Published in final edited form as:

J Allergy Clin Immunol. 2013 April ; 131(4): 1136–1145. doi:10.1016/j.jaci.2012.12.667.

Hypomorphic Janus kinase 3 mutations result in a spectrum of immune defects, including partial maternal T-cell engraftment

Federica Cattaneo, MD^{#a}, Mike Recher, MD^{#b,c}, Stefania Masneri, MS^d, Sachin N. Baxi, MD^c, Claudia Fiorini, PhD^a, Francesca Antonelli, BS^d, Christian A. Wysocki, MD, PhD^e, Jose G. Calderon, MD^e, Hermann Eibel, PhD^f, Angela R. Smith, MD, MS^g, Francisco A. Bonilla, MD, PhD^c, Erdyni Tsitsikov, PhD^h, Silvia Giliani, PhD^d, Luigi D. Notarangelo, MD^{b,c,i}, and Sung-Yun Pai, MD^{a,j}

^aDivision of Hematology-Oncology, Boston Children's Hospital, Boston, Mass

^bManton Center for Orphan Disease Research, Boston Children's Hospital, Boston, Mass

^cDivision of Immunology, Boston Children's Hospital, Boston, Mass

^d"A. Nocivelli" Institute for Molecular Medicine, University of Brescia and Laboratory of Genetic Disorders of Childhood, Spedali Civili, Brescia;

^eSection of Allergy and Clinical Immunology, Yale University School of Medicine, Yale–New Haven Hospital, New Haven;

^fCentre of Chronic Immunodeficiencies, University Medical Center Freiburg;

^gDivision of Pediatric Blood and Marrow Transplantation, University of Minnesota, Minneapolis;

^hDepartment of Laboratory Medicine, Boston Children's Hospital, Boston, Mass;

ⁱDepartment of Pathology, Harvard Medical School, Boston;

^jDepartment of Pediatric Oncology, Dana-Farber Cancer Institute, Boston.

[#] These authors contributed equally to this work.

Abstract

Background: Mutations in Janus kinase 3 (*JAK3*) are a cause of severe combined immunodeficiency, but hypomorphic *JAK3* defects can result in a milder clinical phenotype, with residual development and function of autologous T cells. Maternal T-cell engraftment is a common

Corresponding author: Sung-Yun Pai, MD, Boston Children's Hospital, One Blackfan Circle, Karp Family Research Laboratories, Rm 08214, Boston, MA 02115. sung-yun.pai@childrens.harvard.edu.

Clinical implications: Persistence of *JAK3*-deficient B cells might preclude successful reconstitution of humoral immunity after transplantation. Coexistence of autologous and maternally engrafted T cells should be sought in patients with hypomorphic mutations in *JAK3*.

Disclosure of potential conflict of interest: M. Recher has been supported by one or more grants from the Swiss National Science Foundation (SNSF/SSMBS; NrZ PASMP3–127678). C. A. Wysocki has been supported by one or more grants from the National Institutes of Health. L. D. Notarangelo has received one or more grants from the National Institutes of Health and from the Manton Foundation, is a Board member for the Immune Disease Institute, is employed by Boston Children's Hospital, has received one or more grants from or has one or more grants pending with the Wiskott-Aldrich Foundation and with the March of Dimes, and has received royalties from UpToDate. S.-Y. Pai has been supported by one or more grants from the National Institute of Allergy and Infectious Diseases and from Translational Research Program at Boston Children's Hospital. The rest of the authors declare that they have no relevant conflicts of interest.

finding in infants with severe combined immunodeficiency but is not typically observed in patients with residual T-cell development.

Objective: We sought to study in detail the molecular, cellular, and humoral immune phenotype and function of 3 patients with hypomorphic *JAK3* mutations.

Methods: We analyzed the distribution and function of T and B lymphocytes in 3 patients and studied the *in vitro* and *in vivo* responses of maternal T lymphocytes in 1 patient with maternal T-cell engraftment and residual production of autologous T lymphocytes.

Results: B cells were present in normal numbers but with abnormal distribution of marginal zone–like and memory B cells. B-cell differentiation to plasmablasts *in vitro* in response to CD40 ligand and IL-21 was abolished. In 2 patients the T-cell repertoire was moderately restricted. Surprisingly, 1 patient showed coexistence of maternal and autologous T lymphocytes. By using an mAb recognizing the maternal noninherited HLA-A2 antigen, we found that autologous cells progressively accumulated *in vivo* but did not compete with maternal cells *in vitro*.

Conclusion: The study of 3 patients with hypomorphic *JAK3* mutations suggests that terminal B-cell maturation/ differentiation requires intact JAK3 function, even if partially functioning T lymphocytes are present. Maternal T-cell engraftment can occur in patients with *JAK3* mutations despite the presence of autologous T cells.

Keywords

Severe combined immunodeficiency; cytokine signaling; maternal engraftment

Null mutations in genes critical to T-cell development typically result in severe combined immunodeficiency (SCID) disease.^{1,2} Defects of the IL-2 receptor common γ chain (*IL2RG*, γc),^{3,4} Janus kinase 3 (*JAK3*),^{5,6} and IL-7 receptor α (*IL7R*)⁷ genes affect cytokine signaling, account for a large proportion of SCID,¹ and are typically associated with a T⁺B⁺ phenotype.⁸ Failure of T-cell development in patients with SCID often allows the engraftment of maternal T lymphocytes *in utero*.^{9–11} These maternal T cells are typically oligoclonal, poorly functional *in vitro*, nonprotective *in vivo*, and can cause graft-versus-host disease (GVHD) or graft rejection after T cell–depleted, unconditioned, haploidentical hematopoietic stem cell transplantation from a donor other than the mother.^{9,11–15}

In contrast, missense and splice-site mutations in SCID-causing genes can lead to partial preservation of protein expression, function, or both, allowing residual development of autologous T lymphocytes. Omenn syndrome (OS), which is characterized by erythroderma, hepatosplenomegaly, and lymphadenopathy and associated with oligoclonal and activated autologous T cells, is associated with hypomorphic mutations of SCID-causing genes. In other cases, often referred to as “atypical” or “leaky” SCID, autologous T cells of varying number are observed in the absence of clinical features of OS. Most cases of OS and of atypical SCID in human subjects are caused by hypomorphic recombination-activating gene 1 (*RAG1*) or *RAG2* mutations.^{16,17} However, in a recent series hypomorphic Abbreviations used mutations in genes encoding components of the γc -dependent signaling pathway accounted for 15 of 73 patients with atypical SCID.¹⁸ In particular, hypomorphic *IL2RG* mutations have been demonstrated in patients with clinical features of combined

immunodeficiency, low to normal numbers of poorly functional autologous T cells, and survival that can extend into late childhood or even adulthood.^{19–22} An even broader range of clinical phenotypes has been described in patients with hypomorphic *JAK3* mutations,^{23–28} including presentation in infancy with features of SCID, milder infectious history later in childhood, delayed-onset immunodeficiency, lymphoproliferative disorder, persistent warts, and even asymptomatic presentation in young adulthood. Interestingly, significant clinical and immunologic heterogeneity has been noted in siblings carrying the same *JAK3* mutations,²⁵ suggesting that modifying genes or environmental factors can influence the phenotype.

We studied 3 patients with hypomorphic *JAK3* mutations and a spectrum of humoral and cellular function. Surprisingly, in 1 patient the presence of maternally engrafted T lymphocytes was associated with autologous T cells that retain some residual function *in vitro* and *in vivo*.

METHODS

Subjects

Medical record review and research blood samples were obtained with written consent from parents or guardians and approved by the Boston Children's Hospital Institutional Review Board.

Cell purification and culture

PBMCs were purified by using Ficoll gradient. B-lymphoblast cell lines (B-LCLs) and T-cell lines were derived by means of immortalization with EBV and herpesvirus saimiri, respectively. Cells were cultured in RPMI medium supplemented with 10% FCS and penicillin-streptomycin. Where indicated, IL-2 (National Institutes of Health Biorepository, Rockville, Md) at 100 U/mL, IL-4 (eBioscience, San Diego, Calif) at 25 ng/mL, CD40 ligand (CD40L) at 5 mg/mL, and/or IL-21-Fc were added to the culture.²⁹ For mixed lymphocyte culture, magnetically purified T cells were cultured 1:1 with irradiated PBMCs from the indicated donors for 7 days, including tritiated thymidine during the last 18 hours.

Flow cytometry

Immunophenotyping was performed with the LSR II or FACSCanto flow cytometer (BD Biosciences, San Jose, Calif) by using the FACSDiva (BD Biosciences) and FlowJo software (TreeStar, Ashland, Ore). The following mAbs were used: CD3 (UCHT1 or HIT3a), CD4 (OKT4), CD8 (SK1), CD19 (HIB19), CD24 (ML5), CD38 (HIT2), CD27(0323), IgM (MHM-88), CD45RA (HI100), HLA-A2 (BB7.2), and HLA-DR (L243) from BioLegend (San Diego, Calif) and CD8 (SK1 or RPA-T8), CD38 (HIT2), IgD (IA6-2), HLA-DR (L243), and Ki67 (B56) from BD Biosciences. Percentages of transitional (CD19⁺CD24^{hi}CD38^{hi}), marginal zone-like (CD19⁺CD24^{hi}CD38^{lo}), and switched memory (CD19⁺CD27⁺IgD⁺) B cells were compared with published age-specific normal values.^{30,31}

Expression of T-cell receptor (TCR) V β families was studied with the IOTest Beta Mark kit (Beckman Coulter, Indianapolis, Ind).

T-cell receptor excision circle analysis

T-cell receptor excision circle (TREC) levels were determined by using real-time quantitative PCR of genomic DNA with RNase P as the control gene^{32,33} and newborn screening dried blood spot specimens retrieved with informed consent (patient 1) or in the course of universal pilot screening (patient 2). Determination of TREC levels in patient 3 was performed at age 23 months by using quantitative PCR of genomic DNA from PBMCs with albumin as the internal control of genomic DNA amplification.³⁴

In vitro cell proliferation and plasmablast differentiation

For analysis of T-cell proliferation, PBMCs were incubated with 5 mmol/L carboxyfluorescein diacetate succinimidyl ester (CFSE) and then cultured as indicated with medium only or with soluble anti-CD3 (clone OKT3, eBioscience) plus soluble anti-CD28 (eBioscience) in the presence or absence of 100 U/mL IL-2 (NIH Biorepository) and analyzed after 3 days, gating on CD3, CD4, CD8, HLA-A2, and/or HLA-DR. For analysis of B-cell proliferation, PBMCs were incubated with 5 mmol/L CFSE, cultured as indicated, and analyzed after 5 days, gating on CD19⁺ cells. *In vitro* plasmablast differentiation in response to CD40L plus IL-21 was measured, as previously described.³⁵

Sequence and cDNA analysis

RNA was isolated from B-LCLs with the mirVana miRNA isolation kit (Ambion from Applied Biosystems, Foster City, Calif). Reverse transcription was performed with qScript cDNA SuperMix (QuantaBioSciences, Gaithersburg, Md) with 1 µg of RNA. *JAK3* cDNA (ENST00000527670) was amplified with the primers JAK3–1353F and JAK3–1838R (exons 9–13) or JAK3–1353F and JAK3–2167R (exons 9–15). Sequences and amplification conditions are available on request. PCR products were analyzed after cycle sequencing (Big-Dye Terminator, Applied Biosystems) on an ABI3130 Genetic analyzer (Applied Biosystems). For real-time PCR analysis, TaqMan primers and probes spanning exon 22–23 were used (Hs00169663_m1; Life Technologies, Grand Island, NY).

Protein analysis

After stimulation with 1200 µg/mL IL-2 for 12 minutes at 37°C, cells were lysed in cold buffer (300 mmol/L NaCl, 50 mmol/L Tris-HCl [pH 7.4], 0.5% Triton, 2 mmol/L EDTA [pH 8], and protease inhibitors; Roche, Mannheim, Germany) on ice for 30 minutes. Western blotting of cytoplasmic cell extracts was performed with antibodies to phosphotyrosine signal transducer and activator of transcription (STAT) 5 (pY694) and STAT5 (BD Biosciences), JAK3 (C-21; Santa Cruz Biotechnology, Santa Cruz, Calif), b-actin (A5060; Sigma-Aldrich, St Louis, Mo), horseradish peroxidase–conjugated anti-mouse or anti-rabbit IgG, and the ECL system (Amersham Biosciences, Piscataway, NJ).

RESULTS

Clinical and immunologic findings

Patients 1 and 2 are sister and brother born to nonconsanguineous Northern European parents. Patient 1 had eczema at 1 month of age. Lymphopenia was first demonstrated at age

9 months and persisted (Table I). Skin rash and high IgE levels improved within several months with an elimination diet and topical therapy. She did not make protective titers to tetanus after the primary vaccination series, and despite repeated boosting, she did not maintain protective titers to pneumococcus (Table I and data not shown). She was started on sulfamethoxazole-trimethoprim and intravenous immunoglobulin substitution. With this therapy, she has been thriving without infections up to the current age of 4 years.

Patient 2 was found to have undetectable TRECs and very severe T-cell lymphopenia at birth.³² He was placed in protective isolation at home and treated with sulfamethoxazole-trimethoprim starting at age 6 weeks. At 76 days of age, he underwent 9/10 antigen HLA-A-mismatched unrelated donor bone marrow transplantation (BMT) after busulfan, cyclophosphamide, and antithymocyte globulin conditioning. Cyclosporine, methotrexate, and corticosteroids were administered as GVHD prophylaxis. He is now 19 months after BMT and off immunosuppression and off immunoglobulin substitution, with 100% donor chimerism in CD3, CD15, and CD19 lineages after BMT, and he lives at home free of infections and without signs of GVHD.

Patient 3 was born to nonconsanguineous South Asian parents and was an only child. He had multiple infections while living in India, including disseminated mycobacterium not further speciated, *Pneumocystis jiroveci*, and disseminated varicella zoster infection soon after vaccination with live attenuated varicella zoster vaccine. He was started on immunoglobulin substitution and moved to the United States at approximately 18 months of age. He had and cleared respiratory syncytial virus infection after treatment with ribavirin. At the age of 25 months, he underwent fully matched, unrelated donor BMT after conditioning with busulfan, cyclophosphamide, and antithymocyte globulin. Cyclosporine and methotrexate were administered as GVHD prophylaxis. He is now 12 months after BMT and off immunosuppression and immunoglobulin substitution with mixed donor chimerism; he is free of infections and GVHD.

Mutational and expression analysis of *JAK3*

Patient 1 had marked T lymphopenia and normal numbers of B cells for age, and therefore the possibility of a defect in *JAK3* was considered. Sequencing of the *JAK3* gene in patient 1 and her parents revealed compound heterozygous mutations: c.578G>A on the maternal allele predicted to result in p.Cys193Tyr and c.17861+3G>T within the donor splice site of intron 12 of the paternal allele (Fig 1, *A and B*). The same mutations were confirmed in patient 2. Sequencing of the *JAK3* gene in patient 3 revealed compound heterozygosity for the following mutations: c.1767C>T, which was predicted to result in a splicing defect,²³ and c.678_679delCT, which was predicted to result in a frame-shift with premature termination (p.Cys227fsX49; Fig 1, *B*).

We further analyzed the expression and function of JAK3 protein in patient 1. Western blotting of B-LCLs from patient 1 revealed reduced JAK3 protein expression with 2 bands, one of normal size and one smaller (Fig 1, *C and D*). Stimulation of B-LCLs from patient 1 with IL-2 induced detectable but markedly reduced levels of phosphorylated STAT5 (Fig 1, *D*). Quantification of *JAK3* mRNA by means of real-time PCR in B-LCLs from patient 1 revealed approximately 10% of the normal level of message (Fig 1, *E*). We hypothesized that

the lower-molecular-weight JAK3 band was derived from a splicing defect associated with the c.17861+3G>T allele; however, whether the normal-sized band was derived from the missense allele or was also generated by residual correct splicing of the c.17861+3G>T allele was unclear. We analyzed cDNA from patient 1 by using 2 different pairs of primers (Fig 1, *B*), one spanning exons 9 to 13 (expected product, 503 bp) and one spanning exons 9 to 15 (expected product, 832 bp). Amplification performed with the exon 13 reverse primer resulted in a single normal-sized band. On the contrary, amplification with the exon 15 reverse primer resulted in 2 major bands in patient 1, one the same size as in the healthy control subject and a lower one approximately 200 bp smaller (Fig 1, *F*). Sequencing of the lower band revealed complete skipping of both exon 12 and exon 13 (Fig 1, *G*), which are presumed to cause an in-frame deletion of 71 amino acids (Ser568-Leu638). Overall, these data demonstrate that the *JAK3* mutations in patient 1 dramatically affect the mRNA levels and allow expression of a low amount of full-length protein and a more abundant smaller-sized variant, reflecting the splicing defect.

Phenotypic and functional abnormalities of *JAK3*-deficient B cells

Circulating B cells from the 3 patients were present in normal numbers (Table I). At 15 and 25 months of age, respectively, both patient 1 and patient 3 had transitional B-cell percentages similar to published normal values for age (Fig 2, *A*).³⁰ The proportion of marginal zone–like B cells was in the normal range for age in patient 1 (8.8% vs 4.6% to 15%) but significantly decreased in patient 3 (2.2% vs 5.1% to 12.3%; Fig 2, *A*). Switched memory B cells were nearly absent in patient 3 but present in the normal range in patient 1 (Table I). These findings imply partial function of *JAK3* in patient 1, which is consistent with low levels of residual STAT5 phosphorylation in B-LCLs in response to IL-2 (Fig 1, *D*).

Patient 1 had low serum IgG levels and did not mount an antigen-specific antibody response, despite boosting. We therefore examined *in vitro* plasmablast differentiation and immunoglobulin secretion in response to a γ c-dependent stimulus. As shown in Fig 2, *B*, PBMCs from patient 1 cultured in the presence of CD40L and IL-21 did not proliferate. Furthermore, PBMCs from both patient 1 and patient 3 did not differentiate into plasmablasts in the presence of CD40L and IL-21 (Fig 2, *C*). This result might reflect the low number of memory B cells in patient 3; however, a normal number of class-switched memory B cells (defined as CD19⁺CD27⁺IgD[−]) was present in patient 1, yet this patient did not generate plasmablasts *in vitro*. Therefore these data indicate that integrity of γ c-JAK3 signaling is required for terminal differentiation of B lymphocytes in response to T-dependent signals, which is consistent with our recent observations.³⁵

TCR V β repertoire and T-cell function

Patient 2 had a classic SCID phenotype and had few T cells to examine. In contrast, patient 1 had proportional lymphopenia in both CD4⁺ and CD8⁺ T cells, whereas patient 3 had CD4⁺ T-cell lymphopenia with expansion of CD8⁺ T cells (Table I). Analysis of the TCRV β repertoire showed that in patient 1 the V β families were initially represented in normal proportions but that both CD4⁺ and CD8⁺ T-cell repertoires became aberrant with time (Fig 3). In patient 3, 3 and 10 V β families were underrepresented in CD4⁺ and CD8⁺ T cells,

respectively (Fig 3). In keeping with the clinical phenotype, patient 1, who has had no infections, has low but not absent proliferation to PHA and tetanus toxoid. In contrast, patient 3, who has had many infections, has greatly reduced proliferation to both PHA and tetanus toxoid. Markedly reduced T-cell responses were observed in patient 2, who presented with a classic SCID phenotype (Table I).

Partial maternal engraftment

Maternal engraftment typically occurs in young infants with classic SCID. Short tandem repeat–based analysis of maternal engraftment in patient 3 from whole blood revealed no maternal DNA (Table I). In patient 1, however, short tandem repeat genotyping of magnetically purified CD3⁺ lymphocytes surprisingly showed 34% maternal chimerism (Table I). Unlike 2 previous reports of long-term maternal engraftment,^{36,37} HLA typing showed that patient 1 and her mother are haploidentical (Fig 1, A). To confirm this finding, we analyzed HLA-A2 expression in patient 1's lymphocytes to distinguish autologous (HLA-A2[−]) cells from maternally engrafted (HLA-A2⁺) T lymphocytes. As shown in Fig 4, A, partial maternal engraftment of both CD4⁺ and CD8⁺ T cells was confirmed, with 30.7% and 48.4% of the cells being of maternal origin, respectively. As expected, CD19⁺ B cells were entirely of autologous origin. Serial measurement over time showed persistence of maternal engraftment, albeit with a lower percentage, at 33 months (Table I). Her younger brother, patient 2, had 80.4% and 97.5% of CD4⁺ and CD8⁺ T cells of maternal origin, respectively (Fig 4, A).

Analysis of autologous and maternal T cells

The excellent clinical status in patient 1, with no opportunistic infections, prompted us to analyze the phenotype of autologous and maternal T cells *in vivo*. We hypothesized that CD4⁺ T cells with a naive phenotype would be entirely derived from the patient. Indeed, CD4⁺ CD45RA⁺ naive T cells were only found within the HLA-A2[−] subset and were therefore of autologous origin (Fig 4, B). Furthermore, most of the very few CD4⁺ CD45RA⁺ T cells expressed CD31, which is typical of recent thymic emigrants, implying a low level of ongoing active thymopoiesis *in vivo* (Fig 4, C). We hypothesized that autologous T cells, although few in number, would have a polyclonal repertoire, whereas maternally engrafted cells would be less diverse, as previously reported.³⁸ Indeed, autologous HLA-A2[−] T cells had a diversified TCR Vβ repertoire (Fig 4, D). In contrast, among maternally engrafted T cells, 9 of the 24 TCR Vβ families were underrepresented or overrepresented compared with published normal values (Fig 4, D). T cells that have responded to antigen recently *in vivo* should show evidence of activation and proliferation. Autologous CD4⁺ and CD8⁺ CD45RA[−] T cells were effector memory cells (CD45RA[−], CD62L[−]), central memory cells (CD45RA[−], CD62L⁺) and activated T cells (HLA-DR⁺). In contrast, maternally derived CD4⁺ and CD8⁺ CD45RA⁺ T cells were effector memory and central memory phenotypes and were HLA-DR[−] (Fig 4, E). Only autologous T cells expressed the Ki67 proliferation antigen (Fig 4, F). Thus we concluded that only autologous T cells were proliferating and activated *in vivo*.

IL-2 receptor signaling through JAK3 is required for proliferation and survival of T cells, and mutations in *JAK3* lead to increased apoptosis.²⁴ We reasoned that *JAK3* mutant cells

might have increased susceptibility to activation-induced cell death compared with maternal cells. Freshly isolated autologous T cells from patient 1 had more expression of Annexin V *ex vivo* than maternally engrafted T cells (Fig 5, *A*). Annexin V expression was prominent in both patient-derived cells and maternally engrafted cells after 72 hours of culture with anti-CD3 and anti-CD28 compared with that seen in control cells (Fig 5, *B*). Consistent with the finding of partial *JAK3* function, we also saw partial rescue of apoptosis in both autologous and maternally engrafted T cells when high doses of IL-2 were added (Fig 5, *B*).

Next we compared the proliferative capacity of the maternal and autologous T cells cultured together or separately. When PBMCs from patient 1 were labeled *in vitro* with CFSE and then stimulated with agonistic anti-CD3 and anti-CD28 mAb for 72 hours, both autologous (HLA-A2⁻) and maternally engrafted (HLA-A2⁺) CD4⁺ and CD8⁺ T cells diluted CFSE vigorously (Fig 5, *C*), which is contrary to reports of poor *in vitro* proliferation of maternally engrafted cells.¹¹ This finding was confirmed when HLA-A2⁺ and HLA-A2⁻ T cells from the patient were purified magnetically (>94% purity), loaded with CFSE, and cultured separately (Fig 5, *C*). These data suggest that both autologous and maternally engrafted T cells retained short-term functional capacity *in vitro*, which is in contrast to what we observed *in vivo*, where only freshly isolated HLA-A2⁻ autologous T cells expressed Ki67 (Fig 4, *F*). In long-term culture, however, *JAK3*-deficient T cells in patient 1 ultimately did not compete with maternal T cells. All polyclonally generated herpesvirus saimiri T-cell lines were HLA-A2⁺ and hence exclusively maternally derived, indicating that maternal T cells outcompeted autologous T cells *in vitro* (data not shown).

Finally, we examined the allogeneic responsiveness of T cells from patient 1 in mixed lymphocyte culture to investigate the hypothesis that partial function of these cells could explain the patient's excellent clinical status and lack of infections. At the time of testing, at 3 years and 8 months of age, maternal T cells in peripheral blood had waned to almost nil (data not shown). Purified T cells from patient 1 cultured in mixed lymphocyte culture with irradiated PBMCs from 3 different normal donors proliferated, on average, 51% (37% to 53%) compared with T cells from her mother (Fig 5, *D*). Of note, T cells from patient 1 also exhibited similar proliferation in response to irradiated PBMCs of her mother (Fig 5).

DISCUSSION

JAK3 mutations generally result in complete absence of T-cell development but partial function with a leaky phenotype, and different phenotypes in siblings with the same mutation have been described.²⁵ Here we show that marginal zone–like B-cells, memory B-cells, T-cells, and T-cell *in vitro* proliferation to mitogens and antigens were variably deficient in 3 patients with *JAK3* mutations. STAT5 phosphorylation in response to IL-2 was severely reduced but detectable in B-LCLs from patient 1. Plasma-blast formation in response to CD40L and IL-21 was absent in both patient 1 and patient 3, despite patient 1 having a normal percentage of circulating switched memory B cells. From these data, it is tempting to speculate that the requirement for *JAK3* function is least stringent for T-cell generation and *in vitro* proliferation and most stringent, perhaps absolute, for terminal B-cell differentiation and function. This intrinsic B-cell defect in patients with the γc -*JAK3* defect might account for the poor reconstitution of humoral immunity that is typically observed

after unconditioned, haploidentical hematopoietic stem cell transplantation, with donor T-cell chimerism and persistence of autologous B cells.^{39–41} Our recent data in a series of patients undergoing transplantation with γc or *JAK3* defects support this hypothesis.³⁵

To our knowledge, this is the first definitive demonstration of the long-term coexistence of autologous T cells with a high level of engraftment of haploidentical maternal T cells. Long-term engraftment of maternal cells has been previously described in 2 patients with SCID, one of whom carried *JAK3* mutations; however, in both cases coexistence of autologous and maternal T cells was associated with a high degree of sharing of HLA alleles. In particular, Tezcan et al³⁶ reported an 8-year-old boy with repeated infections, severe failure to thrive, and dermatitis, who nevertheless maintained normal total immunoglobulin levels and residual specific antibody production. The authors speculated that a high degree of matching between patient and mother (7/8 in the GVHD direction and 5/8 in the rejection direction caused by shared alleles and crossover) might have played a role in the persistence and possibly the function of these cells. Al-Muhsen³⁷ reported the case of a 9-year-old boy with chronic pneumonia, bronchiectasis, and failure to thrive who also manifested long-term maternal engraftment of both T cells and a small granulocyte percentage in the setting of full HLA compatibility between mother and patient. Our finding defies the dogma that engraftment of haploidentical maternal T cells can only occur in the complete absence of host T cells.^{11,15} Although we did not discern any evidence that maternally engrafted T cells were functional *in vivo*, it is possible that patient 1's excellent clinical status and freedom from infection to date has been mediated in part by maternal T-cell function. However, we favor the hypothesis that this is due to residual function and a diversified repertoire of autologous, *JAK3*-deficient T cells in addition to intravenous immunoglobulin substitution and antimicrobial prophylaxis. In fact, we demonstrated approximately half-normal allogeneic responsiveness of T cells from patient 1 against third-party donor and also against maternal PBMCs.

Our data add to the spectrum of clinical and immunologic phenotypes associated with *JAK3* mutations in human subjects and demonstrate that long-term maternal engraftment can occur despite the presence of host T cells. Hypomorphic mutations in *JAK3* should be considered in all patients with T-cell lymphopenia in association with poor antigen-specific antibody responses.

We thank the patients and their families for participating in the research; Ms Amelia Sabadini, Mr Gregory Hopkins, and Dr Ottavia Delmonte for technical assistance; Dr Raif S. Geha for helpful discussion; and Dr Anne M. Comeau for assistance with TREC assays from newborn dried blood spots.

Acknowledgments

Supported by the Translational Investigator Service at Boston Children's Hospital (to S.-Y.P.), the Manton Foundation (to L.D.N.), the National Institutes of Health (U54-AI082973 to S.-Y.P. and L.D.N.), and Fondazione Nocivelli (to S.G). M.R. was supported by the Swiss National Science Foundation (SNSF/SSMBS) grant PASMP3-127678.

Abbreviations used

B-LCL	B-lymphoblast cell line
BMT	Bone marrow transplantation
CD40L	CD40 ligand
CFSE	Carboxyfluorescein diacetate succinimidyl ester
GVHD	Graft-versus-host disease
JAK3	Janus kinase 3
OS	Omenn syndrome
SCID	Severe combined immunodeficiency
STAT	Signal transducer and activator of transcription
TCR	T-cell receptor
TREC	T-cell receptor excision circle

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FIG 1.

Analysis of patients with *JAK3* mutations. **A**, Pedigrees of patient 1 (P1), patient 2 (P2) and patient 3 (P3). **B**, Schematic representation of the *JAK3* gene with the location of primers used to amplify cDNA and of mutations (*patients 1 and 2; #patient 3). **C**, Expression of *JAK3* protein by Western blotting on B-LCLs of a null *JAK3* patient, a positive control subject (C), and patient 1 (Pt). **D**, Phosphorylated STAT5 by Western blotting with or without stimulation with IL-2 of B-LCLs from patient 1 (Pt), a *JAK3* null patient (*JAK3*^{-/-}), and a healthy control subject (C). **E**, Quantification of *JAK3* message by means of real-time PCR in B-LCLs from patient 1 (Pt) and a control subject (C). **F**, RT-PCR analysis of *JAK3* RNA spanning exons 9 to 13 and 9 to 15, showing a lower-sized band in the patient. **G**, Direct sequencing of the lower-sized band obtained by using RT-PCR in patient 1 revealed the complete skipping of exons 12 and 13 of the *JAK3* cDNA.

FIG 2.

Phenotypic and functional studies of peripheral B cells. **A**, Percentages of transitional (CD24^{hi}CD38^{hi}) and marginal zone-like (CD24^{hi}CD38^{low}) B cells for adult control subjects, patient 1 (P1), and patient 3 (P3). **B**, Percentage of CD19⁺ B cells from an adult control subject (mother of patient 1) or patient 1 that diluted CFSE in response to the stimuli indicated. **C**, Percentage of plasmablasts (CD19⁺CD27⁺CD38⁺⁺) from an adult control subject, patient 1 (P1; left panel), and patient 3 (P3; right panel) expressing high levels of CD27 and CD38 after culture with the indicated stimuli.

FIG 3.

TCR V β repertoire in peripheral T cells from patient 1 (P1) and patient 3 (P3). The percentages of CD3⁺, CD3⁺CD4⁺, or CD3⁺CD8⁺T cells expressing the indicated TCR V β families are depicted compared with published norms for adults. V β families were expressed at less than (open circles), within (solid squares), or greater than (open triangles) the normal range.

FIG 4.

Phenotypic characteristics of maternally engrafted and host-derived autologous peripheral T cells in patient 1 (P1) and patient 2 (P2). A, The left 2 panels show that CD3⁺ cells from patients 1 and 2 contain both HLA-A2⁺ (maternally derived) and HLA-A2⁻ (autologous) populations. The proportion of maternally derived and autologous CD4⁺, CD8⁺, and CD19⁺ cells in patients 1 and 2 is shown in the right 8 panels. B, HLA-A2⁺ and HLA-A2⁻ cells from patient 1 were electronically gated, and expression of CD45RA is shown (left panel). Control cells from an HLA-A2⁺ adult is shown for comparison (right panel). C, CD45RA and CD31 expression in CD4 cells from patient 1 and an adult control subject are shown. D, CD3⁺ T cells from patient 1 were electronically gated according to HLA-A2 expression, and Vβ repertoire is depicted as in Fig 3. E, CD4⁺ CD45RA⁻ T cells and CD8⁺ CD45RA⁻ T cells from patient 1 (left panel) were analyzed for expression of HLA-A2, HLA-DR, and CD62L. Control cells from an HLA-A2⁺ adult are shown in the right panel. F, CD3⁺ T cells from patient 1 were analyzed for expression of HLA-A2 and Ki67.

FIG 5.

Analysis of apoptosis and proliferation of maternally engrafted and autologous peripheral T cells in patient 1 (P1). A, Staining with Annexin V reagent in freshly isolated CD4⁺ and CD8⁺ T cells from patient 1. B, Staining of CD4⁺ and CD8⁺ T cells with Annexin V reagent after 3 days of culture in the indicated stimuli for an adult control subject (red), HLA-A2⁻ autologous cells from patient 1 (blue), and HLA-A2⁺ maternally engrafted cells from patient 1 (green). C, Cells from an adult control subject, patient 1 cultured in bulk, or patient 1 magnetically separated into HLA-A2-expressing and nonexpressing fractions were cultured for 3 days after CFSE labeling. HLA-A2 expression profile after culture (left) and CFSE and HLA-DR expression (right). D, Proliferation of T cells from patient 1 and her mother to irradiated allogeneic targets from the indicated donors in mixed lymphocyte culture.

TABLE I.

Immunologic phenotype

	Patient 1 (female)			Patient 2 (male)	Patient 3 (male)	Normal values
Age (mo)	9	15	33	1	23	—
Lymphocytes						
CD3 ⁺ cells/ μ L	546	302	541	10	5,670	1,900–6,200
CD4 ⁺ cells/ μ L	304	192	191	4	400	1,300–4,300
CD8 ⁺ cells/ μ L	228	101	276	8	3,263	500–2,000
CD19 ⁺ cells/ μ L	886	546	675	1,010	2,132	300–3,000
CD56 ⁺ cells/ μ L	141	89	58	55	53	170–1,100
γ/δ TCR ⁺ (%)	—	—	—	—	45	
Naive CD4 (%)	—	3.1	0.7	—	1	63–95
Naive CD8 (%)	—	1.3	0.4	—	20	71–99
Proliferation (cpm)						
PHA	—	35,545	25,376	2,313	1,871	>41,000
Tetanus *	—	2,344	6,788	—	346	—
Normal for laboratory	—	>4,000	>4,000	—	>2,000	—
TRECs		Less than limit of detection [†]		Less than limit of detection [†]	Less than limit of detection [‡]	[†] , [‡]
Maternal engraftment (%)						
CD3 ⁺	—	39	5	84	0	0
CD4 ⁺	—	31	12	79	—	0
CD8 ⁺	—	48	1	97	—	0
Immunoglobulins						
IgG (mg/dL)	974 (300–1,500)	571 (400–1,300)	681 [§] (400–1,300)	423 (280–750)	1200 [§] (400–1,600)	—
IgA (mg/dL)	264 (30–327)	224 (16–100)	85 (20–230)	—	155 (15–200)	—
IgM (mg/dL)	146 (25–115)	117 (25–115)	43 (30–120)	—	83 (40–200)	—
IgE (IU/mL)	10,965 (0–30)	2,915 (0–30)	78 (0–30)	—	<2 (0–115)	—
Specific antibody						
Tetanus (IU/mL)	0.05	—	—	—	<0.07	0.15–7.0
Pneumococcus	—	0/7	—	—	0/14	¶
B-cell subpopulation (%)						
CD27 [−] (naive)	—	82.7	83.5		99.1	71.2 to 86.6
CD27 ⁺ IgD ⁺	—	5.9	5.5	—	0.6	4.6 to 15
CD27 ⁺ IgD [−] (switched)	—	8.4	9.5	—	0.3	3.9 to 8.5

* The adult control subject assessed on the same day showed 68,151, 60,217, and 10,968 cpm, respectively.

[†] Measured in a newborn dried blood spot (normal value, >252 copies/mL whole blood).

[‡] Measured in peripheral blood (normal, >801 copies/million PBMCs).

[§] Value while undergoing IgG replacement therapy.

// Measured at 24 months of age.

¶ Value expressed as the number of serotypes with protective titers over the total number of serotypes tested.

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