGTGG
No.5	TT CCTC <u>CACCCGCCAAC</u>	c.284-24_284-14del11ins CACCCGCCAA
No.6	TT CCTCT CAGCC	c.284-23_284-18delTCCTC

reversions found in the patient’s T cells must have occurred before or around the CD4ISP stage. Differences were observed in reversion genotypes between the TCRαβ+ cells and TCRγδ+ cells. TCRγδ+ cells had only one of the second-site reversions found in TCRαβ+ cells in addition to a true-back reversion (No.3 in Table V). The identification of fewer reversions in the patient’s TCRγδ+ compared to TCRαβ+ cells may reflect the greater abundance of TCRαβ+ cells, increasing the likelihood of the stochastic occurrence of additional reversions [18]. Although no reversions in the patient’s B cells or monocytes were observed, it is possible that the reversions occurred in the progenitor at the stage before commitment to T cells and may reflect the unique nature of T cell proliferation and expansion [19].

Reversion mosaicism has previously been reported in SCID-X1 patients with *IL2RG* mutations, but it was accompanied by reduced T cell number and low proliferative response to mitogens [2, 3]. The paradoxical nature of the patient’s cellular immunity, a history of uneventful varicella

infection, and the occurrence of widespread warts with very few naïve T cells prompted an evaluation of his T cell function. The TCR Vβ repertoire analysis of CD4+ and CD8+ T cells revealed comparable diversity to the normal controls (data not shown). CDR3 spectratyping analysis revealed the patient CD4+ T cells had as much variety as the normal controls, but his CD8+ T cells displayed restricted diversity (Fig. 3a). To evaluate the antigen-specific response of the patient’s T cells, response to VZV was measured. The DTH reaction to subcutaneous VZV antigen and the IFN-γ production from VZV antigen-stimulated PBMCs measured by an ELISPOT assay were comparable to those of normal controls (Table I and Fig. 3b). These data suggested that the patient maintained normal cellular immune responses *in vivo* as well as having normal *in vitro* IFN-γ production ability against VZV antigen. Multiple reversions from intronic mutations could provide a sufficient number of normally functioning T cells and may improve the clinical phenotype compared to previously reported cases with single reversions. However, the number of TRECs in the patient’s PBMCs (<10 copies/μg DNA) suggested a low level of recent thymic output, and the restricted diversity of TCRs observed in the patient’s CD8+ cells might reflect the exhaustion of the T cell reservoir. To gain further insight, the long-term immunologic status of the patient was evaluated prospectively for 5 years. Absolute counts of CD4+ and CD8+ T cells as well as mitogen-induced T cell proliferation responses were measured every 2–5 months (Fig. 4). Unexpectedly, the number of both CD4+ and CD8+ T cells and mitogen-induced T cell proliferation responses were stable and the patient remained healthy over this period. In recent years, the effector memory subset of CD8+ T cells (CD8+/CD45RA+/CCR7-) has been taken as a marker of cell exhaustion or replicative senescence [20]. However, the majority of CD8+ T cells of the patient were memory CD8+ T cells (CD8+/CD45RA-/CCR7±) and the population of effector memory CD8+ T cells was very small (Table II). These data

Table V Clonal analysis of somatic mosaicism of the *IL2RG* gene in various cell lineages

	Wild type	Inherited mutation	No.1	No.2	No.3	No.4	No.5	No.6
TCRαβ+	21 % (7/33)	12 % (4/33)	9 % (3/33)	12 % (4/33)	21 % (7/33)	9 % (3/33)	6 % (2/33)	9 % (3/33)
TCRγδ+	2 % (1/42)	2 % (1/42)	0 % (0/42)	0 % (0/42)	95 % (40/42)	0 % (0/42)	0 % (0/42)	0 % (0/42)
CD3+	5 % (2/39)	5 % (2/39)	26 % (10/39)	13 % (5/39)	38 % (15/39)	3 % (1/39)	10 % (4/39)	0 % (0/39)
CD4+	32 % (25/79)	3 % (2/79)	13 % (10/79)	19 % (15/79)	16 % (13/79)	3 % (2/79)	3 % (2/79)	13 % (10/79)
CD8+	10 % (7/73)	4 % (3/73)	21 % (15/73)	19 % (14/73)	25 % (18/73)	8 % (6/73)	12 % (9/73)	1 % (1/73)
CD14+	0 % (0/33)	100 % (33/33)	0 % (0/33)	0 % (0/33)	0 % (0/33)	0 % (0/33)	0 % (0/33)	0 % (0/33)
CD19+	0 % (0/30)	100 % (30/30)	0 % (0/30)	0 % (0/30)	0 % (0/30)	0 % (0/30)	0 % (0/30)	0 % (0/30)

Data represent the percentages of each additional mutant subclone in each lineage. The ratio indicates the number of each mutant clone in various cell lineages as compared to the total number of clones analyzed, based on subcloning and sequencing analysis

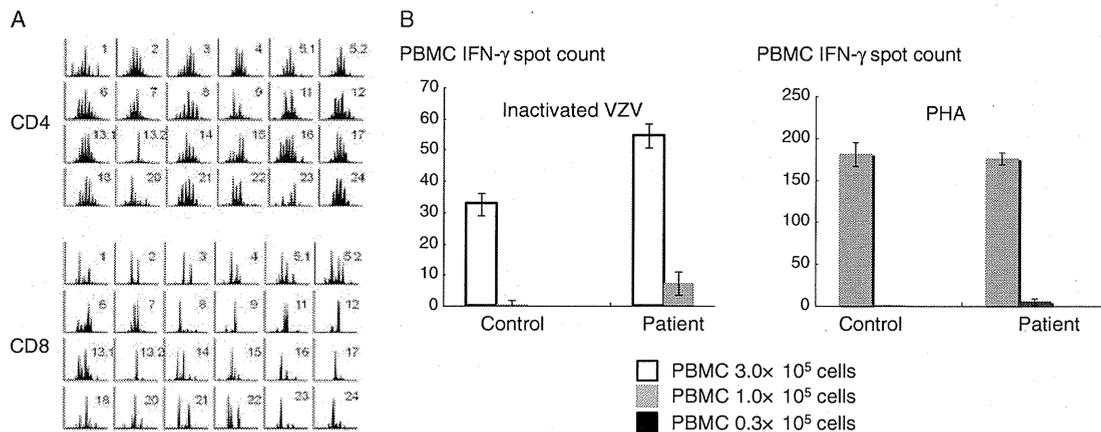


Fig. 3 Functional evaluation of T cells at 9 years old. **a** CDR3 spectratyping of the TCRVβ chain. Each TCRVβ fragment was amplified from cDNA with one of 24Vβ-specific primers (each Vβ chain is indicated). The size distribution of the PCR products was determined by an automated sequencer and GeneScan software. The CDR3 size distribution in CD4+ and CD8+ T cells from the patient is shown. **b**

Elispot analysis of IFN-γ production as a measure of T cell function. (LEFT) Varicella-specific immune response to varicella zoster (VZV) antigen *in vitro*. Patient and control (from a healthy with a previous history of varicella infection) PBMCs ($0.3-3 \times 10^5$) were stimulated with inactivated VZV antigen or (RIGHT) PHA. Data are shown as mean ± SD

demonstrated that the patient maintained a certain level of T cell immunity for over a decade, despite the fact that the supply of fresh T cells from the thymus was limited and the patient suffered from generalized warts. Further follow up is required to determine if the patient can continue to maintain long-term T cell immunity.

In conclusion, this study indicates that it is critical to determine the NK cell number to avoid overlooking reversion mosaicism of SCID-X1. In addition, it has been shown that a number of *IL2RG* gene reversions can restore T cell functions and maintain T cell immunity against viral infection for at least 14 years.

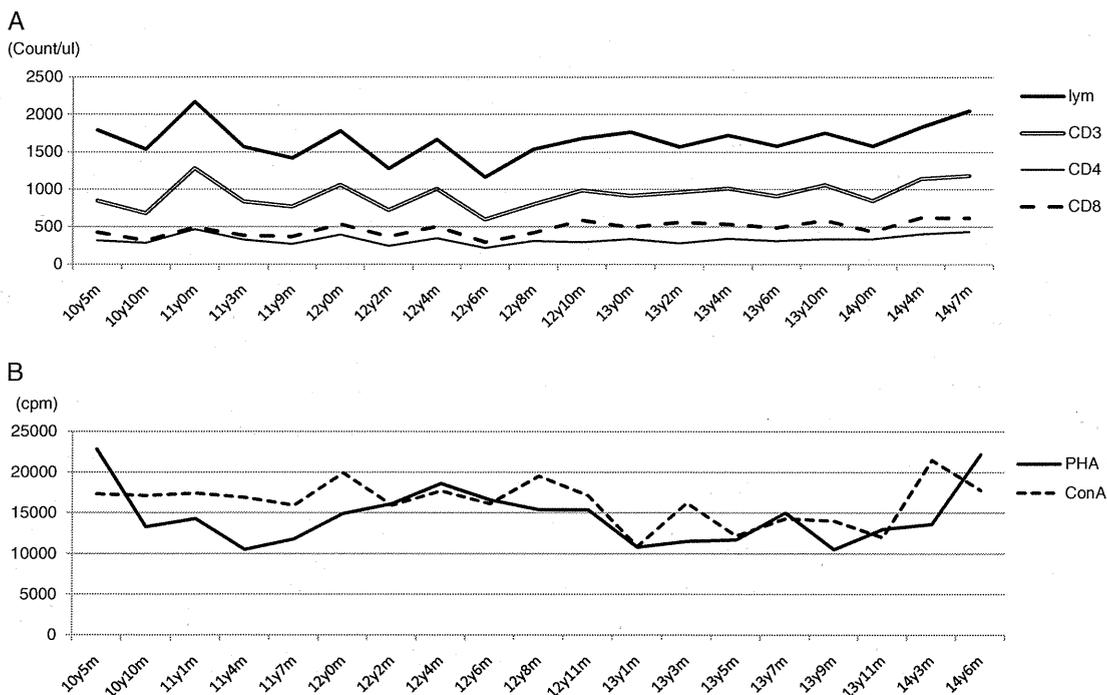


Fig. 4 Long term evaluation of T cell number and mitogen-induced proliferative response. **a** Absolute counts (per μ l) of total lymphocytes (lym), CD3+ cells, CD4+ cells and CD8+ cells were measured for

4 years. **b** T cell proliferation in response to PHA (solid line) and Con A (dotted line). Healthy control values for PHA range from 20,500 to 56,800 cpm and for Con A from 20,500 to 65,700 cpm

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Authors' contributions T.K. performed experiments and wrote the paper. M.S. and Ka.I. performed experiments. R.N. designed the research, wrote the paper and analyzed data. Y.T. wrote the paper and analyzed data. T.M. treated the patient and analyzed data. S.O., Y.M., N.N., Ko.I., S.N., T.W. and A.Y. performed experiments and discussed the research. T.H. and T.N. designed the research.

Conflict of Interests The authors declare no competing financial interests.

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*Delayed onset adenosine deaminase
deficiency associated with acute
disseminated encephalomyelitis*

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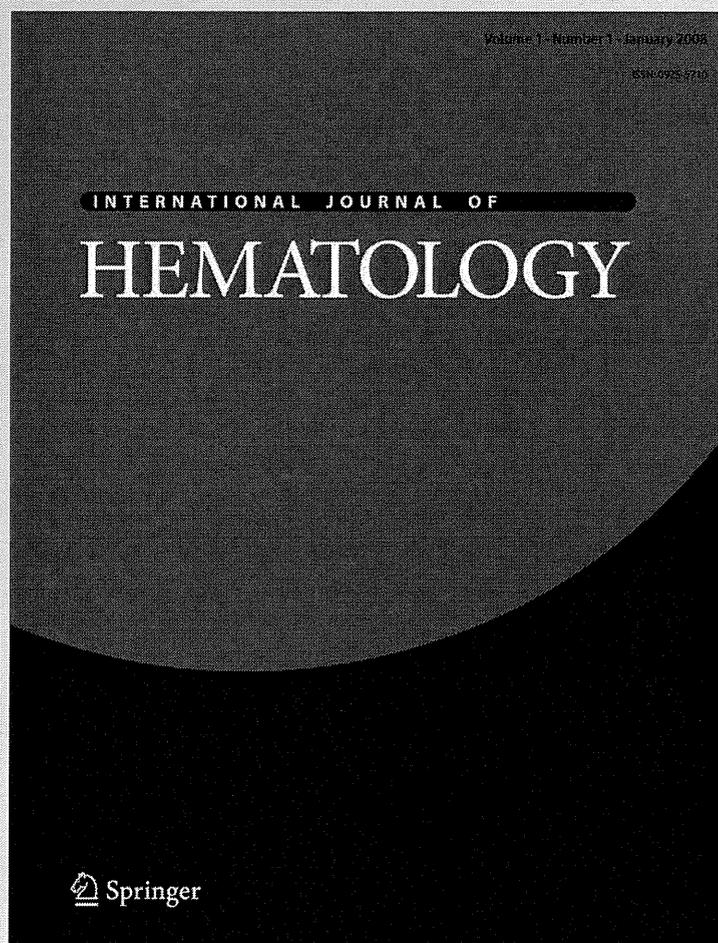
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CASE REPORT

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 proapoptotic 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.

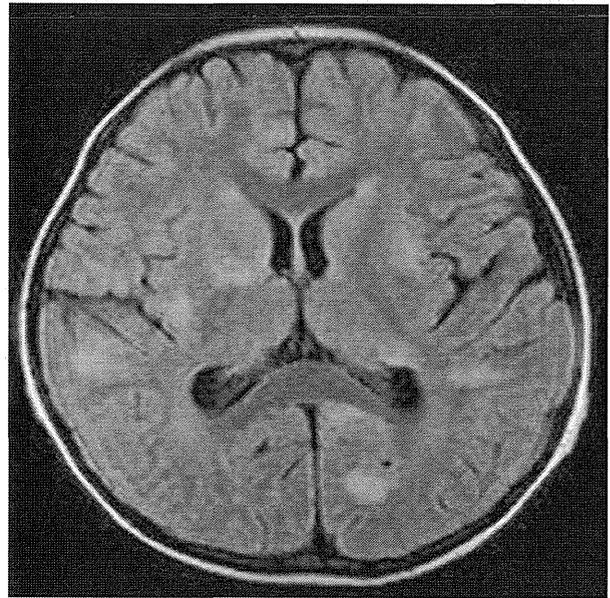


Fig. 1 Axial T2-weighted MRI of the patient's brain. Several high intensity signals are observed in the brain, as indicated

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 SpO₂ 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