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RAG deficiency with ALPS features successfully treated with TCR $\alpha\beta$ /CD19 cell depleted haploidentical stem cell transplant

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Recombination activating genes 1 and 2 (RAG) proteins are involved in generating diversity of T- and B-cell receptors via recombination of variable, diversity, and joining (VDJ) gene segments. *RAG* mutations can manifest with a variety of clinical presentations. Some patients with near complete RAG deficiency, often due to bi-allelic nonsense or frameshift mutations, develop severe infections within the first year of life and are diagnosed with

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severe combined immune deficiency. Patients with near complete RAG deficiency lack T and B cells, but have preserved natural killer (NK) cells (T-B-NK + SCID) [1]. Omenn syndrome (OS), characterized by high numbers of oligoclonal T-cells, generalized erythroderma, lymphadenopathy, hepatomegaly, and infections often results from *RAG* mutations with minimally preserved function. Other categories of severe RAG deficiency, SCID with maternal fetal engraftment and atypical SCID/OS, have also been reported [1]. On the other hand, patients with hypomorphic *RAG* mutations with a moderate decrease in recombinase activity present later in life with autoimmune complications and sometimes granulomas, even prior to infections [2–4]. Autoimmune cytopenias are common in RAG deficiency and may be diagnosed before recognition of an underlying primary immune deficiency (PID). Thirteen percent of children with autoimmune hemolytic anemia (AIHA) [5] and up to 50% with multi-lineage cytopenias (Evans syndrome) may be diagnosed with PID [6]. Timely diagnosis of PID in patients with autoimmune cytopenia is essential to secure proper treatment and a favorable outcome. A patient who presented with AIHA and features of autoimmune lymphoproliferative syndrome (ALPS) due to a novel homozygous hypomorphic *RAG1* mutation is described here. The study was approved by the Institutional Review Board of Istituto Giannina Gaslini (Protocol number 002REG2015).

A previously healthy 30-month-old male developed Coombs positive AIHA and interstitial pneumonia. He had positive cytomegalovirus (CMV) serology and normal immunoglobulin levels on initial evaluation. A full immune evaluation was not performed prior to his referral to a tertiary care center late in his disease course. He was treated with glucocorticoids and ganciclovir and achieved a temporary (10 month) remission. AIHA relapsed at 40 months. CMV reactivation (viral load: 47,000 copies/ml) was associated with development of macrophage activation syndrome. Primary hemophagocytic lymphohistiocytosis was excluded based on normal natural killer cell function by cytotoxicity assay and flow cytometry for signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) and perforin. He responded to ganciclovir and rituximab with a brief remission. At 42 months he developed persistent hypogammaglobulinemia (attributed to rituximab), hepatosplenomegaly, seropositive autoimmune neutropenia and hypereosinophilia. At this point he was referred to a tertiary care center. Mycophenolate mofetil was started. Cytopenias improved, but at 51 months, he developed severe H1N1 influenza pneumonia and CMV relapse, requiring admission to the intensive care unit. He had concomitant relapse of AIHA. He was noted to have a slightly increased fraction (2% of 1100 total lymphocytes) of TCR $\alpha\beta$ +double CD4- CD8- T cells (DNT). On treatment with oseltamivir and sirolimus, cytopenias improved and influenza resolved, but CMV persisted. ALPS was considered in the differential diagnosis due to increased fraction of DNT cells, hepatosplenomegaly, a positive FAS-induced apoptosis test, and clinical history of multilineal autoimmune cytopenias (AIHA and autoimmune neutropenia). Due to refractory cytopenias and persistent CMV, at 63 months, the patient underwent TCR $\alpha\beta$ /CD19 cell depleted haploidentical hematopoietic stem cell transplant (HSCT) from his mother as donor and is doing well 2 years post-transplantation with no graft versus host disease. He did not receive GVHD prophylaxis. Following transplant, a hypomorphic homozygous *RAG1* mutation (R507G) was found via next generation sequencing. The activity of this mutation was assessed by Abelson-immortalized murine *Rag1*^{-/-} pro-B cells transduced with vectors

encoding either mutant or wild type human RAG1 [2]. The enzymatic activity of this mutation was found to be 19.2% (± 1.8 SD %) compared to wild type RAG1. A similar point mutation, R507W, has demonstrated residual recombinase activity of 16% [2].

The favorable outcome of this case is attributed to prompt treatment with antiviral medications, lack of live vaccinations, and HSCT. The patient was treated with ganciclovir early, and he did not receive live virus vaccinations for varicella, measles, mumps, or rubella. Table 1 describes transplant features including the conditioning regimen used in this patient. Haploidentical HSCT protocols have advanced over the past three decades to include features that make them a particularly appealing option for leaky or atypical SCID patients, in which transplant must be performed promptly despite persistent infections and inflammation. Infection and inflammation increase the risk of graft failure and transplant-related mortality (TRM), but transplant is often the only long-term solution in this otherwise fatal disease, despite these challenges. The first haploidentical HSCT protocols relied on CD34+ selection of hematopoietic stem cell progenitors. CD34+ positive selection has advantages including low rates of graft-versus-host disease (GVHD) and high engraftment rate, but also has disadvantages, including slow immune reconstitution and increased risk of transplant-related mortality. Selective ex vivo depletion of TCR $\alpha\beta$ cells and CD19+ B cells, preserving the TCR $\gamma\delta$ and NK cell subsets in the graft, is a promising approach for treating both malignant and non-malignant disorders [7–9]. Regimens have not been standardized for patients with late onset combined immunodeficiency secondary to RAG deficiency [10]. The haploidentical HSCT protocol with preserved TCR $\gamma\delta$ cell subset in this partial RAG deficient patient contained the ongoing CMV infection. Both donor and recipient were CMV positive. The patient did have several episodes of CMV viremia post-transplant, beginning at Day +96 and ending 11 months post-transplant, but he exhibited no signs of CMV-related disease. Episodes of viremia were treated preemptively with antiviral medications. In the challenging field of HSCT for RAG deficiency, ex vivo depletion of TCR $\alpha\beta$ cells and CD19+ B cells could be considered for similar cases in the future. This case highlights the fact that hypomorphic mutations in *RAG* genes may manifest first as autoimmune cytopenia with ALPS-like features rather than infections alone.

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Abbreviations

AIC	Autoimmune cytopenia
AIHA	Autoimmune hemolytic anemia
ALPS	Autoimmune lymphoproliferative syndrome
CMV	Cytomegalovirus

DNT	Double negative T-cell
GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplantation
NK	Natural killer
OS	Omenn Syndrome
PID	Primary immunodeficiency
RAG	Recombination activating gene
SAP	Signaling lymphocytic activation molecule (SLAM)-associated protein
SD	Standard deviation
SLAM	Signaling lymphocytic activation molecule
SCID	Severe combined immune deficiency
TCR	T cell receptor
TRM	Transplant-related mortality
VDJ	Variable, diversity, and joining segments

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Table 1

Transplant features and outcome.

✓ Age at transplant	5 years and 3 months
✓ Conditioning regimen	Thiotepa 8 mg/kg at day -7 Treosulfan 42 mg/m ² in 3 days (from d -6 to d -4) Fludarabine 160 mg/m ² in 4 days (from d -6 to d -3) ATG Fresenius 4 mg/kg/dose, 3 days (from d -4 to d -2) Rituximab 200 mg/m ² at day -1
✓ Donor type	Haploidentical (mother)
✓ Mobilization schedule	G-CSF 6 µg/kg twice daily from day -4
✓ Stem cell source	Peripheral blood
✓ Ex-vivo graft manipulation	TCRαβ ⁺ /CD19 ⁺ negative selection by Miltenyi Clinimacs device
✓ Graft composition	TNC: 14×10^8 /kg CD34 ⁺ cells: 22×10^6 /kg αβ ⁺ T cells: 0.28×10^5 /kg γδ ⁺ T cells: 960×10^5 /kg CD19 ⁺ cells: 0.99×10^5 /kg
✓ Timing of engraftment	Neutrophils at day +14 Platelets at day +12
✓ Chimerism	Full donor since day +12

ATG: antithymocyte globulin; HPC: hematopoietic progenitor cell; G-CSF: granulocyte-colony stimulating factor; HPC: hematopoietic progenitor cells; TNC: total nucleated cell.