Long-term survival after hematopoietic stem cell transplantation for complete STAT1 deficiency

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Abstract

Purpose. Complete Signal Transducer and Activator of Transcription 1 (STAT1) deficiency is a rare autosomal recessive condition characterized by impairment of intracellular signaling from both type I and type II interferons (IFN). Affected patients are prone to early severe mycobacterial and viral infections, which usually result in death before 18 months of age. We previously reported a patient affected by complete STAT1 deficiency who underwent hematopoietic stem cell transplantation (HSCT). Here we describe the transplantation procedures and long-term outcomes.
Methods. The patient, who had suffered multiple life-threatening mycobacterial and viral infections in the first years of life, underwent HSCT at 4 years of age from a partially-matched (HLA compatibility 8/10) unrelated donor after a myeloablative conditioning regimen consisting of busulfan, cyclophosphamide, and anti-thymocyte globulin.
Results. Hematological reconstitution was detected at d+15, with full donor engraftment demonstrated by molecular analysis of leukocytes. Several complications occurred in the post-transplantation phase, including acute graft versus host disease, posterior reversible encephalopathy, thrombotic thrombocytopenic purpura, bilateral keratoconjunctivitis with complete loss of vision, and chronic lower limb lymphedema. Analysis of STAT1 in $CD3^+$ cells at 90 and 120 days after HSCT by flow cytometry showed normal STAT1 phosphorylation levels in response to IFN-α.
Conclusions. Notably, no severe infections occurred after discharge (day +90) during a 9-year follow-up, suggesting that normal response to IFNs in hematopoietic cells is sufficient to provide protection in humans.

Keywords: hematopoietic stem cell transplantation, STAT1 deficiency, primary immunodeficiency, signaling

Introduction

Signal Transducer and Activator of Transcription 1 (STAT1, encoded by *STAT1* gene) is an intracellular protein which has a fundamental role in signal transduction from both type I (IFN-α and IFN-β) and type II (IFN-γ) interferons (IFNs), and is therefore involved in immune response to virus and other intracellular pathogens [1,2]. STAT1 deficiency is a genetic condition characterized by a heterogeneous clinical phenotype [3,4]. Partial deficiency of STAT1 may be inherited in an autosomal recessive or dominant manner. Partial-recessive STAT1 deficiency is characterized by reduced expression of STAT1 leading to decreased but not abolished transcription of type I and type II IFN-induced genes. This condition is associated with increased susceptibility to severe but curable intracellular bacterial and viral diseases [5,6].

Partial-dominant STAT1 deficiency is characterized by impaired IFN-γ signaling but normal response to type I IFN, leading to increased susceptibility to infections by weakly virulent mycobacteria as well as by *Salmonella* spp. but not to viral infections, thus representing one of the causes of Mendelian Susceptibility to Mycobacterial Diseases (MSMD) [7–10]. Complete STAT1 deficiency, on the other hand, is an autosomal recessive condition that results in complete functional impairment of STAT1-dependent interferon response (both type I and II) [11,12]. Affected patients are prone to early fatal mycobacterial and viral infections. So far, only six patients from four unrelated families with complete STAT1 deficiency have been reported, including the present one. All patients, apart from the present one, died before 18 months of age from mycobacterial or viral infections [3]. In one case, hematopoietic stem cell transplantation (HSCT) was attempted, however, the patient died of multiorgan failure associated with Epstein Barr Virus (EBV) infection, three months after transplantation [12].

We previously reported a patient who carried a novel homozygous mutation affecting a splicing site of the *STAT1* gene, leading to exon 3 skipping and to synthesis of a lower molecular weight STAT1 protein.[13] Functional characterization revealed marked reduction of STAT1 phosphorylation, and

a complete defect of DNA-binding activity, resulting in complete impairment of peripheral blood mononuclear cell (PBMCs) functional response to both IFN-γ and IFN-α, as well as partial impairment of NK functional activity. Here we report the clinical course and outcome of HSCT in this patient, demonstrating for the first time that HSCT may be an effective option for treatment of complete STAT1 deficiency.

Methods

Flow cytometry. PBMCs were obtained from heparinized blood by Ficoll-Hypaque centrifugation after obtaining informed consent from the patient's parents. Cells were left unstimulated or stimulated with IFN-α (40000U/ml for 30 minutes) and were stained simultaneously using a fluorescein isothiocyanate (FITC)-conjugated mouse anti-CD3 IgG mAb. Cells were fixed and permeabilized, according to the BD protocol (Protocol III), and stained with phycoerythrin (PE)-conjugated mouse anti-pSTAT1-Tyr-701 (BD Pharmigene) or isotype-matched mAb PE (BD Bioscence). Cells were analyzed by flow cytometry after gating $CD3^+$ cells by FACSCalibur flow cytometer (BD Bioscence) and analyzed by the FlowJo version 7.5 Software (TreeStar). The extent of STAT1 phosphorylation was calculated as Mean Fluorescence Intensity (MFI).

Real Time PCR. Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For the RT-PCR analysis, Assays-on-Demand™ Products and Taqman Master Mix from Applied Biosystems were used according to the instruction manual to analyze *STAT1, IFNγ, IL2RA, SOCS1*,*SOCS3,* and *GAPDH* gene expression. Target gene expression was normalized to the housekeeping gene (GAPDH) expression and presented as *n*-fold increase over those in the healthy control by using $2^{-\Delta\Delta C}{}_T$ evaluation.

Results

Here we describe a male patient born at term via an uncomplicated cesarean section from consanguineous parents who were originally from Pakistan. His father harbored a balanced

chromosomal translocation of uncertain clinical significance [46XY, t(6;11) (q27; q14.3)]. His mother's karyotype was normal. An older brother, who had a multiple malformative syndrome of unknown etiology (including brain, skeletal, anorectal, and renal anomalies), died at nine months of age from respiratory failure. Two previous pregnancies resulted in stillbirth. Notably, the mother reported that five of her brothers died in infancy, from unspecified causes. As previously described, from 10 months of age our patient developed several life-threatening infections (Table 1) [13]. All standard immunological evaluations performed in the first years of life, including lymphocyte subsets analysis, response to mitogens, T-Cell receptor excision circles (TRECs) and K-deleting recombination excision circles (KRECs) counts, serum immunoglobulins, antibody response against vaccine antigens, DHR-123 testing for chronic granulomatous disease, and CD11b/CD18 expression were normal. However, PBMCs response to IFN-γ showed defective production of tumor necrosis factor (TNF) alpha. Genetic analysis of *STAT1* gene revealed a homozygous mutation inherited from heterozygous parents (372G>C), resulting in exon 3 skipping and synthesis of a lower molecular weight STAT1 protein. Further functional and molecular characterization of this defect has been previously reported elsewhere.[13]

At the age of 4 years and 7 months the patient underwent HSCT from a partially-matched unrelated donor (MUD). HLA compatibility was 8/10 (HLA-C mismatch)). Pre-procedure testing showed a low number of CMV copies (500/mL) and absent Epstein Barr Virus (EBV). CMV infection was successfully treated with ganciclovir. Blood testing of the donor showed normal immunoglobulin response against HSV, CMV and EBV, suggesting normal immunization against herpetic viruses. A myeloablative conditioning regimen was used, including busulfan 5 mg/kg/day on day (d)–10 to d–7, cyclophosphamide 50 mg/kg/day on d–6 to d–3, and anti-thymocyte globulin 2.5 mg/kg/day on d–6 to d–3. Graft versus host disease (GVHD) prophylaxis included cyclosporine 5 mg/kg/day from d–1. On the day of transplantation, the patient received 1.87 x 10^9 cells from erythrocyte-depleted marrow (3.93 x 10^6 $CD34^+$ cells/kg, 35.17 x 10^6 $CD3^+$/kg). Because of AB0 incompatibility

between patient (B+ before HSCT) and donor (A+), on d+10 he developed Coombs-positive hemolytic anemia, which required intravenous methylprednisolone therapy at 5 mg/kg.

Hematological reconstitution was detected at d+15, with full donor engraftment demonstrated by molecular analysis of leukocytes. Hematological reconstitution was associated with acute grade III cutaneous and intestinal GVHD, requiring an increase of methylprednisolone therapy at 10 mg/kg for three days. On d+20, he developed severe bilateral ulcerative keratoconjunctivitis, resulting in corneal scarring and complete loss of vision. Tests for viral pathogens were negative. On d+35 rituximab therapy was started following the detection of EBV in blood (358 copies/mL). Treatment was associated with generalized status epilepticus. Computed tomography demonstrated bilateral subcortical hypodense areas, consistent with posterior reversible encephalopathy syndrome (PRES). On d+53 thrombotic thrombocytopenic purpura developed, with severe anemia (6.2 g/dL), thrombocytopenia (5000/mL) and peripheral schistocytes. Defibrotide infusions and repeated plasmaphereses were performed, while mycophenolate mofetil (MMF) was started and cyclosporine was stopped.

Analysis of STAT1 phosphorylation in response to IFN-α (40000U/ml for 30 minutes) in $CD3^+$ cells at 90 and 120 days after HSCT by flow cytometry as compared to pSTAT1 levels in response to IFN-α before HSCT showed normal STAT1 phosphorylation levels in response to the cytokine already at day +90 (Figure 1), suggesting a normal reconstitution of STAT1 signaling in lymphocytes. Functional studies of PBMC response to cytokines signaling through STAT1 revealed normal upregulation of MXA and STAT1 mRNAs in response to IFN-α (1000 U/ml) and TNFA and IDO mRNAs in response to IFN-γ (1000 U/ml).

On d+90 the patient was discharged from the bone marrow transplantation unit, in good general condition and without signs of GVHD. MMF was discontinued. On d+112 III grade cutaneous GVHD relapsed on palms and soles, and was successfully treated increasing prednisone, restarting oral MMF, and applying topical tacrolimus. Ten months after HSCT, he developed progressive left lower limb edema. An uncertain history of trauma to the left knee was present. Ultrasonography

showed post-phlebitis changes in the left common femoral vein and in the external iliac vein. In the following months, he developed progressive swelling of both lower limbs as well as of the scrotal sac and the penis, which was ultimately attributed to bilateral lymphedema. Compressive stockings were proposed, with only partial benefit. Follow-up ophthalmologic evaluations confirmed complete loss of vision, secondary to bilateral vascularized corneal leukoma.

Child growth was normal until 4 years of age (high 109.5 cm, 75-90° percentile, weight 6.7 kg, 25-50° percentile). It briefly declined shortly after HSCT (5.5 years height 110.4 cm, 25-50° percentile, weight 157 kg 3-10° percentile). However, his growth returned to normal in the following years (at 13 years of age; height 160.7 cm, 75-90° percentile; weight 41.2 kg, 25-50° percentile).

At the last available follow up visit, performed at 13 years of age, he was in good general condition, although he still suffered from complete blindness and bilateral lower limbs lymphedema. Complete blood count and lymphocytes subsets were within normal limits, as well as TRECs, KRECs, and lymphocyte proliferative response to mitogens. Serum immunoglobulins were within normal levels. Protective antibody response against vaccine antigens was documented. Molecular evaluation of neutrophils and lymphocytes confirmed full donor chimerism. Notably, no severe infections occurred after discharge from transplantation unit following HSCT.

Discussion

We report the outcome of a patient with complete functional absence of STAT1 who underwent HSCT from an unrelated donor. To our knowledge, this is the first patient to be successfully cured from this condition. Like other patients with complete STAT1 deficiency, the clinical course of our patient prior to HSCT was characterized by severe viral and mycobacterial infections, suggesting the necessity of HSCT. A previous report by Chapgier et al. had not shown favorable outcome after HSCT in another patient with complete STAT1 deficiency [12]. This patient had received HSCT from matched sibling donor at 8 months of age after a conditioning regimen with alemtuzumab, fludarabine, and melphalan, plus prednisolone and tacrolimus for GVHD prophylaxis. Engraftment

in this case was slow and likely incomplete, since $CD3^+$ count 2.5 months after HSCT was low (300/μL), and regular platelet transfusions were required. At 70 days post-transplantation, he developed EBV infection associated with respiratory failure. Despite treatment with rituximab and donor T cell infusions resulting in EBV clearance, the patient eventually died from multiorgan failure at 91 days post-transplant.

HSCT in our patient was performed after a myeloablative conditioning regimen using busulfan and cyclophosphamide, while in the patient reported by Chapgier et al. a reduced-intensity conditioning regimen was adopted. While reduced-intensity regimens are commonly used in HSCT for immune deficiencies, this may have increased the risk of graft failure. A clear recommendation on which regimen to adopt in these patients cannot be made from our limited experience. The post-transplant clinical course in our patient was characterized by multiple and severe complications, including Coombs-positive hemolytic anemia, acute grade III cutaneous and intestinal GVHD, rituximab-associated PRES, thrombotic thrombocytopenic purpura (HSCT-related thrombotic microangiopathy), bilateral keratoconjunctivitis, and bilateral lower limb lymphedema, resulting in long-term disabilities. It is not clear whether the observed complications may have been influenced by the patient's genetic condition. Nevertheless, considering also the experience by Chapgier et al., it may be prudent to consider these patients at high risk of complications during HSCT. Partial HLA-mismatch (8/10) has probably contributed to the development of cutaneous and intestinal GVHD, while keratoconjunctivitis and lower limb lymphedema have an uncertain cause. Interestingly, lymphedema predisposition has been observed in patients with *GATA2* mutations associated with Emberger syndrome, a disorder characterized susceptibility to myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) [14,15]. In this condition, *GATA2* loss of function mutations can affect the transcription of genes involved in lymphatic vessel valve development, resulting in predisposition to lymphedema [16]. Although *STAT1* loss of functions mutations have never been linked to risk of lymphedema, cytokines signaling trough STAT1, such as IL-27, can regulate lymphatic endothelial cell proliferation [17].

In our patient HSCT allowed durable, long-term full donor chimerism as well as full immunological reconstitution, with normalization of STAT1 phosphorylation in lymphocytes, as demonstrated by flow cytometry. STAT1 is required for IFN response both in hematopoietic cells as well as in other cell types (e.g. epithelial cells) following intracellular and viral infections. In mice, lack of both type I and type III IFN-mediated signaling only in epithelial cells but not in hematopoietic cells results in lethal infection by influenza virus [18]. HSCT transplantation has been effective in our patient in the correction of the genetic defect in hematopoietic cells, while it could not have any effect on STAT1 deficiency in epithelial or endothelial cells. Remarkably, our STAT1-deficient patient did not present evidence of susceptibility to viral infections after receiving HSCT, suggesting that immune response against viral infections might be more redundant in the human system as compared to mice, since normal IFN response in hematopoietic cells but not in the epithelia may be sufficient to provide protection.

Authorship contributions

Samuele Naviglio reviewed the study and wrote the manuscript, Elena Soncini designed the study and revised the manuscript; Donatella Vairo performed functional studies and revised the manuscript; Arnalda Lanfranchi performed studies for functional reconstitution and revised the manuscript, Raffaele Badolato supervised the project and helped to write the manuscript; Fulvio Porta made substantial contributions to interpretation of data and revised the manuscript.

Conflict of interest disclosure

The authors declare no conflict of interest.

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Table 1

Infectious diseases prior to HSCT.

Age	**Events**
10 months	Severe disseminated *Mycobacterium kansasii* infection, possibly associated with *Aspergillus fumigatus* co-infection
16 months	Laryngitis and pulmonary infection of unknown etiology with right upper lobe atelectasis
17 months	Right lung pneumonia, unidentified agent
20 months	Acute infectious mononucleosis complicated by cutaneous Herpes simplex infection
23 months	CMV pneumonia
25 months	Sepsis from an unidentified microbial agent, associated with refractory seizures
27 months	Pneumonia from unknown agent
35 months	CMV pneumonia
4 years	Enterovirus meningitis complicated by cerebral venous sinus thrombosis. No persistent neurological disabilities were present at recovery, brain MRI showed multiple subarachnoid liquoral collections and mild hydrocephalus from aqueductal stenosis
	Lymphocyte subsets: lymphocytes 11,634 cells /µl; CD3 81,5%; CD4 30,7%; CD8 43,4%; CD4/CD45RA 14,7%; CD4/CD45R0 11,4%; CD19 14%; CD16 4,9%.

Figure legend

Figure 1. Functional reconstitution of STAT1 mediated signaling after bone marrow transplantation. We performed flow cytometric analysis of STAT1 phosphorylation (Y701) of peripheral blood $CD3^+$ cells after treatment with IFN-α (40000U/µl) or medium alone for 30 minutes at 37°C using intracellular staining with an anti-phospho-STAT1-PE before HSCT (panels A) and 8 years after HSCT (panels B) in healthy donor (left panels) and patient (right panels). Functional response to IFN-α (panels C) and IFN-γ (panels D) were evaluated by stimulation of PBMCs from a healthy donor and from patient, 8 years after HSCT, for 24 hours. MXA and STAT1 mRNAs upregulation in response to IFN-α (1000 U/ml) and TNFA and IDO mRNAs in response to IFN-γ (1000 U/ml) were measured by real time PCR.