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LETTER TO THE EDITOR

Hematopoietic stem cell transplantation for X-linked thrombocytopenia from mild symptomatic carrier

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The prognosis of classic Wiskott–Aldrich syndrome (WAS) with complete lack of WAS protein (WASP) expression in the absence of hematopoietic stem cell transplantation (HSCT) is poor,[1,2] whereas WASP-positive patients with the X-linked thrombocytopenia (XLT) phenotype, which is characterized by thrombocytopenia and small platelets, also have a poor long-term outcome without HSCT.[3] If there is a suitable donor, HSCT is the treatment of choice for WASP-positive patients with XLT because of their high frequency of complications, including intracranial hemorrhage, autoimmune diseases and IgA nephropathy.[3] Two-thirds of WASP-positive patients with splice-site mutations exhibit XLT and show the highest incidence of lymphomas, particularly for intron 6 splice-site mutations.[4]

We reported earlier several members of a single family diagnosed with XLT.[5] Briefly, the present patient was a 9-year-old boy with a history of severe cranial hematoma at birth and purpura at the age of 2 months. He had no history of eczema, severe infection or autoimmune disease. Platelet count was 19×10^9/l with small platelets (mean platelet volume [MPV]: 4.8 fl) at age 9 years. Direct sequencing of the *WASP* gene revealed a splice-site G-to-A mutation at position +5 in intron 6. WASP expression levels in the patient were one-third those in the normal control, and the mother was a heterozygous carrier of the same mutation. His sister was diagnosed as a heterozygous symptomatic carrier of XLT at age 6 years, and her maternally derived X-chromosome was randomly inactivated.[5] Her platelet counts increased gradually from 39×10^9/l with low MPV at diagnosis to 80×10^9/l with normal MPV at age 15 years, which suggested a proliferation/survival advantage in cells with active chromosomes bearing normal *WASP* or spontaneous *in vivo* reversion of the *WASP* mutation would develop.[6]

He received HSCT with non-manipulated bone marrow from the 15-year-old sister, whose platelet count was 80×10^9/l with normal MPV and who was the only HLA-identical individual in the patient's family. The donor and recipient lymphocyte subsets and *in vitro* lymphocyte proliferation in response to mitogens (phytohemagglutinin and concanavalin A) were normal at the time of HSCT. The conditioning regimen consisted of oral busulfan 1 mg/kg × 16 doses over 4 days, followed by intravenous cyclophosphamide 50 mg/kg/day for 4 days. Busulfan achieved a steady-state concentration of 680 ng/ml. Clonazepam and mesna were used for prophylaxis against convulsion and hemorrhagic cystitis, respectively, during conditioning. GVHD prophylaxis was short-term methotrexate administered intravenously at a dose of 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6 and 11, and cyclosporine A until day +97. G-CSF was used from days +5 to +17. Hematopoietic reconstitution was rapid, with an absolute neutrophil count of $>0.5 \times 10^9$/l on day +14 and platelet engraftment was achieved on day +16. Complete donor chimerism of bone marrow cells was maintained from day 21 over the 24 months according to fluorescence *in situ* hybridization. He did not develop acute or chronic graft-versus-host disease, regimen-related complications or infection peri-HSCT. At 24 months after HSCT, lymphocyte subsets and *in vitro* lymphocyte proliferation in response to mitogens were normal, although platelet counts remained at 40×10^9/l with normal MPV; these were approximately half the platelet counts in the donor.

We used two-color flow cytometry (FCM)-WASP to serially detect WASP expression in the recipient and donor before and after HSCT (Figure 1).[7,8] We obtained approval for this study from the Dokkyo Medical University Institutional Review Board. Before HSCT, the recipient scarcely expressed intracellular WASP (WASP^{dim}) in CD3$^+$, CD19$^+$ and CD56$^+$ cells, but in the donor at HSCT, intracellular populations with both WASP (WASP^{bright}) and WASP^{dim} were noted, with the WASP^{bright} population being predominant. At 12 months after HSCT, the recipient's proportion of WASP^{bright} to WASP^{dim} among CD3$^+$ cells was comparable to that of the donor, whereas CD19$^+$ and CD56$^+$ cells were predominant among the WASP^{dim} population. At 24 months after HSCT, the proportion of WASP^{dim} in CD19$^+$ and CD56$^+$ cells was lower in the recipient, but remained present. Over time, the recipient has begun to show a similar pattern to that of the donor and now has WASP^{bright} cell populations that were absent before HSCT.

The X-chromosome inactivation patterns of CD3$^+$, CD19$^+$ and CD56$^+$ cells in the donor and recipient were investigated by methylation-specific polymerase chain reaction assay, which was performed using the human androgen receptor (HUMARA) locus involving a methylation-specific polymerase chain reaction technique, as described earlier.[9] The donor was found to be heterozygous at the *HUMARA* locus and showed a random pattern that was consistent with those reported earlier,[5] indicating a smaller allele (X1) and lager allele (X2) carrying the mutated *WASP* and normal *WASP*, respectively. The recipient at 24 months after HSCT showed a random inactive X-chromosome pattern and the ratio of inactive X1 to X2 in CD3$^+$ cells was approaching that in the donor,

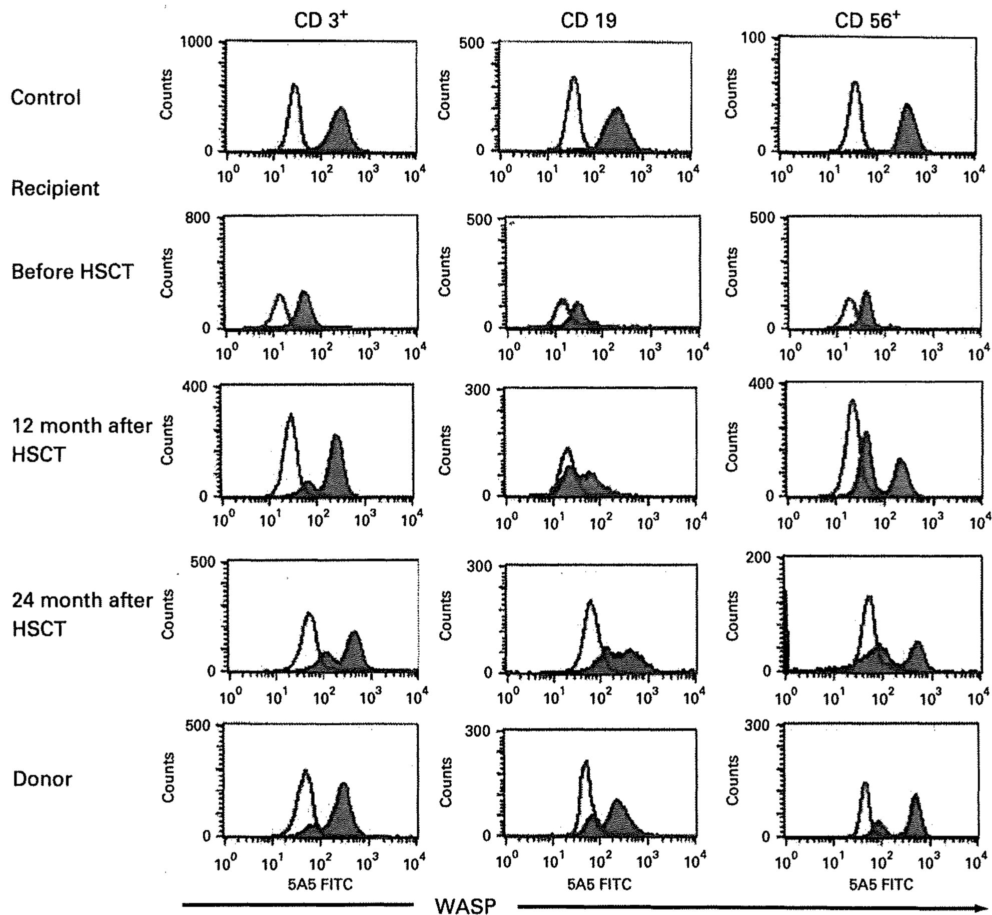

Figure 1 Results of FCM-WASP in lymphocytes before and after HSCT. The *x* axis represents WASP expression; the *y* axis represents cell numbers. The open histogram indicates isotype-control staining; the solid histogram indicates specific staining for WASP. Shown is WASP expression in subpopulations of lymphocytes from healthy control (top panel), the patient before HSCT (second panel), the patient at 12 months after HSCT (third panel), the patient at 24 months after HSCT (fourth panel) and the donor (bottom panel).

being 58:42 in the recipient and 70:30 in the donor (Figure 2, left columns). However, CD19$^+$ and CD56$^+$ cells showed suppressive inactivation (that is, preferential activation) of the mutated allele (X1) at 24 months after HSCT in the recipient, with ratios of 41:59 and 33:67, whereas CD19$^+$ and CD56$^+$ cells showed preferential inactivation of the mutated allele (X1) in the donor, with ratios of 66:34 and 53:47 (Figure 2, middle and right columns). Interestingly, these results were consistent with the recipient and donor results for WASP^{dim} predominance in CD19$^+$ and CD56$^+$ cells obtained by FCM-WASP. On the basis of these results, we speculate that all lymphocyte lineages in the patient will slowly develop into the same pattern as the donor, who showed a predominantly WASP^{bright} population due to the proliferation/survival advantage of the cells with normal WASP.

Long-term follow-up after HSCT is required to assess the clinical course of the patient by FCM-WASP and methylation-specific polymerase chain reaction. It is important to observe how long the acquired random X-inactivation by HSCT will be maintained in this patient.

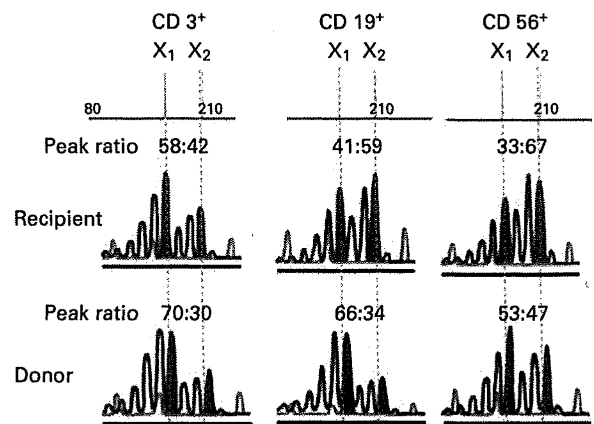

Figure 2 Results of X-chromosome inactivation ratio after HSCT. X-chromosome inactivation using *HUMARA* gene by methylation-specific polymerase chain reaction (M-PCR). Peaks on M-PCR assay of lymphocyte subpopulations show X-inactivation in the patient at 24 months after HSCT (upper panel) and the donor (lower panel). X1 allele and X2 allele refer to X-chromosomes carrying mutated *WASP* and normal *WASP*, respectively. Peak ratio numbers indicate the X-inactivation ratios of X1 and X2, respectively.

Conflict of interest

The authors declare no conflict of interest.

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Hemophagocytosis after bone marrow transplantation for JAK3-deficient severe combined immunodeficiency

Hashii Y, Yoshida H, Kuroda S, Kusuki S, Sato E, Tokimasa S, Ohta H, Matsubara Y, Kinoshita S, Nakagawa N, Imai K, Nonoyama S, Oshima K, Ohara O, Ozono K. Hemophagocytosis after bone marrow transplantation for JAK3-deficient severe combined immunodeficiency. Pediatr Transplantation 2009. © 2009 John Wiley & Sons A/S.

Abstract: HSCT is the optimal treatment for patients with SCID. In particular, HSCT from a HLA-identical donor gives rise to successful engraftment with long survival. We report a six-month-old girl with JAK3-deficient SCID who developed hemophagocytosis after BMT without conditioning from her HLA-identical father. She had suffered from pneumonia and hepatitis before BMT. Prophylaxis for GVHD was short-term methotrexate and tacrolimus. On day 18 after BMT, the patient developed hemophagocytosis in bone marrow when donor lymphocytes were increasing in peripheral blood. Analysis of chimerism confirmed host origin of macrophages and donor origin of lymphocytes. Thus, host macrophage activation was presumably induced in response to donor lymphocytes through immunoreaction to infections and/or alloantigens. HSCT for SCID necessitates caution with respect to hemophagocytosis.

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Key words: bone marrow transplantation – hemophagocytosis – JAK3 mutation – severe combined immunodeficiency

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SCID is a uniformly fatal disease unless promptly treated with HSCT, which reconstitutes a normal immune system (1–3). Patients with SCID have often been affected by various kinds of infections prior to HSCT and the presence of pulmonary infection is a powerful predictor of death after HSCT (1). In addition, hemophagocytosis has been reported as an important complication early after HSCT (4–7). This phenomenon is in many cases triggered by infections (4, 5) and in some cases by an alloimmune response (6, 7). We report a girl with JAK3-deficient SCID who developed hemophagocytosis after BMT without conditioning from her HLA-identical father, where donor lymphocytes presumably activated host macrophages.

Case report

A five-month-old girl, born to consanguineous Chinese parents, had repeatedly developed viral and bacterial bronchitis and oral candidiasis

Abbreviations: γc, γ chain; BCG, Bacille de Calmette et Guérin; BM, bone marrow; BMT, bone marrow transplantation; CMV, cytomegalovirus; EBV, Epstein-Barr virus; FISH, fluorescence *in situ* hybridization; GVHD, graft-versus-host disease; HHV, human herpes virus; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; m-PSL, methyl PSL; NK, natural killer; PCR, polymerase chain reaction; PSL, prednisolone; RT, reverse transcription; SCID, severe combined immunodeficiency; TNF, tumor necrosis factor; TRECs, T-cell-receptor excision circles; VNTR, variable number of tandem repeat.

from two months of age. She had received no BCG vaccination. White blood cell count was 2690/μL (63.5% neutrophils, 27.1% lymphocytes, 1.6% eosinophils, 0% basophils, 7.8% monocytes). Serum IgG, IgA, and IgM levels were 213, 1, and 34 mg/dL, respectively. Lymphocyte subset analysis showed absence of T lymphocytes (0.6% $CD3^+$, 0.3% $CD4^+$, 1.2% $CD8^+$) and NK cells (1.6% $CD16^+$, 0.6% $CD56^+$) with normal numbers of B lymphocytes (96.9% $CD19^+$, 97.3% $CD20^+$). A diagnosis of $T^-B^+NK^-$SCID was made, and genetic analysis revealed a novel homozygous non-sense mutation of JAK3: a C to T point mutation at nucleotide 623 that changed amino acid 175 in the JH6 domain from arginine to a stop codon (C623T; R175X) (Fig. 1).

The clinical course of the patient is summarized in Fig. 2. When she was referred to our

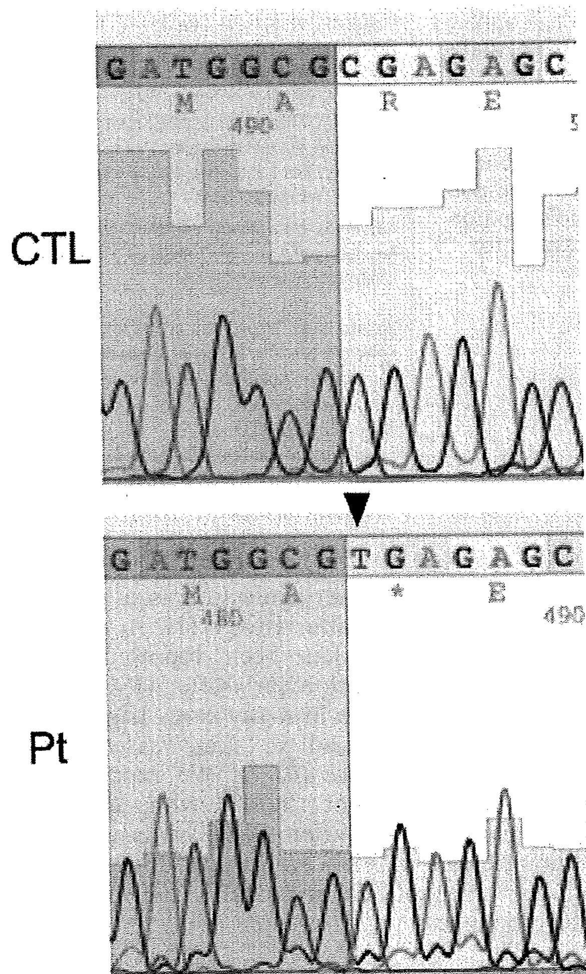

Fig. 1. Sequence analysis showing a non-sense mutation. The JAK3 gene of the patient showed a C to T point mutation (C623T) as shown by arrowhead. CTL, control; Pt, patient.

hospital, she suffered from severe interstitial pneumonia and liver dysfunction. No sign of infection was observed on studies by RT-PCR of her serum for CMV, HHV6, HHV7, adenovirus, HSV-1 or -2, and EBV genomes. *Aspergillus* spp. and *Pneumocystis jiroveci* were not detected in her sputa by PCR analysis. β-D-glucan was not detected in her serum. She received oxygen therapy and infusion of hyperalimentation because of poor feeding.

At the age of six months, she underwent unmanipulated BMT from her genotypically HLA-identical father without conditioning. Both the patient and her father had the same HLA genotype: HLA-A*0101/3001-B*1302/3701-C*0602-DRB1*0701/1501.

Prophylaxis for GVHD was short-term methotrexate (days 1, 3, 6, and 11) and tacrolimus. She developed grade 3 acute GVHD with watery diarrhea (stage 2) and skin eruption (stage 1) on day 9 after BMT, for which she was treated with 2 mg/kg/day of PSL. On day 16, her interstitial pneumonia deteriorated in both lungs on chest X-ray. RT-PCR analysis of sputa showed negative results of CMV, *Aspergillus* spp., and *P. jiroveci.*

Her WBC count decreased to 330/μL on day 18 and BM aspiration revealed hypoplastic marrow with hemophagocytosis by activated macrophages (nuclear cell count, 4000/μL; megakaryocyte count, 0/μL) (Fig. 3). Serum ferritin level was 715 ng/mL and serum soluble IL-2 receptor level was 3295 U/mL. Hemophagocytosis improved three days after administration of etoposide 30 mg/m^2 and pulsed m-PSL 30 mg/kg/day on day 18. VNTR analysis revealed that donor cells were almost completely absent from whole cells and macrophages ($CD14^+$ cells) of the BM cells on days 18 and 20, respectively (Fig. 4). Meanwhile, donor cells were detected in peripheral blood cells on days 20 and 24, including T lymphocytes ($CD3^+$ cells) on day 24 (Fig. 4). A serial flow cytometric analysis of lymphocyte-gated cells also demonstrated that $CD3^+$ cells with predominance of $CD4^+$ cells, most likely donor cells, increased to 3.59% and 5.03% on days 18 and 21, respectively (Table 1). Furthermore, FISH analysis of sex chromosome detected donor cells in 10.8% and 8.6% of peripheral blood cells on days 20 and 28, respectively (data not shown).

As her respiratory condition deteriorated, she received repeated courses of pulsed m-PSL (30 mg/kg/day) therapy and underwent mechanical ventilation on day 20. Despite intensive therapy, she died on day 32 due to respiratory failure. Lung necropsy showed necrotized cells

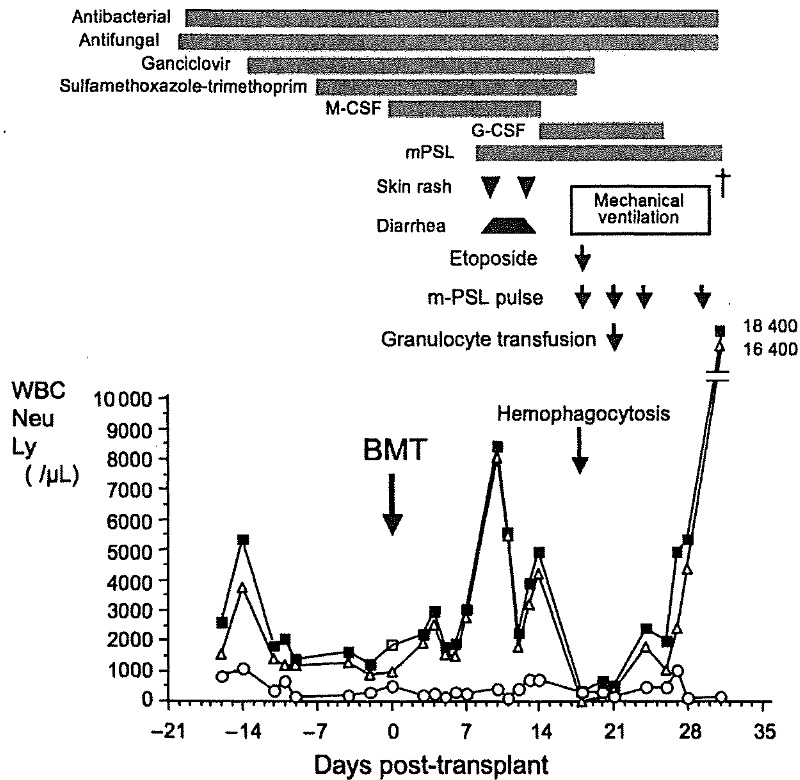

Fig. 2. Clinical course and changes in white blood cell counts. WBC, white blood cells (solid squares); Neu, neutrophil (open triangles); Ly, lymphocyte (open circles); M-CSF, macrophage-colony stimulating factor; G-CSF, granulocyte-colony stimulating factor.

without inflammatory cells. No bacterial, viral, or fungal components were detected in the tissue.

Discussion

SCID is a rare syndrome with heterogeneous genetic inheritance. Common γc mutations have been identified in X-linked SCID, characterized by lack of T cells and NK cells with presence of B cells ($T^-B^+NK^-$SCID). JAK3 mutations have been identified in some patients of autosomal SCID, which shares similar clinical features to X-linked SCID but with normal γc (8–10). JAK3,

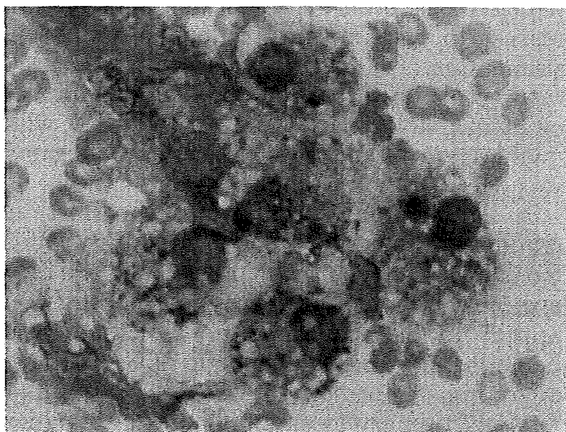

Fig. 3. Bone marrow aspiration on day 18 showing aggregate of activated macrophages. On the far right an erythroblast appears to be undergoing endocytosis.

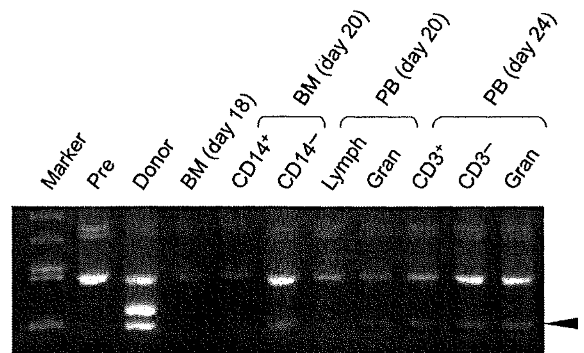

Fig. 4. VNTR analysis. Specific primers designed to flank the repetitive unit, D1S80, were used for the PCR (17). Amplified DNA was electrophoresed and visualized with ethidium bromide. $CD14^+$ or $CD3^+$ cells were purified by magnetic cells sorting enrichment kit (MACS: Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Arrowhead indicates a donor-specific band. Prespecific bands cannot be separated from donor-specific bands.

Table 1. Flow cytometric analysis of lymphocyte-gated cells in peripheral blood

	Lymphocytes (/μL)	CD19 (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD56 (%)
Pre (Day −15)	838	96.7	0.55	NE	NE	1.46
Day +7	243	87.9	2.82	1.31	0.44	0.28
Day +11	111	98.4	0.53	NE	NE	0.34
Day +13	707	98.6	0.39	NE	NE	0.15
Day +18	330	91.6	3.59	3.28	ND	0.24
Day +21	167	88.3	5.03	4.15	0.52	0.88

NE, not evaluable; ND, not done.

a member of the Janus family intracellular protein kinases, associates with intracellular domain of γc and is required for signal transduction from γc-containing receptors (8–10). To date, more than 30 mutations of JAK3 have been reported according to RAPID (Resource of Asian Primary Immunodeficiency Database) (http://rapid.rcai.riken.jp/RAPID/mutation?pid_id=AGID_86); most of them are sporadic and lacking preferential hot spots.

The JAK3 gene has an open reading frame of 3372 bp that is translated into a 1124 amino acid protein (10). In our patient, we identified a novel non-sense homozygous mutation (C623T; Arg157X) leading to a premature stop codon in the JH6 domain. Although we did not evaluate protein expression, this non-sense mutation, nearer to the amino-terminus, probably resulted in abrogated protein expression. The homozygosity was in line with other reported cases with parental consanguinity (8).

Prompt HSCT is an effective life-saving treatment modality for reconstitution of T-cell immunity in this defect (1–3). Our patient therefore underwent BMT immediately after diagnosis from her genotypically HLA-identical father without conditioning. A large European study (1), which analyzed 475 HSCTs for SCID from 1968 to 1999, showed 81% and 72% three-yr survival after HSCT in patients after HSCT from genotypically and phenotypically HLA-identical related donors, respectively. This study furthermore reported 96% sustained engraftment from HLA-identical HSCT, and better engraftment at 93% in SCID with B-cell-positive phenotype, i.e., γc- or JAK3-deficient SCID, compared with 88% in SCID with B-cell-negative phenotype. Recent studies also showed successful HSCT outcome with >90% survival with engraftment in SCID including γc- or JAK3-deficient SCID (2, 3).

Hemophagocytosis early after HSCT has been reported as an important complication (4–7), which is thought to be caused by infections (4, 5) or an alloimmune response (6, 7). The previous reports did not show any detailed analysis of macrophage origin, and the exact mechanism of macrophage activation remains unclear. Moreover, hemophagocytosis after HSCT for SCID as the cause of the graft failure has been reported in only some cases. Norris et al. (11) reported hemophagocytosis after three months HSCT in a T^-B^+ SCID patient who had received T cell-depleted HSCT from an HLA-haploidentical donor without a conditioning regimen. They demonstrated that the hemophagocytosis occurred as a result of donor T-cell engraftment with incomplete immune function, since B-cell reconstitution and tri-lineage hematopoiesis including macrophages showed host type. In our case, hemophagocytosis also occurred after donor T-cell engraftment.

Our patient developed hemophagocytosis and respiratory distress, accompanied by unexpected slow and low engraftment of donor cells. Hemophagocytosis was caused by host macrophages when donor lymphocytes were increasing. Since the patient congenitally had no functioning T cells, it is most probable that donor lymphocytes responded to host cells or resident infectious organisms, leading to IFN-γ production and to activation of host macrophages (12, 13). In SCID patients, maternal engraftment of T cells can lead to GVHD of the skin and liver. Dvorak et al. (14) reported that the T(-)B(+) NK(-) SCID patient with complete CD132 deficiency represented hemophagocytosis without GVHD and that hemophagocytosis was most likely caused by maternal perforin-expressing CD8 T cells. In our case, maternal T cells were not detected pre-SCT (Table 1), which suggests that paternal CD8 T cells or NK cells were involved in hemophagocytosis.

Monocyte function in JAK3-deficient SCID patients has been reported to be intact with respect to cytokine production in response to stimulation (15). The activated macrophages, in turn, probably produced the pro-inflammatory cytokines, TNFα, IL-1β, and IL-6 (12, 13), which might have caused the lung injury as no organism was detected by post-mortem examination.

A conditioning regimen is generally not administered to SCID patients during HSCT from HLA-identical related donors (1–3). However, in our patient, residual macrophages would appear to play an important role in causing hemophagocytosis, which might have led to poor engraftment. Furthermore, Cavazzana et al. (16) analyzed primary T-cell-immunodeficient patients who had undergone HSCT and demonstrated that all patients having undergone full myeloablation had donor myeloid cells and persistent

thymopoiesis, as evidenced by the presence of naive T cells carrying TRECs, which indicates the importance of the complete absence of thymic progenitors by myeloablative conditioning in providing a favorable environment for thymic seeding by early progenitor cells. Our results lead us to surmise that, even when transplanted from an HLA-identical donor, some kind of immunosuppressive conditioning is needed to prevent hemophagocytosis.

In conclusion, we describe a child with JAK3-deficient SCID who developed hemophagocytosis after HSCT from her HLA-identical father. Host macrophage activation would appear to be induced by donor lymphocytes through immune reaction to alloantigen or infectious organisms. HSCT for SCID necessitates caution with respect to hemophagocytosis.

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Edaravone Inhibits DNA Peroxidation and Neuronal Cell Death in Neonatal Hypoxic-Ischemic Encephalopathy Model Rat

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ABSTRACT: Neonatal hypoxic-ischemic encephalopathy (HIE) is the most frequent neurologic disease in the perinatal period. Its major cause is oxidative stress, which induces DNA peroxidation and apoptotic neuronal death. We examined 8-hydroxy-2′-deoxyguanosine (8-OHdG) expression to evaluate brain damage in neonatal HIE and the therapeutic effect of edaravone, a free radical scavenger. Using HPLC and immunohistochemistry, the 8-OHdG levels of neonatal HIE model Sprague-Dawley rats that were subjected to left common carotid artery ligation and 2-h hypoxia significantly increased after 24–48 h of hypoxic-ischemic (HI) insult, but decreased after 72 h. Moreover, the number of apoptotic cells with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling and karyorrhexis significantly increased after 24–72 h of HI insult. In a therapeutic experiment, edaravone was administered i.p. (9 mg/kg) after HI insult every 24 h. Edaravone reduced both the apoptotic neuronal cell number and 8-OHdG expression after 24–48 h of HI. From a double immunofluorescent study, DNA peroxidation occurred in apoptotic neuronal cells with 8-OHdG expression. Edaravone may inhibit the number of apoptotic neuronal cells and 8-OHdG expression within 48 h after HI insult. ***(Pediatr Res* 65: 636–641, 2009)**

Neonatal asphyxia, which is defined as impairment of gas exchange with metabolic acidosis, often develops into hypoxic-ischemic encephalopathy (HIE) (1). Neonatal HIE is sometimes accompanied with the neurologic sequelae, such as cerebral palsy, mental retardation, and intractable epilepsy, although most cases have no such complication. However, the morbidity of neonatal asphyxia is estimated to be 0.22% and 13.8% of the babies afflicted reportedly have neurologic sequelae in Japan (2). Another report estimates the incidence of neonatal asphyxia at 0.1–0.8% in live births (3). The number of victims is not small. Hence, it is necessary to rescue the immature brain from asphyxia and to assure development without neurologic sequelae.

The pathophysiology of neonatal HIE is mainly glutamate neurotoxicity due to the high concentration of free glutamate in intersynaptic spaces and reduction of energy supply from blood (4). As a result of the latter, mitochondrial release of cytochrome *c* is induced, which activates apoptosis through the caspase-pathway (5). Actually, asphyxiated brains often demonstrate apoptotic neuronal death (6). Additionally, it was recently found that in the early stage after hypoxic-ischemic (HI) insult, reactive oxygen species (ROS), such as nitric oxide and hydroxyl radicals, contribute to apoptotic neuronal death (7–11). ROS, which are induced by oxidative stress, are an important factor in initiating DNA peroxidation, which can directly trigger DNA fragmentation and thereby initiate apoptosis (12). 8-Hydroxy-2′-deoxyguanosine (8-OHdG), which is caused by pathologic oxygen radicals, is a hydroxylated form of guanosine of DNA structure (13,14). Therefore, 8-OHdG measurement may be useful to evaluate DNA damage and neuronal apoptosis in the early stage of HIE (15).

Animal models of neonatal HIE have demonstrated apoptotic neuronal death, and its pathologic mechanism has been discussed. Furthermore, several studies have shown a therapeutic approach. Recently, antioxidative stress agents have been successful in cases of brain circulation insufficiency (16,17). Among them, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is thought to be the most beneficial agent for patients with acute ischemic stroke (18). Edaravone can interact with both peroxyl and hydroxyl radicals, followed by the formation of a stable oxidation product through a radical intermediate (19). This scavenging effect cannot only inhibit neuronal apoptosis (20) and lipid peroxidation (21) but may also protect neurons against DNA peroxidation by excess production of ROS.

In this study, we reveal that the 8-OHdG amount is useful to evaluate DNA peroxidation and neuronal cell death in the acute stage of neonatal HIE model rat and demonstrate that edaravone is useful to reduce brain damage.

MATERIALS AND METHODS

Neonatal hypoxic-ischemic encephalopathy model rat. All experiments were performed with the approval of the Animal Experiment Ethical Committee of the National Center of Neurology and Psychiatry. We used unsexed Sprague-Dawley rat pups (15–21 g; CLEA Japan Inc., Tokyo, Japan) on postnatal-day 7. A model of neonatal HIE was developed at postnatal-day 7 (22). Under anesthesia using isoflurane (Forane; Dainippon Pharma, Osaka, Japan) inhalation, the left common carotid artery was isolated, double ligated, and cut. After recovery for 2–4 h with their mothers, the pups were exposed to 8% oxygen with an ambient temperature 36°C for 2 h and then returned to their mothers. The pups were kept in a room (12 h/12 h light/dark cycle) until they were killed. The same mother littermates as age-matched controls

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Abbreviations: 8-OHdG, 8-Hydroxy-2′-deoxyguanosine; **EdG,** edaravone group; **HI,** hypoxic-ischemic; **HIE,** hypoxic-ischemic encephalopathy; **LIC,** left ipsilateral cortices; **RCC,** right contralateral cortices; **VehG,** vehicle group

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ingestion (by hand-to-mouth activity) of powder residues (3,4).

In conclusion, this case illustrates how drug testing not only in conventional matrices, which account for acute exposure, but also in nonconventional matrices can shed light on past and possibly chronic exposure and can disclose the situation of an infant living in an unsafe and high-risk environment in which exposure to drugs of abuse takes place. The hair is always available and gives information on the past exposure as it grows by approximately 1 cm per month and accumulates drugs (5). It is particularly useful if the results of urine analysis are negative during acute investigation. The accurate assessment of both acute and chronic exposure of a young child to drugs of abuse through the objective use of a biomarker is of major importance because it provides the basis for appropriate immediate treatment, adequate medical follow-up and social intervention. In case of any acute exposure to drugs of abuse or of postnatal withdrawal syndrome, we suggest to investigate further the possibility of chronic exposure.

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A case of glycogen storage disease type Ib presenting with prolonged neonatal hypoglycaemia and minimal metabolic abnormalities

Sir,

The symptoms of hypoglycaemia in glycogen storage disease (GSD) patients usually do not manifest during the newborn period, because frequent feeding obviates the need for the gluconeogenic process. We herein report a case of neonatal hypoglycaemia with GSD type Ib that showed minimal metabolic abnormalities, thus making it difficult to make a definitive diagnosis.

A boy, the first child of healthy unrelated parents, was born at 38 weeks gestation and his birth weight was 2,686 g. At day 1, the patient showed tachypnea and hypoglycaemia and the intravenous infusion of glucose was started. On day 4, repeated episodes of hypoglycaemia were noted and he was transferred to our hospital. On physical examination, only the palpable edge of the liver was noted. Blood chemistry showed an elevated level of triglyceride (504 mg/dL) and lactate (36.3 mg/dL). Total cholesterol, free fatty acid, uric acid, carnitine profile and blood gas analysis were all within the normal level. A serum sample at hypoglycaemia showed a measurable level of insulin (insulin and glucose were 2.4 IU/mL and 34 mg/dL respectively), and ketoacidosis was not noted.

To differentiate between hyperinsulinemic hypoglycaemia and defects in glycogenolysis, a glucagon stimulation test was performed, showing no glycaemic response (Figure 1). The glucose administration (2 g/kg) showed a decrease in the lactate level (from 32.8 to 19.6 mg/dL). These results suggested a defect in glycogenolysis, and we started to give the patient formula milk under a tentative diagnosis of GSD. However, the patient could not show a stable blood glucose level. Furthermore, a gene analysis of GSD-Ia/b focused on hot spot mutations (1) revealed no mutation. Although the serum insulin level was not so high, diazoxide was added to suppress insulin secretion, which resulted in a poor glycaemic response.

From 3 months of age, diarrhoea and repeated episodes of bronchitis were noted. Simultaneously, the blood count showed a decreased neutrophil level. A complete gene analysis of *glucose-6-phosphate transporter 1* was performed, and a compound heterozygous mutation IVS1 + 1G>A and c.1016G>A (Gly339Asp) in exon 7 was found.

The median age of the presenting signs is an age from 4 to 6 months in GSD-I (2), while some neonatal cases have also been reported (3). GSD-Ib is a rare but important cause of neonatal hypoglycaemia, and it may be present with minimal accompanying metabolic abnormalities.

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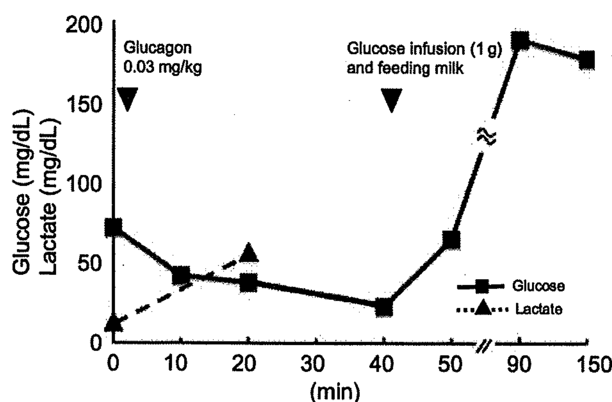

Figure 1 Glucagon stimulation test showed the absence of a glycaemic response and a rise in lactate level.

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G6PC mutations in two patients with glycogen storage disease type Ia in Thailand

Dear Sir,

Glycogen storage disease type Ia (GSD Ia) is characterized by hepatomegaly and hypoglycaemia and caused by mutations in the glucose-6-phosphatase gene (*G6PC*). Mutations unique to several ethnic groups have been described (1). However, no reports on Thai patients have been published. Here, we present the first description of *G6PC* mutations in Thailand.

Two Thai patients with GSD Ia, described elsewhere (2), were studied. In brief, patient 1 was aged 11 years and patient 2 was aged 9 years. Both showed hepatomegaly and treated with uncooked cornstarch to prevent hypoglycaemia. Parents of patient 1 had a consanguineous marriage. Sequence analysis of *G6PC*, as described previously (3), showed that patient 1 was a homozygote for p.R83H (c.248 G>A) and that patient 2 was a compound heterozygote for p.R83H and c.648G>T, previously described as G727T mutation. Two mutations were verified by PCR-restriction fragment length polymorphism (RFLP) analyses: RFLP with restriction enzyme *Hga* I for detection of p.R83H and RFLP with *Bsr*GI for c.648G>T as described (3). In patient 2, c.648G>T allele had 1176C polymorphism as well as in Japanese and Chinese patients (4,5).

R83H is frequently detected in Chinese patients (26% of alleles studied) and c.648G>T is prevalent in Japanese (91%), Korean (75%) and Chinese (54%) patients (1). Our results suggest that Thai GSD Ia patients share the same mutations as Asian patients. Other examples of founder mutations in Asians include phenylketonuria (6) and complement component C9 deficiency (7).

Clinically, instead of performing an invasive liver biopsy, a rapid and non-invasive DNA testing using PCR-RFLP enables us to make a precise diagnosis of GSD Ia in Thailand. Our report expands the spectrum of Asian-specific *G6PC* mutations to Thailand.

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Correction was added on 14 October 2009: the first author's name was amended.

Increased levels of Monocyte Chemoattractant Protein-1 in cerebrospinal fluid with gamma globulin induced meningitis

Dear Sir,

Intravenous immunoglobulin G (IVIG) is widely used in the treatment of severe infectious diseases, immune thrombocytopenic purpura (ITP) in children. Complications of IVIG therapy include acute generalized reactions and aseptic meningitis (1). Although IVIG-induced meningitis is recognized, the mechanisms remain unknown. We applied this technique to investigate multiple cytokines and chemokines in gamma globulin-induced meningitis, as compared with mumps meningitis.

An 11-year-old boy was admitted because of bleeding tendency. We diagnosed him as having ITP and initiated gamma globulin for wet purpura. The day after the start of IVIG therapy, he complained of severe headache and exhibited vomiting with nuchal stiffness. Cerebrospinal fluid findings (CSF) revealed mild pleocytosis and we diagnosed IVIG-induced meningitis.

All specimens were collected for diagnostic tests and the remainder of these specimens were used for cytokine investigation. The Institutional Review Board approved the collection and investigation of samples, and written informed consent was obtained from all subjects. Cytokine measurement in CSF was performed simultaneously for 17 different cytokines (Interlekin-1β, -2, -4, -5, -6, -7, -8, -10, -12, -13, -17, Granulocyte-colony stimulating factor, Granulocyte-Monocyte colony stimulating factor, interferon-γ, Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage inflammatory protein-1β), and Tumour Necrosis Factor-α using BioPlex Cytokine Assay System (Bio-Rad Laboratories, Tokyo, Japan).

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bjh research paper

Quantitative assessment of *PTPN11* or *RAS* mutations at the neonatal period and during the clinical course in patients with juvenile myelomonocytic leukaemia

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Summary

To evaluate minimal residual disease (MRD) after chemotherapy and haematopoietic stem cell transplantation in juvenile myelomonocytic leukaemia (JMML), a locked nucleic acid-allele specific quantitative polymerase chain reaction (LNA-AS-qPCR) was developed for 13 patients (four types of *PTPN11* mutation and four types of *RAS* mutation). The post-transplant MRD detected by LNA-AS-qPCR analysis was well correlated with chimerism assessed by short tandem repeat PCR analysis. Non-intensive chemotherapy exerted no substantial reduction of the tumour burden in three patients. There was no significant difference in the quantity of *RAS* mutant DNA after spontaneous haematological improvement in 4 patients with *NRAS* or *KRAS* 34G > A during a 2- to 5-year follow-up. *PTPN11*, *NRAS*, or *KRAS* mutant DNA was detected from Guthrie card dried blood in five of seven patients (who were aged <2 years at diagnosis) at a level of $1{\cdot}0–6{\cdot}5 \times 10^{-1}$ of the values at diagnosis. Accordingly, these five patients might have already reached a subclinical status at birth. Considering the negative correlation between mutant DNA level in neonatal blood spots and age at diagnosis, JMML patients with a larger tumour burden at birth appeared to show earlier onset.

Keywords: juvenile myelomonocytic leukaemia, minimal residual disease, *PTPN11*, *RAS*, allele-specific quantitative PCR.

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Rapid and simultaneous detection of 6 types of human herpes virus (herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, human herpes virus 6A/B, and human herpes virus 7) by multiplex PCR assay

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ABSTRACT

A multiplex PCR assay was developed that enabled the simultaneous detection of DNA from 6 types of human herpes virus, HSV-1/2, VZV, EBV, CMV, HHV-6A/B, and HHV-7, using appropriate primer sets and conventional PCR techniques and instruments, with PCR products for each type of virus designed to be easily distinguishable by size. Electropherograms obtained from conventional agarose gels showed that, for each type, the observed number of base pairs corresponded to the intended product and that bands were easily distinguishable from each other. A minimum of 20 copies of viral DNA in a reaction was sufficient to confirm the existence of each of the 6 types of human herpes virus. Comparison of the data obtained from this method and the data obtained from conventional TaqMan PCR using clinical specimens from various sources showed consistent results. The multiplex PCR method reported here for the detection and differentiation of human herpes viruses did not require special equipment or techniques such as hybridization analysis and sequencing analysis and, therefore, enabled us to easily and rapidly detect and identify the 6 types of human herpes virus using conventional methods.

Eight types of human herpes virus have been identified: herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes virus 6 (HHV-6), human herpes virus 7 (HHV-7), and human herpes virus 8 (HHV-8) (20). Infection with a herpes virus can remain latent for decades and eventually cause various clinical symptoms; in many cases, the infection manifests itself as an opportunistic disease (4, 8, 10, 14, 17, 26, 27). Several anti-viral drugs that are effective for treating herpes virus infections have been developed and are now routinely used in treatment (1, 2, 18, 19, 21). However, because different human herpes viruses often elicit similar clinical symptoms, there is an increasing need for the accurate and timely diagnosis of herpes infections in order for these powerful anti-viral drugs to be used effectively (5, 7, 14, 15, 22, 26).

Recently, a polymerase chain reaction (PCR) method has been developed that is specific for each virus and serves as a rapid diagnostic method. Real-time PCR is also now available for quantitative genome-specific analysis of viral infections (9, 11–13, 23, 24). The conventional PCR method requires the individual examination of each virus, which leads to problems with respect to time and cost. Although PCR techniques that can simultaneously detect human herpes viruses have been reported, amplification product analysis requires complicated methods such as southern blot hybridization (28) and microarray technology (29) to detect many viruses. Furthermore, there are few reports of the 6

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Primary immunodeficiencies: 2009 update

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More than 50 years after Ogdeon Bruton's discovery of congenital agammaglobulinemia, human primary immunodeficiencies (PIDs) continue to unravel novel molecular and cellular mechanisms that govern development and function of the human immune system. This report provides the updated classification of PIDs that has been compiled by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies after its biannual meeting in Dublin, Ireland, in June 2009. Since the appearance of the last classification in 2007, novel forms of PID have been discovered, and additional pathophysiology mechanisms that account for PID in human beings have been unraveled. Careful analysis and prompt recognition of these disorders is essential to prompt effective forms of treatment and thus to improve survival and quality of life in patients affected with PIDs.

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Key words: *Primary immunodeficiencies, T cells, B cells, severe combined immunodeficiency, predominantly antibody deficiencies, DNA repair defects, phagocytes, complement, immune dysregulation syndromes, innate immunity, autoinflammatory disorders*

Since 1970, a committee of experts in the field of primary immunodeficiencies (PIDs) has met every 2 years with the goal of classifying and defining these disorders. The most recent meeting, organized by the Experts Committee on Primary Immunodeficiencies of the International Union of Immunological Societies, with support from the Jeffrey Modell Foundation and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, took place in Dublin, Ireland, in June 2009. In addition to members of the expert committee, the meeting gathered more than 30 speakers and more than 200 participants from 6 continents. Recent discoveries on the molecular and cellular bases of PID and advances in the diagnosis and treatment of these disorders were discussed. At the end of the meeting, the International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies met to update the classification of PIDs, presented in Tables I to VIII.

The general outline of the classification has remained substantially unchanged. Novel PIDs, whose molecular basis has been identified and reported in the last 2 years, have been added to the list. In Table I (Combined T and B–cell immunodeficiencies), coronin-1A deficiency (resulting in impaired thymic egress) has been added to the genetic defects causing T^-B^+ severe combined immunodeficiency (SCID). The first case of DNA-activated Protein Kinase catalytic subunit (DNA-PKcs) deficiency has also been reported and adds to the list of defects of nonhomologous end-joining resulting in T^-B^- SCID. Among calcium flux defects, defects of Stromal Interaction Molecule 1 (STIM-1), a Ca^{++} sensor, have been reported in children with immunodeficiency, myopathy, and autoimmunity. Mutations of the gene encoding the dedicator of cytokinesis 8 protein have been shown to cause an autosomal-recessive combined immunodeficiency with hyper-IgE, also characterized by extensive cutaneous viral infections, severe atopy, and increased risk of cancer. Also in Table I, mutations of the adenylate kinase 2 gene have been shown to cause reticular dysgenesis, and mutations in DNA ligase IV (LIG4), adenosine deaminase (ADA), and γc have been added to the list of genetic defects that may cause Omenn syndrome.

In Table II (Predominantly antibody deficiencies), mutations in Transmembrane Activator and CAML Interactor (TACI) and in B

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Abbreviations used
ADA: Adenosine deaminase
PID: Primary immunodeficiency
SCID: Severe combined immunodeficiency

cell activating factor (BAFF)-receptor have been added to the list of gene defects that may cause hypogammaglobulinemia. However, it should be noted that only few TACI mutations appear to be disease-causing. Furthermore, variability of clinical expression has been associated with the rare BAFF-receptor deficiency. Table III lists other well defined immunodeficiency syndromes. Post-Meiotic Segregation 2 (PMS2) deficiency and immunodeficiency with centromeric instability and facial anomalies syndrome have been added to the list of DNA repair defects, whereas Comel-Netherton syndrome is now included among the immune-osseous dysplasias, and hyper-IgE syndrome caused by dedicator of cytokinesis 8 (*DOCK8*) mutation has also been added. Interleukin-2 Inducible T cell Kinase (ITK) deficiency has been included among the molecular causes of lymphoproliferative syndrome in Table IV (Diseases of immune dysregulation). Also in Table IV, CD25 deficiency has been listed to reflect the occurrence of autoimmunity in this rare disorder. Progress in the molecular characterization of congenital neutropenia and other innate immunity defects has resulted in the inclusion of Glucose-6-phosphate Transporter 1 (*G6PT1*) and Glucose-6-phosphate catalytic subunit 3 (*G6PC3*) defects in Table V (Congenital defects of phagocyte number, function, or both) and of MyD88 deficiency (causing recurrent pyogenic bacterial infections) and of CARD9 deficiency (causing chronic mucocutaneous candidiasis) in Table VI (Defects in innate immunity). Tables V and VI also include 2 novel genetic defects that result in clinical phenotypes distinct from the classical definition of PIDs. In particular, mutations of the Colony Stimulating Factor 2 Receptor Alpha (*CSF2RA*) gene, encoding for GM-CSF receptor α, have been shown to cause primary alveolar proteinosis as a result of defective surfactant catabolism by alveolar macrophages (Table V). Mutations in Apolipotein L 1 (*APOL1*) are associated with trypanosomiasis, as reported in Table VI. It can be anticipated that a growing number of defects in immune-related genes will be shown to be responsible for nonclassic forms of PIDs in the future. Along the same line, the spectrum of genetically defined autoinflammatory disorders (Table VII) has expanded to include NLR family pyrin domain-containing 12 (*NLRP12*) mutations (responsible for familial cold autoinflammatory syndrome) and Interleukin-1 receptor antagonist (*IL1RN*) defects (causing deficiency of the IL-1 receptor antagonist). Again, it is expected that a growing number of genetic defects will be identified in other inflammatory conditions. Finally, defects of ficolin 3 (which plays an important role in complement activation) have been shown to cause recurrent pyogenic infections in the lung (Table VIII).

Although the revised classification of PIDs is meant to assist with the identification, diagnosis, and treatment of patients with these conditions, it should not be used dogmatically. In particular, although the typical clinical and immunologic phenotype is reported for each PID, it has been increasingly recognized that the phenotypic spectrum of these disorders is wider than originally thought. This variability reflects both the effect of different mutations within PID-causing genes and the role of other genetic, epigenetic, and environmental factors in modifying the phenotype. For example, germline hypomorphic mutations or somatic mutations in SCID-related genes may result in atypical/leaky SCID or Omenn syndrome, with the latter associated with significant immunopathology. Furthermore, infections may also significantly modify the clinical and immunologic phenotype, even in patients who initially present with typical SCID. Thus, the phenotype associated with single-gene defects listed in the revised classification should by no means be considered absolute.

Finally, a new column has been added to the revised classification to illustrate the relative frequency of the various PID disorders. It should be noted that these frequency estimates are based on what has been reported in the literature because with few exceptions, no solid epidemiologic data exist that can be reliably used to define the incidence of PID disorders. Furthermore, the frequency of PIDs may vary in different countries. Certain populations (and especially, some restricted ethnic groups of geographical isolates) have a higher frequency of specific PID mutations because of a founder effect and genetic drift. For example, DNA cross-link repair protein 1C (*DCLRE1C*) (Artemis) and Z-associated protein of 70 kD (*ZAP70*) defects are significantly more common in Athabascan-speaking Native Americans and in members of the Mennonite Church, respectively, than in other populations. Similarly, MHC class II deficiency is more frequent in Northern Africa. The frequency of autosomal-recessive immunodeficiencies is higher among populations with a high consanguinity rate.

TABLE I. Combined T and B-cell immunodeficiencies

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features/atypical presentation	Inheritance	Molecular defect/presumed pathogenesis	Relative frequency among PIDs†
1. T^-B^+ SCID*							
(a) γc Deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells Leaky cases may present with low to normal T and/or NK cells	XL	Defect in γ chain of receptors for IL-2, IL-4, IL-7, IL-9, IL-15, IL-21	Rare
(b) JAK3 deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells Leaky cases may present with variable T and/or NK cells	AR	Defect in Janus activating kinase 3	Very rare
(c) IL-7Rα deficiency	Markedly decreased	Normal or increased	Decreased	Normal NK cells	AR	Defect in IL-7 receptor α chain	Very rare
(d) CD45 deficiency	Markedly decreased	Normal	Decreased	Normal γ/δ T cells	AR	Defect in CD45	Extremely rare
(e) CD3δ/CD3ε/CD3ζ deficiency	Markedly decreased	Normal	Decreased	Normal NK cells No γ/δ T cells	AR	Defect in CD3δ CD3ε or CD3ζ chains of T-cell antigen receptor complex	Very rare
(f) Coronin-1A deficiency	Markedly decreased	Normal	Decreased	Detectable thymus	AR	Defective thymic egress of T cells and T-cell locomotion	Extremely rare
2. T^-B^- SCID*							
(a) RAG 1/2 deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination May present with Omenn syndrome	AR	Defect of recombinase activating gene (RAG) 1 or 2	Rare
(b) DCLRE1C (Artemis) deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination, radiation sensitivity May present with Omenn syndrome	AR	Defect in Artemis DNA recombinase-repair protein	Very rare
(c) DNA PKcs deficiency	Markedly decreased	Markedly decreased	Decreased	[widely studied *scid* mouse defect]	AR	Defect in DNAPKcs Recombinase repair protein	Extremely rare
(d) ADA deficiency	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Costochondral junction flaring, neurologic features, hearing impairment, lung and liver manifestations Cases with partial ADA activity may have a delayed or milder presentation	AR	Absent ADA, elevated lymphotoxic metabolites (dATP, S-adenosyl homocysteine)	Rare
(e) Reticular dysgenesis	Markedly decreased	Decreased or normal	Decreased	Granulocytopenia, deafness	AR	Defective maturation of T, B, and myeloid cells (stem cell defect) Defect in mitochondrial adenylate kinase 2	Extremely rare
3. Omenn syndrome‡	Present; restricted heterogeneity	Normal or decreased	Decreased, except increased IgE	Erythroderma, eosinophilia, adenopathy, hepatosplenomegaly	AR (in most cases)	Hypomorphic mutations in RAG1/2 , Artemis, IL-7Rα, RMRP, ADA, DNA ligase IV, γc	Rare
4. DNA ligase IV deficiency	Decreased	Decreased	Decreased	Microcephaly, facial dysmorphisms, radiation sensitivity May present with Omenn syndrome or with a delayed clinical onset	AR	DNA ligase IV defect, impaired nonhomologous end joining (NHEJ)	Very rare

(Continued)

TABLE I. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features/atypical presentation	Inheritance	Molecular defect/presumed pathogenesis	Relative frequency among PIDs†
5. Cernunnos deficiency	Decreased	Decreased	Decreased	Microcephaly, *in utero* growth retardation, radiation sensitivity	AR	Cernunnos defect, impaired NHEJ	Very rare
6. CD40 ligand deficiency	Normal	IgM^+ and IgD^+ B cells present, other isotypes absent	IgM increased or normal, other isotypes decreased	Neutropenia, thrombocytopenia; hemolytic anemia, biliary tract and liver disease, opportunistic infections	XL	Defects in CD40 ligand (CD40L) cause defective isotype switching and impaired dendritic cell signaling	Rare
7. CD40 deficiency	Normal	IgM^+ and IgD^+ B cells present, other isotypes absent	IgM increased or normal, other isotypes decreased	Neutropenia, gastrointestinal and liver/ biliary tract disease, opportunistic infections	AR	Defects in CD40 cause defective isotype switching and impaired dendritic cell signaling	Extremely rare
8. Purine nucleoside phosphorylase deficiency	Progressive decrease	Normal	Normal or decreased	Autoimmune hemolytic anemia, neurological impairment	AR	Absent purine nucleoside phosphorylase deficiency, T-cell and neurologic defects from elevated toxic metabolites (eg, dGTP)	Very rare
9. CD3γ deficiency	Normal, but reduced TCR expression	Normal	Normal		AR	Defect in CD3 γ	Extremely rare
10. CD8 deficiency	Absent CD8, normal CD4 cells	Normal	Normal		AR	Defects of CD8 α chain	Extremely rare
11. ZAP-70 deficiency	Decreased CD8, normal CD4 cells	Normal	Normal		AR	Defects in ZAP-70 signaling kinase	Very rare
12. Ca^{++} channel deficiency	Normal counts, defective TCR-mediated activation	Normal counts	Normal	Autoimmunity, anhydrotic ectodermic dysplasia, nonprogressive myopathy	AR AR	Defect in Orai-1, a Ca^{++} channel component Defect in Stim-I, a Ca^{++} sensor	Extremely rare
13. MHC class I deficiency	Decreased CD8, normal CD4	Normal	Normal	Vasculitis	AR	Mutations in *TAP1*, *TAP2*, or *TAPBP* (tapasin) genes giving MHC class I deficiency	Very rare
14. MHC class II deficiency	Normal number, decreased CD4 cells	Normal	Normal or decreased		AR	Mutation in transcription factors for MHC class II proteins (*C2TA*, *RFX5*, *RFXAP*, *RFXANK* genes)	Rare
15. Winged helix deficiency (Nude)	Markedly decreased	Normal	Decreased	Alopecia, abnormal thymic epithelium, impaired T-cell maturation [widely studied nude mouse defect]	AR	Defects in forkhead box N1 transcription factor encoded by *FOXN1*, the gene mutated in nude mice	Extremely rare
16. CD25 deficiency	Normal to modestly decreased	Normal	Normal	Lymphoproliferation (lymphadenopathy, hepatosplenomegaly), autoimmunity (may resemble IPEX syndrome), impaired T-cell proliferation	AR	Defects in IL-2Rα chain	Extremely rare
17. STAT5b deficiency	Modestly decreased	Normal	Normal	Growth-hormone insensitive dwarfism, dysmorphic features, eczema, lymphocytic interstitial pneumonitis, autoimmunity	AR	Defects of STAT5b, impaired development and function of γδT cells, regulatory T and NK cells, impaired T-cell proliferation	Extremely rare
18. Itk deficiency	Modestly decreased	Normal	Normal or decreased		AR	EBV-associated lymphoproliferation	Extremely rare

(Continued)

TABLE I. *(Continued)*

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features/atypical presentation	Inheritance	Molecular defect/presumed pathogenesis	Relative frequency among PIDs†
19. DOCK8 deficiency	Decreased	Decreased	Low IgM, increased IgE	Recurrent respiratory infections. Extensive cutaneous viral and bacterial (staphylococcal) infections, susceptibility to cancer, hypereosinophilia, severe atopy, low NK cells	AR	Defect in *DOCK8*	Very rare

ADA, Adenosine deaminase; *AR*, autosomal-recessive inheritance; *ATP*, adenosine triphosphate; *C2TA*, class II transactivator; *EBV*, Epstein-Barr virus; *FOXN1*, forkhead box N1; *GTP*, guanosine triphosphate; *IL* (interleukin); *JAK3*, Janus associated kinase 3; *NHEJ*, non homologous end joining; *RFX*, regulatory factor X; *RMRP*, RNA component of mitochondrial RNA processing endonuclease; *NK*, natural killer; *RAG*, Recombinase Activating Gene; *SCID*, severe combined immune deficiency; *STAT*, signal transducer and activator of transcription; *TAP*, transporter associated with antigen processing; *TCR*, T cell receptor; *XL*, X-linked inheritance;
*Atypical cases of SCID may present with T cells because of hypomorphic mutations or somatic mutations in T-cell precursors.
†Frequency may vary from region to region or even among communities, ie, Mennonite, Innuit, and so forth.
‡Some cases of Omenn syndrome remain genetically undefined.
****Some metabolic disorders such methylmalonic aciduria may present with profound lymphopenia in addition to their typical presenting features.

TABLE II. Predominantly antibody deficiencies

Disease	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/presumed pathogenesis	Relative frequency among PIDs
1. Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells					
(a) Btk deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	XL	Mutations in *BTK*	Rare
(b) μ heavy chain deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in μ heavy chain	Very rare
(c) λ5 deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in *IGLL1* (λ5)	Extremely rare
(d) Igα deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in Igα	Extremely rare
(e) Igβ deficiency	All isotypes decreased	Severe bacterial infections normal numbers of pro-B cells	AR	Mutations in Igβ	Extremely rare
(f) BLNK deficiency	All isotypes decreased	Severe bacterial infections normal numbers of pro-B cells	AR	Mutations in *BLNK*	Extremely rare
(g) Thymoma with immunodeficiency	All isotypes decreased	Bacterial and opportunistic infections; autoimmunity	None	Unknown	Rare
2. Severe reduction in at least 2 serum immunoglobulin isotypes with normal or low numbers of B cells					

(Continued)

TABLE II. *(Continued)*

Disease	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/presumed pathogenesis	Relative frequency among PIDs
(a) Common variable immunodeficiency disorders (CVIDs)*	Low IgG and IgA and/or IgM	Clinical phenotypes vary: most have recurrent bacterial infections, some have autoimmune, lymphoproliferative and/or granulomatous disease	Variable	Unknown	Relatively common
(b) ICOS deficiency	Low IgG and IgA and/or IgM	—	AR	Mutations in *ICOS*	Extremely rare
(c) CD19 deficiency	Low IgG, and IgA and/or IgM	—	AR	Mutations in *CD19*	Extremely rare
(d) TACI deficiency**	Low IgG and IgA and/or IgM	—	AD or AR or complex	Mutations in *TNFRSF13B* (TACI)	Very common
(e) BAFF receptor deficiency**	Low IgG and IgM	Variable clinical expression	AR	Mutations in *TNFRSF13C* (BAFF-R)	Extremely rare
3. Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells					
(a) CD40L deficiency***	IgG and IgA decreased; IgM may be normal or increased; B cell numbers may be normal or increased	Opportunistic infections, neutropenia, autoimmune disease	XL	Mutations in *CD40L* (also called *TNFSF5* or *CD154*)	Rare
(b) CD40 deficiency***	Low IgG and IgA; normal or raised IgM	Opportunistic infections, neutropenia, autoimmune disease	AR	Mutations in *CD40* (also called *TNFRSF5*)	Extremely rare
(c) AID deficiency****	IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centers	AR	Mutations in *AICDA* gene	Very rare
(d) UNG deficiency****	IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centers	AR	Mutation in *UNG*	Extremely rare
4. Isotype or light chain deficiencies with normal numbers of B cells					
(a) Ig heavy chain mutations and deletions	One or more IgG and/or IgA subclasses as well as IgE may be absent	May be asymptomatic	AR	Mutation or chromosomal deletion at 14q32	Relatively common
(b) κ chain deficiency	All immunoglobulins have lambda light chain	Asymptomatic	AR	Mutation in κ constant gene	Extremely rare
(c) Isolated IgG subclass deficiency	Reduction in one or more IgG subclass	Usually asymptomatic; may have recurrent viral/ bacterial infections	Variable	Unknown	Relatively common
(d) IgA with IgG subclass deficiency	Reduced IgA with decrease in one or more IgG subclass;	Recurrent bacterial infections in majority	Variable	Unknown	Relatively common
(e) Selective IgA deficiency	IgA decreased/absent	Usually asymptomatic; may have recurrent infections with poor antibody responses to carbohydrate antigens; may have allergies or autoimmune disease A few cases progress to CVID, others coexist with CVID in the same family.	Variable	Unknown	Most common

(Continued)

TABLE II. *(Continued)*

Disease	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/presumed pathogenesis	Relative frequency among PIDs
5. Specific antibody deficiency with normal Ig concentrations and normal numbers of B cells	Normal	Inability to make antibodies to specific antigens	Variable	Unknown	Relatively common
6. Transient hypogammaglobulinemia of infancy with normal numbers of B cells	IgG and IgA decreased	Recurrent moderate bacterial infections	Variable	Unknown	Common

AD, Autosomal-dominant inheritance; *AID*, activation-induced cytidine deaminase; *AR*, autosomal-recessive inheritance; *BLNK*, B-cell linker protein; *BTK*, Bruton tyrosine kinase; *ICOS*, inducible costimulator; *Ig(κ)*, immunoglobulin of κ light-chain type; *UNG*, uracil-DNA glycosylase; *XL*, X-linked inheritance.
*Common variable immunodeficiency disorders: there are several different clinical phenotypes, probably representing distinguishable diseases with differing immunopathogeneses.
**Alterations in *TNFRSF13B (TACI)* and *TNFRSF13C (BAFF-R)* sequence may represent disease-modifying mutations rather than disease-causing mutations.
***CD40L and CD40 deficiency are also included in Table I.
****Deficiency of AID or UNG present as forms of the hyper-IgM syndrome but differ from CD40L and CD40 deficiencies in that the patients have large lymph nodes with germinal centers and are not susceptible to opportunistic infections.

TABLE III. Other well defined immunodeficiency syndromes

Disease	Circulating T cells	Circulating B cells	Serum immunoglobulin	Associated features	Inheritance	Genetic defects/ presumed Pathogenesis	Relative frequency among PIDs
1. Wiskott-Aldrich syndrome (WAS)	Progressive decrease, abnormal lymphocyte responses to anti-CD3	Normal	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE	Thrombocytopenia with small platelets; eczema; lymphomas; autoimmune disease; IgA nephropathy; bacterial and viral infections XL thrombocytopenia is a mild form of WAS, and XL neutropenia is caused by missense mutations in the GTPase binding domain of WASP	XL	Mutations in *WAS*; cytoskeletal defect affecting hematopoietic stem cell derivatives	Rare
2. DNA repair defects (other than those in Table I)							
(a) Ataxia-telangiectasia	Progressive decrease	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	Ataxia; telangiectasia; pulmonary infections; lymphoreticular and other malignancies; increased α fetoprotein and X-ray sensitivity; chromosomal instability	AR	Mutations in *ATM*; disorder of cell cycle check-point and DNA double-strand break repair	Relatively common

(Continued)