

Letter to the Editor

A novel hypomorphic mutation in *STIM1* results in a late-onset immunodeficiency*To the Editor:*

Autosomal-recessive mutations in the Ca^{2+} -sensing protein stromal interaction molecule 1 (*STIM1*) result in combined immunodeficiency (CID), immunodysregulation, ectodermal dysplasia, and nonprogressive myopathy.¹ *STIM1* senses decreases in Ca^{2+} concentration in the endoplasmic reticulum and activates ORAI calcium release-activated calcium modulator 1, the pore-forming subunit of calcium release-activated calcium channels in the plasma membrane. Lack of store-operated Ca^{2+} entry (SOCE) is responsible for the CID owing to a pronounced defect in T-cell activation. So far, 6 patients with *STIM1* deficiency have been reported.¹⁻³ All patients with *STIM1* deficiency had autoimmune cytopenia, 5 had lymphoproliferation, and 3 had eczema.¹⁻³ Chronic diarrhea was seen in 2 patients.^{1,3} Other manifestations included enamel defects (5 of 5), anhydrosis (2 of 2), and iris hypoplasia/mydriasis (5 of 5).³ Only 2 of the 6 patients are currently alive after hematopoietic stem cell transplantation (HSCT) at the age of 1.2 and 6 years (patients 3

and 5). The other 4 patients died at 1.5, 1.7, 2, and 9 years because of encephalitis (patient 2), sepsis (patient 6), Kaposi sarcoma (patient 4), and complications of HSCT (patient 1), respectively.¹⁻³

We present 2 siblings born to consanguineous parents from Moroccan origin. The 21-year-old index patient (patient 7) presented at the age of 6 months with recurrent infections, failure to thrive, and delayed motor development. At the age of 1 year, he developed generalized lymphadenopathy and hepatosplenomegaly. Primary infection with varicella-zoster virus was complicated by cellulitis requiring intravenous antibiotics at age 3 years. He had a *Streptococcus pneumoniae* sepsis at age 4 years. Chest computed tomography at age 6 years showed bronchiectasis. From the age of 10 years, he suffered from colitis poorly responsive to steroids, 5-aminosalicylic acids, azathioprine, and metronidazole. Histopathology showed ulceration and crypt abscesses, with an infiltrate consisting mainly of neutrophils but not eosinophils. No granulomata were found. No infectious cause for colitis could be demonstrated (see the [Case reports](#) section in this article's Online Repository at www.jacionline.org). Other clinical findings are extensive enamel hypoplasia, anhydrosis, disabling nonprogressive myopathy (wheelchair bound), and psoriasis. He recently experienced an

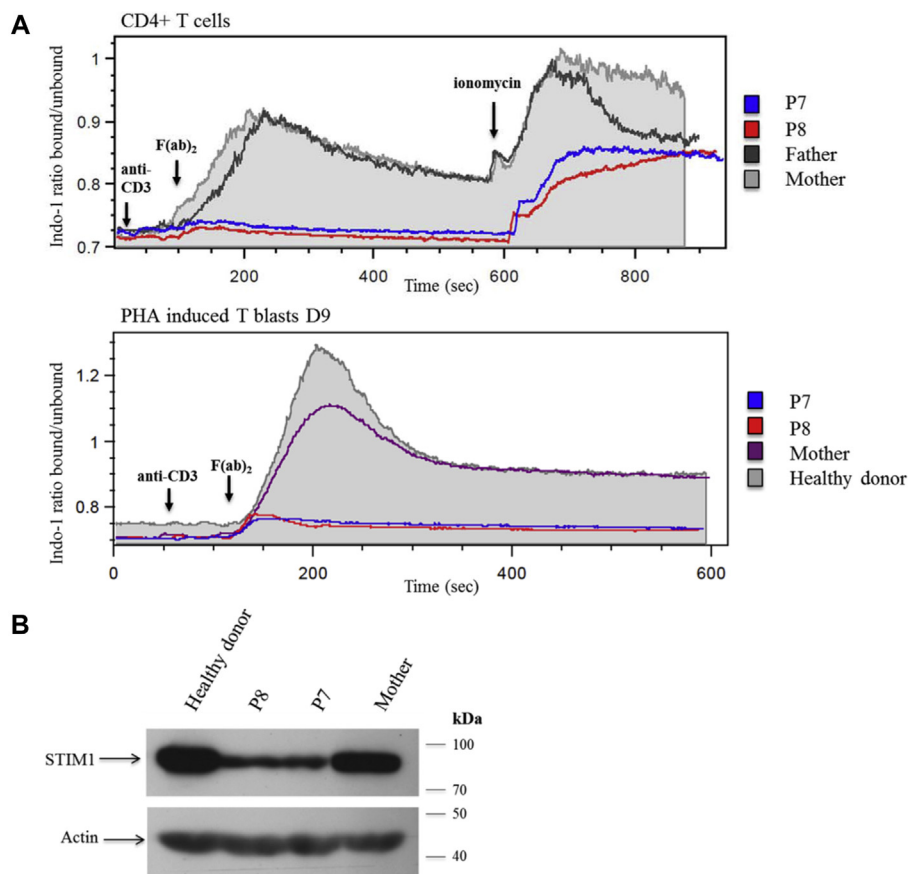


FIG 1. Strongly reduced store-operated calcium entry with residual *STIM1* protein expression. **A**, Ca^{2+} flux in PBMCs and PHA-induced T-cell blasts from patient 7 (P7), patient 8 (P8), the mother, the father, and a healthy control in response to stimulation with anti-CD3 and antimouse IgG crosslinker (F(ab)₂). **B**, Western blot analysis of lysates from T blasts, a healthy control, patient 8, patient 7, and the heterozygous mother incubated with anti-*STIM1* and antiactin antibodies.

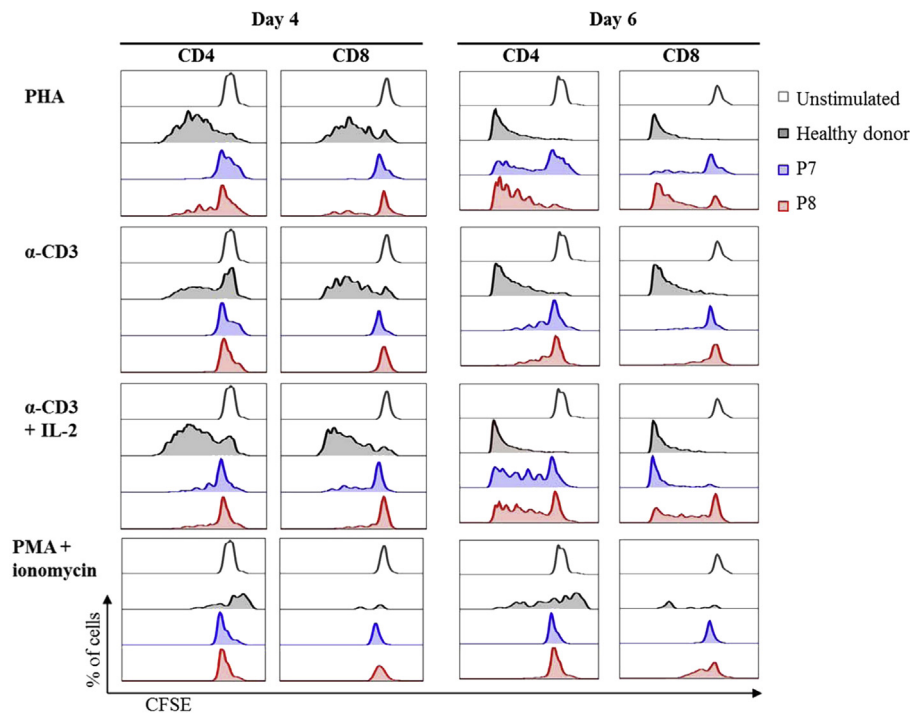


FIG 2. Partially impaired T-cell proliferation in patients with STIM1 deficiency. CFSE proliferation assay using PBMCs from a healthy donor, patient 7 (P7), and patient 8 (P8) after stimulation with PHA, anti-CD3, anti-CD3 + IL-2, and PMA + ionomycin for 4 and 6 days. The histograms are gated on CD3⁺CD4⁺ cells and CD3⁺CD8⁺ cells. CFSE, Carboxyfluorescein succinimidyl ester; PMA, phorbol 12-myristate 13-acetate.

episode of Scabies crustosa complicated by *Staphylococcus aureus* plus *Streptococcus pyogenes* sepsis at the age of 20 years and again suffered from Scabies crustosa at the age of 21 years.

His 8-year-old sister (patient 8) experienced bacterial pneumonia and sepsis at the age of 3 months, recurrent upper respiratory tract infections and chronic dermatitis with superinfection. At the age of 4 years, intravenous immunoglobulin treatment was initiated and she has not experienced any severe infection since. Furthermore, she suffers from enamel hypoplasia (see Fig E1, A, in this article's Online Repository at www.jacionline.org), brittle nails, recurrent dental abscesses, and myopathy. She had no episodes of lymphoproliferation. Neither patient 7 nor patient 8 had autoimmune cytopenia. Iris hypoplasia was also absent in both patients 7 and 8.

Guided by the clinical phenotype, sequencing of *STIM1* gene was performed on genomic DNA from patients 7 and 8 and revealed a novel homozygous missense mutation c.494C>A in exon 4 in both patients (see Fig E1, B), resulting in a proline to glutamine substitution, p.165P>Q. Pedigree analysis is shown in Fig E1, C.

The functional impact of this mutation was investigated by studying the Ca²⁺ influx in PBMCs and in PHA-induced T cells after stimulation by anti-CD3 (see the Methods section in this article's Online Repository at www.jacionline.org). A decreased T cell receptor-mediated Ca²⁺ influx in T-cell blasts was found in both patients' cells (Fig 1, A). Western blot analysis with an antibody against the C-terminus of STIM1 revealed residual but reduced expression of STIM1 in the patients' T-cell blasts (Fig 1, B). These data indicate that the p.165P>Q mutation leads to reduced STIM1 protein expression and function.

The immunologic features of patients 7 and 8 are presented in Table E1 in this article's Online Repository at www.jacionline.org.

Of note, the patients have normal regulatory T-cell (Treg) numbers, yet nearly absent invariant natural killer T (iNKT) cells and decreased number of mucosal-associated invariant T (MAIT) cells. The antipneumococcal antibody response was deficient in patient 7. Both patients 7 and 8 exhibited impaired activation-induced cell death and impaired degranulation of CD8⁺ T cells and NK cells (see Table E1 and Fig E2 in this article's Online Repository at www.jacionline.org). However, proliferation of T cells was only partially impaired (Fig 2).

Phenotypically, patients 7 and 8 largely fit the clinical image of previously reported patients with STIM1 deficiency, but there are some differences. First, there is the prolonged survival up to 8 years and 21 years without HSCT. Four previously reported patients with STIM1 deficiency (patients 1-4) had a complete lack of STIM1 protein production.^{1,2} Two siblings (patients 5 and 6) have been described with residual STIM1 protein expression, but completely abolished SOCE in their T cells.³ In the patients reported herein, reduced STIM1 expression may lead to minimal residual SOCE, sufficient for some T-cell responses, explaining the prolonged survival of these patients. Second, neither patient displayed autoimmune cytopenia. This may be related to normal Treg-cell numbers in both patients, in contrast to other patients with STIM1 deficiency^{1,3} and Stim1/Stim2-deficient mice.⁴ Third, psoriasis and colitis were not previously reported in patients with STIM1 deficiency. Because no infectious cause of colitis could be demonstrated in patient 7, both manifestations might be explained by immune dysregulation. Immune dysregulation has been reported in other CIDs with partial defects in T-cell activation, for example, hypomorphic mutations in *ZAP70* and *LCK*.^{5,6} Interestingly, decreased MAIT cells in the patients parallel the recent finding of low blood MAIT cells in inflammatory bowel disease.⁷ Alternatively, it is possible that

abnormal Treg-cell function causes immune dysregulation. In mice with T-cell-specific deletion of Stim1/Stim2, both number and function of Treg cells were reduced.⁸

Patient 7, reported here, as well as patient 5³ showed no response to unconjugated pneumococcal vaccine, although normal T cell-independent responses were found in Stim1/Stim2-deficient mice with absent SOCE.⁸ Whether this defect is due to a role of SOCE in human B-cell development and function or due to other populations involved in B-cell help (eg, iNKT cells) needs further study. Indeed, both patients 7 and 5 had absent iNKT cells.³

In conclusion, we report a novel homozygous missense mutation in *STIM1* gene in a young adult and in his 8-year-old sibling. Thus, a hypomorphic *STIM1* mutation can lead to CID with longer survival, without autoimmune cytopenia but with other inflammatory manifestations such as psoriasis and colitis. Particular immunologic findings such as absent iNKT cells, low MAIT cells, and deficient pneumococcal polysaccharide antibody response inspire further research into the role of STIM1 and SOCE in the immune system.

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CASE REPORTS

Both patients were never immunized with BCG, and their vaccination with the live Measles-Mumps-Rubella vaccine was uncomplicated.

No infectious cause for colitis could be demonstrated in patient 7. Repeated stool cultures showed no growth of enteropathogens. No parasites were detected on microscopy and PCR for *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum/hominis* was negative. Cryptosporidium antigen and toxine were not detected. PCR on stools for enteroviruses, rotavirus, adenovirus, and norovirus were negative.

METHODS

Genetic analysis

The exons of STIM1 were amplified from genomic DNA extracted from EDTA blood and sequenced with the primers listed by Picard et al^{E1} using standard procedures. A novel homozygous missense mutation c.494C>A in exon 4 was identified in both patients (see Fig E1, B). The resulting proline to glutamine substitution, p.165P>Q, is predicted to be probably damaging *in silico* according to PolyPhen-2 and SIFT Bioinformatics (sift.jcvi.org/ and genetics.bwh.harvard.edu/pph2/). This variant was not detected in the dbSNP138, Exome Variant Server databases (EVS, <http://evs.gs.washington.edu/EVS/>), and 1000 genomes (<http://www.1000genomes.org/data>).

Cells

PHA-induced T-cell blasts were obtained following standard protocols.

Ca²⁺ flux analysis

Ca²⁺ responses were assessed by flow cytometry, as previously described.^{E2} PBMCs or T-cell blasts (10⁶ cells) were labeled with Indo-1 AM (Life Technologies, Carlsbad, Calif; 5 μ M) in 2 mL RPMI-HEPES with 20 μ L Powerload concentrate (Life Technologies). The cells were subsequently stained with CD4-Alexa Fluor 647 (Biolegend, San Diego, Calif) and CD8-fluorescein isothiocyanate (FITC) (BD Biosciences, San Jose, Calif) in RPMI and 5% FCS. The cells were adjusted to a concentration of 10⁶/mL. To induce store depletion, cells were preincubated with antihuman Functional Grade Purified CD3 (eBioscience, San Diego, Calif) and then stimulated with AffiniPure Rabbit antimouse IgG (Jackson ImmunoResearch, Westgrove, Pa). Ionomycin was finally added to the cells at a concentration of 1 μ M (Sigma-Aldrich, St Louis, Mo). The measurement was performed using a FACS Aria flow cytometer (BD Biosciences) and analyzed with FlowJo software, version 9.3.2 (Tree Star Ashland, Ore). Intracellular Ca²⁺ levels correspond to the normalized ratio of Ca²⁺-bound to Ca²⁺-free Indo-1 fluorescence and are plotted as a function of time.

Immunoblots

Cell lysates from T blasts and immunoblots were performed according to standard protocols. Anti-STIM1 (clone D88E10) mAb was from Cell Signaling (Danvers, Mass). An antibody against beta-actin (clone AC-74, Sigma-Aldrich) was used to demonstrate equal protein loading for each sample.

Flow cytometry

NK T cells were detected from stained PBMCs with monoclonal anti-V β 11 conjugated to FITC (Beckman Coulter, Brea, Calif) and monoclonal anti-V α 24 conjugated to phycoerythrin-Cy7 (Beckman Coulter). CD161-Brilliant Violet 421 (Sony Biotechnology, Inc, San Jose, Calif) and Va7.2-Peridinin-Chlorophyll Protein Cy5,5 (Sony Biotechnology, Inc) were used for staining of MAIT cells.

Proliferation assay

PBMCs were labeled with carboxyfluorescein succinimidyl ester (CFSE) (0.5 μ M; Invitrogen, Carlsbad, Calif) for 8 minutes at 37°C. Cells were stimulated with Functional Grade anti-CD3 (1 μ g/mL; eBioscience) $\pm$ IL-2 (100 IU/mL), PHA (2.5 μ g/mL; Sigma-Aldrich), or phorbol 12-myristate 13-acetate (1 ng/mL; Sigma-Aldrich) + ionomycin (1 μ g/mL; Sigma-Aldrich). The measurement of CFSE was performed using a Canto II cytometer (BD Biosciences) and analyzed with FlowJo software, version 10 (Tree Star).

Degranulation assay

Degranulation of T-blast cells and NK cells was assessed following standard procedures. T-cell blasts were incubated for 12 hours at 4°C with coated antihuman Functional Grade Purified CD3 (eBioscience). The cells were then stained with CD107a-FITC and CD107b-FITC, CD3-BV541 (Sony Biotechnology, Inc), and CD8-BV786 (Sony Biotechnology, Inc). CD107 positivity in CD8 T cells was measured using a FACS Fortessa LSR cytometer (BD Biosciences) and analyzed with FlowJo software, version 9.3.2 (Tree Star). For NK-cell degranulation, 2 $\times$ 10⁵ PBMCs were mixed with 2 $\times$ 10⁵ target cells (K562) in 200 μ L complete medium or dissolved in 200 μ L complete medium with 2.5 μ g/mL PHA and then immediately stained with CD107a-phycoerythrin (BD Biosciences). The cells were incubated for 23 hours at 37°C. Thereafter, the cells were stained with CD3-FITC, CD56-allophycocyanin (APC), and CD8-APC-H7, all from BD Biosciences and analyzed on a FACS Canto flow cytometer (BD Biosciences).

Apoptosis assay

The assay was performed on PHA-induced T-cell blasts at day 8. Apoptotic cells were detected by propidium iodide staining 36 hours after stimulation with coated anti-CD3 according to standard protocols. The percentage of induced apoptosis was calculated with the formula: 100 $\times$ (% of experimental apoptotic cells – % of spontaneously apoptotic cells)/100 – % of spontaneously apoptotic cells).

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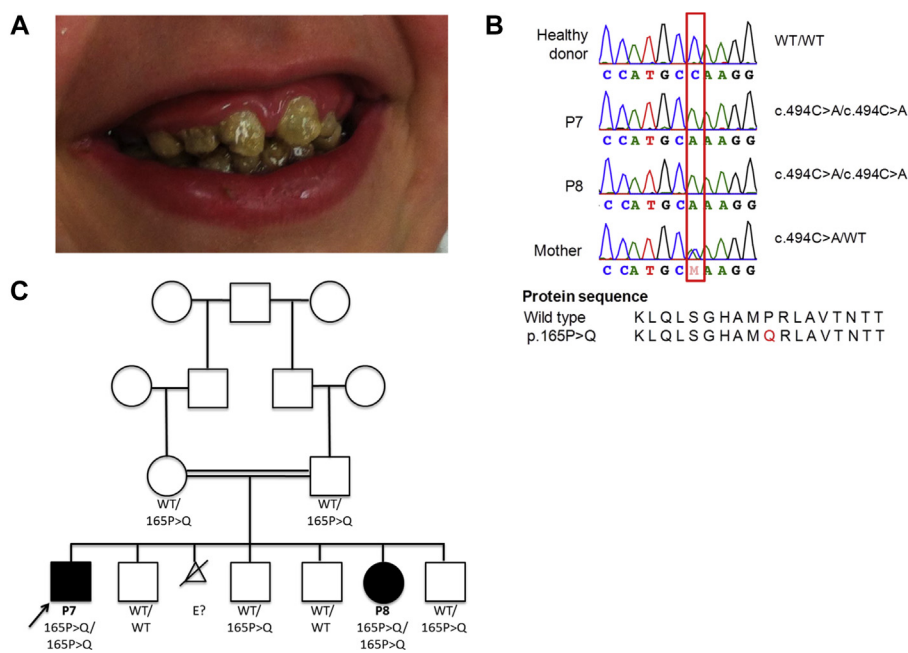


FIG E1. STIM1 deficiency caused by a novel homozygous missense mutation c.494C>A. **A**, Enamel hypoplasia in patient 8 (P8). **B**, Sanger sequencing of genomic *STIM1* DNA revealed the mutation c.494C>A, homozygous in patient 7 (P7) and P8, heterozygous in the mother. **C**, The pedigree of the index patient (P7) and his affected sister (P8). The *STIM1* genotypes of patients and family members are listed under their symbols.

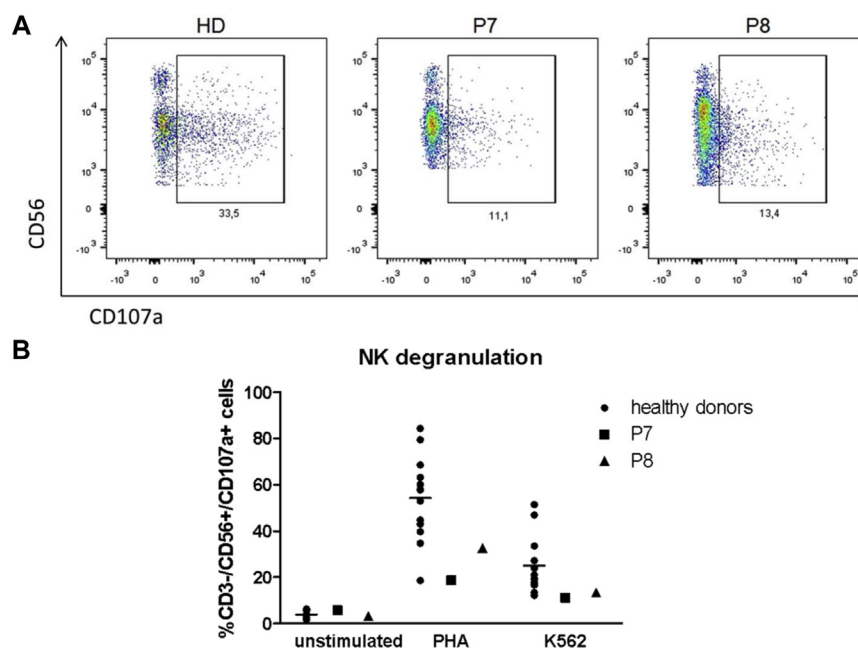


FIG E2. Impaired NK-cell degranulation due to *STIM1* mutation. **A**, CD107a expression in PBMCs from a healthy donor (HD) and patient 7 (P7) and patient 8 (P8) with *STIM1* deficiency, stimulated with K562 cells for 23 hours at 37°C. CD56 is plotted against CD107a in CD3(–)CD56(+) cells. **B**, CD107a expression in CD3(–)CD56(+) cells in unstimulated condition, after stimulation with PHA (2.5 µg/mL) and after stimulation with K562 target cells.

TABLE E1. Immunologic characteristics of patients with STIM1 deficiency

Immunologic features	Patient 7 (at 21 y)	Patient 8 (at 7 y)	Patients 1-6*
Lymphocyte count, $\times 10^9/L$ (normal range)	2.83 (1.5-2.7)	3.31 (1.8-5.0)	6/6 normal
CD3 ⁺ (T cells), % (normal range)	72.2 (55-83)	72.4 (55-78)	6/6 normal
CD4 ⁺ , % (normal range)	42.1 (28-57)	46.3 (27-53)	6/6 normal
CD27 ⁺ CD45RA ⁺ (naive)	↓ 9.02 (49.4-71.9)	↓ 47.5 (55.6-75.8)	1/2 low
CD27 ⁺ CD45RA ⁻ (memory)	↑ 78.1(24.3-42.7)	↑ 44.1(22.5-37)	NA
CD27 ⁻ CD45RA ⁻ (effector)	↑ 13.5 (2.2-9.6)	↑ 8.1 (1.6-11)	NA
CD25 ⁺ CD127 ⁻ (Treg cells)	8.3 (5-12)	7 (2.3-7.7)	2/2 low
CD8 ⁺ , % (normal range)	25.1 (10-39)	21 (19-34)	2/6 low
CD27 ⁺ CD45RA ⁺ (naive)	↓ 20.2 (48.6-87.5)	59 (57-83.7)	1/1 low
CD27 ⁺ CD45RA ⁻ (memory)	↑ 39.4 (9.8-37.6)	22.2 (9.2-22.6)	NA
CD27 ⁻ CD45RA ⁻ (memory effector)	↑ 9.7 (0.2-6.9)	7.4 (0.7-14)	NA
CD27 ⁻ CD45RA ⁺ (effector)	↑ 16.7 (0.8-14)	3.1 (0.9-17.8)	1/1 high
CD19 ⁺ (B cells)	11.3 (7.8-15.1)	15.7 (8.5-20.2)	1/6 low
CD27 ⁺ IgM ⁺ IgD ⁺	↓ 1.4 (7.4-13.8)	↓ 4.3 (7.5-12.5)	NA
CD27 ⁺ IgM ⁻ IgD ⁻	11.1 (7.2-12.7)	↓ 2.8 (5.2-12.1)	NA
CD27 ⁻ IgM ⁺ IgD ⁺	76.6 (65.6-79.6)	↑ 86.7 (69.4-80.4)	NA
CD56 ⁺ CD16 ⁺ (NK cells)	15 (7-31)	10.9 (4-26)	4/4 normal
CD3 ⁺ V α 24 ⁺ V β 11 ⁺ (iNKT cells), count/ 10^6 T cells (mean healthy donors)	↓ 19-47 (189)	↓ 21-33 (189)	1/1 absent
CD3 ⁺ V α 7.2 ⁺ CD161 ⁺⁺ (MAIT cells), % of T cells (mean healthy donors)	↓ 0.31 (3.9)	↓ 0.30 (3.9)	NA
T-cell V β repertoire	Polyclonal	NA	1/1 normal
T lymphocyte proliferation test (CFSE) (see Fig E2)	PHA: ↓ α -CD3: ↓ α -CD3 + IL2: ↓ PMA + ionomycin: ↓	PHA: ↓ α -CD3: ↓ α -CD3 + IL2: ↓ PMA + ionomycin: ↓	PHA: ↓3/3 α -CD3: ↓3/3 α -CD3 + IL2: ↓2/2 PMA + ionomycin: ↓1/1 Tetanus toxoid: ↓1/1 VZV: ↓1/1
AICD on 10 μ g/mL α -CD3 (mean healthy controls)	11.71% (17.87%)	0.71% (17.87%)	NA
Degranulation CD8 ⁺ T cells with 3 μ g/mL α -CD3 (healthy control)	20.3% (48.3%)	9.9% (48.3%)	NA
Degranulation NK cells with K562 cells (mean healthy controls)	11.1% (28%)	13.4% (28%)	↓ 1/1
IgG (g/L) (normal range)	↑ 23.9 (7.51-15.6)	↓ 5.42† (5.58-12.54)	↑ 2/6 , ↓ 1/6
IgA (g/L) (normal range)	↑ 5.26 (0.82-4.53)	↓ 0.25† (0.29-3.84)	↑ 1/6 , ↓ 1/6
IgM (g/L) (normal range)	0.61 (0.46-3.04)	↓ 0.27† (0.34-1.42)	↓ 1/6
IgE	<2 U/L	NA	3/3 normal
Pneumococcal antibody response (before Pneumo23 to after Pneumo23; >1.3 mg/L postvaccination is normal)	PS8: 1.0-1.1 mg/L PS9N: 1.2-1.1 mg/L PS12F: 0.2-0.2 mg/L	IgG treatment	Abnormal 1/1
Allohemagglutinins of blood groups (IgM)	BG A+: anti-B IgM 1/16	NA	NA
Specific IgG antibodies to vaccination antigens	Polio-	IgG treatment	HiB- 2/2 , MM- 1/1
Specific IgG antibodies after infection	VZV+, EBV+, CMV+ PCR CMV-, PCR EBV 4.85 log copies/mL	IgG treatment, PCR EBV 2.22 log copies/mL	EBV+ 1/2, CMV+ 2/2, HSV+ 2/2

Reference values are within parentheses. Values that are outside the normal ranges for age are given in boldface.

AICD, Activation-induced cell death; CFSE, carboxyfluorescein succinimidyl ester; HiB, *Haemophilus influenzae* type B; HSV, herpes simplex virus; IV, intravenous; MM, measles and mumps; NA, not assessed; PMA, phorbol 12-myristate 13-acetate; VZV, varicella-zoster virus.

*References E1, E3, and E4.

†Through levels at 4 weeks past IV immunoglobulin replacement treatment.