

FIG E5. TREC and KREC quantification classifies patients with SCID, AT, NBS, or ataxia-telangiectasia-like disease (*ATLD*) into 4 groups. **A**, Patients with B⁺SCID (n = 20) were classified as group C, and patients with B⁻SCID (n = 8) were classified as group D; these patients were included in the previous studies.^{5,6} **B**, Although most patients with AT (n = 23) and patients with NBS (n = 4) were classified as group D, TRECs were detected in peripheral blood samples (n = 4 in patients with AT and n = 2 in patients with NBS) and neonatal Guthrie cards (n = 3) of some patients with AT, who were classified as group B. Patients with ATLD with *MRE11A* mutations were classified as group A.

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Virologically Confirmed Acyclovir-Resistant
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Neonatal Herpes Encephalitis Caused by a Virologically Confirmed Acyclovir-Resistant Herpes Simplex Virus 1 Strain

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A neonate with herpes simplex virus 1 encephalitis was treated with intravenous acyclovir. During the course of therapy, the infection became intractable to the treatment and a mutation in the viral thymidine kinase gene (nucleotide G375T, amino acid Q125H) developed. This mutation was demonstrated *in vitro* to confer acyclovir resistance.

CASE REPORT

A 13-day-old boy was admitted to National Defense Medical College Hospital due to lethargy and failure to thrive. He was born at 39 weeks 5 days of gestation and 2,558 g birth weight to a healthy 35-year-old mother (gravida 2, para 2). Group B streptococcus (GBS) was detected from the mother's vagina in the third trimester, but the baby's bacterial culture tests performed at birth, including throat, skin, and blood analyses, were negative for GBS. The mother did not have a history of genital herpes. Her herpes simplex virus 1 (HSV-1) and HSV-2 serostatus was not examined, and her history of acyclovir (ACV) use was not clear. Furthermore, the genital swab culture examination for HSV was not performed. On admission, physical examination of the boy revealed skin blisters on the forehead and upper lip. A swab from the blister showed positive and negative reactions for the specific antigens of HSV-1 and HSV-2, respectively, in a direct immunofluorescent antibody assay (Denka Seiken Co. Ltd., Tokyo, Japan) performed by a qualified clinical laboratory (SRL Inc., Tokyo, Japan). A serum sample collected on admission showed positive and negative reactions in the enzyme-linked immunosorbent assay for detection of anti-HSV IgM and IgG antibody (SRL Inc.), respectively. A lumbar puncture revealed pleocytosis (547 cells/ μ l) and an elevated protein level (168 mg/dl) in the cerebrospinal fluid (CSF). The CSF was also positive for HSV-1 DNA, which was determined by a previously reported method (1) in PCR testing (SRL Inc.). The boy was diagnosed as having neonatal herpes encephalitis (NHE), and intravenous high-dose ACV (60 mg/kg/day) treatment was initiated. His general status improved with resolution of the skin lesions within a few days of the beginning of treatment. However, the viral load in the CSF determined by TaqMan-based quantitative real-time PCR (SRL Inc.), which dropped temporarily, increased again after 4 weeks from the initiation of ACV treatment (Fig. 1A) without obvious deterioration in clinical symptoms. Because the standard dose of ACV was given and drugs which have antagonistic effects for ACV were not used, we assumed that an ACV-resistant HSV-1 strain had developed. The ACV concentration in the CSF was not measured. Foscarnet, an antiviral drug recommended for treatment of ACV-resistant HSV infections (2), was not immediately available. Therefore, vidarabine

(15 mg/kg/day) was added to the therapeutic regimen from the fifth week of the treatment course. Subsequently, HSV-DNA in the CSF decreased to a level that was finally undetectable; hence, the antiviral drug treatment was terminated. Because virus isolation from the CSF of the patient was unsuccessful, as is common in herpes encephalitis cases (3), we could not perform a plaque reduction assay to test the *in vitro* inhibition concentration of ACV. Neuroimaging showed residual necrotic changes of the bilateral temporal lobes. Unfortunately, neurodevelopmental sequelae remained in this patient.

To reveal the mechanism of the clinical ACV resistance, sequencing analysis of the viral thymidine kinase (*vTK*) gene was conducted using the CSF samples collected at two different time points. As denoted by the arrows in Fig. 1A, sample 1 and sample 2 were collected before the initiation of and at the 5th week of ACV treatment, respectively. Full-length *vTK* genes were successfully amplified from both samples by a previously reported nested PCR method (4). By direct sequencing, one nucleotide substitution, G375T, leading to a Q125H amino acid substitution was detected. CSF sample 2 contained a mixture of *vTK* genes with and without this mutation (Fig. 1B). To examine whether or not this mutation induced HSV-1 ACV resistance, further analysis was conducted.

The analysis was performed according to a method developed by our group (4). The concept for the novel assay system is to assess the sensitivity of the HSV-1 to ACV and other *vTK*-associated drugs by measuring the replication capacity of the *vTK*-deficient and highly ACV-resistant HSV-1 TAR strain (5) in 293T cells expressed with the recombinant *vTK* protein of the HSV-1 strain of interest. In this study, *vTK* expression plasmid vectors were constructed using pTARGET (Promega, Madison, WI). A *vTK* expression plasmid without the G375T mutation, which was inserted with the *vTK* PCR product from sample 1, was constructed

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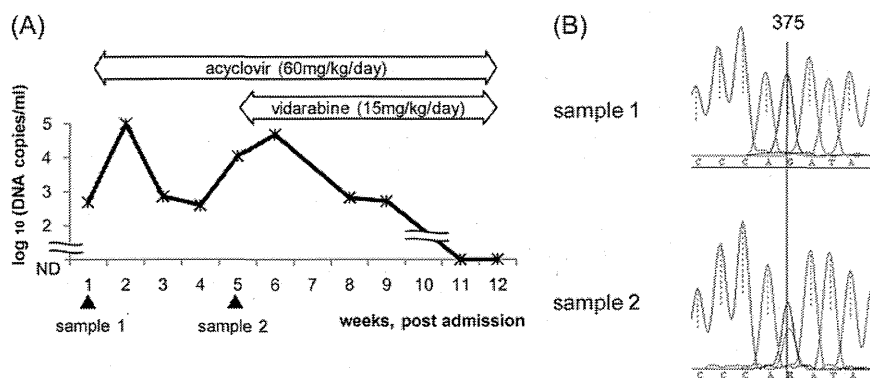


FIG 1 (A) Sequential changes in the HSV-1 DNA level in the CSF determined by quantitative real-time PCR. Arrows below the chart represent the time points of the CSF sample collection for sequencing analysis. ND, not detected. (B) Chromatogram of the *vTK* genes amplified from sample 1 and sample 2. A nucleotide substitution (G375T) was found in sample 2 (lower panel).

and designated *vTK*-375G. Subsequently, a G375T-mutant *vTK* expression plasmid was constructed by site-directed mutagenesis using the following primers, reagents, and PCR conditions and designated *vTK*-375T. Primers 5'-ATATAACAATGGGCATGCC TTATGCC and 5'-GGGCGCTTGTCAATTACCAC were designed for the inverse PCR (the underlined T is the targeted nucleotide), and *vTK*-375G was used as the template. The reaction was performed using a PrimeSTAR GXL DNA polymerase kit (TaKaRa Bio, Otsu, Japan), and the amplification conditions included an initial denaturation step of 2 min at 94°C, followed by 10 cycles of 10 s at 98°C, 15 s at 55°C, and 7 min at 68°C. Digestion and self-ligation were performed with a KOD mutagenesis kit (Toyobo, Osaka, Japan). G375T substitution without other nucleotide changes was confirmed by sequencing analysis. The expression plasmid for the ACV-sensitive HSV-1 *vTK* TAS strain (5) was constructed as a positive control and designated *vTK*-TAS. Empty pTARGET served as a negative control. In the final stage of the assay, the titers of the replicated TAR were determined by the standard plaque assays. Then, $\Delta\log_{10}$ PFU values were calculated as follows: $\Delta\log_{10}$ PFU = \log_{10} (PFU per milliliter of the replicated TAR at each ACV concentration) - \log_{10} (PFU per milliliter of the replicated TAR at the ACV concentration of 0 μ g/ml). This value represents the inhibitory effect of ACV on TAR replication, which is brought about by the transfection. Thus, the higher the value is, the lower the activity of the expressed *vTK*. In this way, the *vTK*-related resistance of HSV-1 can be judged from the $\Delta\log_{10}$ PFU values. The sensitivities to ganciclovir (GCV; Sigma-Aldrich Chemical Company, St. Louis, MO), penciclovir (PCV; Wako), and brivudine (BVDU; Sigma-Aldrich) were also tested in the same way.

TAR replication in 293T cells transfected with a negative control was not affected by any concentrations of any antiviral compounds (Fig. 2). When ACV was used, $\Delta\log_{10}$ PFU values elicited by *vTK*-375G transfection were at almost the same level as those elicited by *vTK*-TAS transfection, indicating that HSV-1 in sample 1 and TAS had nearly equal levels of sensitivity to ACV. However, $\Delta\log_{10}$ PFU values elicited by *vTK*-375T transfection were significantly higher than those elicited by *vTK*-375G transfection (Welch's *t* test; $P = 0.004$, <0.001 , and $= 0.045$, at ACV concentrations of 0.4, 4, and 40 μ g/ml, respectively), indicating that the HSV-1 with the G375T mutation in the *vTK* gene had acquired ACV resistance (Fig. 2A). When GCV, PCV, and BVDU were

used, transfection of *vTK*-375G, *vTK*-375T, and *vTK*-TAS resulted in almost the same level of $\Delta\log_{10}$ PFU values (Fig. 2B to D). The HSV-1 G375T mutant was therefore considered to be sensitive to these drugs.

To our knowledge, this is the first report of a patient with ACV-resistant neonatal HSV-1 disease. Neonatal HSV infection is estimated to occur in 1 in every 3,500 to 5,000 deliveries (6). Approximately 30% of the patients are diagnosed as having NHE (7). Although the introduction of ACV has significantly improved the prognosis, NHE is still a severe disease with a mortality rate of 6%, and 70% of the survivors suffer from moderate-to-severe neurological abnormalities (7, 8). ACV-resistant HSV mainly threatens immunocompromised patients, and the prevalence among them is reported to range from 3.5% to 10%. In immunocompetent individuals, the prevalence of ACV-resistant HSV is far lower, ranging from 0.1% to 0.7% (2). Neonatal ACV-resistant HSV infections are quite rare, and all the cases previously described have been caused by ACV-resistant HSV-2 (9–11).

The present study also showed for the first time that a Q125H amino acid substitution in the *vTK* polypeptide induces ACV resistance. Using a method previously described (4), it was confirmed that the Q125H mutation was not a part of natural polymorphism. Q125 of HSV-1 TK has been shown to be located above the nucleotide binding pocket in the three-dimensional (3D) structure of the *vTK* protein (12). Several studies have shown that substitution of Q125 to other amino acids changes *vTK* activity; Q125E and Q125L are associated with resistance to ACV, and Q125N leads to hypersensitivity to ACV (13, 14). Interestingly, the Q125H mutation did not induce cross-resistance to GCV, PCV, and BVDU, suggesting that these drugs may be effective with respect to this specific mutant.

This study showed also for the first time a central nervous system infection caused by a virologically confirmed ACV-resistant HSV-1 strain. There is one report of a possibly ACV-resistant HSV-1 encephalitis adult patient (15). In that report, virus isolation from the CSF failed, but an amino acid substitution of R41H found in the *vTK* polypeptide was suspected to be responsible for the ACV resistance, although it has not been virologically confirmed whether the mutation confers ACV resistance. The method

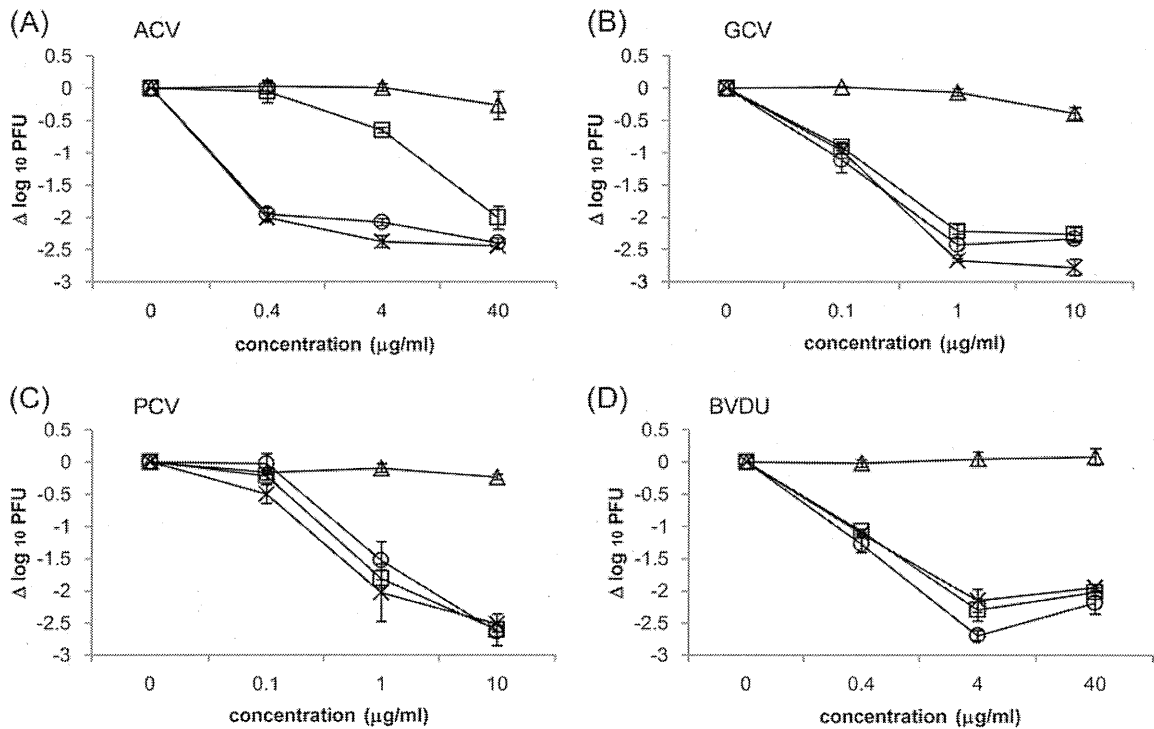


FIG 2 The inhibitory effects of antiviral compounds on replication of TAR in 293T cells transfected with each of the plasmids vTK-375G (\circ), vTK-375T (\square), vTK-TAS (\times), and empty pTARGET (Δ). Each experiment was performed in triplicate, and the error bars indicate standard deviations.

used in the present study may be suitable for such a condition. However, it should be kept in mind that the method can be applied only for vTK-related ACV resistance and not for DNA polymerase-related resistance.

ACV-resistant HSV is usually seen in patients with a history of ACV treatment (16). In the present case, administration of ACV may possibly have induced the appearance of the ACV-resistant HSV-1 strain. Although a recent study showed a benefit of oral ACV suppressive therapy for survivors of NHE (17), emergence of ACV-resistant HSV during the suppressive therapy was also reported (18). Thus, sensitivity of the causative HSV to antiviral drugs should be carefully monitored. This patient did not receive the suppressive therapy because he suffered from NHE before the beneficial effect of the therapy was reported.

It is recommended to repeat the lumbar puncture after 21 days from the initiation of ACV administration in the treatment of NHE (19): On the other hand, persistence of CSF HSV DNA is reported to be associated with poor neurodevelopmental outcomes of NHE patients (20). CSF HSV DNA quantification was conducted weekly in this patient to monitor the HSV-1 genome level in a real-time manner. We considered that the practice was beneficial, although further discussion is needed. In fact, the frequent monitoring enabled us to treat NHE with an appropriate choice of antiviral drugs.

In conclusion, ACV-resistant HSV-1 was virologically confirmed for the first time in a NHE patient. A nucleotide mutation, G375T in the HSV-1 TK gene, leading to a Q125H amino acid substitution, conferred ACV resistance.

Nucleotide sequence accession numbers. The vTK DNA sequence data have been deposited in the DNA Data Bank of Japan

(DDBJ) under accession no. AB713519 (CSF sample 1) and AB713520 (CSF sample 2).

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We have no conflicts of interest.

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REFERENCES

- Kimura H, Shibata M, Kuzushima K, Nishikawa K, Nishiyama Y, Morishima T. 1990. Detection and direct typing of herpes simplex virus by polymerase chain reaction. *Med. Microbiol. Immunol.* 179:177–184.
- Piret J, Boivin G. 2011. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob. Agents Chemother.* 55:459–472.
- Nahmias AJ, Whitley RJ, Visintine AN, Takei Y, Alford CA Jr. 1982. Herpes simplex virus encephalitis: laboratory evaluations and their diagnostic significance. *J. Infect. Dis.* 145:829–836.
- Shiota T, Lixin W, Takayama-Ito M, Iizuka I, Ogata M, Tsuji M, Nishimura H, Taniguchi S, Morikawa S, Kurane I, Mizuguchi M, Saijo M. 2011. Expression of herpes simplex virus type 1 recombinant thymidine kinase and its application to a rapid antiviral sensitivity assay. *Antiviral Res.* 91:142–149.
- Saijo M, Suzutani T, Itoh K, Hirano Y, Murono K, Nagamine M, Mizuta K, Niikura M, Morikawa S. 1999. Nucleotide sequence of thymidine kinase gene of sequential acyclovir-resistant herpes simplex virus type 1 isolates recovered from a child with Wiskott-Aldrich syndrome: evidence for reactivation of acyclovir-resistant herpes simplex virus. *J. Med. Virol.* 58:387–393.
- Whitley RJ. 1993. Neonatal herpes simplex virus infections. *J. Med. Virol.* 1993(Suppl 1):13–21.
- Kimberlin DW, Lin CY, Jacobs RF, Powell DA, Frenkel LM, Gruber WC, Rathore M, Bradley JS, Diaz PS, Kumar M, Arvin AM, Gutierrez K, Shelton M, Weiner LB, Sleasman JW, de Sierra TM, Soong SJ, Kiell

- J, Lakeman FD, Whitley RJ. 2001. Natural history of neonatal herpes simplex virus infections in the acyclovir era. *Pediatrics* 108:223–229.
8. Kimberlin DW, Lin CY, Jacobs RE, Powell DA, Corey L, Gruber WC, Rathore M, Bradley JS, Diaz PS, Kumar M, Arvin AM, Gutierrez K, Shelton M, Weiner LB, Slesman JW, de Sierra TM, Weller S, Soong SJ, Kiell J, Lakeman FD, Whitley RJ. 2001. Safety and efficacy of high-dose intravenous acyclovir in the management of neonatal herpes simplex virus infections. *Pediatrics* 108:230–238.
 9. Levin MJ, Weinberg A, Leary JJ, Sarisky RT. 2001. Development of acyclovir-resistant herpes simplex virus early during the treatment of herpes neonatorum. *Pediatr. Infect. Dis. J.* 20:1094–1097.
 10. Nyquist AC, Rotbart HA, Cotton M, Robinson C, Weinberg A, Hayward AR, Berens RL, Levin MJ. 1994. Acyclovir-resistant neonatal herpes simplex virus infection of the larynx. *J. Pediatr.* 124:967–971.
 11. Oram RJ, Marcellino D, Strauss D, Gustafson E, Talarico CL, Root AK, Sharma PL, Thompson K, Fingerroth JD, Crumpacker C, Herold BC. 2000. Characterization of an acyclovir-resistant herpes simplex virus type 2 strain isolated from a premature neonate. *J. Infect. Dis.* 181:1458–1461.
 12. Evans JS, Lock KP, Levine BA, Champness JN, Sanderson MR, Summers WC, McLeish PJ, Buchan A. 1998. Herpesviral thymidine kinases: laxity and resistance by design. *J. Gen. Virol.* 79(Pt 9):2083–2092.
 13. Kussmann-Gerber S, Kuonen O, Folkers G, Pilger BD, Scapozza L. 1998. Drug resistance of herpes simplex virus type 1—structural considerations at the molecular level of the thymidine kinase. *Eur. J. Biochem.* 255:472–481.
 14. Vogt J, Perozzo R, Pautsch A, Prota A, Schelling P, Pilger B, Folkers G, Scapozza L, Schulz GE. 2000. Nucleoside binding site of herpes simplex type 1 thymidine kinase analyzed by X-ray crystallography. *Proteins* 41: 545–553.
 15. Schulte EC, Sauerbrei A, Hoffmann D, Zimmer C, Hemmer B, Muhlau M. 2010. Acyclovir resistance in herpes simplex encephalitis. *Ann. Neurol.* 67:830–833.
 16. Chen Y, Scieux C, Garrat V, Socie G, Rocha V, Molina JM, Thouvenot D, Morfin F, Hocqueloux L, Garderet L, Esperou H, Selimi F, Devergie A, Leleu G, Aymard M, Morinet F, Gluckman E, Ribaud P. 2000. Resistant herpes simplex virus type 1 infection: an emerging concern after allogeneic stem cell transplantation. *Clin. Infect. Dis.* 31:927–935.
 17. Kimberlin DW, Whitley RJ, Wan W, Powell DA, Storch G, Ahmed A, Palmer A, Sanchez PJ, Jacobs RE, Bradley JS, Robinson JL, Shelton M, Dennehy PH, Leach C, Rathore M, Abughali N, Wright P, Frenkel LM, Brady RC, Van Dyke R, Weiner LB, Guzman-Cottrill J, McCarthy CA, Griffin J, Jester P, Parker M, Lakeman FD, Kuo H, Lee CH, Cloud GA. 2011. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N. Engl. J. Med.* 365:1284–1292.
 18. Kimberlin D, Powell D, Gruber W, Diaz P, Arvin A, Kumar M, Jacobs R, Van Dyke R, Burchett S, Soong SJ, Lakeman A, Whitley R. 1996. Administration of oral acyclovir suppressive therapy after neonatal herpes simplex virus disease limited to the skin, eyes and mouth: results of a phase I/II trial. *Pediatr. Infect. Dis. J.* 15:247–254.
 19. Kimberlin DW, Lakeman FD, Arvin AM, Prober CG, Corey L, Powell DA, Burchett SK, Jacobs RE, Starr SE, Whitley RJ. 1996. Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J. Infect. Dis.* 174: 1162–1167.
 20. Mejias A, Bustos R, Ardura MI, Ramirez C, Sanchez PJ. 2009. Persistence of herpes simplex virus DNA in cerebrospinal fluid of neonates with herpes simplex virus encephalitis. *J. Perinatol.* 29:290–296.

A Phenotypic Approach for IUIS PID Classification and Diagnosis: Guidelines for Clinicians at the Bedside

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Abstract The number of genetically defined Primary Immunodeficiency Diseases (PID) has increased exponentially, especially in the past decade. The biennial classification published by the IUIS PID expert committee is therefore quickly expanding, providing valuable information regarding the disease-causing genotypes, the immunological anomalies, and the associated clinical features of PIDs. These are grouped in eight, somewhat overlapping, categories of immune dysfunction. However, based on this immunological classification, the diagnosis of a specific PID from the clinician's observation of an individual clinical and/or immunological phenotype remains difficult,

especially for non-PID specialists. The purpose of this work is to suggest a phenotypic classification that forms the basis for diagnostic trees, leading the physician to particular groups of PIDs, starting from clinical features and combining routine immunological investigations along the way. We present 8 colored diagnostic figures that correspond to the 8 PID groups in the IUIS Classification, including all the PIDs cited in the 2011 update of the IUIS classification and most of those reported since.

Keywords Primary immunodeficiency · classification · IUIS · diagnosis tool

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Abbreviations

αFP	Alpha- fetoprotein	EDA-ID	Anhidrotic ectodermal dysplasia with immunodeficiency
Ab	Antibody	EO	Eosinophils
AD	Autosomal dominant inheritance	FA	Frequency of attacks
ADA	Adenosine deaminase	FCAS	Familial cold autoinflammatory syndrome
Adp	Adenopathy	FISH	Fluorescence in situ hybridization
AIHA	Auto-immune hemolytic anemia	GI	Gastrointestinal
AML	Acute myeloid leukemia	Hib	<i>Haemophilus influenzae</i> serotype b
Anti PSS	Anti- pneumococcus polysaccharide antibodies	HIDS	Hyper IgD syndrome
AR	Autosomal recessive inheritance	HIES	Hyper IgE syndrome
BL	B lymphocyte	HIGM	Hyper Ig M syndrome
CAPS	Cryopyrin-associated periodic syndromes	HLA	Human leukocyte antigen
CBC	Complete blood count	HSM	Hepatosplenomegaly
CD	Cluster of differentiation	Hx	Medical history
CGD	Chronic granulomatous disease	Ig	Immunoglobulin
CID	Combined immunodeficiency	IL	Interleukin
CINCA	Chronic infantile neurologic cutaneous and articular syndrome	LAD	Leukocyte adhesion deficiency
FCM*	Flow cytometry available	MKD	Mevalonate kinase deficiency
CMML	Chronic myelo-monocytic leukemia	MSMD	Mendelian susceptibility to mycobacteria disease
CNS	Central nervous system	MWS	Muckle-Wells syndrome
CVID	Common variable immunodeficiency disorders	N	Normal, not low
CT	Computed tomography	NK	Natural killer
CTL	Cytotoxic T-lymphocyte	NKT	Natural killer T cell
DA	Duration of attacks	NN	Neonate
Def	Deficiency	NOMID	Neonatal onset multisystem inflammatory disease
DHR	DiHydroRhodamine	NP	Neutropenia
Dip	Diphtheria	PAPA	Pyogenic sterile arthritis pyoderma gangrenosum, Acne syndrome
EBV	Epstein-barr virus	PMN	Neutrophils
EDA	Anhidrotic ectodermal dysplasia	PT	Platelet
		SCID	Severe combined immune deficiencies

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Sd	Syndrome
SLE	Systemic lupus erythematosus
SPM	Splenomegaly
Subcl	IgG subclass
TCR	T-cell receptor
Tet	Tetanus
TL	T lymphocyte
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome
WBC	White blood cells
XL	X-linked

Introduction

Primary Immunodeficiency Diseases (PID) comprise at least 200 genetically-defined inborn errors of immunity

[1–3]. The International Union of Immunological Societies (IUIS) PID expert committee has proposed a PID classification [1], which facilitates clinical care and clinical research studies world-wide; it is updated every other year to include new information. The PIDs are grouped into eight categories based on the principal mechanism in each disease, though if more than one mechanism is involved, there are diseases that could appear in more than one category. For each individual PID, the genotype, immunological and clinical phenotypes are briefly described. Since the number of disorders is quickly increasing every year [4–6], at an even faster pace since the advent of next-generation sequencing, the classification and these tables are therefore cumbersome. They offer limited assistance to most physicians at the bedside, especially those outside the field of PIDs and those in training; clinicians in regions

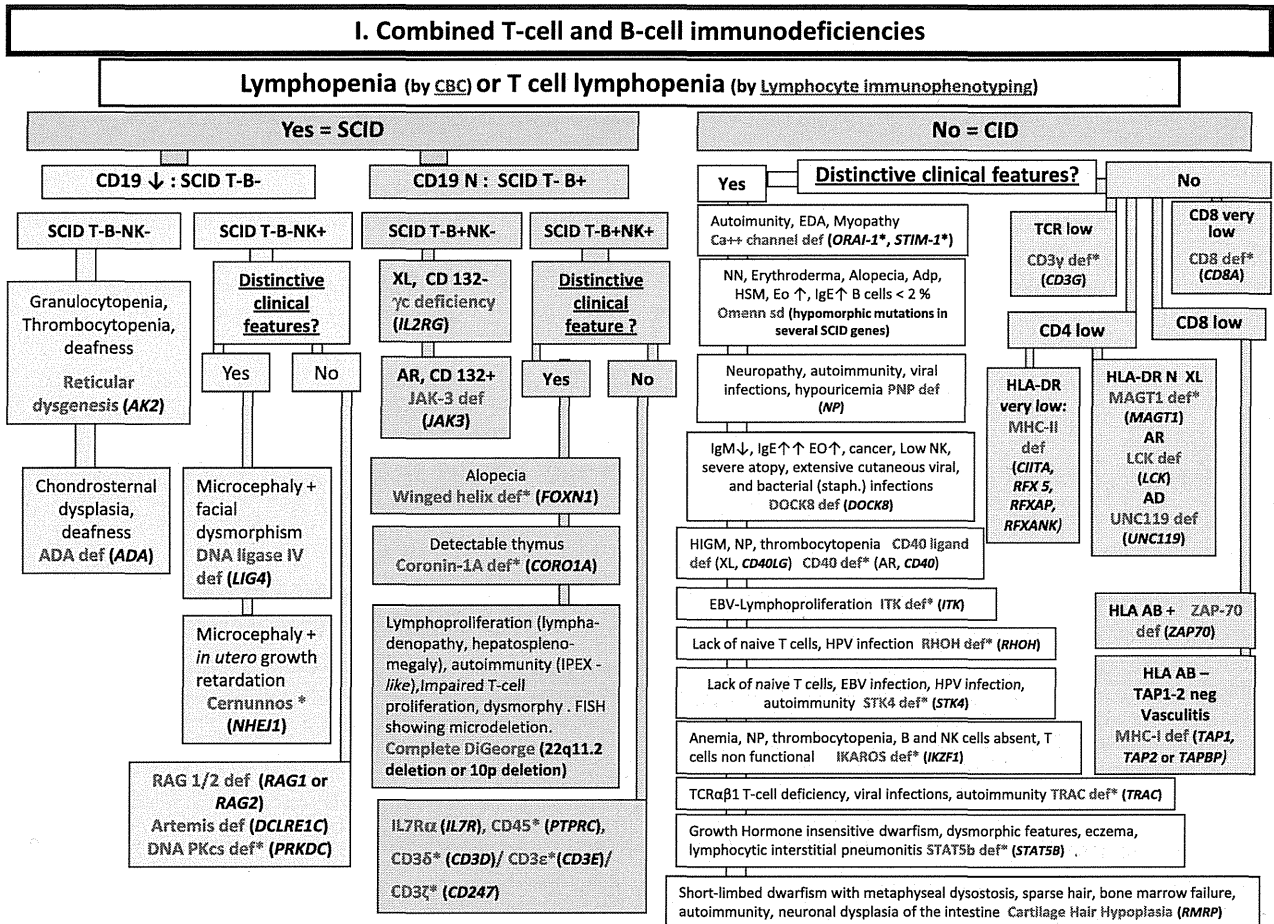


Fig. 1 Combined T- and B- cell immunodeficiencies. ADA: Adenosine Deaminase; Adp: adenopathy; AIHA: Auto-Immune Hemolytic Anemia; AR: Autosomal Recessive inheritance; CBC: Complete Blood Count; CD: Cluster of Differentiation; CID: Combined Immunodeficiency; EBV: Epstein-Barr Virus; EDA: Anhidrotic ectodermal dysplasia; EO: Eosinophils;

FISH: Fluorescence in situ Hybridization; HIGM: Hyper IgM syndrome; HLA: Human Leukocyte Antigen; HSM: Hepatosplenomegaly; Ig: Immunoglobulin; N: Normal, not low; NK: Natural Killer; NN: Neonate; NP: Neutropenia; PT: Platelet; SCID: Severe Combined ImmunoDeficiency; TCR: T-Cell Receptor; XL: X-Linked

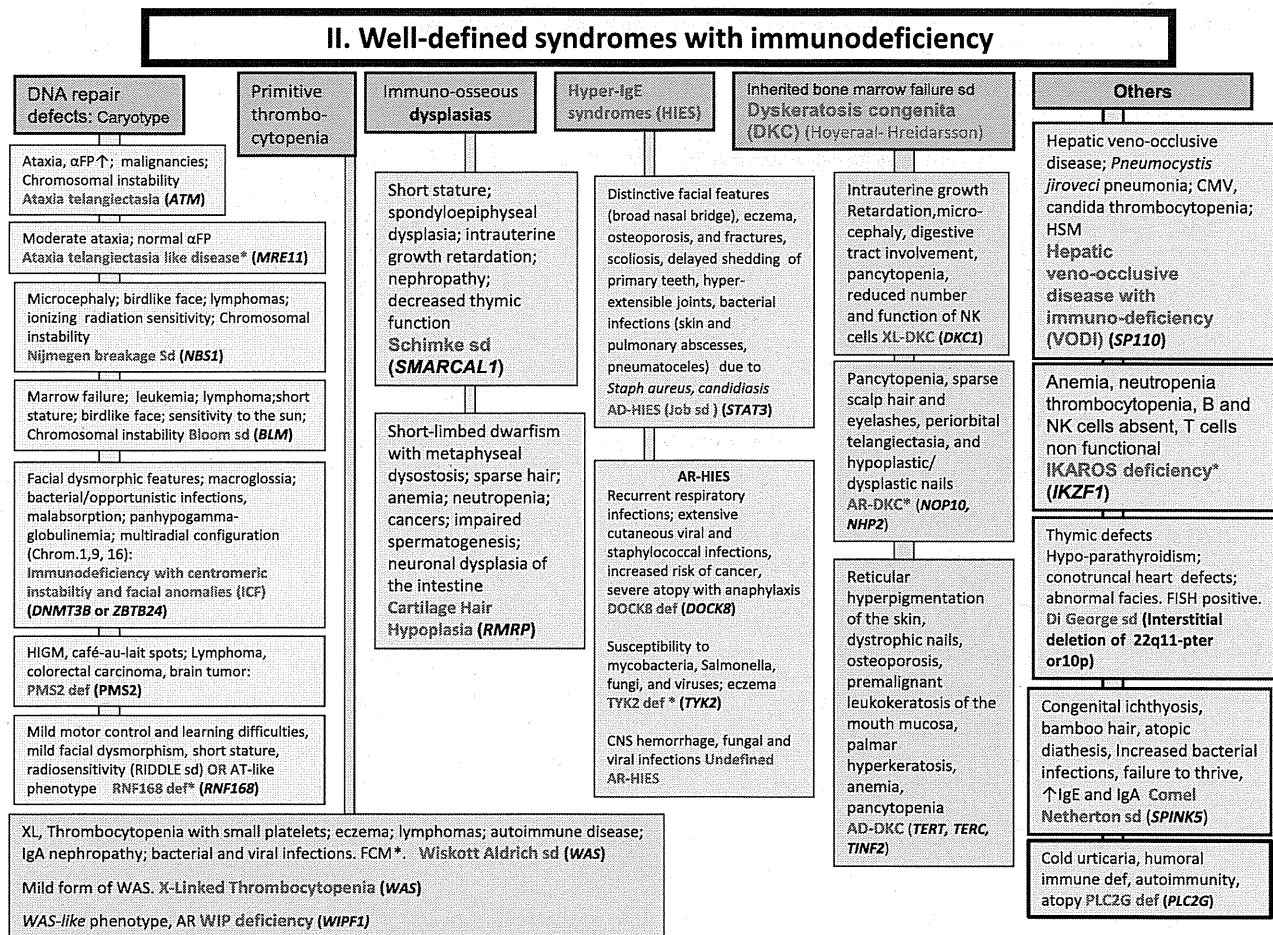


Fig. 2 Well-defined syndromes with immunodeficiencies. These syndromes are generally associated with T-cell immunodeficiency. α FP: alpha-fetoprotein; AD: Autosomal Dominant inheritance; AR: Autosomal Recessive inheritance; CNS: Central Nervous

System; FCM*: Flow cytometry available; FISH: Fluorescence in situ Hybridization; HSM: Hepatosplenomegaly; Ig: Immunoglobulin; NK: Natural Killer; XL: X-Linked inheritance

of the world where awareness for PIDs is limited may also find the tables tricky.

Patients with a PID may first present to many types of medical and surgical disciplines and this is likely to be increasingly common given the growing number of patients with known or suspected PIDs [7]. Such physicians, who may lack familiarity with PIDs, need a classification that is based on a clinical and/or biological phenotype that they observe. This prompted IUIS PID experts to work on a simplified classification, based on simple clinical and immunological phenotypes, in order to provide some easy-to-follow algorithms to diagnose a particular PID or group of PIDs. This will optimize collaboration between primary centers and specialized centers, particularly for genetic

studies, and will lead to faster and more precise molecular diagnosis and genetic counseling, paving the way to more appropriate management of affected patients and families. This work presents a user-friendly classification of PIDs, providing a tree-based decision-making process based on the observation of clinical and biological phenotypes.

Methodology

We included all diseases from the 2011 update of IUIS PID classification [1]. To stay up-to-date, we also included new diseases described in the last 2 years [2]. However, there may be other genes associated with

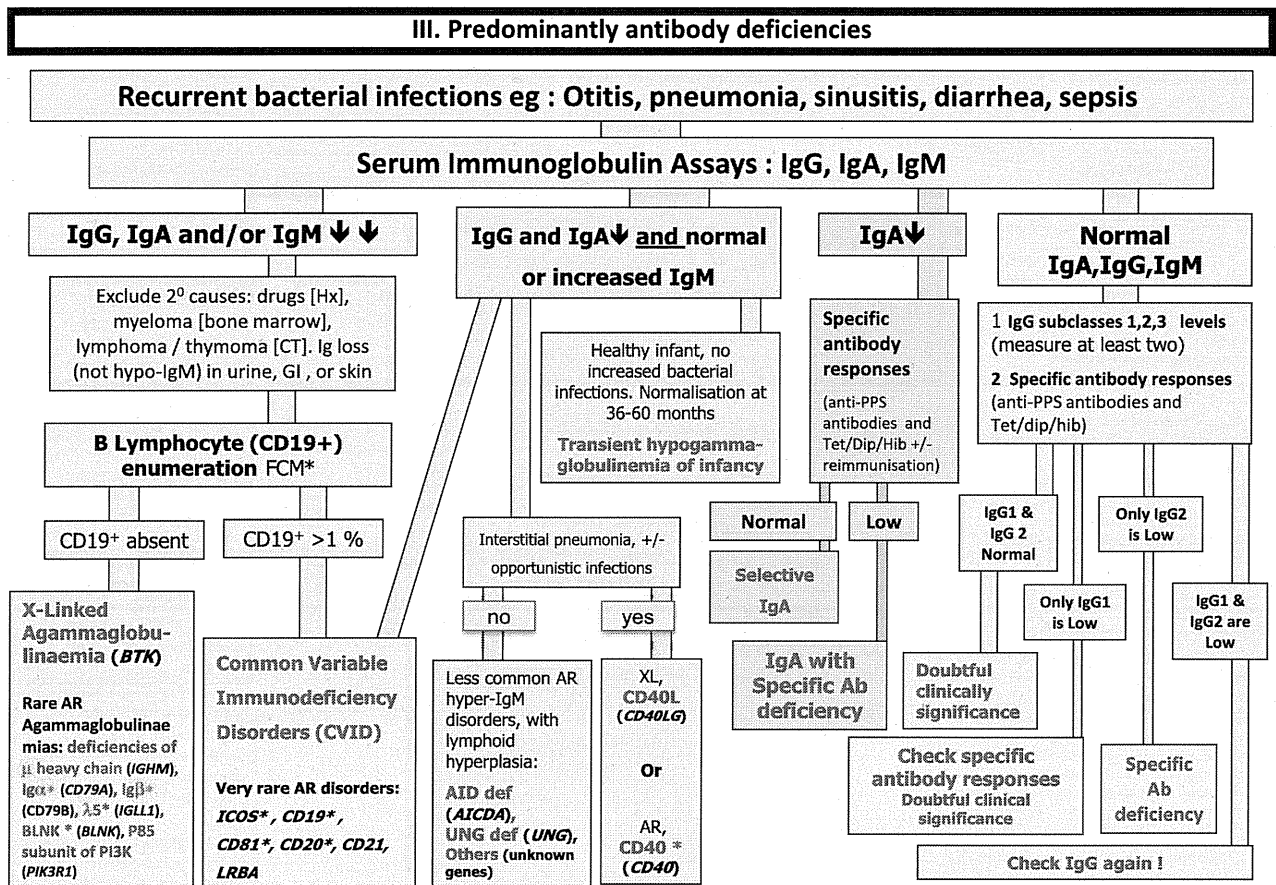


Fig. 3 Predominantly antibody deficiencies. Ab: Antibody; Anti PPS: Anti- pneumococcal polysaccharide antibodies; AR: Autosomal Recessive inheritance; CD: Cluster of Differentiation; CVID: Common Variable Immunodeficiency Disorders; CT:

Computed Tomography; Dip: Diphtheria; FCM*: Flow cytometry available; GI: Gastrointestinal; Hib: *Haemophilus influenzae* serotype b; Hx: medical history; Ig: Immunoglobulin; subcl: IgG subclass; Tet; Tetanus; XL: X-Linked inheritance

PIDs that are not included here to be faithful to our inclusion criteria. An algorithm was assigned to each of the eight main groups of the classification. We used the same color for each group of similar conditions. Disease names are written in red. As in the IUIS Classification, an asterisk is added to highlight extremely rare disorders (less than 10 cases reported in the medical literature). These algorithms were first established by a small committee; then validated by one or two experts for each figure.

Results

A classification validated by the IUIS PID expert committee is presented in Figs. 1, 2, 3, 4, 5, 6, 7 and 8.

Discussion

These figures are diagnostic tools that represent a modified and simplified version of the 2011 IUIS classification [1]. They are based on patients' clinical and biological phenotypes and are mostly presented as decision trees for diagnostic orientation. These figures serve as diagnostic orientation tools for the typical forms of PID; the more atypical presentations of PIDs are not covered in these figures. These figures do not therefore aim to replace decisional trees or diagnostic protocols proposed by other teams or scientific societies [8–11]. Rather they aim at being a user-friendly first approach to the IUIS classification [1]. These figures enable non-PID specialists to select the most appropriate diagnostic tree and to undertake some preliminary

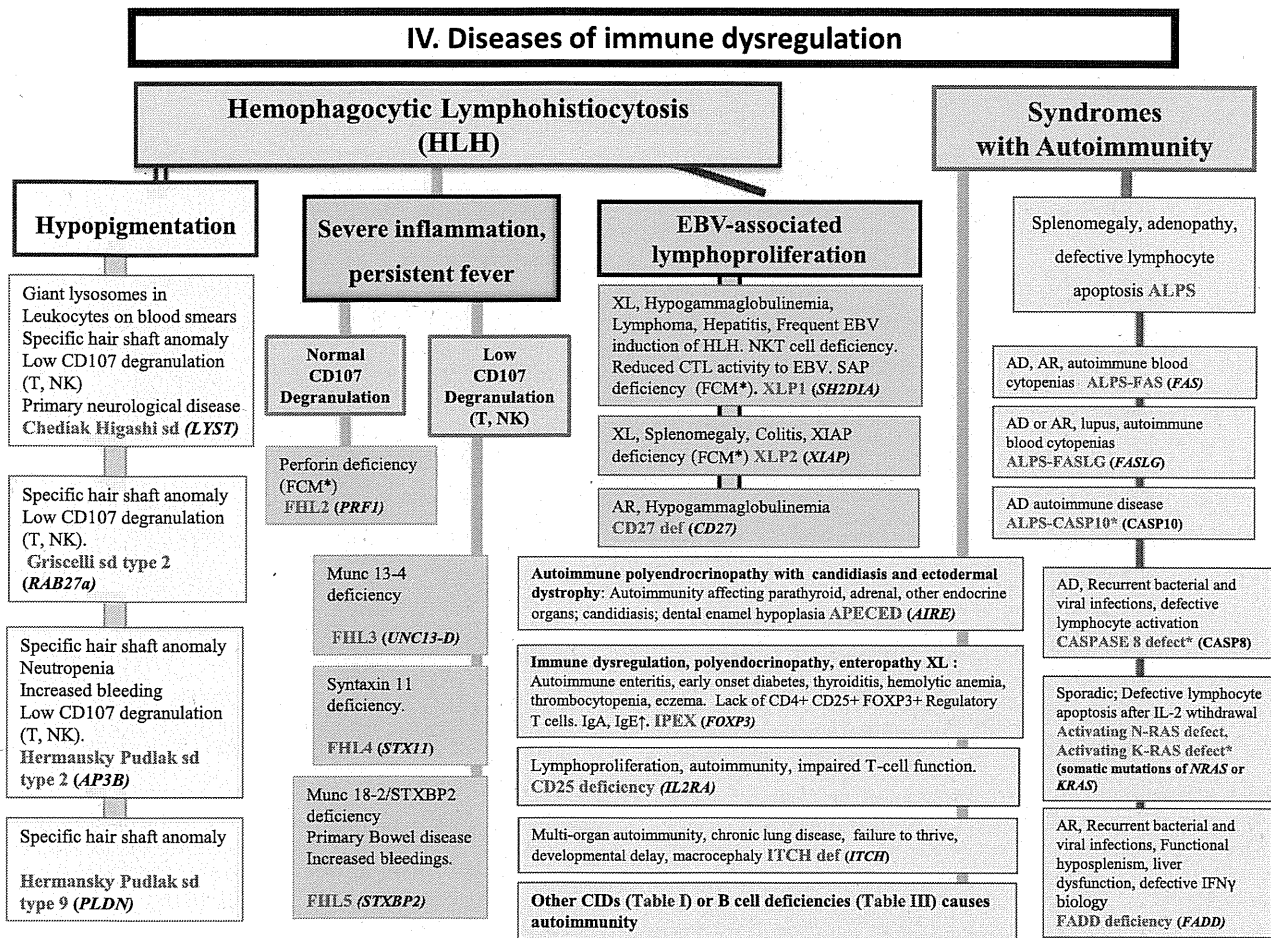


Fig. 4 Diseases of immune dysregulation. AD: Autosomal Dominant inheritance; AR: Autosomal Recessive inheritance; CD: Cluster of Differentiation; CTL: Cytotoxic T-Lymphocyte; EBV: Epstein-Barr Virus; FCM*: Flow cytometry available; HSM:

Hepatosplenomegaly; Ig: Immunoglobulin; IL: interleukin; NK: Natural Killer; NKT: Natural Killer T cell; TL: T lymphocyte; XL: X-Linked inheritance

investigations, whilst contacting an expert in PIDs. They may also help in the selection of the center or expert to whom the patient should be referred, given the patient's particular phenotype. In all cases, whether a tentative diagnosis can be made based on these figures or not, we recommend that the practitioner outside the field who sees a patient with a possible PID seeks specialist advice.

To simplify our figures, we did not systematically include all data from the IUIS classification (OMIM number, presumed pathogenesis, affected cells or function...) [1]. In order to present the 24 pages from the IUIS classification in only 8 figures, we used common abbreviations familiar to most physicians (explained in footnotes). The use of a color code makes these figures

easy to follow, so that they could be hung, in larger format, in clinical wards. This is also suitable for informing young clinicians and students.

To make these figures easier to use by clinicians and biologists, we highlighted the clinical and biological features, adding to the data from the IUIS classification some other features typical of the PID in question. This allows an initial orientation towards a particular disease or group of diseases. Whenever it was possible, we have focused on clinical or routine laboratory features that distinguish disorders that are closely related. Example: A female infant with an opportunistic infection in whom lymphocyte subpopulation investigation reveals profound CD3 and CD16/56 lymphopenia without CD19/20 lymphopenia

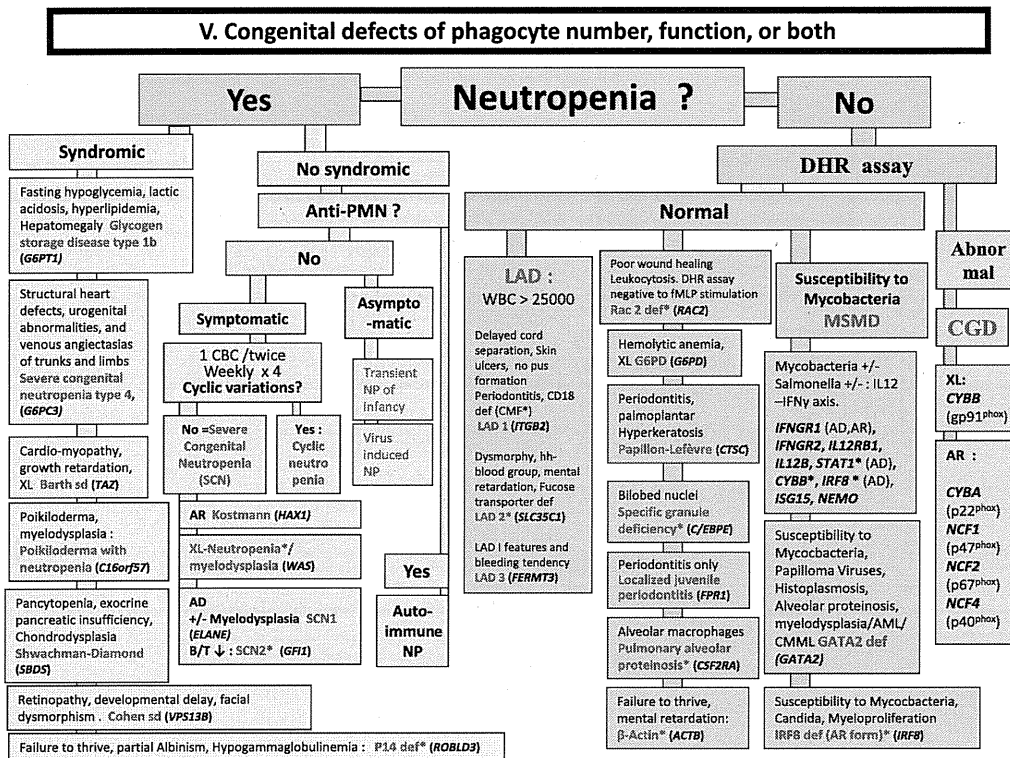


Fig. 5 Congenital defects of phagocyte number, function, or both. For DHR assay, the results can distinguish XL-CGD from AR-CGD, and gp40pHox defect from others AR forms. AD: Autosomal Dominant inheritance; AML: Acute Myeloid Leukemia; AR: Autosomal Recessive inheritance; CBC: Complete Blood Count; CD: Cluster of

Differentiation; CGD: Chronic Granulomatous Disease; CMML: Chronic Myelo-monocytic Leukemia; DHR: DiHydroRhodamine; LAD: Leukocyte Adhesion Deficiency; MSMD: Mendelian Susceptibility to Mycobacteria Disease; NP: Neutropenia; PNN: Neutrophils; WBC: White Blood Cells; XL: X-Linked inheritance

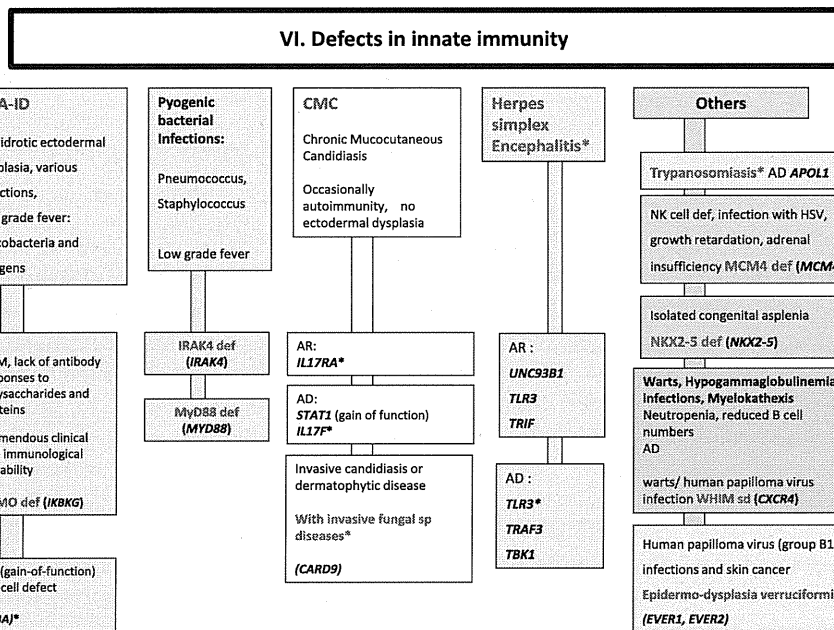


Fig. 6 Defects in innate immunity. AD: Autosomal Dominant inheritance; AR: Autosomal Recessive inheritance; BL: B lymphocyte; EDA-ID: Anhidrotic Ectodermal Dysplasia with Immunodeficiency; Ig: Immunoglobulin; PNN: Neutrophils; XL: X-Linked inheritance

VII. Auto-inflammatory disorders.

Usual age at onset

Neonatal		Infancy	Childhood / Early Adult	
AR DA : Continuous FA : Continuous Sterile multifocal osteomyelitis, Folliculitis. IL1: Unopposed effect Deficiency of IL-1 Receptor Antagonist (DIRA)* (<i>IL1RN</i>)	AD DA : Continuous, often worse in the evenings FA : Often daily Ethnic group : North European Urticaria , Deafness, Conjunctivitis Amyloidosis. Muckle Wells syndrome (CAPS) (<i>NLRP3</i>)	AR DA : > 3–7 days FA : 1–2 monthly Cervical adenopathy Oral aphthosis. Diarrhea Elevated IgD and IgA, acute phase response and mevalonate aciduria during attacks MKD (HIDS) (<i>MVK</i>)	AR DA : 1–4 days. FA : Variable. Polyserositis, Abdominal pain, Arthritis, Amyloidosis Colchicine-responsive +++ Erysipelas-like erythema Marked acute-phase response during attacks Familial Mediterranean Fever (FMF) (<i>MEFV</i>)	AD DA : 1–4 weeks FA : Variable, continuous Serositis, rash, Periorbital edema and conjunctivitis; Amyloidosis. Acute-phase response during attacks. Low levels of soluble TNF-R1 when well TRAPS (<i>TNFRSF1A</i>)
AR DA : Few days FA : 1-3 / month Chronic recurrent Multifocal osteomyelitis, severe pain, tender soft tissue swelling, Transfusion-dependent anemia, MAJEED* (<i>LPIN2</i>)	AR Early onset enterocolitis, Enteric fistulas, Perianal abscesses, Chronic folliculitis. ↑ TNFα EOIBD: Early onset inflammatory bowel disease (<i>IL10 / IL10R</i>)	AD , Sporadic DA : Continuous FA : Continuous Urticarial rash. Aseptic and chronic meningitis Deforming arthropathy Sensorineural deafness Mental retardation Visual loss. Acute-phase response most of the time CINCA (NOMID, CAPS) (<i>NLRP3</i>)	AD DA : 24-48 H Cold exposure. Non pruritic urticaria, arthritis, chills Conjunctivitis. Familial Cold Autoinflammatory Syndrome (CAPS) (<i>NLRP3</i>)	AD DA : 5 days FA : Fixed interval :4-6 weeks Sterile pyogenic oligo-arthritis, Pyoderma gangrenosum, Myositis. Acute-phase response during attacks PAPA (<i>PSTPIP1</i>)
AD, DA : Continuous. FA : Continuous. Uveitis, Granulomatous synovitis, Campodactyly, Rash, Cranial neuropathies, Crohn disease. Sustained modest acute-phase response BLAU syndrome (<i>NOD2</i>)				
Others : 1- AR , early-onset pustular dermatitis, short and broken hair, paronychia, frequent cutaneous bacterial infections, and diarrhea , high IL-1 and IL-6 production. Lack of TNF-α was considered partly responsible for their increased susceptibility to infection and development of cardiomyopathy. Inflammatory skin and bowel disease (<i>ADAM17</i>) 2- AR , life-threatening, multisystemic inflammatory disease characterized by episodic widespread, diffuse erythematous pustular rash associated with high fever, malaise, and leukocytosis. Generalized pustular psoriasis (<i>IL-36Ra</i>)				

Fig. 7 Autoinflammatory disorders. AD: Autosomal Dominant inheritance; AR: Autosomal Recessive inheritance; CAPS: Cryopyrin-Associated Periodic syndromes; CINCA: Chronic Infantile Neurologic Cutaneous and Articular syndrome; DA: Duration of Attacks; FA: Frequency of Attacks; FCAS: Familial Cold Autoinflammatory Syndrome; HIDS: Hyper IgD syndrome; Ig: Immunoglobulin; IL: interleukin; MKD: Mevalonate Kinase deficiency; MWS: Muckle-Wells syndrome; NOMID: Neonatal Onset Multisystem Inflammatory Disease; PAPA: Pyogenic sterile Arthritis, Pyoderma gangrenosum, Acne syndrome; SPM: Splenomegaly; TNF: Tumor Necrosis Factor; TRAPS: TNF Receptor-Associated Periodic Syndrome

has a SCID T-B+NK- phenotype, which strongly suggests Jak3 deficiency (Fig. 1). After discussion with a team specialized in the diagnosis and treatment of SCID patients, an analysis of the *JAK3* gene will be arranged as a priority, while expert advice will be given on the appropriate management for the infant.

Though atypical forms of PID are increasingly reported in the literature [12–15], typical presentations of these conditions remain predominant, permitting this classification to be useful in most of cases. Moreover, the genetic heterogeneity of most PIDs is high and patients with almost any PID may lack coding mutations in known disease-causing genes. This manuscript will therefore be up-dated

every other year along with the IUIS classification. Meanwhile, we hope that this phenotypic approach to diagnosis of PID can constitute a useful tool for physicians or biologists from various related specialties, especially in the setting of pediatric and adult medicine (internal medicine, pulmonology, hematology, oncology, immunology, infectious diseases, etc...) who may encounter the first presentation of PID patients.

Conclusion

The strengths of this algorithmic approach to the diagnosis of PID are its simplified format, reliance on phenotypic

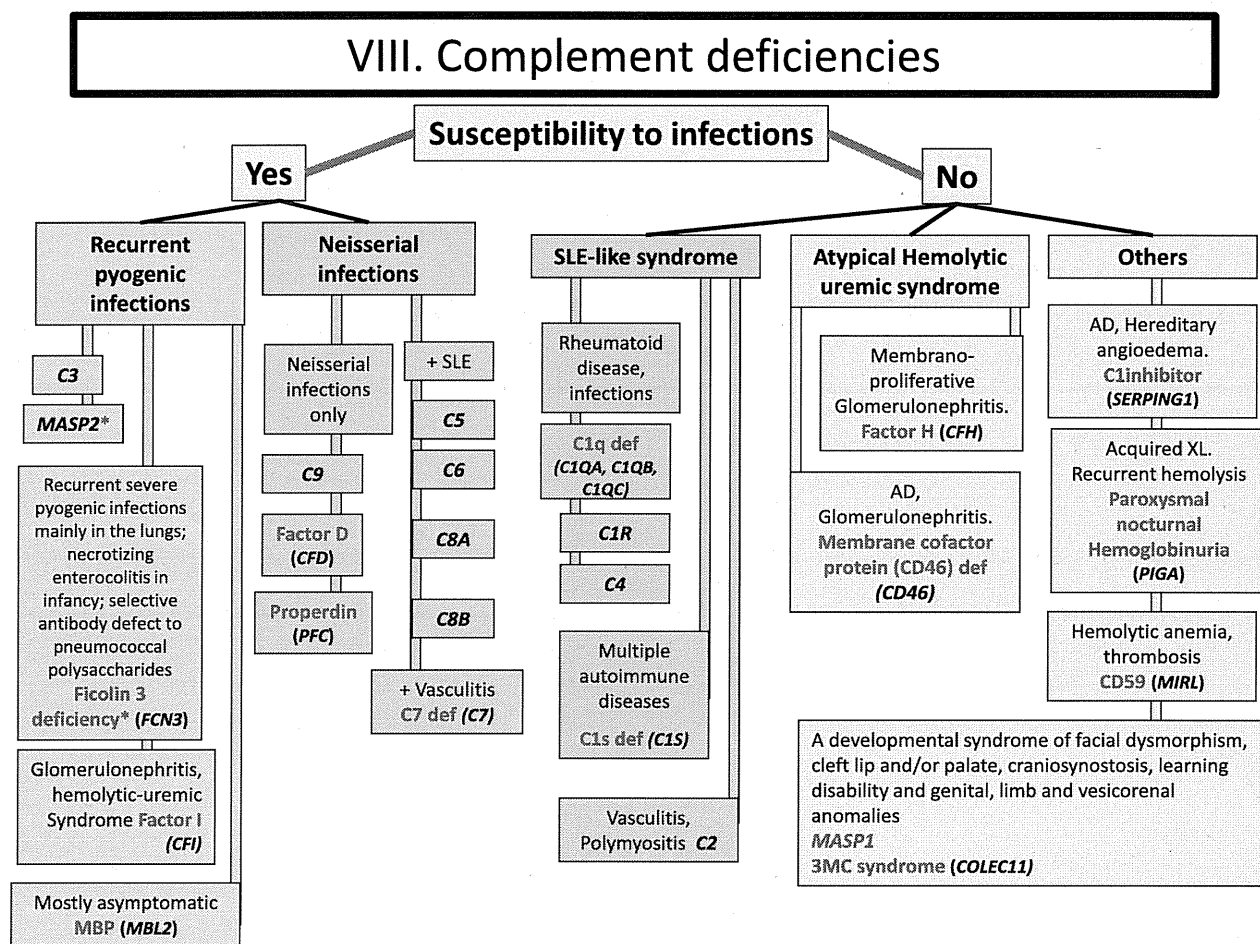


Fig. 8 Complement deficiencies. Def: deficiency; SLE: Systemic Lupus Erythematosus

features, presentation in user-friendly pathways, and validation by a group of PID experts. We hope they will be useful to physicians at the bedside in several areas of pediatrics, internal medicine, and surgery. While these algorithms cannot be comprehensive, due to the tremendous genetic and phenotypic heterogeneity of PIDs, they will be improved over time with progress in the field as well as by feedback from users. They will also be expanded with the discovery of new PIDs and the refined description of known PIDs.

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References

- Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Front Immunol*. 2011;2:54.
- Parvaneh N, Casanova JL, Notarangelo LD, Conley ME. Primary immunodeficiencies: a rapidly evolving story. *J Allergy Clin Immunol*. 2013;131(2):314–23.
- Ochs HD, Smith CIE, Puck JM. Primary immunodeficiency diseases: a molecular & cellular approach. 2nd ed. New York: Oxford University Press; 2007.
- Casanova JL, Abel L. Primary immunodeficiencies: a field in its infancy. *Science*. 2007;317:617–9.
- Notarangelo LD, Casanova JL. Primary immunodeficiencies: increasing market share. *Curr Opin Immunol*. 2009;21:461–5.
- Conley ME, Notarangelo LD, Casanova JL. Definition of primary immunodeficiency in 2011: a “dialogue” among friends. *N Y Acad Sci*. 2011;1238:1–6.
- Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova JL, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. *J Clin Immunol*. 2012. doi:10.1007/s10875-012-9751-7.
- de Vries E, European Society for Immunodeficiencies (ESID) members. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. *Clin Exp Immunol*. 2012;167(1):108–19.
- Oliveira JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. *J Allergy Clin Immunol*. 2010;125:S297–305.
- Admou B, Haouach K, Ailal F, Benhsaien I, Barbouch MR, Bejaoui M, et al. Primary immunodeficiencies: diagnosis

- approach in emergent countries (African Society for Primary Immunodeficiencies). *Immunol Biol Spec*. 2010;25(5–6):257–65.
11. Samarghitean C, Ortutay C, Vihinen M. Systematic classification of primary immunodeficiencies based on clinical, pathological, and laboratory parameters. *J Immunol*. 2009;183(11):7569–75.
 12. Perreault S, Bernard G, Lortie A, Le Deist F, Decaluwe H. Ataxia-telangiectasia presenting with a novel immunodeficiency. *Pediatr Neurol*. 2012;46(5):322–4.
 13. Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K, et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. *Haematologica*. 2010;95(12):2080–7.
 14. Pessach I, Walter J, Notarangelo LD. Recent advances in primary immunodeficiencies: identification of novel genetic defects and unanticipated phenotypes. *Pediatr Res*. 2009;65(5 Pt 2):3R–12.
 15. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, Abinun M, Abrahamsen TG, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. *Blood*. 2001;97(1):81–8.

B-cell function after unrelated umbilical cord blood transplantation using a minimal-intensity conditioning regimen in patients with X-SCID

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Abstract Patients with X-linked severe combined immunodeficiency (X-SCID) suffer from severe and persistent infections, and usually die early in life unless treated by hematopoietic stem cell transplantation. If a patient has an HLA-identical sibling donor, preparative conditioning is not necessary for T-cell engraftment and B-cell function. However, in the absence of such a donor, long-term reconstitution of full B-cell function is often problematic, leading in many cases to a lifetime requirement for immunoglobulin replacement therapy. Preparative myeloablative conditioning has been shown to improve long-term B-cell function, but may aggravate pre-existing infection and transplant-related toxicity. It is thus

important to determine the minimum intensity of conditioning that assures immunoglobulin production. In the present study, we performed reduced-intensity conditioning (RIC), consisting of fludarabine 125 mg/m² and melphalan 80 mg/m², prior to unrelated umbilical cord blood transplantation (UCBT) for five patients with X-SCID, none of them had an HLA-identical donor. Four patients survived more than 4 years without sequelae, and none required long-term immunoglobulin replacement therapy. One patient succumbed to sepsis in conjunction with severe GVHD. Our result demonstrates that the RIC regimen described above in combination with UCBT is an effective and less toxic conditioning to correct B-cell function in patients with X-SCID.

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Keywords X-SCID · Reduced-intensity conditioning · Umbilical cord blood transplantation · Fludarabine/melphalan

Introduction

X-linked severe combined immunodeficiency (X-SCID), which accounts for approximately half the cases of SCID, is caused by mutations of the γ c chain. Immunological characteristics of this disease include profound impairment of both cellular and humoral immunity due to the absence or diminished numbers of T cells and natural killer (NK) cells, and abnormal B-cell function in spite of normal or elevated numbers of B cells. Therefore, patients with X-SCID suffer from severe and persistent infections, including opportunistic pathogens, and usually die early in life unless treated by hematopoietic stem cell transplantation (HSCT) or gene therapy [1]. Previous reports demonstrated excellent results of HLA-identical BMT with

survival rate over 90 %, and full restoration of T- and B-cell function [2, 3]. Since most patients do not have an HLA-identical sibling donor, HLA-haploidentical bone marrow transplantation (BMT) was developed in the early 1980s, when better T-cell depletion methods became available. However, the survival rate was lower, at around 60–80 %, and about half of the patients required life-long immunoglobulin replacement therapy despite normal T-cell immunity with or without pre-transplant conditioning [2–4]. These results suggested that T-cell-depleted, HLA-haploidentical bone marrow cells might not be a suitable source of HSCT for correcting B-cell function. Another strategy to treat this condition is to use unrelated donor HSCT with conventional myeloablative conditioning regimens, which leads to stable reconstitution of T- and B-cell function [5]. However, this approach has been associated with significant treatment-related toxicities and aggravation of pre-existing infections. To avoid these problems, reduced-intensity conditioning (RIC) regimens have been developed. Recently, Rao et al. [6] reported that a RIC regimen using a total dose of 150 mg/m² of fludarabine, 140 mg/m² of melphalan, and Campath 1H or ATG resulted in an improved survival and reduced transplantation-related mortality, compared with myeloablative conditioning, in children with primary immunodeficiency (PID) undergoing unrelated BMT. They used the same regimen for patients with X-SCID who would be expected to need less intensive conditioning because their immune system is already profoundly impaired. Based on their report, we performed unrelated umbilical cord blood

transplantation (UCBT) with pre-transplant conditioning using a further reduced dosage of fludarabine and melphalan, in the absence of Campath 1H or ATG, to investigate whether a minimally intensive conditioning regimen could assure correction of B-cell function in X-SCID patients.

Patients and methods

Patients

Five patients with typical X-SCID received unrelated UCBT because they had no HLA-matched sibling donor. As shown in Table 1, mutations in the γ c chain gene were detected in all patients. Patient 3 suffered from pneumonia caused by *Pneumocystis jiroveci* at the time of diagnosis of X-SCID. All patients except for patient 3 were diagnosed with X-SCID at birth because their brothers had the same disease. Immunoglobulin replacement therapy was initiated once hypogammaglobulinemia was confirmed, and IgG trough levels were maintained over 500 mg/dL. This study was performed with the approval of Institutional Review Board at each university and with the written informed consents of the parents.

Conditioning regimen and GVHD prophylaxis

Pre-transplant conditioning for all patients consisted of fludarabine (25 mg/m² per day) from day -7 to day -3

Table 1 Patient characteristics

Patient	1	2	3	4	5
Age at diagnosis (months)	0	0	4	0	0
Age at UCBT (months)	3	3	10	3	3
Mutations in the γ c chain	868 G > A	691 G > A	c.735 741*	IVS4 + 2 T > A	568A > G
HLA identity	6/6	5/6	6/6	5/6	5/6
Nucleated cell dose ($\times 10^7$ /kg)	7.1	10.0	5.0	9.5	11.2
CD34+ cell dose ($\times 10^5$ /kg)	1.09	3.65	ND	3.50	1.68
Hematological recovery					
Nt > 500/ μ L	30	20	36	20	12
Plt > 5 $\times 10^4$ / μ L	10	16	95	17	16
Ret > 1 %	18	16	38	20	15
Complications at UCBT	None	None	Pneumonia	None	None
Additional infections during UCBT	None	None	Sepsis	CMV	Sepsis
GVHD					
Prophylaxis	CyA	FK + sMTX	FK	FK + sMTX	CyA + PSL
Acute (grade)	0	0	II	II	III
Chronic	–	–	Extensive	–	Extensive
Therapy	–	–	FK + mPSL	FK + PSL	FK + MMF + PSL

c.735 741* c.735_741delAGCCACC→insGGGAGCAACTACTT, ND not determined, Nt neutrophils, Plt platelets, Ret reticulocytes, sMTX short-term methotrexate

(total dose 125 mg/m²) and melphalan (40 mg/m² per day) from day -4 to day -3 (total dose 80 mg/m²). Neither ATG nor Campath 1H was included in the conditioning regimen.

Prophylaxis for acute GVHD was performed with either cyclosporine A (CyA) with/without prednisolone or FK506 with/without short-term methotrexate as shown in Table 1.

Graft characteristics

As shown in Table 1, UCB units were either serologically full-matched or one locus mismatched at 6/6 (A, B, DR) HLA loci. Infused nucleated cell doses were 5.0×10^7 /kg– 11.2×10^7 /kg (mean 8.6×10^7 /kg), which contained CD34+ stem cells, ranging from 1.09×10^5 /kg to 3.65×10^5 /kg (mean 2.48×10^5 /kg) except for patient 3, whose information on CD34+ cells was not available.

Chimerism studies

Hematological recovery was defined as achievement of absolute neutrophil count (ANC) >500/ μ L for 3 consecutive days and a platelet count > 5.0×10^4 / μ L for 7 consecutive days without need for further transfusion. Chimerism was analyzed at Human Leukocyte Antigen Laboratory (Kyoto, Japan) as described previously [7]. Briefly, T cells, B cells and NK cells were separated by anti-CD3, anti-CD19 and anti-CD56 microbeads (Invitrogen Dyanl AS, Oslo, Norway), respectively. Donor- and recipient-specific polymorphic short tandem repeats (STR) were amplified by PCR, and subsequently analyzed by SDS-PAGE.

Immunological reconstitution studies

Immunological reconstitution status after transplantation was monitored by serum immunoglobulin levels (IgG, IgA, IgM and IgE), isoheamagglutinin, and specific antibodies, and by flow cytometry analyses of peripheral mononuclear cells for CD3, CD4, CD8, CD19, CD16 and CD56.

Results

The age at transplantation was 3 months in four patients and 10 months in one patient (Table 1). All patients received UCBT using fludarabine (125 mg/m²) and melphalan (80 mg/m²) as a pre-transplant conditioning. They all achieved engraftment of ANC > 500 μ L and platelets > 5.0×10^4 / μ L at a mean of 23.6 days (range 12–36 days) and 30.8 days (range 10–95 days), respectively. All but one survived more than 4 years without complication. One patient, patient 5, succumbed to sepsis in conjunction with severe GVHD.

Infections

Patient 3 suffered pneumonia due to *P. jiroveci* infection prior to admission and intravenous trimethoprim/sulfamethoxazole therapy was initiated. The pneumonia resolved with the engraftment of donor cells. He also experienced an episode of sepsis due to enterococci after UCBT, which was cured by appropriate antibiotics. Patients 1, 2, 4, and 5 were diagnosed with X-SCID at birth by sequencing of the γ c chain because their brothers had the same disease. They had been protected in a clean environment soon after birth and they did not experience any infection until UCBT. Patient 5 developed sepsis due to a catheter infection, which was the cause of death at day 491 after UCBT.

Regimen-related toxicity and GVHD

Mild mucositis and myelosuppression were observed with this reduced-intensity conditioning, and no other regimen-related toxicity was noted.

Patient 3 developed acute GVHD grade II (skin stage 3) and extensive chronic GVHD, while patient 4 developed acute GVHD grade II (skin stage 3, liver stage 1 and gut stage 1). Symptoms in both cases resolved on prednisolone and FK506. Patient 5 developed acute GVHD grade III (skin grade 1, liver grade 3 and gut stage 3), followed by extensive chronic GVHD. He succumbed to sepsis in conjunction with uncontrolled GVHD, although he was treated with prednisolone, FK506 and mycophenolate mofetil (MMF).

Chimerism

Median follow-up was 68 months (range 48–73 months). As shown in Table 2, all survivors had complete donor T-cell chimerism. One survivor, patient 3, also had complete lymphocyte and granulocyte chimerism, which was confirmed by day 52. The others demonstrated mixed chimerism in these cell lineages. The percentage of the donor cells in each cell lineage had been stable since day 168 after UCBT in patient 1. In patients 2 and 4, detailed chimerism using fractionated cells was analyzed only the date indicated in Table 2. Donor cells of patient 5 constituted only 5 % of his peripheral blood nucleated cells at day 420 after UCBT, although T cells were 100 % of donor origin.

Immune reconstitution

Table 3 shows the results of immunologic evaluation at the most recent follow-up after UCBT in all survivors. Absolute numbers of lymphocytes were normal after

Table 2 Leukocyte chimerism

Patient	1	2	3	4	5				
Days after UCBT ^a	168	1620	60	1098	52	2021	90	2078	420
T cell (donor %)	100	>95	90	94		100	100	92	100
B cell (donor %)	24	20	20	8		100	70	50	ND
NK cell (donor %)	55	69	15	33		100	90	84	ND
Granulocyte (donor %)	65	59	18	48	>95	100	20	13	ND
Lymphocyte (donor %)					>95				

ND not determined

^a Days after UCBT when chimerism was determined

Table 3 Immune reconstitution

Patient	1	2	3	4	5 (at day 470)
WBC (/μL)	7700	7400	4710	9200	1000
Lymphocyte (/μL)	3700	4370	4120	4100	400
CD3 (%)	82.2	60.4	63.7	81.3	38.6
CD4 (%)	48.4	24.4	35.3	42.0	33.0
CD8 (%)	27.8	28.7	25.7	32.2	11.5
CD19 (%)	13.4	37.9	32.0	15.1	0.0
CD16/56 (%)	1.8	0.6	4.0	2.7	13.0
B-cell function					
IgG (mg/dL)	937	531	692	1157	660 (under i.v.Ig)
IgA (mg/dL)	58	32	55	101	89
IgM (mg/dL)	117	77	115	231	112
IgE (IU/mL)	37	<3	4.2	1	ND
Isohemagglutinin	+	+	-	+	-
Specific antibody	-	+	+	+	ND
T-cell function					
PHA stimulation (SI)	164.4	243.6	220.9	1213.4	1.1
ConA stimulation (SI)	897.7	322.1	225.5	713.1	1.1
NK activity (%)	15	4	10	19	ND

Normal values; PHA stimulation (SI) >100, ConA stimulation (SI) >75, NK activity 18–40 %

ND not determined, SI stimulation index

transplantation (Table 3). Numbers of CD3+, CD4+, CD8+ T cells and CD19+ B cells were within normal ranges, and T-cell function was normal by assessment with PHA and ConA stimulation. Immunoglobulin serum levels were within normal ranges of age-matched controls in all four patients, and none requires IgG substitution (Tables 3, 4). Also each patient had a positive antibody response. NK activity was lower than normal in all but patient 4.

Growth and psychomotor development

As shown in Table 4, all survivors have shown normal height, body mass index (BMI), psychomotor development and performance status to date.

Discussion

We report the outcome of unrelated UCBT in five patients with X-SCID using a RIC regimen. The most important result of this study is all four survivors are free from immunoglobulin replacement therapy.

Previous studies showed that about two-thirds of SCID patients required immunoglobulin replacement therapy after T-cell-depleted, HLA-haploidentical BMT from related donors without pre-transplant conditioning [2, 8]. In Europe, about half of SCID patients who received HLA-haploidentical related marrow cells were conditioned mostly with busulfan (8 mg/kg) and cyclophosphamide (200 mg/kg) [3]. However, the mortality rate for this type of conditioning was higher than that of patients without conditioning. Further, pre-transplant conditioning in combination with HLA-haploidentical related marrow cells did not always result in correction of B-cell function, and about one-third of the SCID patients continue to require immunoglobulin replacement therapy. In contrast, all surviving SCID patients, who had received bone marrow cells from unrelated donors after conventional conditioning with busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg), did not require immunoglobulin replacement therapy [5, 6]. This conventional conditioning regimen, however, has been associated with a significant mortality rate due to treatment-related toxicities such as profound pancytopenia, severe organ toxicity, and exacerbation of pre-existing infections. In addition, children treated with myeloablative regimens often suffer from delayed effects such as infertility, hormonal dysfunction, growth failure and secondary malignancies [9]. Recently, Rao et al. [6] reported the outcome of 33 patients with primary immunodeficiency (PID) [SCID ($n = 6$) and non-SCID ($n = 27$)] who received unmodified unrelated donor marrow grafts following reduced-intensity conditioning consisting of fludarabine (150 mg/m²), melphalan (140 mg/m²), and alemtuzumab (Campath 1H) or anti-thymocyte globulin (ATG). All patients had primary engraftment, and most patients achieved normal immunoglobulin production and B-cell function, although it is not clear whether patients with SCID were on immunoglobulin replacement therapy or not. From these