PERSISTENT MYCOBACTERIUM AVIUM INFECTION FOLLOWING NON-MYELOABLATIVE ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR INTERFERON-γ RECEPTOR-1 DEFICIENCY

Running Title: SCT for Interferon-γ receptor deficiency

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ABSTRACT

Interferon-γ receptor-1 (IFNγR1) deficiency is a rare inherited immunodeficiency. We performed a nonmyeloablative allogeneic stem cell transplantation on a boy with complete IFNγR1 deficiency and refractory disseminated *Mycobacterium avium* infection. Despite the patient’s profound immune defect, early donor stem cell engraftment was low. Full donor engraftment was accomplished only following multiple donor lymphocyte infusions. Detection of IFNγR1 expression on peripheral blood monocytes and neutrophils corresponded with establishment of stable, complete donor hematopoietic chimerism. However, expression of, and signaling through IFNγR1 disappeared shortly thereafter. Disseminated *Mycobacterium avium* infection persisted and the patient died. Co-culture of *Mycobacterium avium* with normal myeloid cells resulted in an IFNγ signaling defect similar to that observed in vivo. Active disseminated *Mycobacterium avium* infection may significantly compromise normal immune reconstitution following allogeneic stem cell transplantation. Patients with IFNγR1 deficiency should be transplanted before developing refractory mycobacterial infections.
Interferon-γ receptor-1 deficiency (IFNγR1) is a rare immunodeficiency in which patients are susceptible to a broad range of pathogenic and non-pathogenic mycobacteria, Salmonellae, Listeria monocytogenes, histoplasma, and severe viral infections.1-4 Patients with autosomal recessive IFNγR1 deficiency typically have a complete lack of interferon-γ responsiveness and usually die of disseminated mycobacterial infections in childhood.1

Allogeneic stem cell transplantation (SCT) has the potential of restoring normal immune function by providing normal interferon-γ responsive myeloid cells.5 The potential for allogeneic stem cell transplantation to facilitate clearance of an established mycobacterial infection has not previously been tested. Therefore, we performed a non-myeloablative SCT on a chronically ill boy with autosomal recessive IFNγR1 deficiency and refractory disseminated Mycobacterium avium. Following engraftment, the donor cells acquired a defect in IFNγR1 surface expression and signaling. In vitro studies suggest a role for Mycobacterium avium in the signaling defect.

Study Design

With parental informed consent, the child was enrolled as a compassionate exemption to National Institutes of Health protocol 98-I-0104 (Non-myeloablative stem cell transplantation for Chronic Granulomatous Disease). Using high-resolution typing, the patient’s mother was found to be identical at the HLA-A, B, DRβ1, DQβ1 and DRβ loci. Although heterozygous for the IFNγR1 mutation, maternal peripheral blood mononuclear cells demonstrated normal in vitro IFNγR1 expression and signaling.6 Non-myeloablative conditioning consisted of intravenous Cyclophosphamide (60mg/kg on days −7 and −6),
Fludarabine (25mg/m² on days –5 to –1) and equine anti-thymocyte globulin (40mg/kg on days –5 to –2). Following conditioning, a G-CSF mobilized, T-cell depleted (Isolex®, Nexell Therapeutics Inc., Irvine Ca.) peripheral blood stem cell graft consisting of 52.2 x 10^6 CD34+ cells/kg and 5.4 x 10^4 CD3+ cells/kg (recipient body weight) was infused. Donor chimerism was determined by quantitative PCR amplification of informative microsatellites using DNA isolated from CD14+ and CD15+ cells that were purified using immunomagnetic beads. Cyclosporine (target trough levels of 200-350 µg/l) was administered until day 100 as prophylaxis for graft versus host disease (GvHD) and graft rejection. Donor lymphocyte infusions (DLI) were performed on days +30 (2 x 10^6 CD3+ cells/kg), +60 (1 x 10^7 CD3+ cells/kg) and +100 (5.3 x 10^8 CD3+ cells/kg) to augment donor stem cell engraftment. Cells for the day +30 and day +60 DLI were obtained from a dedicated lymphocyte apheresis of the donor. Cells for the day +100 DLI were obtained following G-CSF mobilization of the donor and contained 20.4 x 10^6 CD34+ cells/kg. Anti-mycobacterial therapy consisting of amikacin, azithromycin, ethambutol, levofloxacin and rifabutin was continued throughout the SCT. Lineage specific chimerism analysis was performed by microsatellite analysis on CD14+ monocytes, CD15+ neutrophils and CD3+ T cells.7

*M. avium* co-culture (5 x 10^5 organisms/ml) was performed on normal peripheral blood mononuclear cells (1 x 10^6 cells/ml) in RPMI supplemented with 10% human AB serum for 6 days. The *M. avium* isolate used in these experiments was derived from the patient. Flowcytometric analysis was performed using anti-IFNγR1 (Genzyme, Cambridge MA) and anti phosphorylated STAT1 (New England Biolabs, Beverly MA) as previously described.8

Results and Discussion
The patient was a chronically ill appearing 5 year old boy with marked hepatosplenomegaly, renal insufficiency and severe restrictive and reversible obstructive airway disease. All organ pathology was felt to be a consequence of a disseminated *Mycobacterium avium* infection originally diagnosed at 8 months of age. Blood, sputum and urine cultures grew *Mycobacterium avium* (colonies too numerous to quantify) despite aggressive antimicrobial therapy. The patient’s genetic defect has been previously described. In *vitro* analysis of peripheral blood demonstrated complete unresponsiveness to interferon-γ and absence of IFNγR1 expression by flow cytometry.

The conditioning regimen was tolerated without significant complications. The patient’s absolute neutrophil count was below 500/µl for 6 days and the platelet count nadir was 74,000/µl. Full donor stem cell engraftment was accomplished following the DLI given on day 100 of the transplant (figure 1). Coincident with engraftment was development of grade 3 GvHD involving the skin and intestine that resolved with corticosteroid and cyclosporine therapy. Despite resolution of GvHD, the patient had continued debilitating generalized inflammation characterized by cachexia, metabolic acidosis, azotemia, bronchoconstriction, splenomegaly and chylous ascites. High-level mycobacterial bacteremia persisted. In addition, multi-focal calcification developed in the spleen, atria, and cerebral vessels. Prednisolone at doses that ranged from 0.5 to 3 mg/kg was essential to maintain clinical stability. Approximately 218 days following SCT, we first observed decline of surface IFNγR1 expression (figure 1). This was progressive and became complete (figure 2a). Concurrent with loss of surface expression of IFNγR1 was loss of normal phosphorylation of STAT 1 in response to interferon-γ stimulation in engrafted cells, but not in cells collected...
directly from the donor (figure 2a-d). The patient died 12 months after SCT of acute polymicrobial sepsis. An autopsy was not permitted.

This is the first reported case of allogeneic SCT of a patient with primary immunodeficiency who is actively infected with *Mycobacterium avium*. Using a non-myeloablative bone marrow conditioning regimen and delayed DLI, complete donor stem cell engraftment was achieved with minimal conditioning-related toxicity. Prolonged corticosteroid therapy was mandated not by refractory GvHD, but by a profound systemic inflammatory state that may have been part of the appropriate donor cell response against disseminated *Mycobacterium avium*. The acquired defect in IFNγR1 surface expression and signaling through STAT 1 by the previously normal donor cells was likely caused by, and contributed to the persistent *Mycobacterium avium* infection in this patient. Co-culture of mononuclear cells from two normal volunteers with a concentration of *Mycobacterium avium* similar to what had been found in our patient’s blood simulated the *in vivo* defect in STAT 1 phosphorylation (figure 2e,f). This observation is consistent with the findings of Hussain et al. who demonstrated down-regulation of STAT1 phosphorylation and interferon-γ inducible genes following interferon-γ stimulation in murine macrophages infected with *Mycobacterium avium*.9 That we can replicate the *in vivo* finding of defective IFNγ receptor signaling *in vitro* argues against the possibility that the patient developed blocking antibodies to IFNγR1 following SCT. Furthermore, formation of such antibodies would be highly unlikely given the prolonged use of immunosuppressive medications.

This case demonstrated two important points. First, transplantation of normal functioning hematopoietic stem cells into a *Mycobacterium avium* infected host resulted in a sustained, steroid responsive inflammatory response. In the early days following stem cell
engraftment, the inflammatory response was indistinguishable from classic GvHD. What role the *Mycobacterium avium* infection played in initiating the GvHD is unknown, however, conventional GvHD treatment (corticosteroids and cyclosporine) resulted in resolution of typical manifestations. The subsequent symptomatology characterized by high fever, multifocal calcifications, nephritis and chylous ascites are atypical for GvHD. Second, reconstitution of the interferon-γ responsive pathways responsible for host defense was impaired by the ongoing high-grade *Mycobacterium avium* infection. Our experience suggests that restoration of normal immune function with allogeneic SCT in patients with IFNγR1 deficiency may not be possible in the setting of disseminated *Mycobacterium avium* infection. This case has important implications for other SCT candidates with mycobacterial infections as a consequence of primary or secondary (chemotherapy induced) immunodeficiency. Further studies are needed to characterize the mechanism by which *Mycobacterium avium* affects interferon gamma signaling in human cells.
REFERENCES


Figure Legends:

Figure 1. Engraftment of CD14+ donor monocytes (■), CD15+ donor neutrophils (●) and percent cells positive for surface expression of interferon-γ receptor-1 on donor CD14+ monocytes (▲). Graft rejection was prevented by infusion of escalating doses of donor lymphocytes. Following full donor stem cell engraftment, surface expression of the interferon-γ receptor progressively diminished. Downward pointing arrows indicate microsatellite chimerism data prior to transplant (accompanied by the donor pattern), day 134 and day 296 following the transplant.

Figure 2. Surface interferon-γ receptor-1 expression and STAT1 phosphorylation. (A) Down-regulation of surface expression of IFNγR1 on mononuclear cells from the patient 322 days after allogeneic stem cell transplantation (160 days after complete donor stem cell engraftment). (B) Down-regulation of STAT1 phosphorylation following stimulation with IFNγ of cells from patient at the same time as in panel A. (C) Normal IFNγR1 expression on donor mononuclear cells and (D) normal STAT1 phosphorylation following IFNγ stimulation of donor cells. (E) STAT-1 phosphorylation in response to IFNγ stimulation in normal cells cultured in the absence of Mycobacterium avium and (F) in the presence of Mycobacterium avium.
Figure 1

Transplant Day

Percent

0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 0 25 50 75 100

DLI DLI Lymphocyte Stem Cell Infusion

Recipient Pre-Transplant

CD14 Day 194

CD14 Day 266

Donor
Figure 2

A

Patient

B

Normal (No M. avium)

C

D

Normal (M. avium)

E

Fluorescence

F

Patient

Donor

Donor

Normal (M. avium)
Persistent *Mycobacterium avium* infection following non-myeloablative allogeneic peripheral blood stem cell transplantation for Interferon-γ receptor-1 deficiency

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