

Letter to the Editor

Combined immunodeficiency due to JAK3 mutation in a child presenting with skin granuloma*To the Editor:*

We describe a child with a combined immunodeficiency due to a JAK3 mutation partially affecting protein expression and presenting primarily with persistent skin granuloma.

The patient, a 2-year-old female, born of consanguineous parents, was hospitalized because of pleuropneumonia and diarrhea. In her clinical history, there was an uneventful chickenpox infection at age 2 months, frequent respiratory infections, including 1 episode of pneumonia treated at home, and recurrent diarrhea. She had normal growth. At the age of 16 months, persistent papular-nodular lesions appeared on her face and arms. The skin biopsy revealed a noninfectious, nonnecrotizing granulomatous dermatitis (see Fig E1, A, in this article's Online Repository at www.jacionline.org).

Investigations did not identify any notable etiology for recurrent pneumonia and diarrhea but revealed lymphopenia fluctuating over time (lymphocytes, 970-1900/mm³) for which she was referred to our unit. Immunological analysis showed a marked decrease in CD3⁺ cells, an increased proportion of B cells, and normal natural killer (NK) cells.

Although she had normal serum immunoglobulins, a detailed analysis demonstrated an impaired humoral immunity with absence of isohemoagglutinins, lack of response to pneumococcal vaccine, IgG subclass deficiency, low memory B cells with markedly reduced switched B cells, and abnormal response to cytosine-phosphate-guanosine (CpG)-DNA stimulation (see Table E1 and Fig E2 in this article's Online Repository at www.jacionline.org). Interestingly, protective antibody titers to chickenpox infection and to measles immunization were detected.

Lymphocytes failed to normally proliferate in response to antigens and mitogens, even with addition of IL-2 and anti-CD28. Reduced naive T cells, recent-thymic-emigrants, and T cell receptor excision circles (TRECs), associated with skewed CD3⁺TCRV β spectratyping (see Fig E3 in this article's Online Repository at www.jacionline.org), were found. Indeed, a substantial number of CD45RO⁺/HLA-DR⁺-activated T lymphocytes and a high percentage of CD4⁺ cells producing IFN- γ were detected (see Table E1 in the Online Repository). Chimerism analysis excluded maternal engraftment. EBV-viremia was present and remained stable over time although this did not result in a complete seroconversion.

Sulfamethoxazole-trimethoprim prophylaxis and intravenous immunoglobulin substitution were started with clinical improvement but without significant amelioration of skin lesions. During follow-up, a focal swelling appeared bilaterally on ankles consistent with a diagnosis of tenosynovitis. Autoantibodies (antinuclear antibodies and antineutrophil cytoplasmic antibody) were negative. The tendon biopsy revealed a lymphohistiocytic infiltration with a predominance of T cells, mainly CD8⁺ lymphocytes, in the absence of infectious microorganisms, including EBV. A similar picture was found in the skin biopsy (see Fig E1). Both lesions improved after treatment with steroids.

Adenosine deaminase, purine nucleoside phosphorylase, IL-7R deficiency, and syndromes associated with defective DNA repair were excluded. Analysis of IL-2 signal transducer and activator of transcription 5 (STAT5) phosphorylation by flow cytometry on peripheral blood revealed a reduced but detectable percentage of phospho-STAT5⁺ CD4⁺CD45RO⁺ population (Fig 1, A, I-II).

A decreased STAT5-phosphorylation was confirmed on the patient's EBV-transformed B-cell line in response to IL-2 and IL-15 (Fig 1, A, III). These data suggested a defect in the JAK3 pathway despite the presence of NK cells. The JAK3-western blot analysis on peripheral blood and EBV-transformed B-cell line showed reduced protein expression (Fig 1, B, I). In the tyrosine-phosphorylation immunoblot, JAK3 phosphorylation appeared very low, consistent with reduced JAK3 levels (Fig 1, B, II). Sequence analysis of JAK3 revealed a homozygous missense mutation c.1208 G>A that causes the substitution p.R403H in the SH2 domain. Both parents and brother were heterozygous for the mutation and were clinically normal and showed a STAT5 phosphorylation comparable to that of healthy control (Fig 1, A).

The R403H mutation is present in the Catalogue of Somatic Mutations In Cancer database and was found to be associated with severe combined immunodeficiency (SCID) in a single Chinese patient in whom it was present in a compound heterozygous state (the other mutation being R1085Q).¹ The R403H mutation is predicted to affect the protein stability by MuStab and MUpPro Web servers and is defined as a disease-related variant by the SNP&GO server. Because of the severe immunological alterations associated with worsening of immunodysregulation and organ infiltration by autoreactive T cells, we decided to perform HLA-partially matched hematopoietic stem cell transplantation (HSCT) depleted of TCR- $\alpha\beta$ ⁺/CD19⁺ lymphocytes using the father as donor. A reduced-toxicity conditioning regimen consisting of thiotepa (8 mg/kg in 2 doses), fludarabine (160 mg/m² over 4 days), treosulfan (42 g/m² over 3 days), antithymocyte globulin (ATG) (12 mg/kg over 3 days), and rituximab (200 mg/m² in single dose) was used.² The patient did not receive any pharmacological graft-versus host disease prophylaxis after the allograft. A full donor engraftment was achieved, with the median time to reach neutrophil ($\geq 0.5 \times 10^9/L$) and platelet ($\geq 20 \times 10^9/L$) recovery being 16 and 9 days after transplantation, respectively. No serious adverse events occurred, but she experienced a cytomegalovirus viremia early after HSCT that required treatment with ganciclovir and foscarnet. Five months after HSCT, the patient is in good general condition and shows a considerable improvement in skin lesions. At last evaluation, the absolute lymphocyte count was $118 \times 10^9/L$, with $0.5 \times 10^9/L$ and $0.3 \times 10^9/L$ $\alpha\beta$ ⁺ and $\gamma\delta$ ⁺ T cells, respectively. The absolute number of B and NK cells at the time of last evaluation was $0.2 \times 10^9/L$ and $0.25 \times 10^9/L$, respectively.

Although null mutations in genes involved in T-cell development lead to usually fatal SCID, the JAK3 mutation in this patient, similar to other mutations in other SCID-causing genes, results in a partial preservation of protein expression and functionality, giving rise to a different clinical phenotype.³⁻⁶ Indeed, the R403H mutation maintains residual levels of STAT5 phosphorylation, likely contributing to the milder clinical presentation with no

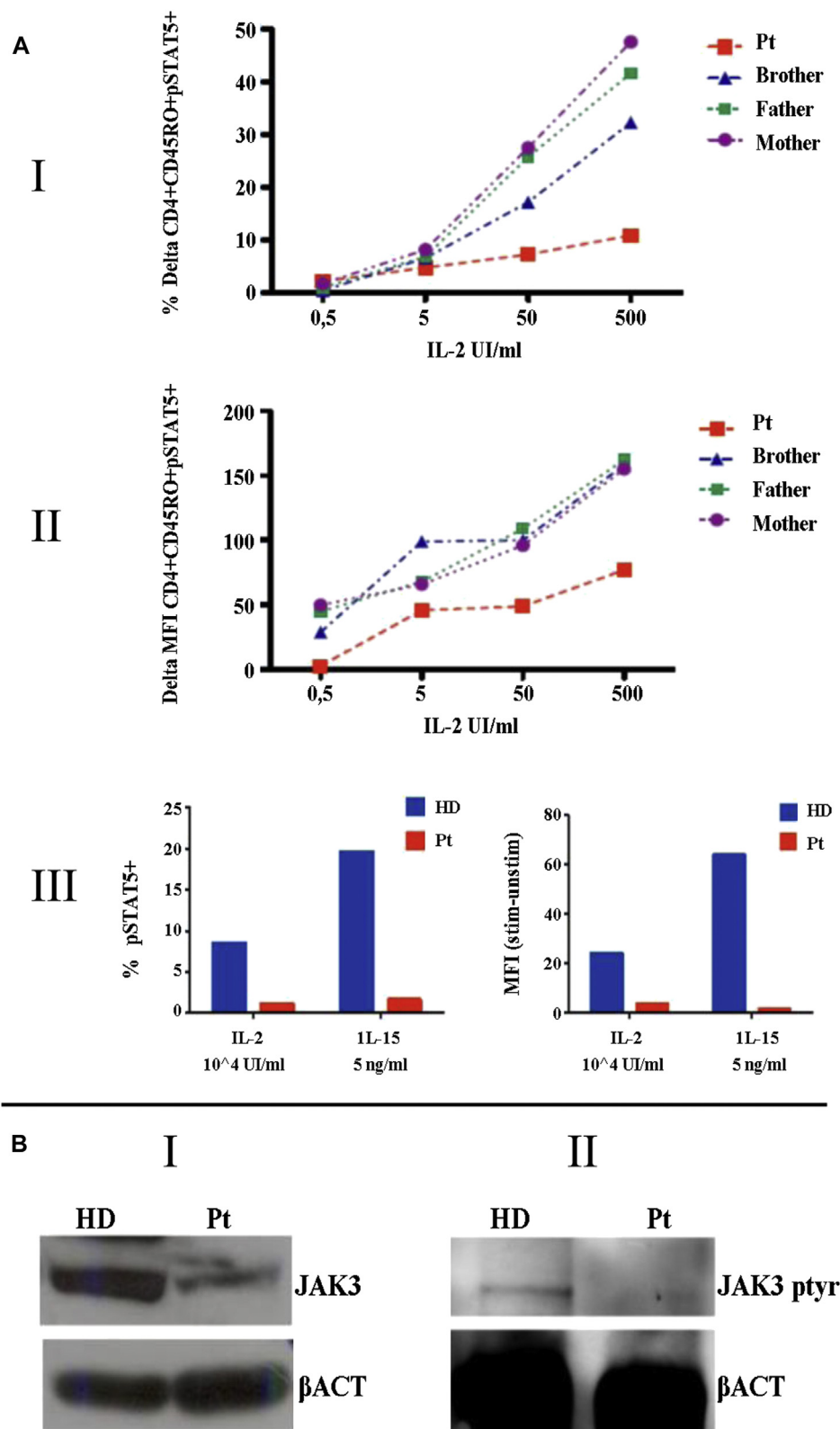


FIG 1. A and B, Defective JAK3 expression and signaling. Fig 1, A, Percentage (I) and median fluorescence intensity (MFI) (II) of STAT5⁺ cells gated on patient's CD4⁺CD45RO⁺ after IL-2 stimulation for 10 minutes at the indicated concentrations compared with those of the parents and brother. Data are shown as difference between stimulated and unstimulated samples. (III) STAT5⁺ cells on EBV cell lines (difference between stimulated and unstimulated) after IL-2 and IL-15 stimulation compared with healthy donor (HD). Fig 1, B, (I) Western blot analysis of whole-cell extracts from EBV-transformed B lymphocytes of JAK3-mutated patient and HD. (II) IL-2 stimulation of JAK3 tyrosine phosphorylation. Lysates were immunoprecipitated with an antibody to JAK3 and then immunoblotted with an antiphosphotyrosine antibody. Pt, Patient.

early and/or severe complications. However, the role of environmental and epigenetic factors cannot be excluded. The residual JAK3 activity may be sufficient to permit NK-cell development and partial immunoglobulin production but was not sufficient to sustain a physiological thymic development of T lymphocytes.^{3,5} Disseminated varicella infection and lack of protective titers to tetanus have been reported in patients with hypomorphic JAK3 mutations.^{3,4} Surprisingly, our patient had a chickenpox infection with an uncomplicated course in the first months of life. One could speculate that maternal immunity has ensured protection against the disseminated chickenpox infection, but the detection of persistent specific antibodies to varicella-zoster virus, measles, and tetanus vaccination suggests a partial preservation of memory function in the initial stages of life. A residual activity of IL21R/γ-chain/JAK3 pathway, mostly stimulated in the case of antigen overload (infection, vaccination), could allow B-cell proliferation, immunoglobulin switching, and plasma cell generation.⁷ Furthermore, a partial T-cell function could have deteriorated over time following the chickenpox and/or a long duration of antigen load (EBV chronic infection), leading to immune dysregulation with sustained induction of pro-inflammatory response. In addition, this patient had a history of undefined skin lesions but was brought to our attention after evidence of lymphopenia. Cutaneous lesions can be one of the earliest signs of primary immunodeficiency. The persistence of undefined skin lesions or unexplained autoinflammatory signs, such as the severe joint swelling in our patient, should raise the suspicion of an underlying immune defect. To our knowledge, this is the first case of cutaneous granuloma associated with JAK3 deficiency. Granuloma formation is described in other primary immunodeficiencies such as chronic granulomatous disease, common variable immunodeficiency, and RAG1/2 and DNA repair deficiencies.⁸

In our atypical case, the diagnosis was suggested by STAT-5 phosphorylation, which is a rapid functional assay to detect abnormalities in the γ-chain/JAK3 pathway, in patients having indistinct immunophenotypes (T^{low}B⁺NK⁺).^{9,10} Although direct molecular approaches such as genome sequencing and exome sequencing are mostly used, functional assays could be a rational and cost-effective approach before gene sequencing in patients with atypical presentations and could help to define the effect of mutations, especially in those cases in which genome sequencing is not available.

This case demonstrates the high clinical and immune heterogeneity of JAK3 deficiency and should alert clinicians to consider hypomorphic mutations in SCID-causing-genes, including JAK3, in all patients with an atypical presentation and T-cell lymphopenia.

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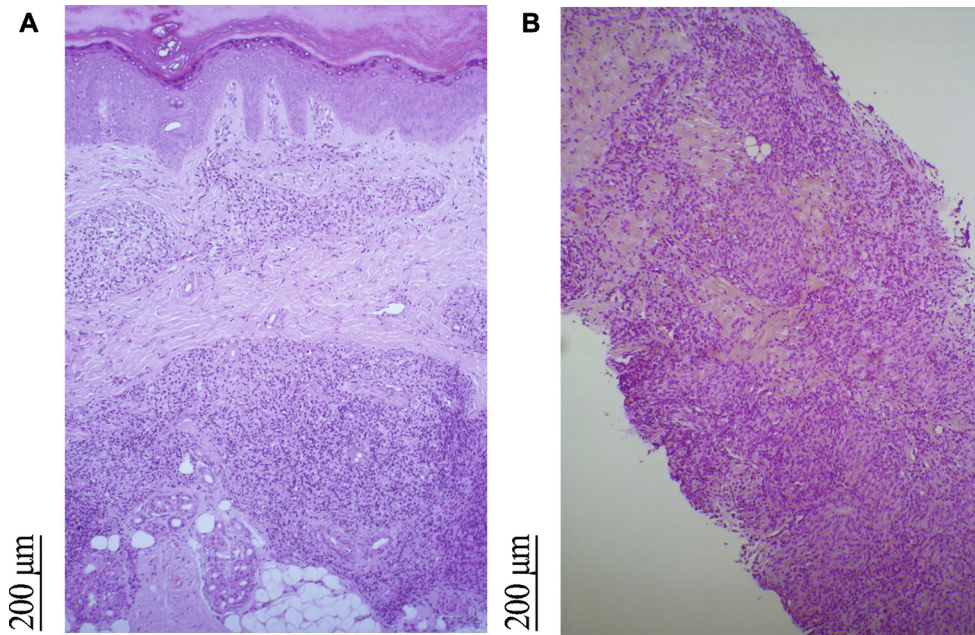


FIG E1. Cutaneous and tendons biopsy (E&E10×). Inflammatory process with lymphohistiocytic infiltration in dermis (**A**) and tendon (**B**) forming nonnecrotizing granuloma.

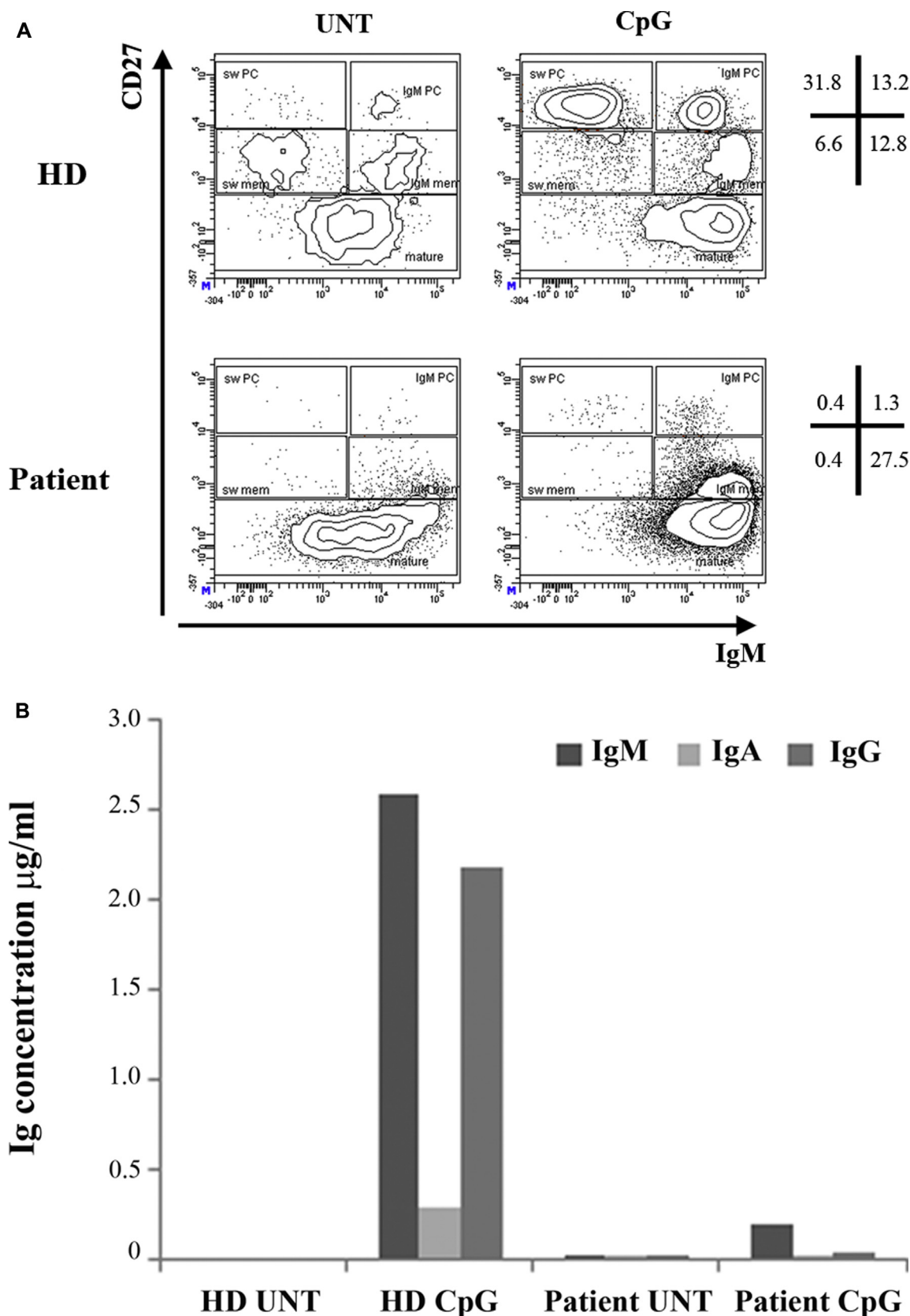


FIG E2. CpG response. **A**, Contour plots show the staining of B cells in the absence or presence of CpG. The gates identify the different B-cell populations: IgM⁺ and IgM⁻ CD27^{bright} plasma cells, CD27⁺ memory B cells, which are either IgM memory (IgM⁺) or switched memory (IgM⁻), and mature B cells. The numbers indicate the percentages of these subpopulations in the B-cell gate. **B**, The histograms represent the concentration ($\mu\text{g/mL}$) of IgM (black column), IgA (white column), and IgG (gray column) antibodies in the supernatants of 7-day cultures with CpG in healthy control and the patient. *UNT*, Unstimulated.

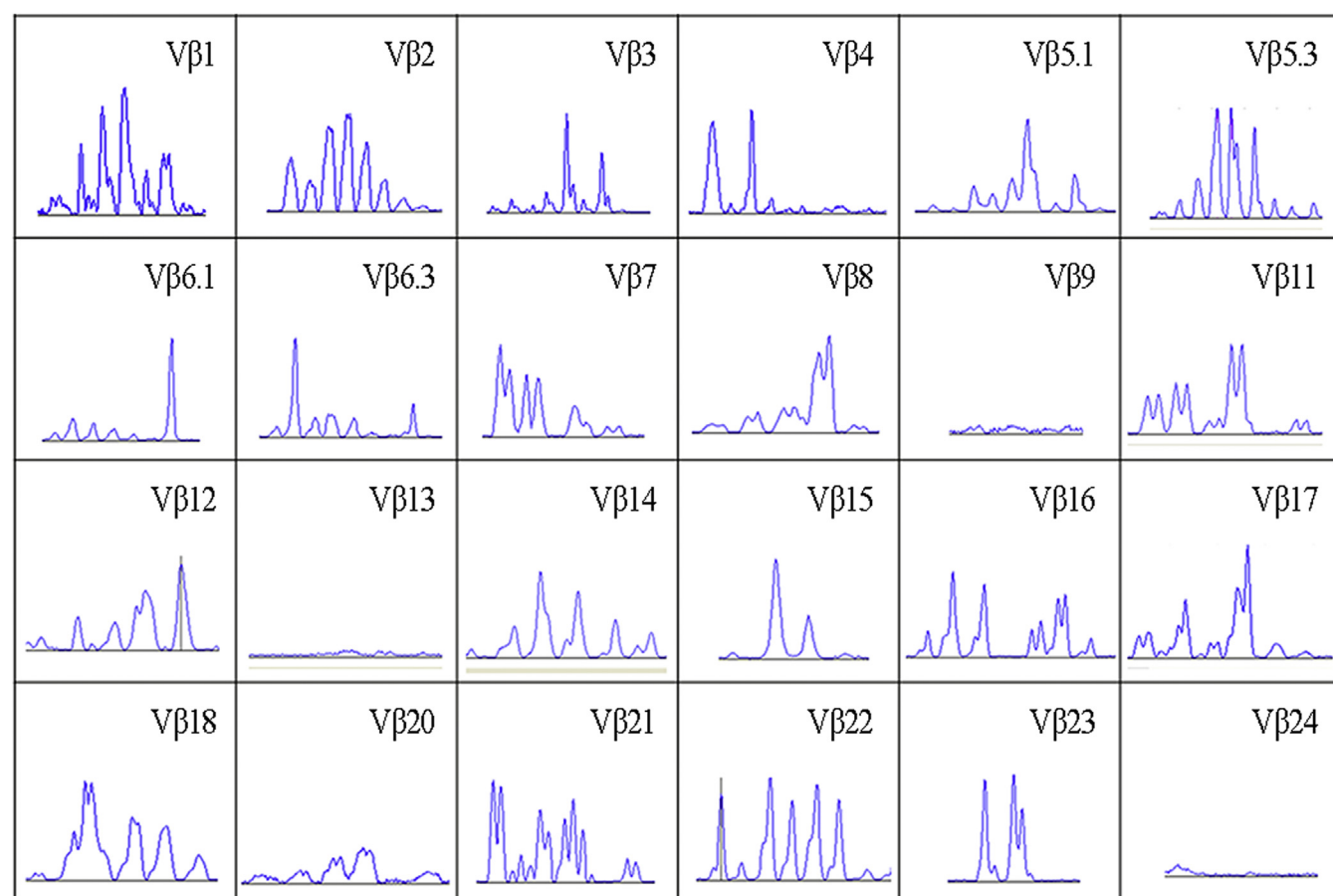


FIG E3. Analysis of TCR diversity. TCRVβ spectratyping of CD3⁺ T cells showed a skewed pattern.

TABLE E1. Summary of immunological data

Parameter	Patient (age, 27 mo)	Normal values for age
White blood cell per mm ³ *	5,830	5,200-11,000
Lymphocyte phenotype (absolute count per mm ³)	30.7% (1.790)	1,400-5,500
CD3 ⁺ †	21.5% (384)	52% to 92% (850-4,300)
CD4 ⁺ †	15% (268)	25% to 66% (500-2,700)
CD8 ⁺ †	4.5% (80.5)	9% to 49% (200-1,800)
CD16 ⁺ CD56 ⁺ †	7.6 % (136)	2% to 25% (61-510)
CD19 ⁺ †	69% (1.236)	8% to 39% (180-1,300)
CD4 ⁺ CD31 ⁺ CD45RA ⁺ (RTE)†	0.3% (0.8)	37% to 100% (190-2,600)
CD4 ⁺ CD27 ⁺ CD45RA ⁺ (naive)†	0.5% (1.34)	52% to 92% (300-2,300)
CD8 ⁺ CD27 ⁺ CD45RA ⁺ CCR7 ⁺ (naive)†	0.2% (0.16)	19% to 100% (53-1,100)
CD4 ⁺ HLADR ⁺ *‡	41%	3% to 12% (30-140)
CD8 ⁺ HLADR ⁺ *‡	56.7%	7% to 37% (50-340)
Regulatory T (CD4 ⁺ CD25 ⁺ CD127 ^{low} FoxP3 ^{high})	6.4%	4.86% to 7.09%
CD3 ⁺ CD4 ⁺ IFNγ ⁺	45.7%	6.9% to 18.1%
Lymphoproliferation test (10 ³ cpm)‡		
Unstimulated	0.8	<2
PHA (2.5 μg/mL)	7.1	>30
PHA + IL-2 (20 U/mL)	9.4	>40
PWM (0.35 μg/mL)	6.9	>18
OKT3 (1.5 μg/mL)	2.8	>20
OKT3 + IL-2	6.3	>30
OKT3 + aCD28	3.1	>25
PMA + IONO	34.1	>20
Unstimulated	0.7	<2
Candida	1	>5
Tetanus	0.8	>5
TCRVβ spectratyping		
Percentage of polyclonal families (CD3 ⁺)	22.8%	>70%
TREC (copies/100 ng DNA)	<25	1,106-15,787
Serum immunoglobulin levels§		
IgG (mg/dL)§	676	462-1,710
IgA (mg/dL)§	24	27-173
IgM (mg/dL)§	26	62-257
IgE (kU/L)	<2	1.1-49.0
Blood group isohemagglutinins (IgM-anti-B)	Absent	(>1:8)
IgG subclasses		
IgG ₁	576	290-970
IgG ₂	17.8	36-214
IgG ₃	10	80-630
IgG ₄	<0.35	
Specific antibodies		
Tetanus toxoid (IU/mL)	0.5	>0.1
Hepatitis B (mIU/mL)	33.26	≥11
<i>Hemophilus influenzae</i> (mg/L)	0.3	>0.15
Pneumococcus (mg/L)¶	<3.3	>35
B-cell subpopulations#		
CD19 ⁺ 27 ⁺	1.8%	8% to 15%
CD27 ⁺ IgM ⁺	1.4%	5% to 11%
CD27 ⁺ IgM ⁻	0.16%	2% to 5%
CD20 ⁺ CD24 ⁺⁺ CD38 ⁺⁺	27.6%	10% to 30%
CpG stimulation	Lymphocyte proliferate but differentiate poorly in plasma cells and produce only IgM ⁺	

Patient data have been collected on the first admission in the Immuno-Infectivology Unit. Absolute values are given within parentheses.

IONO, Ionomycine; OKT3, orthoclone, monoclonal antibody anti-CD3; PHA, phytohaemagglutinin; PMA, phorbol myristate acetate; PWM, pokeweed-mitogen; RTE, recent-thymic-emigrant.

*Normal range for age are taken from Shearer et al.^{E1}

†Normal range for age taken from Schatorjè et al.^{E2}

‡T-cell proliferation at day 3 by radio-labeled thymidine incorporation.

§Normal values according to Ugazio et al.^{E3}

||Normal values according to Plebani et al.^{E4}

¶Specific antibodies were measured 4 weeks after pneumococcal vaccination.

#Normal values of B-cell subsets refer to the 5th and 95th percentile of values collected from 7 age-related healthy children being referred to our hospital for elective procedures.

Other normal values were measured according to internal laboratory references.