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LETTER TO EDITOR

Hematopoietic Stem Cell Transplantation Successfully Treats *CD40LG* Duplication

Di Sun[1] · Carole Le Coz[1] · Nancy Bunin[2] · Neil Romberg[1,3,4]

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To the editor:

CD40LG encodes CD40L, a tightly regulated T-cell coactivation receptor, that binds CD40 on antigen presenting cells such as dendritic cells, macrophages, and B cells. Physiologic CD40L induction promotes T-cell apoptosis and B-cell class switching [1–3]. Supraphysiologic CD40L expression promotes autoantibody production and portends poor prognoses in patients with lymphopenia-associated autoimmune diseases [3].

Recently, we described a boy with infantile-onset Evans syndrome, massive splenomegaly, and IgM deficiency who maternally inherited a 240 kb chromosome X microduplication (ChrX:135,539,461–135,780,648) encompassing *CD40LG* and its regulatory elements [4]. His T lymphocytes, which overexpressed CD40L upon induction, demonstrated accelerated cell death and exuberantly promoted B-cell immunoglobulin class-switching in vivo and in vitro [4]. Although his autoimmune manifestations were successfully managed with cyclosporine, which normalized CD40L expression by inhibiting nuclear factor of activated T cells (NFAT), his T-cell lymphopenia was insidiously progressive. At eight years old, his peripheral blood $CD3^+$ T-cell concentration fell to 334 cells/μL (134 $CD4^+$ cells/μL and 154 $CD8^+$ cells/μL; Supplemental Fig. 1) and he was referred for hematopoietic stem cell transplantation (HSCT).

After myeloablative conditioning with 3.2 mg/kg/dose busulfan (day −9 to −4), 30 mg/m^2/dose fludarabine (day −6 to −3, −2), 3 mg/kg/dose anti-thymocyte globulin (day −5 to −3), and 30 mg/m^2/dose cyclophosphamide (day −3 to −2), the patient received a 9/10 matched unrelated donor T-cell receptor α/β+/$CD19^+$ depleted peripheral stem cell transplant. His only transplant-related adverse event was BK virus cystitis which resolved with aggressive hydration. During conditioning, the patient experienced total and durable resolution of all autoimmune manifestations. Other than standard graft-versus-host disease prophylaxis with mycophenolate mofetil for 45 days post-transplant, he required no further immunosuppressant therapies. One month after HSCT, 99% of the patient's myeloid, B-cell, and NK lineage cells were donor-derived (Fig. 1A). In contrast, T-cell-mixed chimerism was significant. A total of ~40% of T cells were of donor origin a month after transplantation, but the donor fraction slowly increased to 93% by 18 months (Fig. 1A). Despite rapid numerical naive B-cell reconstitution (Supplemental Fig. 1), the patient remained immunoglobulin replacement therapy-dependent after transplantation. In contrast to the excessive in vivo class-switching observed pre-HSCT, circulating class-switched memory B cells (0–1 $CD19^+CD27^+IgM^-$ cells/μL) and all immunoglobulin isotypes were scarce during the post-HSCT period.

To functionally assess correction of the patient's immune defect by HSCT, we subjected patient $CD4^+$ T cells cryopreserved before and 18 months after transplant to a series of functional in vitro studies. Compared to pre-transplant patient $CD4^+$ T cells, which overexpressed CD40L upon activation, CD40L induction by post-transplant patient cells was similar to healthy donor (HD) counterparts (Fig. 1B). Similarly, while 16.7% of naïve HD B cells co-cultured with pre-HSCT patient $CD4^+$ T cells class-switched to IgG, only 10.8% and 7.8–11.8% of naïve HD B cells IgG class-switched when co-cultured with post-transplant patient $CD4^+$ T cells or HD $CD4^+$ T cells, respectively (Fig. 1C). Finally, cell death, measured with annexin V and 7-AAD co-staining after culturing live-sorted $CD4^+$ cells for 48 h in culture, was similar

✉ Neil Romberg
rombergn@chop.edu

1 Division of Immunology and Allergy, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

2 Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

3 Department of Pediatrics, Perelman School of Medicine, Philadelphia, PA 19104, USA

4 Abramson Research Center, Children's Hospital of Philadelphia, Room 1216C, Philadelphia, PA 19104, USA

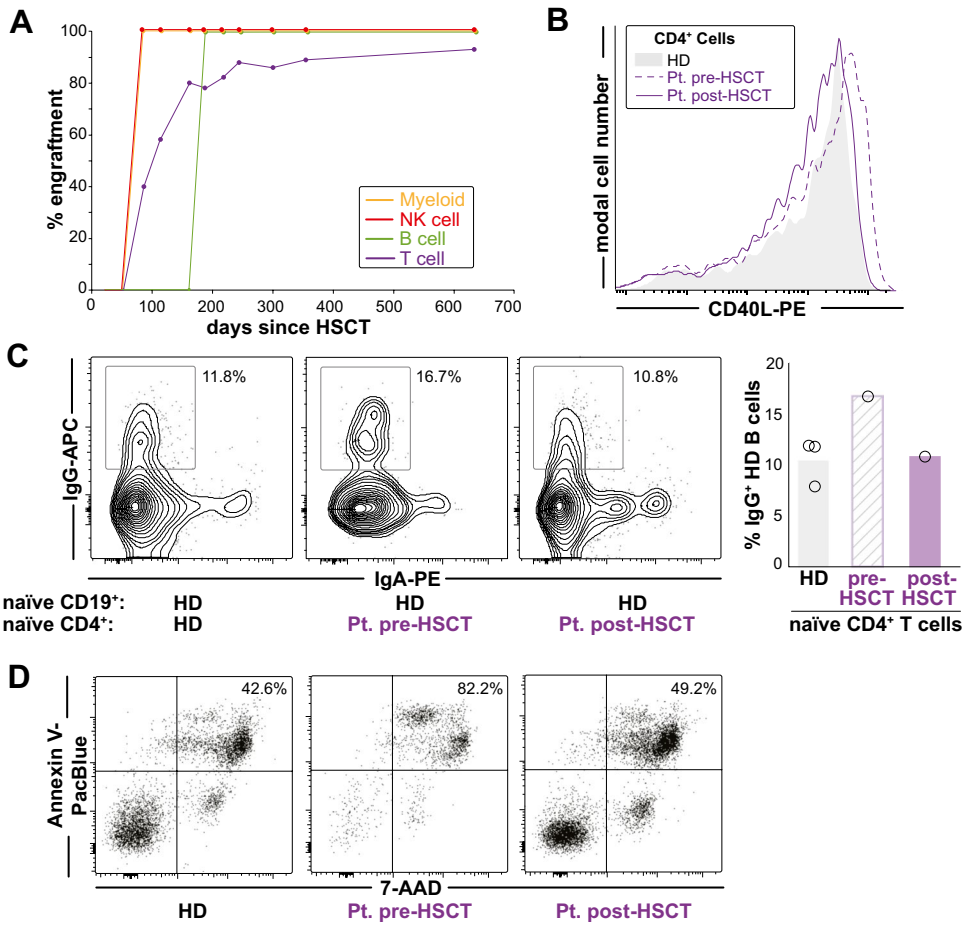

Fig. 1 Lineage engraftment and in vitro functional studies after hematopoietic stem cell transplantation (HSCT). **A** Lineage-specific engraftment following HSCT. **B** CD40L expression on healthy donor (HD) $CD4^+$ T cells (mean fluorescence intensity, MFI 4529) and patient (Pt.) $CD4^+$ T cells either pre- or post-HSCT (MFIs 5850 and 4502, respectively). Assessments were made 8 h after phorbol 12-myristate-13-acetate (PMA) induction. **C** Representative flow cytometry plots of IgG expression on HD naïve B cells following a 7-day co-culture with anti-CD3/CD28-activated $CD4^+CD45RO^-$ T cells from either a different HD, the patient pre-HSCT, or the patient post-HSCT. Bar graph depicts IgG^+ HD B-cell frequencies after co-culture with $CD4^+$ T cells from three HD controls (2 male, 1 female) and the patient pre- and post-HSCT. **D** Annexin V and 7-AAD staining of $CD4^+$ T cells after 48 h in anti-CD3/CD28 antibody activating conditions. At time zero, all cells were verified viable through sorting

in HD and post-HSCT patient cells (42.6% and 49.2%, respectively) (Fig. 1D). In contrast, nearly all (82.2%) pre-HSCT patient cells died in culture.

The clinical phenotype associated with CD40L overexpression—autoantibody-mediated autoimmune disease and lymphopenia—illustrates the two seemingly disparate consequences of CD40/CD40L engagement: B-cell activation or T-cell death. Although our *CD40LG*-duplicated patient's autoimmune disease was responsive to CD40L-modulating cyclosporine, his progressive lymphopenia required more definitive cellular therapy. HSCT works especially well when donor cells enjoy a competitive survival advantage over host cells. Although *CD40LG*-duplicated T cells demonstrate accelerated cell death, our patient's donor T-lineage engraftment was delayed. As thymic epithelial architecture is disrupted in CD40L-overexpressing transgenic mice, it may be that our patient's thymus required a normalization period before it could fully accommodate donor thymocyte development [5]. Regardless, the patient's clinical course and normalization of T-cell function post-HSCT reaffirm CD40L overexpression's role in disease pathogenesis and highlights that *CD40LG* duplication is a transplantable condition.

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Declarations

Consent for Publication All authors have reviewed the manuscript and consented to publication.

Conflict of Interest The authors declare no competing interests.

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