Journal of Clinical Immunology (2019) 39:257–260
https://doi.org/10.1007/s10875-019-00621-8

LETTER TO EDITOR

Interferon-γ Receptor 1 Deficiency Corrected by Umbilical Cord Blood Transplantation

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Received: 25 November 2018 / Accepted: 31 March 2019 / Published online: 5 April 2019

Abbreviations

AUC	Area under the curve
CD	Cluster of differentiation
HLH	Hemophagocytic lymphohistiocytosis
HSCT	Hematopoietic stem cell transplantation
IFN	Interferon
IFNGR	Interferon-gamma receptor
IgG	Immunoglobulin G
IL	Interleukin
IL12R	Interleukin-12 receptor
MSMD	Mendelian susceptibility to mycobacterial diseases
STAT1	Signal transducer and activator of transcription 1

To the Editor:

Interferon-gamma receptor (IFNGR)–related disorders are rare variants of Mendelian susceptibility to mycobacterial diseases (MSMD). Given interferon (IFN)–gamma's crucial role in innate immunity against mycobacterium and intracellular microorganisms, affected individuals are characteristically prone to serious infections with not only weakly virulent mycobacteria species but also *Salmonella*, *Listeria*, *Histoplasma*, and various viruses. Death often occurs in childhood in those with complete receptor deficiencies unless definitive treatment is provided [1–3]. Although treatment with hematopoietic stem cell transplantation (HSCT) has been described and is curative, it is complicated by high rates of delayed or failed engraftment thought to be due to elevated concentrations of IFN-gamma [4–7]. Umbilical cord blood transplantation and reduced-intensity conditioning regimens additionally increase the risk of graft failure [8, 9]. Here, we describe a pediatric patient with a novel *IFNGR1* mutation who successfully underwent reduced-intensity umbilical cord blood transplantation.

A 19-month-old boy of Yemeni descent, born within the USA to non-consanguineous parents, presented with hepatosplenomegaly and extensive lymphadenopathy including a large mediastinal mass. He did not receive bacille Calmette–Guérin immunization. A right cervical lymph node biopsy exhibited reactive lymphoid hyperplasia with follicular regression, and bone marrow evaluation was noted to be normocellular with maturing trilineage hematopoiesis; both were negative for malignancy or mycobacterial infection (assessed via Ziehl–Neelsen and Fite staining). He subsequently presented in septic hypotensive shock requiring vasopressor support, intubation, and continuous renal replacement therapy following the development of daily fevers, frequent diarrhea, and respiratory distress. Initial laboratory analyses were remarkable for white blood count 20.5 K/uL, absolute neutrophil count 15.1 K/uL, absolute lymphocyte count 3.1 K/uL, hemoglobin 6.2 g/dL, platelet count 12 K/uL, erythrocyte sedimentation rate 108 mm and C-reactive protein 24.6 mg/dL. Peripheral blood smear pathologic review was notable for anemia, absolute neutrophilia, reactive lymphocytes, and immature granulocytes and neutrophils with toxic/reactive granules. Blood culture, gastrointestinal pathogen polymerase chain reaction study, endotracheal tube culture, and standard stool culture all subsequently showed the presence of *Salmonella enteritidis*. His evaluation showed a hyperinflammatory state with elevations in ferritin, IFN-gamma, tumor necrosis factor alpha, IL-10, IL-6, and IL-8 levels (Table 1). Maximal ferritin and IFN-gamma levels reached 12,065 ng/mL and 463 pg/mL (normal < 5 pg/mL), respectively. A diagnosis of hemophagocytic lymphohistiocytosis (HLH) was considered with the patient found to meet six of eight clinical HLH criteria (fever, splenomegaly, cytopenias, fasting hypertriglyceridemia, increased ferritin, and elevated soluble IL-2 receptor) [10, 11]. As a result, the patient was initially treated with dexamethasone 10 mg/m^2/day for 7 days

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Table 1 Patient inflammatory markers

	IFN-gamma Nml: < 5 pg/mL	IL-8 Nml: < 5 pg/mL	IL-6 Nml: < 5 pg/mL	IL-10 Nml: < 18 pg/mL	TNF-α Nml: < 22 pg/mL	Ferritin Nml: < 320 ng/mL
Levels at presentation	463	23	3500	1210	93	12,065
Following *Salmonella* sepsis treatment	24	28	6	3	2	79
Prior to HSCT	< 5	< 5	< 5	< 5	3	62

HSCT hematopoietic stem cell transplantation, *IFN* interferon, *IL* interleukin, *TNF-α tumor necrosis factor alpha*

with a rapid decrease in serum ferritin levels. Given the elevation in serum IFN-gamma and disseminated *S. enteritidis*, an evaluation for MSMD was undertaken. Commercial functional testing (Medical College of Wisconsin Clinical Immunodiagnostic and Research lab) revealed expression of IFNGR1 on 23% of monocytes but absent IFN-gamma-stimulated STAT1 phosphorylation within monocytes (stimulation index (SI),1), suggesting defective IFNGR1 signaling (Fig. 1a). IL-12R expression and IL-12-induced STAT4 phosphorylation in lymphocytes were evaluated and were normal (75% expression; SI, 4; respectively); testing of IFNGR2 was not completed. Genetic testing (Invitae Corporation) confirmed a previously unreported homozygous mutation in *IFNGR1* (c.373+2T>C), which is predicted to affect a donor

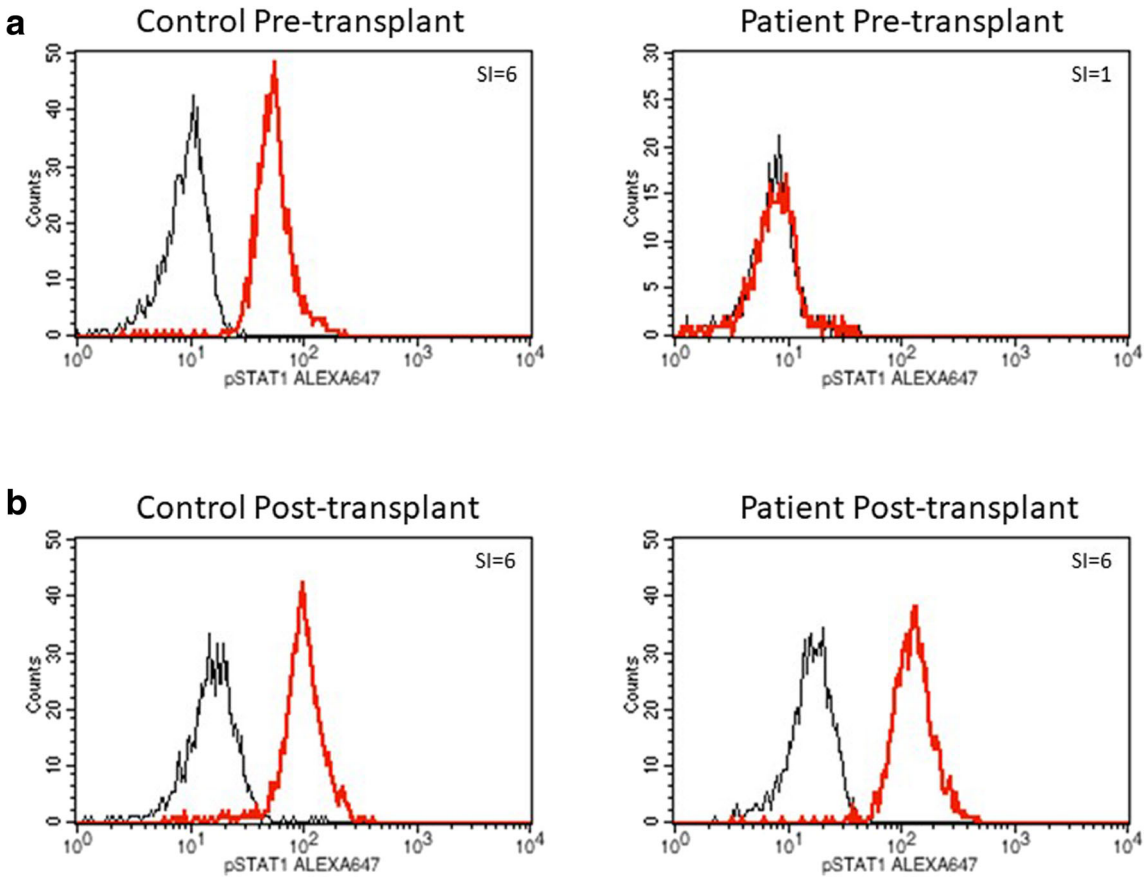

Fig. 1 Functional pSTAT1 flow cytometry studies. Peripheral blood mononuclear cells at a concentration of 1×10^6 cells/condition were stimulated for 15 min with 5 ng/mL IFN-gamma at 37 °C, fixed with paraformaldehyde (final concentration of 2%), washed, and then permeabilized overnight in 100% methanol at − 20 °C. The following day, the cells are washed twice in BD Fix/Perm buffer and then stained with anti-pSTAT1 for 60 min at room temperature. **a** Pre-HSCT flow cytometry showing absent IFN-gamma-stimulated STAT1 phosphorylation within our patient's peripheral blood mononuclear cells. **b** Corrected IFN-gamma-stimulated STAT1 phosphorylation following HSCT within our patient. Studies performed at the Clinical Immunology Research Laboratory at the Medical College of Wisconsin. SI, stimulation index

splice site in intron 3 causing absent protein function, consistent with the lack of STAT1 phosphorylation in response to IFN-gamma.

The patient's blood cultures remained positive for *S. enteritidis* for 16 days, during which time he was treated initially with piperacillin/tazobactam, vancomycin, and tobramycin, then ampicillin, ciprofloxacin, and sulfamethoxazole/trimethoprim. Given a lack of signs or symptoms, family members were not tested for *S. enteritidis.* Dedicated bone imaging to locate a focal infectious source was not performed, but extensive CT imaging and echocardiogram studies showed no evidence of abscess, localized persistent infectious foci, or endocarditis. Upon patient stabilization, a peripherally inserted central catheter was removed with blood cultures subsequently becoming negative, raising the concern for central line colonization with *Salmonella enteritidis* as the cause of prolonged positivity of blood cultures. Once cultures became negative, the patient was transitioned to IV ampicillin to complete 6 weeks of treatment from the last positive culture. Ampicillin therapy was completed 42 days prior to transplant, and the patient was placed on prophylactic azithromycin and sulfamethoxazole/trimethoprim until initiation of the pre-transplant conditioning regimen. Throughout the clinical course, IFN-gamma levels were serially monitored. Given that high levels of IFN-gamma are thought to inhibit engraftment, alemtuzumab (0.2 mg) was given 20 days prior to HSCT to decrease serum IFN-gamma levels prior to transplant as emapalumab was unavailable for commercial use. A reduced-intensity conditioning regimen consisting of alemtuzumab (0.6 mg/kg; days − 8 to − 6), fludarabine (180 mg/m^2; days − 8 to − 3), and busulfan (AUC 55 mg/L × h; days − 5 to − 3) was utilized, and the patient underwent allogeneic HSCT using a 5/6 HLA-matched umbilical cord unit (5/8 allele-level match; antigen mismatch at HLA-B and allele-level mismatches at HLA-A and HLA-C) with a cell dose of 8.5×10^7 total nucleated cells/kg and 10.6×10^5 CD34+ cells/kg. [12] Of note, cord blood was selected as a donor source given that our patient was without siblings and lacked matched or acceptably mismatched unrelated bone marrow donors. GVHD prophylaxis was with tacrolimus until day + 180 and mycophenolate mofetil until day + 100 post-transplant. Plasma IFN-gamma was undetectable prior to starting conditioning and prior to transplant (Table 1).

Antibacterial prophylaxis with levofloxacin was given from day + 1 through engraftment per institutional guidelines. Viral prophylaxis was not given post-transplant; however, weekly PCR monitoring for Epstein–Barr virus, cytomegalovirus, and adenovirus was undertaken. Fungal prophylaxis with voriconazole was given from the start of conditioning until all immunosuppression was discontinued per institutional guidelines. Immunoglobulin replacement was given for IgG levels less than 400 mg/dL. Neutrophil engraftment occurred on day + 14, and chimerism analysis of peripheral blood at 10 months post-transplant showed 100% donor in myeloid cells and 91% donor in CD3+ cells. Repeat functional testing following transplant now shows successful immune reconstitution with expression of IFNGR1 on 93% of monocytes; normal IFN-gamma-stimulated STAT1 phosphorylation (SI, 6) (Fig. 1b); and normal lymphocyte populations (CD3, CD4, CD8, CD19, and CD56). The patient's clinical course was complicated by *Klebsiella pneumoniae* and methicillin-sensitive *Staphylococcus aureus* sepsis approximately 90 days post-transplant. He is now doing well greater than 1 year post-transplantation with no signs of graft versus host disease following withdrawal of immunosuppression.

Here, we report the first successful case of reduced-intensity umbilical cord blood transplantation in a patient with *IFNGR1* deficiency. Children with a complete deficiency of interferon-gamma receptor function can present with high levels of plasma IFN-gamma, and this is hypothesized to increase the risk of graft rejection and failure by direct inhibition of hematopoietic stem cell proliferation by IFN-gamma, although increased Fas-mediated apoptosis may additionally play a role [6, 13, 14]. Despite the high rates of delayed or failed engraftment with HSCT reported in patients with IFNGR1-related disorders, it is possible that the substantial reduction in plasma IFN-gamma levels achieved in our patient prior to transplant combined with control of any active infections contributed to the success of transplant despite using umbilical cord blood as the hematopoietic stem cell source. This suggests that achieving adequate control of inflammation, particularly IFN-gamma, prior to transplant may improve outcomes, even for those undergoing umbilical cord blood transplantation. However, the risks of immunosuppression in the setting of ongoing infection, particularly mycobacterial infection, must be carefully considered. In these settings, alternative medications that directly target IFN-gamma, such as emapalumab, may be preferable to decrease IFN-gamma while avoiding the profound immunosuppression induced by the alemtuzumab-based strategy employed for this patient [15].

One limitation of this report is our inability to directly confirm the exact effect of our patient's genetic variant in *IFNGR1* (c.373+2T>C) on IFN-gamma signaling by functional studies, particularly with respect to IFN-gamma binding or receptor heterodimerization. We expect this mutation to be pathogenic given that donor splice site variants typically lead to a loss of protein function and loss-of-function variants in IFNGR1 have previously been reported to be pathogenic [16–19]. Additionally, a similar genetic variant affecting this splice site (c.373+1G>T) has been reported in homozygous and compound heterozygous individuals who suffer from recurrent mycobacterial infections [20, 21]. The patient's overall clinical picture, presence of a homozygous genetic variant predicted to have deleterious effects on IFN-gamma signaling, and

lack of STAT1 phosphorylation to IFN-gamma suggest that this patient's genetic variant is indeed pathogenic and likely results in a complete functional IFNGR1 deficiency, although a severe partial deficiency cannot be completely excluded.

Continued collection of IFNGR-related disorder patient outcomes following HSCT, such as that currently being performed through EBMT-ESID, will be imperative in advancing our understanding of the optimal management approach for these conditions [22].

Acknowledgments The authors would like to thank the family of our patient for allowing the sharing of his clinical case. We additionally appreciate the assistance of the Clinical Immunology Research Laboratory at the Medical College of Wisconsin, specifically Dr. Jeffrey Woodliff, Dr. John Routes, and Dr. James Verbsky, in performing the diagnostic functional studies that were crucial in characterizing the patient's defect.

Author Contributions Dr. Michniacki drafted the manuscript. Drs. Vander Lugt, Frame, and Walkovich assisted in reviewing and editing the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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