Published in final edited form as:

Pediatr Blood Cancer. 2023 May ; 70(5): e30183. doi:10.1002/pbc.30183.

Refractory, Fatal Autoimmune Hemolytic Anemia due to Ineffective Thymic-derived T-cell Reconstitution Following Allogeneic Hematopoietic Cell Transplantation for Hypomorphic RAG1 deficiency

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Keywords

Hypomorphic RAG1; Transplant; Immune reconstitution; autoimmune hemolytic anemia

To the Editor

Pathogenic recombination-activating gene 1 (*RAG1)* variants cause a phenotypic spectrum ranging from severe combined immunodeficiency (SCID) to combined immunodeficiency with granulomas and autoimmunity (CID-G/A), depending on residual recombinase activity.[1–4] Our patient a 12-year-old African American female with hypogammaglobulinemia and T, B-cell lymphopenia was diagnosed with CID at 5 years of age, with a history of recurrent herpes simplex virus (HSV) gingivostomatitis, bacterial pneumonia, and cutaneous abscess. She tolerated two doses of live measles-mumps-rubella vaccine. She was referred to our facility for steroid-refractory sterile non-caseating cutaneous granulomas (Supplemental Figure 1) affecting posterior thigh and midface, appearing at 9 years. This with progressive lymphopenia (Figure 1A) was concerning for a CID. Next-generation sequencing identified compound heterozygous pathogenic

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*Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Conflicts of Interest: The authors have no conflicts of interests.

RAG1 variants, c.1187G>A, p.Arg396His and c.1566G>T, p.Trp522Cys (NM_000448.2). Residual recombinase activity is estimated at 40–50% for *RAG1*-Trp522Cys and <5–30% for *RAG1* - Arg396His.[2,5,6] Immunofluorescent staining of granulomas identified rubella virus antigen in neutrophils in the necrotic region of deep dermis and a few CD206+ M2 macrophages[7] (Supplemental Figure 2). RT-PCR and sequencing detected vaccine-derived rubella virus (VDRV) genomic RNA in nasopharyngeal secretions, while urine and peripheral blood (PB) were negative. PB markers of persistent rubella infection were detected: rubella-specific IgM antibody and elevated titers of rubella neutralizing antibody (NT_{50}=400)[7,8]. T-cell phenotyping demonstrated reduced frequency of naïve T cells with relative expansion of CD45RO+ memory T cells (Figures 1B, 1C), and evidence of cellular exhaustion (PD1, data not shown). T-cell proliferation to Candida and tetanus toxoid was normal. Class-switched memory B cells were absent, while CD19+CD21^{dim} B cells (anergic autoreactive subset with potential for altered homing, autoimmunity) were expanded. Autoantibodies to IL-12, IFN-α, or IFN-ω, previously reported in CID-G/A were absent.[1,2] CD3+Vα7.2+CD161+mucosal-associated invariant T cells (MAITs) were undetectable (Figure 2A). Degree of loss of MAIT, a sensitive biomarker of TCR-Vα rearrangement defects has been correlated with phenotypic severity in CID-G/A.[1] CD4+CD45RO+memory T cells expressing CD162 and CCR9 (facilitating homing to skin, gastrointestinal mucosa), were significantly reduced (Figure 2B). TCR-Vβ spectratyping confirmed expansion of an oligoclonal T-cell repertoire. Frequency of CD127+ innate lymphoid cells (ILCs) was increased, with relative expansion of ILC2 and ILC3 subsets (Figure 2C). The patient underwent a 10/10 matched-unrelated bone marrow HCT following busulfan/fludarabine/alemtuzumab conditioning with tacrolimus/methotrexate for graft versus host disease (GvHD) prophylaxis[9]. Early post-HCT the granulomas regressed, though infections included HSV gingivostomatitis, BK hemorrhagic cystitis, and adenoviremia (responsive to pharmacotherapy). She achieved full (99%) donor chimerism (FDC) in the myeloid lineage by Day (D) +30 and in CD3+Tcells by D+59. At D+95, she showed persistent T-cell lymphopenia with absent naïve CD4+T cells (Figure 1A). There was evidence of B cell recovery (CD19+ B-cell lymphocytosis, presence of class-switched memory B cells), likely facilitated by the few memory and/or follicular T helper cells. On D+170, she developed warm AIHA with reticulocytopenia and parvovirus reactivation. Bone marrow showed erythroid arrest. Following transient response to intravenous immunoglobulin and corticosteroids, she had multiple relapses of AIHA with emergence of cold agglutinin and Cartwright red-cell-antigen antibodies. Response to daratumumab and eculizumab lasted 4 weeks with no response to subsequent therapies (rituximab, cyclosporine, abatacept). She had no thymic reconstitution with absent naïve T cells. On D+272 she remained at FDC in CD19+ and CD56+ fractions, ruling out expansion of autoreactive recipient clones. Thirteen-months post-HCT, she underwent a 2nd HCT using CD34+ selected PB stem cells (same donor) following therapeutic plasma exchange (TPE). She received fludarabine/alemtuzumab and total lymphocyte irradiation (200cGY) with no GvHD prophylaxis. Infections at the time of 2nd HCT included adenovirus (on cidofovir) and parvovirus DNAemia. Despite FDC status on D+30 post 2nd HCT, she remained lymphopenic with no naïve T cells. Majority of lymphocytes (85%) were CD8+ T cells, with no CD19+ B cells (Figure1A). Following a transient response, two months later, she presented with a hemoglobin of 2.6 gm/dl secondary to AIHA. Despite TPE

and bortezomib she succumbed at D+50 post 2nd HCT to right ventricular failure. This patient exemplifies the phenotypic diversity of *RAG1* variants and complexity of allo-HCT in the context of immune-dysregulation. CID-G/A unlike SCID is characterized by higher T and B-cell counts at birth and may escape detection via TREC-based newborn SCID screening. T and B-cell lymphopenia tends to worsen as peripheral expansion fails to maintain homeostasis, and replicative senescence exacerbates functional cellular defects. These likely caused emergence of rubella virus in the latent period between our patient's MMR and clinical disease.[10] Her granulomas regressed early post-HCT suggesting that the pathogenesis involved dysregulated reactive inflammation[7] Allo-HCT is indicated for all forms of RAG deficiency with overall survival rates of 69%−85% with robust immune reconstitution in younger patients (< 3.5 years).[3,11,12] Myeloablation with serotherapy is employed to deplete dysregulated lymphocytes and promote engraftment. However, ineffective thymic-derived T-cell reconstitution post-HCT along with viral infections can trigger uncontrolled immune-mediated cytopenias (IMC) and the consequent treatment aggravates ineffective immune reconstitution. Post-transplant autoimmunity occurs even in FDCs due to reduced thymic expression of tissue-restricted antigens and survival of recipient long-lived plasma cells.[1,12] B or plasma cell (rituximab, daratumumab) depletion peri-HCT may be considered to decrease risk of IMC[13]. Finally early diagnosis followed by HCT facilitates improved immune reconstitution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

L.D. Notarangelo is supported by the Division of Intramural Research, NIAID, NIH: grant 1 ZIA AI001222-02 (PI: L.D. Notarangelo).

List of Abbreviations

Allo-HCT	Allogeneic hematopoietic cell transplantation
AIHA	Autoimmune hemolytic anemia
YTa	Cartwright red cell antigen
(CID-G/A)	Combined immunodeficiency with granulomas and autoimmunity
FC	Full chimerism
HCT	Hematopoietic cell transplantation
HSV	Herpes Simplex Virus
IMC	Immune-mediated cytopenia
ILCs	Innate lymphoid cells
IL	Interleukin

INF	Interferon
MSSA	Methicillin sensitive staphylococcus aureus
MAITs	Mucosal-associated invariant T cells
NK	Natural Killer
PB	Peripheral blood
RAG1	Recombination-activating gene 1
RT-PCR	Reverse transcription polymerase chain reaction
SCID	Severe combined immunodeficiency
TPE	Therapeutic plasma exchange
TREC	T cell receptor excision circle
VDRV	Vaccine-derived rubella virus

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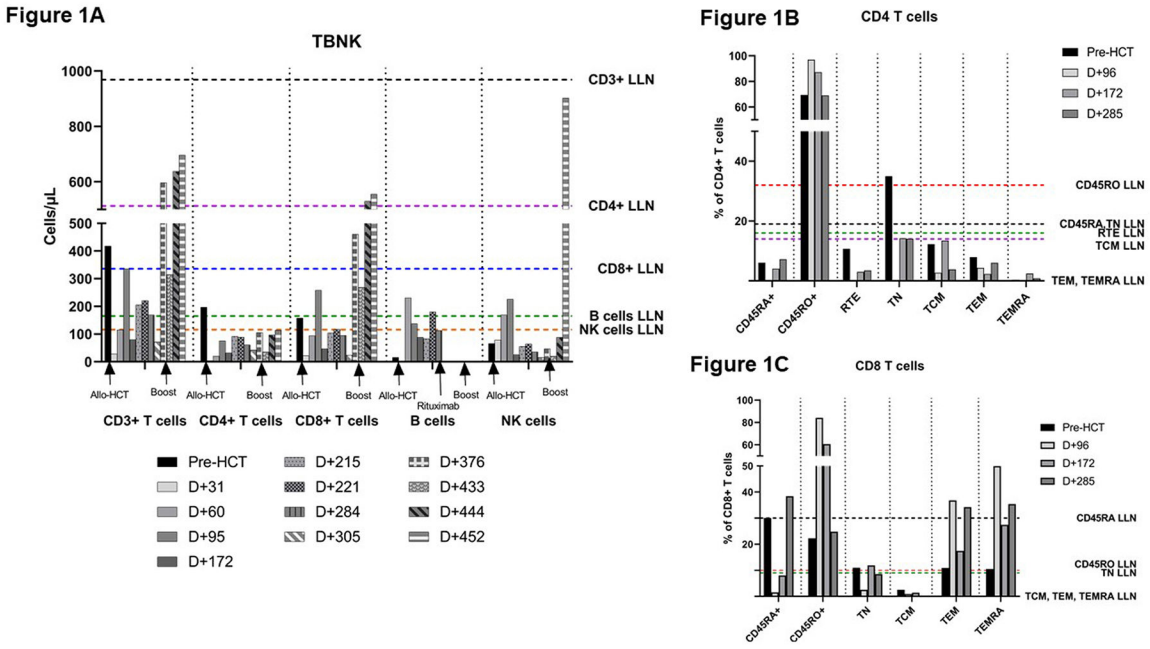


Figure 1.

A) T, B, and NK enumeration over time. The lower limit of normal (LLN) of the performing institution's pediatric reference range is shown as the "reference". Rituximab 375 mg/m^2 weekly x4 doses was given for recalcitrant autoimmune hemolytic anemia. Allo-HCT: Allogeneic hematopoietic cell transplant Boost: CD34 selected Stem cell boost.

B). CD4 T cell naïve and memory subset phenotyping over time.

RTE: Recent thymic emigrant, CD45RA+CD31+

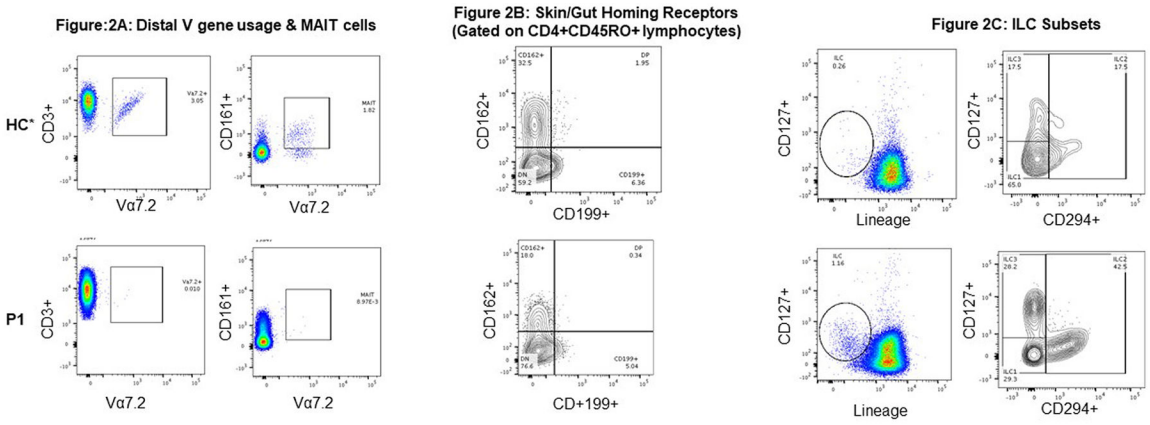
Naïve, CD45RA+CD62L+CCR7+

TCM: Central memory T cell, CD45RO+CD62L+CCR7+

TEM: Effector memory T cell, CD45RO+CD62L−CCR7−

TEMRA: T effector memory re-expressing RA, CD45RA+CD62L−CCR7−

(C) CD 8 T cell naïve and memory subset phenotyping



T Cell Subset Phenotyping		Healthy Control*
Mucosal associated invariant T cells (%CD3+)	0	2
CD4+CD45RO+CD162+CCR9+ (%CD4+CD45RO+)	0.3	2
Innate Lymphoid Cell (ILC) Subset Phenotyping		**Healthy Control***
ILC, CD45+CD127+ (%CD45+)	1	0.3
ILC 1, CD127+CD117-CD294- (%CD127+)	29	65
ILC 2, CD127+CD117-CD294+ (%CD127+)	43	18
ILC 3, CD127+CD117+CD294- (%CD127+)	28	18

Figure 2.

A.) Distal T cell receptor V gene usage and mucosal associated invariant T cell (MAIT) cell enumeration.

B.) Expression of skin and gut homing receptors, CD199 and CD162, on memory CD4+ T cells. C.) Innate Lymphoid Cell (ILC) subset phenotyping.