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LETTER TO THE EDITOR

First report of successful stem cell transplantation in a child with CD40 deficiency

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CD40 deficiency is a rare form of autosomal recessive combined immunodeficiency, caused by mutations in the *TNFRSF5* gene.[1,2] The CD40 transmembrane protein is constitutively expressed by B lymphocytes, monocytes and dendritic cells (DCs), but can also be expressed by some non-immune cells, such as endothelial and neuronal cells. Interaction between CD40-expressing B lymphocytes and activated $CD4^+$ T cells that express CD40 ligand (CD40L) promotes B cell proliferation, triggers class-switch recombination and somatic hypermutation, and induces generation of long-lived plasma cells.[3] Moreover, CD40L/CD40 interaction between activated T cells and DCs is essential to promote T cell priming and interferon-γ secretion and thus to defend against intracellular pathogens.[4] Accordingly, patients with CD40 or with CD40L deficiency share a common and severe phenotype. They have reduced levels of all immunoglobulin isotypes other than IgM, and present with recurrent bacterial and opportunistic infections.[1,5] Moreover, they tend to develop severe neutropenia, which further contributes to susceptibility to infections. In the view of the severe manifestations and outcome associated with defects of the CD40L/CD40 axis, hematopoietic stem cell transplantation (HSCT) has been successfully used to cure CD40L deficiency.[6] However, such strategy has been attempted only once, and unsuccessfully, in CD40 deficiency.[7] In addition to clinical status at transplantation, and previous infection by *Pneumocystis jiroveci* and *Cryptosporidium parvum*, that represent negative prognostic factors for patients with CD40L or with CD40 deficiency,[6,7] additional concerns have been raised on the potential efficacy of HSCT for CD40 deficiency, including the need of myeloablation to reconstitute the myeloid compartment, and inability to correct CD40 deficiency in non-hematopoietic cells.

We report here on the first case in which successful immune reconstitution has been achieved in a child with CD40 deficiency following myeloablative HSCT from her HLA-identical sister.

The patient, a two-year-old Turkish girl, born to second-degree consanguineous parents, was admitted to the Pediatric Immunology Division of the Ege University, Izmir (Turkey), with a history of recurrent pneumonia, otitis media and purulent skin infections. At admission, she appeared as a well-nourished child, with slight hepatomegaly. Laboratory evaluations disclosed marked hypogammaglobulinemia with elevated IgM (Table 1). Specific IgG antibodies to tetanus toxoid were undetectable, in spite of full-course immunization. Lymphocyte sub-populations were normal, however, CD40 expression on the surface of B lymphocytes and monocytes was virtually absent (Figure 1). Molecular analysis at the *TNFRSF5* locus revealed a homozygous 3-nucleotide deletion in exon 2, del(TAA)175–177, resulting in one-amino-acid deletion (del I33) in the extracellular domain of CD40. Both her parents, and the healthy, HLA-matched sibling, were found to be heterozygous for the same mutation, and expressed CD40 at intermediate levels (Figure 1 and data not shown).

In spite of substitution therapy with intravenous immunoglobulins (IVIG), and antibiotic prophylaxis, the patient developed further episodes of upper and lower respiratory tract infections. In addition, at 2.4 years of age, she was found to be profoundly neutropenic (ANC $0.3 \times 10^{-3}/\mu l$), and was treated with human recombinant granulocyte-colony stimulating factor (5 μg/kg 3 days a week).

At 3 years of age, she was admitted to the Department of Pediatrics, University of Brescia (Italy), where she received HSCT from her HLA-identical, CMV-positive healthy carrier sibling. Conditioning regimen included busulfan (4 mg/day/die from days −9 to −6) and cyclophosphamide (50 mg/kg/die from days −5 to −2). A total of unmani-

Table 1 Hematological and immunological reconstitution after HSCT for CD40 deficiency

	At presentation (2 years 1 months)	*18 months post-HSCT (4 years 6 months)*
ANC ($\times 10^{-3}/\mu l$)	0.3 (1.5–8.5)	4.2 (1.5–8.5)
ALC ($\times 10^{-3}/\mu l$)	4.8 (3.0–8.2)	5.6 (2.4–6.0)
$CD3^+$ ($\times 10^{-3}/\mu l$)	3.8 (1.6–4.2)	3.7 (1.6–4.2)
$CD4^+$ ($\times 10^{-3}/\mu l$)	2.4 (0.9–2.9)	2.2 (0.9–2.9)
$CD8^+$ ($\times 10^{-3}/\mu l$)	1.4 (0.6–1.9)	1.3 (0.6–1.9)
$CD19^+$ ($\times 10^{-3}/\mu l$)	0.7 (0.7–1.3)	1.1 (0.7–1.3)
$CD16^+$ ($\times 10^{-3}/\mu l$)	0.9 (0.4–0.9)	0.9 (0.4–0.9)
IgG (mg/dl)	<124 (430–1290)	1130 (528–1490)
IgA (mg/dl)	<6.6 (23–130)	205 (23–205)
IgM (mg/dl)	1990 (36–199)	367 (33–207)
Anti-TT IgG (IU/ml)[a]	Undetectable	4.01
Anti-HBsAg IgG (IU/ml)[a]	ND	347
Anti-Hib IgG (μg/ml)[a]	ND	9.0

Abbreviations: ALC = absolute lymphocyte count; ANC = absolute neutrophil count; HBsAg = hepatitis B surface antigen; Hib = *Haemophilus influenzae* type b; HSCT = hematopoietic stem cell transplantation; TT = tetanus toxoid.

Normal values for age-matched Turkish controls are indicated in parentheses.

[a]Protective titers to TT, HBsAg and Hib are >0.1 IU/ml, >1 μg/ml and $\geqslant$10 IU/ml, respectively.

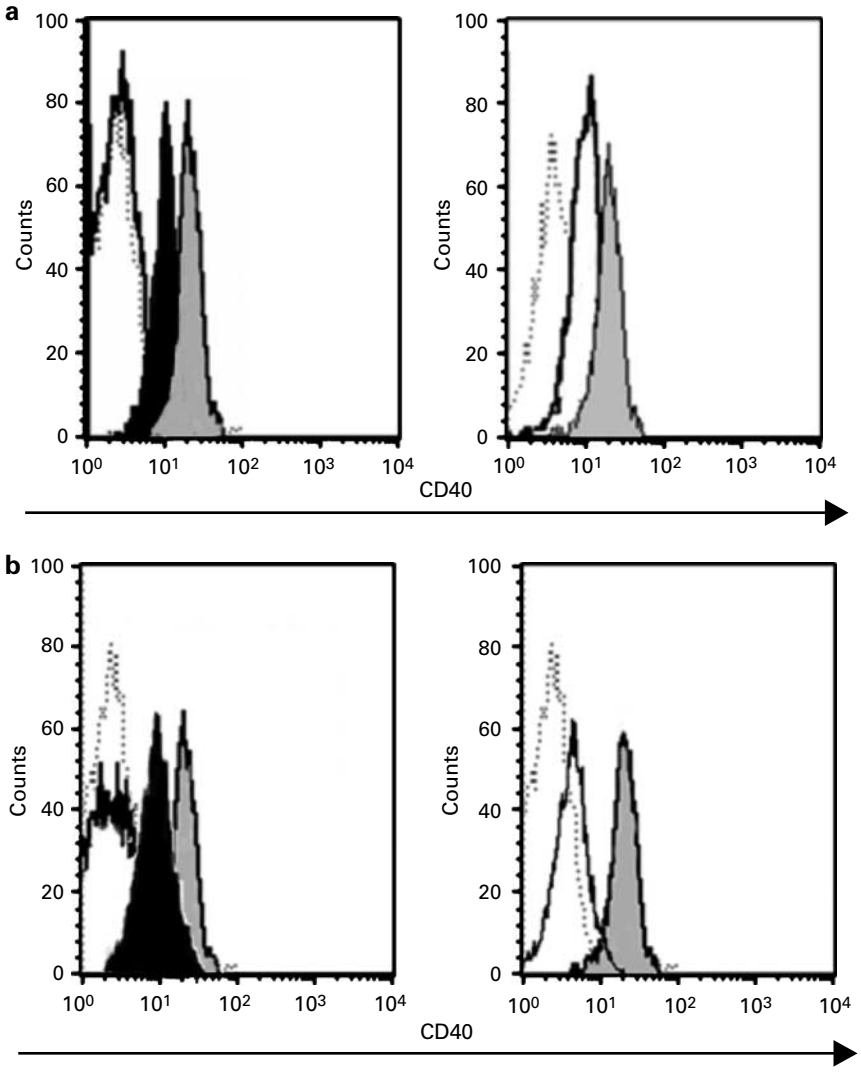

Figure 1 Reconstitution of CD40 expression after HSCT for CD40 deficiency. (**a**) Expression of CD40 on $CD19^+$ cells before (left panel) and after (right panel) HSCT in a child with CD40 deficiency. (**b**) Expression of CD40 on monocytes before (left panel) and after (right panel) HSCT in a child with CD40 deficiency. Gating on monocytes was performed using forward scatter. Filled gray histograms represents expression of CD40 in a healthy control, filled black histograms represent expression of CD40 in the heterozygous healthy donor, dashed line identifies staining with an isotype-matched control immunoglobulin, and solid line identifies expression of CD40 in the patient's cells.

pulated 2.1×10^8/kg bone marrow cells (including 9.9×10^6 $CD34^+$ cells/kg, and 47.5×10^6 $CD3^+$ cells/kg) were injected. Graft-versus-host disease (GvHD) prophylaxis was with cyclosporine A per os. No blood transfusions were required after day +13, and full engraftment was recorded at day +23. Because of CMV viremia, pre-emptive treatment with Foscarnet was administered from days +22 to +45, but was unnecessary thereafter. The clinical course was uneventful for infections and GvHD. Neutropenia has been completely reversed by HSCT. Substitution therapy with IVIG was continued for 7 months after transplant, and was then withdrawn because of consistent detection of serum IgA, and simultaneous reduction of serum IgM.

At 19 months after HSCT, the child lives at home in excellent clinical condition. No significant infections have been recorded after transplant. Stable multilineage full chimerism has been consistently observed by molecular typing with microsatellite markers. CD40 expression is now consistently detectable on the surface of the totality of B lymphocytes and monocytes, at levels that are comparable to what observed in the healthy carrier sibling donor (Figure 1). The immune function is normal. In particular, she has normal IgG and IgA serum levels, and has produced protective titers of specific IgG in response to immunizations (Table 1). No neurological or vascular problems have been observed.

This is the first report of successful HSCT in a patient with CD40 deficiency. This experience has demonstrated that even intermediate levels of CD40 expression (as detected in the healthy carrier donor and in the patient, after HSCT) are sufficient to allow for normalization of immune function. In addition, it also indicates that correction of CD40 expression in hematopoietic cells is

sufficient to prevent disease-specific complications. Although long-term follow-up will be necessary to rule out that persistent defective expression of CD40 by non-hematopoietic cells may lead to decreased resistance to opportunistic pathogens that the patient has not yet encountered (such as *Cryptosporidium* or *Toxoplasma*), such complications have not been observed so far.

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