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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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 reversions in T cells, which restored sufficient cell-mediated immunity to overcome viral infection. Lineage-specific analysis revealed multiple reversions in T cell receptor (TCR) αβ+ and TCRγδ+ T cells. Diversity of the TCRVβ 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/μl, [mean of controls: 1,683± 874]) and CD56+ cells (39/μl [mean of controls: 306± 207]), while CD3+ cells (1,803/μl [mean of controls: 2,997±1,751]), CD8+ cells (1,067/μl [mean of controls: 1,683±874]) and CD19+ cells (1,850/μl [mean of controls: 1,114±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), TCRαβ (clone IP26A), TCRγδ (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 CAGTGTTTTG-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 [^{3}H]-thymidine uptake assays were performed as previously described [8]. T cell receptor (TCR) Vβ 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/μl)	7,400	3,600–9,800
Neutrophil	(count/μl)	4,773	3,000–5,000
Lymphocyte	(count/μl)	2,028	2,500–4,500
Monocyte	(count/μl)	340	200–950
Eosinophil	(count/μl)	252	0–700
Basophil	(count/μl)	7	0–150
Red blood cells	($\times 10^6$ count/μl)	5.15	4.08–5.07
Hemoglobin	(g/dl)	12.5	11.6–14.1
Platelet	($\times 10^3$ count/μl)	275	201–409
Serum Immunoglobulin levels			
IgG	(g/L)	7.69	10.79±2.63
IgA	(g/L)	0.26	2.46±0.91
IgM	(g/L)	1.08	0.83±0.21
IgD	(mg/L)	<6	55±16
IgE	(IU/mL)	<5	<170
isohemagluttinin		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	≧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 ± 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/μl)	Healthy controls (count/μl)
CD3+	1,080	2,813±1,197
CD4+	357	1,699±850
CD8+	582	972±457
TCRαβ+	890	2,154±1,004
TCRγδ+	190	324±182
CD4+CD45RA+CCR7+	8	1,290±756
CD8+CD45RA+CCR7+	25	655±503
CD8+CD45RA+CCR7-	114	221±95.3
CD8+CD45RA-CCR7+	33	30.1±27.6
CD8+CD45RA-CCR7-	410	132±87.4
CD19+	894	1,238±605
CD19+CD27+smIgD-	0.4	86.6±61.3
CD19+CD27+smIgD+	14.3	172±123
CD3-CD56+	Undetectable	271±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 ± 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+/cmIgD-) 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 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
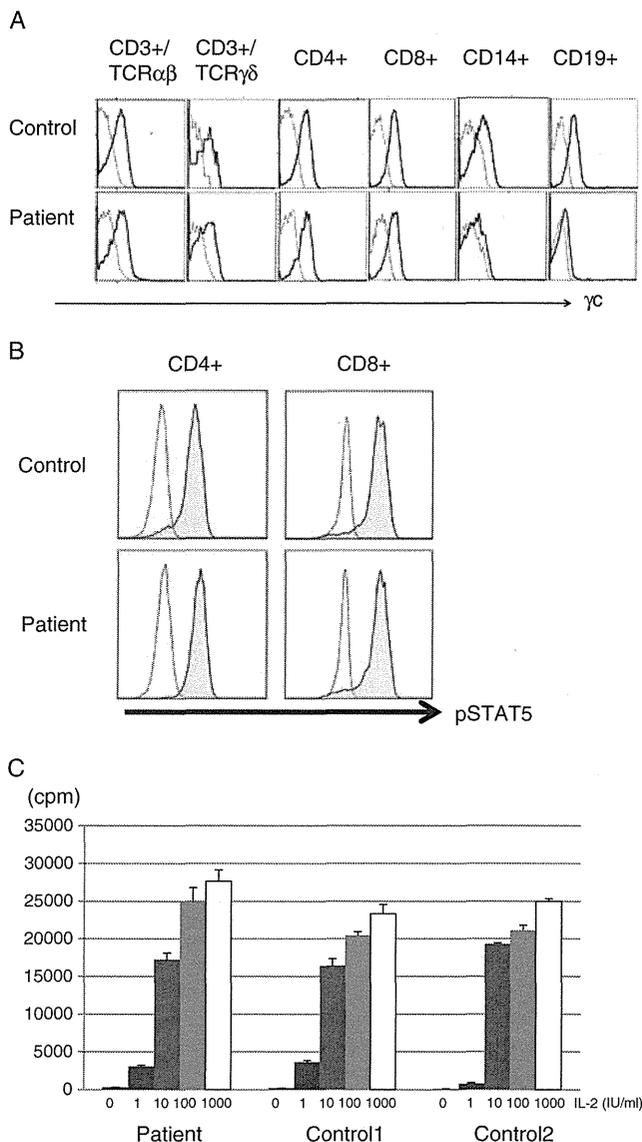

Fig. 1 IL2RG expression and T cell function at 9 years old. **a** Surface expression of IL2RG on PBMCs from the patient and healthy control gated according to the expression of the indicated lineage surface markers. *Black lines* indicate staining for IL2RG (with anti-CD132 Ab) and *gray lines* indicate 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 ± 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. Re characters indicate the inserted 14 bases spanning nucleotide −14 to nucleotide −1 of exon 3
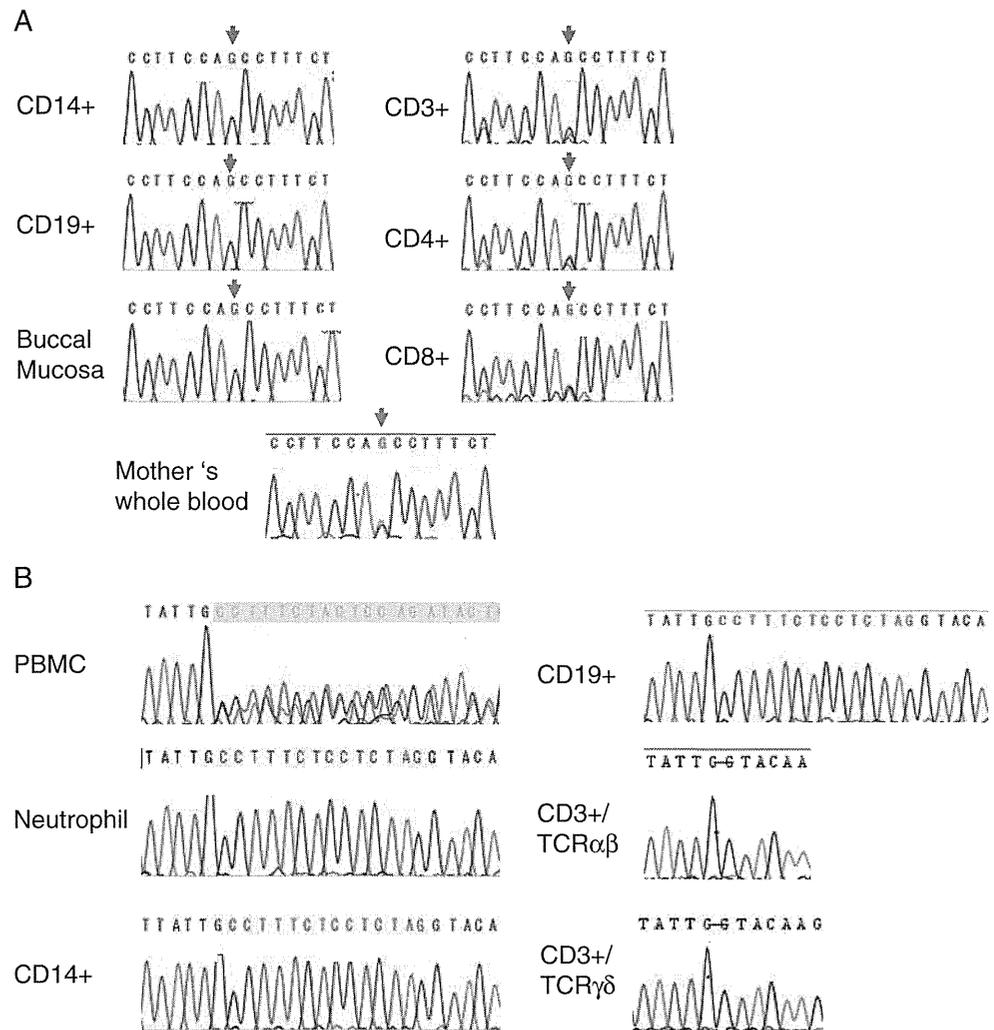
subcloning and sequencing analysis of genomic DNA and cDNA was performed in various cell lineages. In B cells and monocytes, no reversion was detected and all of the cDNA clones had aberrant splicing (Table III). Analysis of TCRαβ+ cells revealed seven reversions, a true-back reversion, two fully compensating 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. TCRVβ 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 TCRVβ 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 CCACCC	c.284-15A>C
No.3	TT CCTCT T CAT T CCAGCC	c.284-15A>G, c.284-21C>A
No.4	TT AGAGTGG CCTCT T CCT T CCAGCC	c.284-15A>G, c.284-29_284-28insAGAGTGG
No.5	TT CCTC CACCCGCCAAC	c.284-24_284-14del11ins CACCCGCCAA
No.6	TT CCTCT CAGCC	c.284-23_284-18delTCCTTC

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
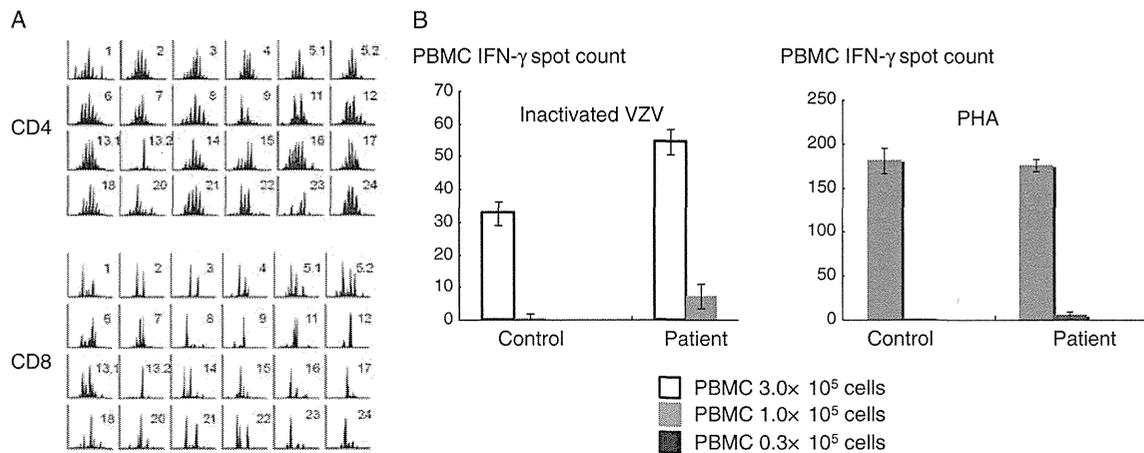
Fig. 3 Functional evaluation of T cells at 9 years old. **a** CDR3 spectratyping of the TCRVβ chain. Each TCRVβ fragment was amplified from cDNA with one of 24Vβ-specific primers (each Vβ chain is indicated). The size distribution of the PCR products was determined by an automated sequencer and GeneScan 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 (0.3-3×10^5) 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.
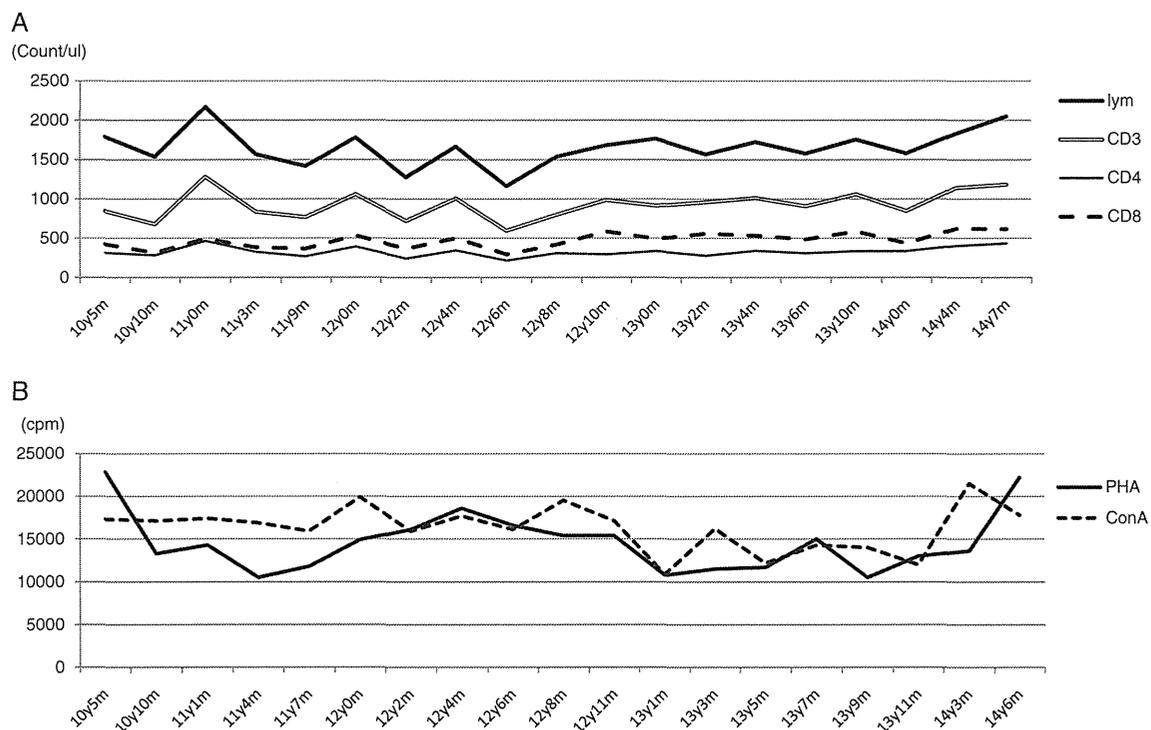

Fig. 4 Long term evaluation of T cell number and mitogen-induced proliferative response. **a** Absolute counts (per μl) of total lymphocytes (lym), CD3+ cells, CD4+ cells and CD8+ cells were measured for

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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Delayed onset adenosine deaminase deficiency associated with acute disseminated encephalomyelitis

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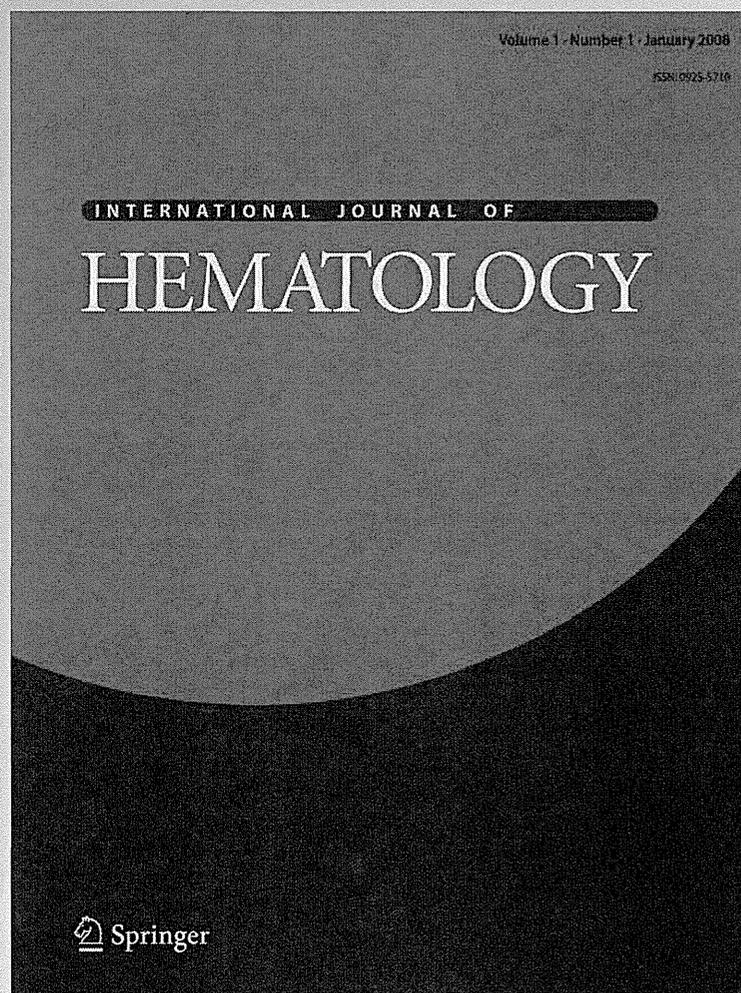
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CASE REPORT

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.

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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.
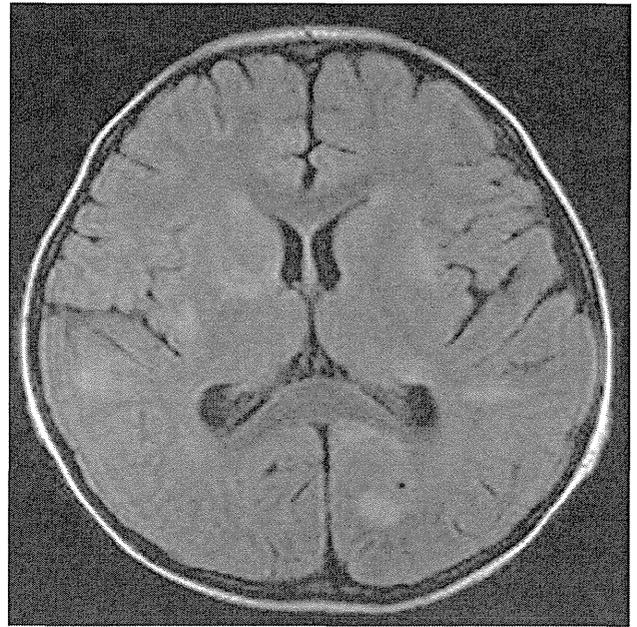

Fig. 1 Axial T2-weighted MRI of the patient's brain. Several high intensity signals are observed in the brain, as indicated

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
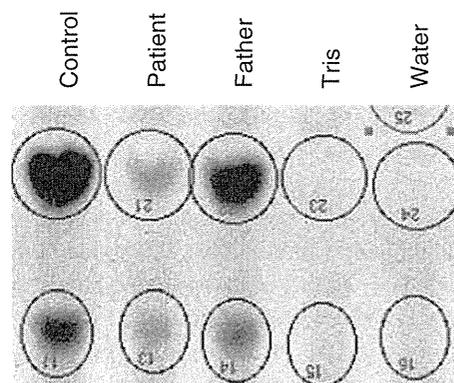

Fig. 2 ADA enzymatic activity. Each lane corresponds to a different sample as follow: control individual (102.6 U), the patient (8.6 U), the patient's father (39.6 U), Tris (1.0 U), and water (1.0 U). U denotes nmol/min/10⁸ cells

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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Letter to the Editor

Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin κ-deleting recombination excision circles

To the Editor:

Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency associated with hypogammaglobulinemia and other various clinical manifestations. CVID was originally reported to be a disease primarily caused by defective B-cell function, with defective terminal B-cell differentiation rendering B cells unable to produce immunoglobulin. However, combined immunodeficiency (CID) involving both defective B and T cells is often misdiagnosed as CVID.[1] Indeed, one study reported that CD4^{+} T-cell numbers were decreased in 29% of 473 patients with CVID[2]; similarly, another study found that naive T-cell numbers were markedly reduced in 44% (11/25) of patients with CVID.[3] These observations indicated that a subgroup of patients with clinically diagnosed CVID is T-cell deficient. Consistently, some patients with CVID have complications that might be related to T-cell deficiency, including opportunistic infections, autoimmune diseases, and malignancies, which is similar to that observed in patients with CID.[1,4] Therefore identifying novel markers to better classify CVID and distinguish CID from CVID will be required to best manage medical treatment for CVID.

We recently performed real-time PCR-based quantification of T-cell receptor excision circles (TREC) and signal joint immunoglobulin κ-deleting recombination excision circles (KREC) for mass screening of severe combined immunodeficiency (SCID)[5] and B-lymphocyte deficiency[6] in neonates. TREC and KREC are associated with T-cell and B-cell neogenesis, respectively.[7] Here we retrospectively report that TREC and KREC are useful for classifying patients with clinically diagnosed CVID.

Hypogammaglobulinemic patients (n = 113) were referred to our hospital for immunodeficiency from 2005-2011, and the following patients were excluded from the CVID pool by estimating their SCID genes based on clinical manifestations and lymphocyte subset analysis: 18 patients with SCID diagnoses; 14 patients less than 2 years of age (transient infantile hypogammaglobulinemia); 10 patients with IgM levels of greater than 100 mg/dL (hyper-IgM syndrome); 26 patients with diseases other than CVID caused by known gene alterations (10 with X-linked agammaglobulinemia and 11 with hyper-IgM syndrome [*CD40L* or *AICDA* mutated]), (2 with DiGeorge syndrome, and 3 with *FOXP3*, *IKBKG*, or *6p* deletions); and 5 patients with drug-induced hypogammaglobulinemia. The remaining 40 patients with decreased IgG (≥2 SDs below the mean for age), IgM, and/or IgA levels, as well as absent isohemagglutinins, poor response to vaccines, or both were included in this study as patients with CVID and analyzed for TREC/KREC levels, retrospectively.

Ages of patients with CVID ranged from 2 to 52 years (median age, 15.5 years). The sex ratio of the patients was 21 male/19 female patients. Serum IgG, IgA, and IgM levels were 370 ± 33 mg/dL (0-716 mg/dL), 30 ± 7 mg/dL (1-196 mg/dL), and 40 ± 6 mg/dL (2-213 mg/dL), respectively. TREC and KREC quantification was performed by using DNA samples extracted from peripheral blood, as reported previously.[5,6] Clinical symptoms were then assessed
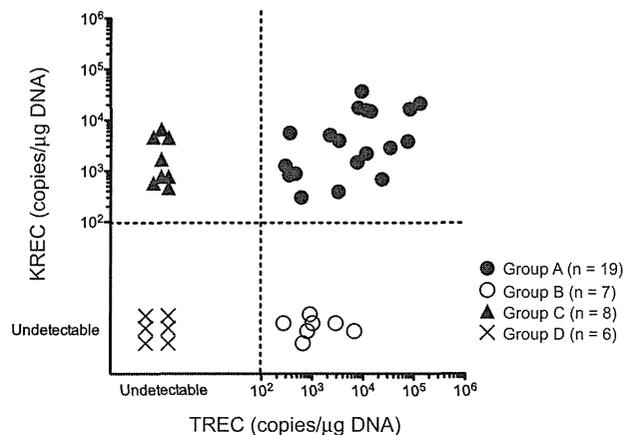

FIG 1. Quantifying TREC and KREC classifies patients with CVID into 4 groups. Patients with CVID were classified as follows: TREC(+)/KREC(+), group A (19 patients); TREC(+)/KREC(−), group B (7 patients); TREC(−)/KREC(+), group C (8 patients); and TREC(−)/KREC(−), group D (6 patients). *Undetectable,* Less than 100 copies/μg DNA.

retrospectively. The study protocol was approved by the National Defense Medical College Institutional Review Board, and written informed consent was obtained from adult patients or parents of minor patients in accordance with the Declaration of Helsinki.

Based on TREC and KREC copy numbers, the 40 patients with CVID were classified into 4 groups (groups A, B, C, and D; Fig 1). Comparing lymphocyte subsets, CD3^{+} T-cell numbers were similar among groups A, B, and D but were significantly lower in group C ($P < .05$; group A, 1806 ± 204 cells/μL; group B, 1665 ± 430 cells/μL; group C, 517 ± 124 cells/μL; and group D, 1425 ± 724 cells/μL; $P = .0019$, Tukey multiple comparison test based on 1-way ANOVA). CD3^{+}CD4^{+}CD45RO^{+} memory T-lymphocyte percentages in groups B, C, and D were significantly higher than those in group A ($P < .0001$; group A, 37% ± 16%; group B, 67% ± 13% [$P = .0006$]; group C, 92% ± 8.2% [$P < .0001$]; and group D: 83% ± 14% [$P < .0001$]; see Fig E1 in this article's Online Repository at www.jacionline.org); additionally, the percentages of these cells in groups C and D were higher than in group B ($P = .0115$). These results indicate that group C and D patients have markedly decreased CD4^{+}CD45RA^{+} naive T-cell counts than group A patients and that counts in group B are also significantly decreased, although less so than in groups C or D, which is consistent with a report showing lower TREC copy numbers in CD4^{+}CD45RO^{+} cells. Some patients in groups B, C, and D exhibited normal CD4^{+}CD45RO^{+} percentages, although TREC levels, KREC levels, or both decreased. This discrepancy indicates that TREC/KREC levels could be independent markers to determine the patient's immunologic status in addition to CD4^{+}CD45RA^{+}; the reasons underlying the discrepancy between CD4^{+}CD45RA^{+} and TREC/KREC levels remain unsolved.

CD19^{+} B-cell numbers in group A were significantly higher ($P < .05$) than those in groups B and D (group A, 269 ± 65 cells/μL; group B, 35 ± 16 cells/μL; group C, 60 ± 11 cells/μL; and group D, 29 ± 16 cells/μL; $P = .0001$). However, B-cell subpopulations, including CD27^{−}, IgD^{+}CD27^{+}, and
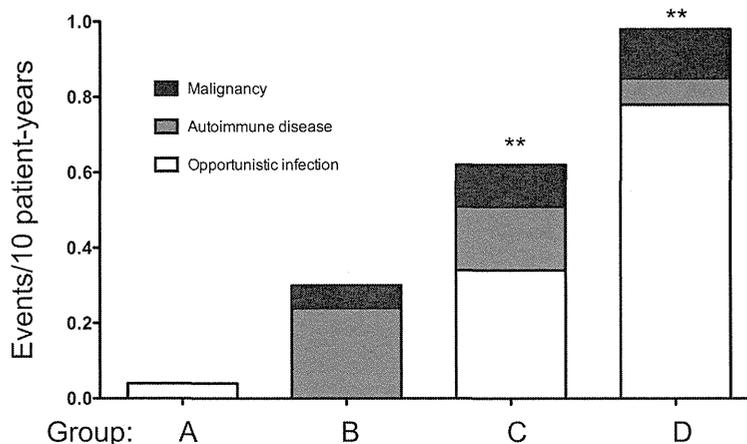
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FIG 2. Cumulative incidence of complication events per 10 patient-years differs among groups. Opportunistic infections, autoimmune diseases, and malignancies were evaluated for each patient group. Complication incidences in group D (0.98 events/10 patient-years), group C (0.63 events/10 patient-years), and group B (0.30 events/10 patient-years) were higher than in group A (0.04 events/10 patient-years). Group A versus group D: **P = .0022; group A versus C: **P = .0092; group A vs group B: P = .0692.

IgD⁻CD27⁺ cells, were not significantly different among the groups. Standardizing KREC copy numbers for each patient by dividing their CD19⁺ by their CD27⁺ percentages revealed the same patient classification as that shown in Fig 1 (data not shown), indicating that the original classification was independent of CD19⁺ B-cell or CD27⁺ memory B-cell percentages.

Because TREC and KREC levels decrease with age (see Fig E2 in this article's Online Repository at www.jacionline.org)[5,6] and age distribution was wide in this study, we compared patients' ages among groups at the time of analysis to determine whether classification was associated with age. TREC/KREC-based classification was independent of both age and sex because age distribution was not significantly different among groups (P > .05; group A, 12.7 ± 2.3 years [2-30 years]; group B, 23.4 ± 4.2 years [6-39 years]; group C, 21.5 ± 6.1 years [4-52 years]; and group D, 25.5 ± 4.4 years [15-46 years]; data not shown) nor was male/female sex ratio (overall, 21/19; group A, 10/9; group B, 2/5; group C, 5/3; and group D, 4/2; P = .4916, χ^2 test; data not shown).

We next evaluated whether any correlation existed between TREC/KREC-based classification and clinical symptoms in each patient group. All patients in the study had been treated with intravenous immunoglobulin (IVIG) substitution at the time of analysis. We found that the cumulative events of complications (opportunistic infections, autoimmune diseases, and malignancies) per 10 patient-years were highest in group D (0.98 events/10 patient-years), followed by group C (0.63 events/10 patient-years), group B (0.30 events/10 patient-years), and group A (0.04 events/10 patient-years), where events in groups D and C were significantly higher than group A (group A vs group D, P = .0022; group A vs group C, P = .0092; group A vs group B, P = .0692; Fig 2). Furthermore, we found similar results when evaluating only patients 19 years old or older for group D (1.01 events/10 patient-years), group C (0.56 events/10 patient-years), group B (0.32 events/10 patient-years), and group A (0.06 events/10 patient-years; group A vs group D, P = .0074; group A vs group C, P = .0407; group A vs group B, P = .1492; data not shown). Categorizing patients by using several different previously reported CVID classifications (focused primarily on separating patients based on levels of circulating B-cell subsets), we found

that no classification scheme showed any significant event increases in any particular group (see Fig E3 in this article's Online Repository at www.jacionline.org). Assessing longitudinal cumulative opportunistic infection incidence among the groups, group D and C values were significantly higher than in group A (see Fig E4, A, in this article's Online Repository at www.jacionline.org; P = .0059). Autoimmune and malignant diseases (P = .5168 and P = .6900, respectively) were observed in groups B and D but not in group A (see Fig E4, B and C). Cumulative events were significantly different between groups (P = .0313, log-rank test; group A, 5.3% and 5.3%; group B, 14.3% and 57.1%; group C, 27.1% and 63.5%; and group D, 33.3% and 83.3% at 10 and 30 years of age, respectively; see Fig E4, D). One patient in group D died of *Pneumocystis jirovecii* pneumonia, and 2 other patients in the same group received hematopoietic stem cell transplantation after complications caused by EBV-related lymphoproliferative disorder.

Assessing these data, TREC/KREC-based classification matches clinical outcomes. Because group D patients exhibited the most frequent complications (opportunistic infections, autoimmune diseases, and malignancies), they could receive a diagnosis of CID based on these symptoms. If they are indeed determined to have CID, then TREC/KREC analysis is helpful to distinguish between CID and CVID. Their TREC(−)/KREC(−) phenotype might relate to defective V(D)J recombination in T- and B-cell development[8] because patients with B-negative SCID (*RAG1*, *RAG2*, *Artemis*, and *LIG4*), as well as patients with ataxia-telangiectasia (AT) and Nijmegen breakage syndrome (NBS; see Fig E5 in this article's Online Repository at www.jacionline.org),[5,6] were also negative for both TREC and KREC; it is intriguing to speculate that an unknown V(D)J recombination gene or genes is responsible. As for treatment, hematopoietic stem cell transplantation should be considered the preferred treatment to "cure" group D patients, as reported in patients with severe CVID/CID, because event-free survival is poor.[9]

In contrast to group D patients, TREC(+)/KREC(+) group A patients treated with IVIG substitution therapy remained healthy. One possible explanation is that these patients harbor

defects only in terminal B-cell differentiation, but not in T cells, and represent typical patients with CVID, as originally reported.

Group C patients had a high frequency of both opportunistic infections and malignancies, suggesting that these TREC(−) patients have T-cell defects. Although group C patients had a similar TREC/KREC pattern to patients with SCID with B cells (*IL2RG* and *JAK3*; see Fig E5, *A*), they do not fulfill the European Society for Immunodeficiencies criteria for SCID, and no mutation was identified in the SCID genes estimated from clinical manifestation and lymphocyte subset analysis. However, from our data, they would likely benefit from undergoing similar treatment to patients with SCID or CID to prevent these complications.

Although opportunistic infections were rare in group B patients, autoimmune diseases were often observed. This is consistent with this group being TREC(+)/KREC(−) and the idea that balance between T and B cells is important to prevent autoimmune diseases in patients with CVID.[1] Intriguingly, a group of patients with AT and NBS were also TREC(+)/KREC(−) (see Fig E4, *B*), which is similar to group B patients. Additionally, CD45RA$^+$CD4$^+$ naive T-cell numbers were reduced in most group B patients, which is similar to the phenotype exhibited by patients with AT and NBS. This finding raises the possibility that although some group B patients are also T-cell deficient, as well as B-cell deficient, and should be treated similarly to patients with CID, other patients have only B-cell deficiency and are effectively treated with IVIG substitution therapy.

By analyzing a large CVID patient cohort, the overall survival rate of patients with more than 1 complication was worse than that for patients without other complications.[4] Our findings indicate that low TREC levels, KREC levels, or both are useful markers that correlate well with the overall survival rate in patients with CVID. Therefore we conclude that TREC and KREC are useful markers to assess the clinical severity and pathogenesis of each patient with CVID and to distinguish CID from CVID. Thus patient classification based on TREC/KREC levels would provide a helpful tool for deciding on an effective treatment plan for each patient with CVID.

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