

Hematopoietic stem cell transplantation outcomes for 11 patients with dedicator of cytokinesis 8 deficiency

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Background: Dedicator of cytokinesis 8 (DOCK8) deficiency can be cured by allogeneic hematopoietic stem cell transplantation (HSCT). Reports of outcomes are still limited.

Objective: We sought to analyze the results of HSCT in patients with DOCK8 deficiency and report whether approaches resulting in mixed chimerism result in clinically relevant immune reconstitution.

Methods: We performed a retrospective chart review of 11 patients with DOCK8 deficiency and measured DOCK8 expression and cytokine production.

Results: Of 11 patients, 7 received HSCT from related and 4 from unrelated donors; 9 patients received busulfan-based conditioning regimens. Survival was excellent (10 [91%] of 11 patients alive), including a patient who had undergone liver transplantation. Patients showed significant improvements in the frequency and severity of infections. Although eczema resolved in all, food allergies and high IgE levels persisted in some patients. Lymphopenia, eosinophilia, low numbers of naive CD8⁺ T cells and switched memory B cells, and T_H1/T_H2 cytokine imbalance improved in most patients. Although the 8 matched related or unrelated donor recipients had full donor chimerism, all 3 recipients of mismatched unrelated donor HSCT had high levels of donor T-cell chimerism and low B-cell and myeloid cell chimerism (0% to 46%). Almost all switched memory B cells were of donor origin. All patients, including those with mixed chimerism, mounted robust antibody responses to vaccination.

Conclusion: Allogeneic HSCT ameliorated the infectious and atopic symptoms of patients with DOCK8 deficiency. In patients with mixed chimerism, selective advantage for donor-derived T cells and switched memory B cells promoted restoration of cellular and humoral immunity and protection against opportunistic infection. (*J Allergy Clin Immunol* 2016;■■■■:■■■-■■■.)

Key words: Primary immunodeficiency, dedicator of cytokinesis 8 deficiency, hematopoietic stem cell transplantation, conditioning, mixed chimerism

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Dedicator of cytokinesis 8 (DOCK8) deficiency is a combined immunodeficiency that was first described as a major genetic cause of autosomal recessive hyper-IgE syndrome in 2009.^{1,2} Two recently published large retrospective cohort studies reported eczema, recurrent respiratory tract infections, persistent viral infections, food allergies, and abscesses as the major clinical features of the disease.^{3,4} Malignancies, typically hematologic or epithelial, are reported in 8% to 17% of DOCK8-deficient patients.^{3,4} Immunologic findings include T-cell lymphopenia, hypereosinophilia, increased IgE levels, low IgM levels, low antibody responses, poor memory B-cell generation, and a T_H1/T_H2 cytokine imbalance.³⁻⁵ DOCK8 deficiency carries high morbidity and mortality, with an overall survival of 50% and an event-free survival of 21% at 20 years.³ Hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment option available.

Published experience with HSCT for patients with DOCK8 deficiency is limited to case reports and small series of

Abbreviations used

AUC: Area under the curve
 DOCK8: Dedicator of cytokinesis 8
 GVHD: Graft-versus-host disease
 HSCT: Hematopoietic stem cell transplantation
 mMURD: Mismatched unrelated donor
 NK: Natural killer

DOCK8-deficient patients.⁶⁻¹⁴ Because the majority of cases reported underwent myeloablative conditioning and had full donor chimerism, whether mixed chimerism is sufficient to improve the symptoms of DOCK8 deficiency is unknown. In this retrospective study of 11 patients, we report survival, complications, lineage-specific chimerism, immune reconstitution, and resolution of clinical and laboratory features of DOCK8 deficiency after both myeloablative and reduced-intensity approaches.

METHODS**Patients and clinical characteristics of DOCK8 deficiency**

We performed a retrospective chart review of 9 patients at Boston Children's Hospital (Boston, Mass) and 2 patients at Great Ormond Street Hospital (London, United Kingdom), with approval by local ethics committees. Research testing was performed with written informed consent from the parent or guardian and assent when appropriate. Eight patients were recruited from the Kuwait National Primary Immunodeficiency Registry under the research protocol titled "Functional and genetic studies of primary immunodeficiency syndromes" and approved by the Research and Ethics Committee of the Ministry of Health in Kuwait. Clinical and laboratory features recorded before and after HSCT included eczema, recurrent sinopulmonary infections, viral infections (herpes simplex virus, varicella zoster virus, EBV, molluscum contagiosum, human papillomavirus, and cytomegalovirus), fungal infections (*Candida* species and others), abscesses, autoimmunity, asthma, food allergies, and malignancies. Mutational analysis and DOCK8 expression by means of Western blotting and flow cytometry were performed, as previously described.^{5,11,13} Mutations for 8 patients have been reported elsewhere.^{5,11,13}

HSCT

We recorded age at HSCT; treatment before transplantation; conditioning regimen, including busulfan exposure, as determined by the cumulative area under the curve (AUC; in milligrams times hour per liter); graft-versus-host disease (GVHD) prophylaxis; donor type; donor cell source; CD34⁺ cell dose; day of neutrophil engraftment; complications after HSCT; acute and/or chronic GVHD; and chimerism in T-cell, B-cell, and myeloid lineages. Chimerism in the various lineages after HSCT was determined by means of short tandem repeat analysis of magnetically separated cells.

Immune reconstitution

Lymphocyte subsets were analyzed with FACSCanto, FACSARIA, or LSR II flow cytometers (BD Biosciences, San Jose, Calif) and FlowJo software (TreeStar, Ashland, Ore). Immune reconstitution was assessed by comparing numbers of CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD16/56⁺, CD4⁺CD45RA⁺ (naïve CD4 T cells), CD8⁺CD45RA⁺ (naïve CD8 T cells), CD19⁺CD27⁺IgD⁺ (switched memory B cells), and CD19⁺CD27⁺IgD⁺ cells (unswitched memory B cells) before HSCT and at the last available follow-up after HSCT. We only included patients who had their last measurement 12 or more months after HSCT. We compared all measurements with age-dependent normal values.¹⁵⁻¹⁷

Patients received 3 doses of protein-conjugated pneumococcus and tetanus vaccine at least 4 weeks apart according to standard guidelines after

discontinuation of immunosuppression and immunoglobulin substitution. Response was determined by measuring specific antibody titers 1 or more months after the last vaccination.

Cytokine production by PBMCs

Culture supernatants of PBMCs stimulated with PHA were tested by means of ELISA for levels of IL-2, IL-4, TNF- α (Beckman Coulter, Villepinte, France) and IFN- γ (Immunotech, Marseille, France) per the manufacturer's instructions. Samples were tested in triplicate at 24 hours for IL-4 and IL-2 and at 96 hours for TNF- α and IFN- γ . Sensitivities of cytokine detection were 3 pg/mL for IFN- γ , 10 pg/mL for TNF- α , 5 pg/mL for IL-2 and 5 pg/mL for IL-4. At the time of testing, there was no clinical evidence of acute infection or allergic reaction, and none of the patients were receiving systemic immunosuppression. Testing was done for the same patients before and after HSCT.

Total and allergen-specific IgE testing

Total and allergen-specific serum IgE levels were assessed with the HYTEC Enzyme Immunoassay (HYCOR Europe Bio Crest B.V., Lohfelden, Germany), according to standard procedures. IgE measurements were compared with age-dependent normal values.

Statistical analysis

Paired *t* tests to compare pre- and post-HSCT cell counts were performed with GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, Calif). A *P* value of .05 or less was considered significant.

RESULTS**Patients' characteristics**

Of 11 patients, 10 had complete absence of DOCK8 protein expression, as determined by using Western blotting, flow cytometry, or both (Table I and see Fig E1 in this article's Online Repository at www.jacionline.org for patients 4 and 7).¹³ All patients were born of consanguineous parents and presented with characteristic manifestations of DOCK8 deficiency, including eczema (11/11), viral infections (11/11), food allergies (10/11), recurrent sinopulmonary infections (9/11), and skin abscesses (6/11). None of the patients had symptomatic autoimmunity.

Patient 4 had end-stage liver disease (cirrhosis, portal hypertension, and ascites) secondary to chronic *Cryptosporidium* species-induced sclerosing cholangitis. She received a living related left lateral segment liver transplantation from her mother, who was 4/6 HLA matched in the GVHD direction, 5/6 matched in the rejection direction, and ABO blood group compatible. Her liver graft function was excellent. Transplant recovery was complicated by a bile leak that became infected with *Candida tropicalis*, requiring percutaneous drainage on postoperative day 30 and antifungal therapy. She began conditioning for HSCT on postoperative day 65 with normal liver graft function. The transplanted liver was functioning well without signs of rejection 3.9 years after transplantation.

HSCT procedure, survival, and transplant-related complications

The median age at transplantation was 6.2 years (range, 2.1-11.0 years). Donors included matched sibling donors (*n* = 7, Table II), fully matched unrelated donors (*n* = 1), or mismatched unrelated donors (mMURDs; *n* = 3; Table II). Two of the mMURD recipients received peripheral blood stem cells, and the remaining 9 patients received bone marrow. Most patients (9/11) received busulfan-based regimens combined either with

TABLE I. Patients' characteristics before HSCT

Clinical manifestations of DOCK8													IVIG Pre- HSCT
Patient no.	Sex	Mutation*	DOCK8 protein expression†	Eczema	Food allergies	Infections					Other		
						Recurrent sinopulmonary infections	Skin abscesses	Viral					
								Cutaneous	Systemic	Fungal			
1	M	+1 IVS44 G>A	Reduced	+	+	+	—	—	EBV	—		—	
2	F	Del ex 29-36	—	+	+	+	—	HSV HPV	—	—	Asthma	+	
3	F	Del ex 1-2	—	+	—	+	+	MC	EBV	+	Asthma, poor dentition	—	
4	F	Del ex 1-23	—	+	+	+	+	HSV HPV	CMV		Asthma, liver failure, cryptosporidium induced sclerosing cholangitis	+	
5‡	F	Del ex 1-5	—	+	+	+	+	HSV	—	—	Poor dentition	+	
6‡	F	Del ex 1-5	—	+	+	+	+	HSV	CMV	+	Asthma, poor dentition, severe bronchiectasis s/p lobar resections	+	
7	M	Del ex 10-12	—	+	+	—	—	HSV	VZV EBV CMV	—	EBV-driven leiomyosarcoma, MRSA pericarditis, chronic cryptosporidium and Giardia	—	
8‡	M	Del ex 1-5	—	+	+	—	+	MC	—	—		+	
9‡	F	Del ex 1-5	—	+	+	+	+	HSV	—	+	Asthma, poor dentition	+	
10‡	M	Del ex 1-5	—	+	+	—	—	MC	—	—		+	
11‡	F	Del ex 1-5	—	+	+	+	—	HSV MC	—	—	Poor dentition	+	

CMV, Cytomegalovirus; Del, deletion; EBV, Epstein-Barr virus; F, female; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin; M, male; MC, molluscum contagiosum; MRSA, methicillin-resistant *Staphylococcus aureus*; VZV, varicella zoster virus.

*All mutations are homozygous.

†DOCK8 protein expression determined by means of either Western blotting or flow cytometry.

‡Related patients (patients 9 and 11 are sisters, and patients 5, 6, 8, 9, 10, and 11 are cousins).

cyclophosphamide or fludarabine. Median busulfan cumulative AUC among all patients was 75.7 mg*h/L (range, 50.1–90.5 mg*h/L). Patients 9 and 11 also received antithymocyte globulin. Notably, patient 4 had normal busulfan excretion after liver transplantation. The remaining 2 patients received a reduced-intensity conditioning regimen, fludarabine, melphalan, and alemtuzumab.

All patients recovered from pancytopenia. Chimerism in the T-cell, B-cell, and myeloid cell lineages was full in 8 patients and mixed in 3 patients when measured 0.5 to 3.2 years after HSCT (Table II). After a median follow-up of 3.2 years (range, 7 months–5.6 years), survival was 10 (91%) of 11; 1 patient died 58 days after HSCT of bacterial sepsis.¹¹ HSCT was generally well tolerated without veno-occlusive disease or acute or chronic GVHD. Complications after HSCT occurred in 7 of 11 patients and were mostly infectious.

Infections and atopy improve or resolve after HSCT

All patients with chronic or difficult-to-treat viral infections cleared these infections after HSCT. Despite full chimerism in all cell lineages, patients 3, 4, and 6 continued to have bacterial infections, including pneumonia, otitis media treated with

tympanostomy tubes, and skin boils, although at a much reduced frequency compared with before HSCT. Patients 4 and 6 have significant residual bronchiectasis.

All patients with eczema had resolution after HSCT. Increased eosinophil counts consistently decreased after HSCT in all patients (see Table E1 in this article's Online Repository at www.jacionline.org). In contrast, total serum IgE levels normalized in 5 patients, but remained increased in 3 patients (patients 4, 5, and 6), despite full donor chimerism (Fig 1, A).

Ten (91%) patients presented with food allergy, which was confirmed by increased food allergen-specific IgE levels. Clinically, food allergies resolved after HSCT in the vast majority of patients (8/10), as confirmed by oral challenges. Patient 4 had an anaphylactic reaction to milk at 3.9 months after HSCT and has not been rechallenged. Patient 6 had an anaphylactic reaction to the *Haemophilus influenzae* type b vaccine but tolerates all foods. Furthermore, allergen-specific IgE levels decreased in all patients tested (data not shown).

Immune reconstitution after HSCT

Of 10 patients who are alive after HSCT, 9 have stopped immunosuppression at a median duration of 7.1 months (range,

TABLE II. HSCT procedure and outcome

Patient no.	Age at HSCT (y)	Before treatment	Conditioning regimen	Bu AUC (mg*h/L)	GVHD prophylaxis	Donor type	Donor cell source	Cell dose ($\times 10^6$ CD34 ⁺ cells/kg)	Day of neutrophil engraftment
1	3.2	Rituximab for EBV viremia	Bu/Cy	67.2	CSA/MTX	MSD	BM	16.57	31
2	8.9		Bu/Cy	67.1	CSA/MTX	MSD	BM	9.39	18
3	10.2	Rituximab for EBV viremia	Bu/Flu	77.3	CSA/MTX	MSD	BM	11.75	27
4	11.0	Living-related liver transplant for liver failure/chronic sclerosing cholangitis	Bu/Flu	90.5	Tacrolimus/MTX	MSD	BM	13.49	28
5	5.8		Bu/Flu	70.8	CSA/MTX	MSD	BM	10.38	20
6	7.0	Lung resections $\times 2$	Bu/Flu	87.8	CSA/MTX	MSD	BM	12.75	27
7	8.0	Rituximab for EBV viremia; resection of leiomyosarcoma	Bu/Flu/rATG	50.1	CSA/MTX	MSD	BM	6.26	29
8	6.2		Alemtuzumab/Flu/melphalan	NA	CSA/MMF	mMURD (8/10)	PB	10	NA
9	3.4		Bu/Flu/eATG	85.6	CSA/MTX/prednisone	MURD	BM	3.65	21
10	2.3		Alemtuzumab/Flu/melphalan	NA	CSA/MMF	mMURD (9/10)	PB	23.72	15
11	2.1		Bu/Flu/eATG	80.6	CSA/MTX/prednisone	mMURD (9/10)	BM	2.26	34

ATG, Anti-thymocyte globulin (equine [e] or rabbit [r]); A&W, alive and well; BM, bone marrow; Bu, busulfan; CMV, cytomegalovirus; CNS, central nervous system; CSA, cyclosporin A; Cy, cyclophosphamide; Flu, fludarabine; HSV, herpes simplex virus; MMF, mycophenolate mofetil; mMURD, mismatched unrelated donor; MSD, matched sibling donor; MTX, methotrexate; MURD, matched unrelated donor; NA, not available; PB, peripheral blood.

5.5-15.5 months) after HSCT, and all are off of immunoglobulin substitution. Patient 4 continues on tacrolimus to prevent rejection of the transplanted liver. We analyzed CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD16/56⁺, CD4/CD8 ratio, CD4⁺CD45RA⁺ (naive CD4 T cells), CD8⁺CD45RA⁺ (naive CD8 T cells), CD19⁺CD27⁺IgD⁻ (switched memory B cells), and CD19⁺CD27⁺IgD⁺ (unswitched memory B cells) cell counts and percentages in patients who had both a pre-HSCT and a post-HSCT measurement 12 or more months after HSCT (see Table E2 in this article's Online Repository at www.jacionline.org).

Before HSCT, CD3⁺, CD4⁺, and CD8⁺ cell counts were abnormally low for age in 6 of 9, 6 of 9, and 5 of 9 patients, respectively; all counts normalized after HSCT, except the CD4⁺ T-cell count in patient 4, which remained low for age. The CD4/CD8 ratio was abnormally low in 6 of 9 patients before HSCT. Of these, 3 continued to have an abnormally low ratio after HSCT (patients 3, 4, and 6). Percentages of naive CD8 T cells ($P = .002$) and switched ($P = .007$) and unswitched ($P = .02$) memory B cells all significantly increased after HSCT and normalized in the majority of patients (Fig 1, B-D).

TABLE II. (Continued)

Complications	GVHD	Chimerism at last measurement (y after HSCT)	Status (y after HSCT)
	—	T cell: >97% B cell: >97% Myeloid: >95% (3.2)	A&W (4.6)
<i>Klebsiella</i> species sepsis	—	T cell: 100% B cell: 97% Myeloid: 100% (0.1)	Patient deceased at day +58
CMV reactivation; persistent and worsening CNS white matter changes (leukoencephalopathy) and neurologic symptoms responsive to corticosteroids; idiopathic pneumonia syndrome (day +91) treated with etanercept	—	T cell: >97% B cell: >97% Myeloid: >97% (2.5)	A&W (4.2) Otitis treated with tympanostomy tubes
Neurologic symptoms of unclear cause, pericardial effusion, presumed viral meningoencephalitis responsive to foscarnet	—	T cell: >97% B cell: >97% Myeloid: >97% (2.7)	A&W (3.7). Pneumonias, asymptomatic EBV viremia
	—	T cell: 96% B cell: >97% Myeloid: >97% (0.6)	A&W (2.0)
Disseminated HSV with Bell palsy	—	T cell: >97% B cell: >97% Myeloid: >97% (2.3)	A&W (2.5). Pneumonias, otitis treated with tympanostomy tubes, abscesses
CMV pneumonitis, EBV viremia, transient pseudotumor cerebri	No acute	T cell: 100% B cell: 100% Myeloid: 100% (0.5)	A&W (0.6)
	—	T cell: 89% B cell: 11% Myeloid: 0% (2.0)	A&W (4.4)
CMV reactivation	—	T cell: 100% B cell: 100% Myeloid: 100% (3.1)	A&W (3.5)
EBV viremia	—	T cell: 82% B cell: 0% Myeloid: 0% (1.8)	A&W (2.8)
HSV viremia, eczema herpeticum, <i>Enterobacter</i> species bacteremia	—	T cell: 97% B cell: 46% Myeloid: 7% (2.5)	A&W (2.8)

We analyzed antibody response to vaccines in the patients who had prevaccination and postvaccination titers available (patients 1, 3, 4, 5, 8, and 11). All 6 patients mounted protective titers to pneumococcus to at least 7 of 14 serotypes and to tetanus, including 2 who had mixed B-cell chimerism (patients 8 and 11). The third patient with mixed chimerism, patient 10, did not have a pre-HSCT vaccine pneumococcal titer available but had protective titers to both pneumococcus and tetanus after vaccination.

Selective advantage for T-cell lineage and switched memory B cells in patients with mixed chimerism

All 3 patients (patients 8, 10, and 11) who had mixed chimerism demonstrated by means of short tandem repeat analysis, flow cytometry, or both (Fig 2 and Table II) received mMURD transplants, and 2 of 3 patients received reduced-intensity conditioning regimens. Patients with mixed chimerism had 82% to 97% donor cells in the T-cell lineage, whereas chimerism in the B-cell and myeloid lineages was 0% to 46%

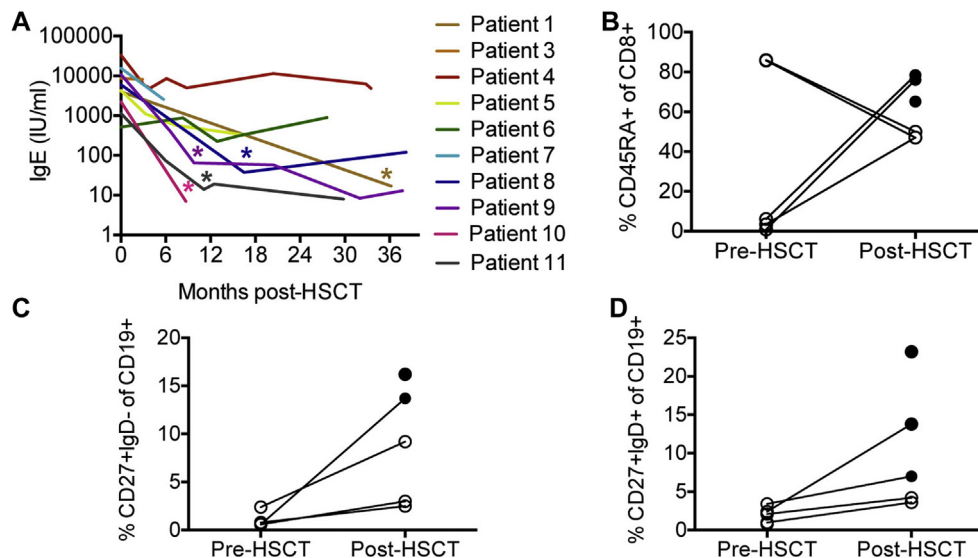


FIG 1. Immune reconstitution after HSCT for DOCK8 deficiency. **A**, Persistence of IgE level increase after HSCT. Asterisks mark the first measurement with an IgE level within the normal range for age. **B**, Naive CD8 cells. **C**, Switched memory B cells. **D**, Unswitched memory B cells. Levels shown are before HSCT and at the last follow-up (≥ 12 months) after HSCT. Open circles indicate that the measured value is abnormal for age, and solid circles indicate that the value is normal.

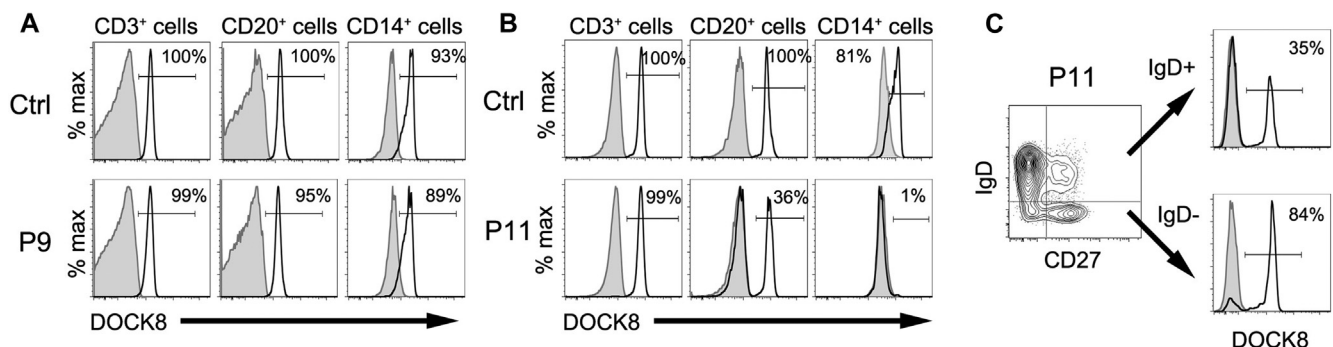


FIG 2. DOCK8 expression determined by mean of flow cytometry after HSCT. **A**, DOCK8 expression in cells from a healthy control and patient P9 after HSCT. **B**, DOCK8 expression in cells from a healthy control and patient P11 after HSCT. **C**, DOCK8 expression in CD27⁺IgD⁻ and CD27⁺IgD⁺ cells from patient P11 after HSCT. Shaded histograms indicate isotype control. Numbers represent the percentage of cells that expressed DOCK8.

and 0% to 7%, respectively, at 1.8 to 2.5 years after HSCT. Patient 11 had high-level donor chimerism in the CD3⁺ T cells, mixed chimerism in the CD20⁺ B lineage, and negligible chimerism in CD14⁺ monocytes, as indicated by flow cytometry for DOCK8 expression, which was in contrast to patient 9 who had high-level donor chimerism in all lineages (Fig 2, A and B). We further found that switched memory CD27⁺IgD⁻ cells of patient 11 had a higher percentage of cells expressing DOCK8 compared with CD27⁺IgD⁺ cells (84% vs 35%; Fig 2, C), suggesting selective advantage for switched memory B cells.

Shift toward T_H1 cytokines after HSCT

Before HSCT, PBMCs of 5 patients tested secreted significantly lower amounts of IL-2, TNF- α , and IFN- γ than those from healthy control subjects in response to stimulation with PHA. Repeat testing after HSCT showed a significant

increase in levels of the T_H1 cytokines IFN- γ and TNF- α ($P = .001$ and $.018$, respectively) and a trend toward a decrease in levels of the T_H2 cytokine IL-4 ($P = .085$, Fig 3). Cytokine production by PBMCs of patients with DOCK8 deficiency who did not undergo transplantation show a T_H2 bias.⁵ Therefore we compared ratios of mean secreted IL-2, TNF- α , and IFN- γ with those of IL-4 in patients undergoing transplantation. Compared with the T_H2 bias before HSCT, the post-HSCT ratios showed a shift toward T_H1 cytokines (Table III).

DISCUSSION

DOCK8 deficiency has high mortality, and HSCT should be considered as early as possible before development of significant organ damage or virus-driven malignancies. In our cohort survival was very good (91% [9/11]), which we attribute to their young age, early identification in several cases based on family

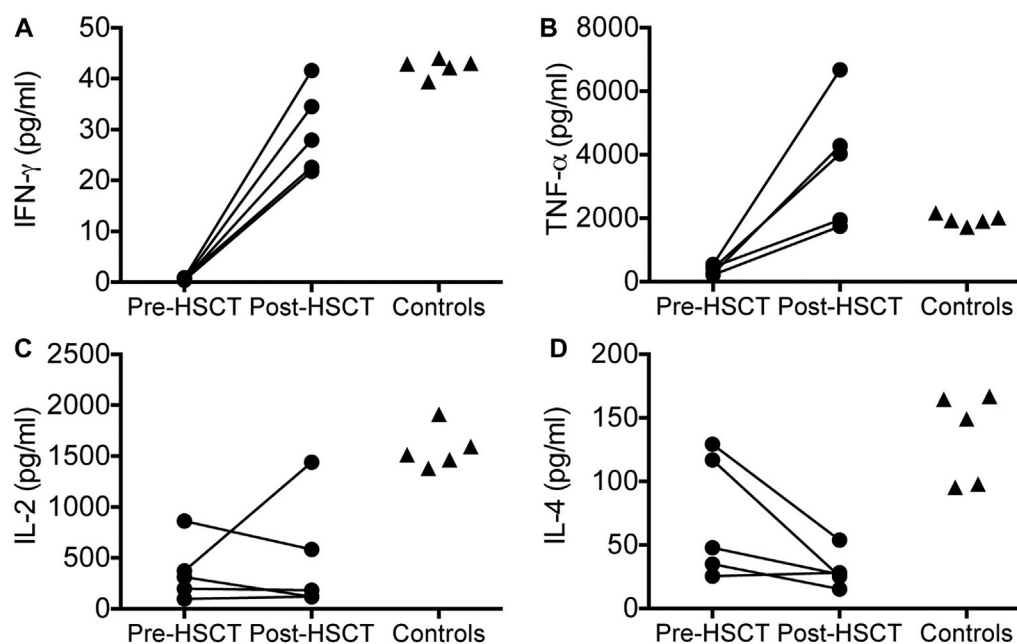


FIG 3. Cytokine production in response to PHA before and after HSCT in 5 patients with DOCK8 deficiency and healthy adult control subjects. **A**, IFN- γ . **B**, TNF- α . **C**, IL-2. **D**, IL-4.

TABLE III. T_H1/T_H2 ratios before and after HSCT in DOCK8-deficient patients

	Before HSCT	After HSCT	Control
IL-2/IL-4	5.20	16.37	11.67
IFN- γ /IL-4	5.34	125	14.44
TNF- α /IL-4	0.009	0.99	0.31

history, and aggressive pre-HSCT management, including liver transplantation in 1 case. We recommend that even patients with significant comorbidities should undergo HSCT as long as these conditions can be aggressively treated before HSCT.

Many patients with DOCK8 deficiency have organ damage necessitating reduced-intensity conditioning approaches, which in turn carry a higher risk of mixed donor chimerism. Indeed, both patients receiving fludarabine, melphalan, and alemtuzumab conditioning had mixed chimerism, whereas patient 11 had mixed chimerism despite receiving a myeloablative dose of busulfan (AUC, >80 mg*h/L). Because all 3 patients with mixed chimerism received a mMURD graft, HLA mismatch might also have played a role. Nevertheless, mixed chimerism was sufficient to improve immunity because of the strong selective advantage for donor-derived T cells and switched memory B cells that express DOCK8. All patients with mixed chimerism had higher donor chimerism in the T than in the B or myeloid lineages, similar to what has been shown in mouse models,^{18,19} thus protecting the patients against life-threatening viral and opportunistic infections. Effective humoral immunity after vaccination in our patients with mixed chimerism is likely mediated by donor-derived switched memory B cells, which also have strong selective advantage, as seen in patient 11 (Fig 2) and in mouse models.²⁰ Based on the finding that DOCK8-deficient patients might have natural killer (NK) cell revertants, we suspect that donor-derived NK cells might exhibit

selective advantage²¹ but did not examine NK chimerism in our cohort. Long-term follow-up is needed to determine whether immunologic reconstitution in these patients will be sustained. Whether the lack of donor-derived myeloid cells has clinical consequences remains unclear.

DOCK8-deficient patients have been shown to have an imbalance of T_H1/T_H2 cytokine production, which might impair their ability to combat viruses because of deficiency of IFN- γ production.⁵ *Ex vivo* cytokine levels measured before and after transplantation in a portion of our cohort showed improved T_H1 cytokine production. These findings might explain the improvement in the severity and frequency of viral infections after HSCT in our cohort. We also saw a trend toward a decrease in IL-4 production. Consistent with the improvement in T_H1/T_H2 balance we observed and with other reports of clinical outcomes,¹⁴ our patients showed resolution of eczema, and most showed a decrease or normalization of eosinophil counts, total IgE levels, and allergen-specific IgE levels after HSCT. Interestingly, 3 patients with full donor chimerism continue to have significantly increased serum IgE levels, presumably generated from recipient-derived long-lived plasma cells. Why some patients continue to produce high levels of IgE after HSCT remains unknown. In the case of patient 4, the known predilection to food allergies in liver transplant recipients might play a role.²² We speculate that ongoing infections related to pre-existing bronchiectasis might cause persistent immune activation and dysregulation, as manifested by the reversed CD4/CD8 ratio, and ongoing IgE production.

In summary, patients with DOCK8 deficiency in our cohort had excellent survival rates and immune reconstitution, despite differences in transplant technique and post-HSCT donor chimerism. For patients with a suitable donor, HSCT is the recommended curative option as long as there is aggressive management of infectious sequelae and end-organ damage before HSCT. The strong selective advantage we demonstrated in T cells

and switched memory B cells implies that correction of a portion of hematopoietic stem cells, such as that achieved with gene therapy, might be sufficient to correct symptoms.

Clinical implications: Early allogeneic transplantation should be pursued in patients with DOCK8 deficiency. Approaches resulting in mixed chimerism nevertheless led to clinically significant immunologic improvement. Food allergies can persist.

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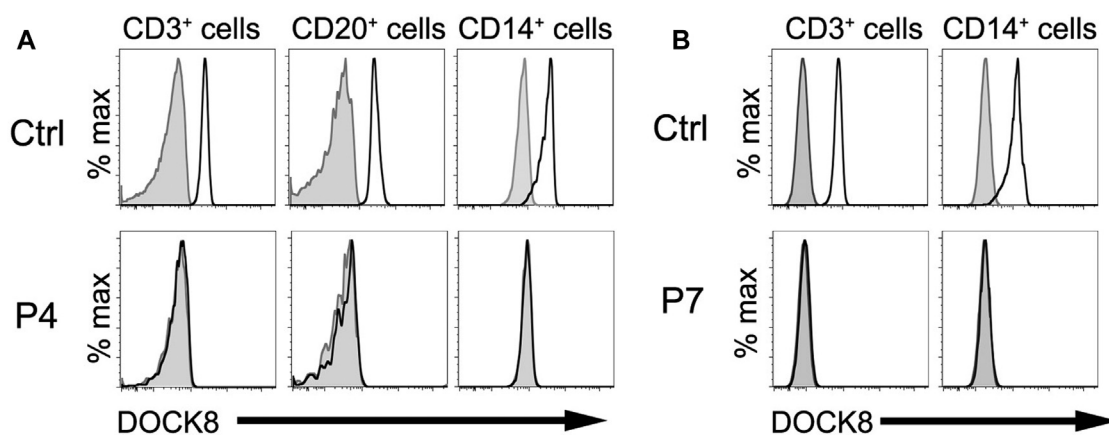


FIG E1. DOCK8 expression determined by using flow cytometry in peripheral blood cells. **A**, Control subject and patient P4 before HSCT. **B**, Control subject and patients P7 (after rituximab) before HSCT. *Shaded histograms indicate isotype control.*

TABLE E1. Eosinophils (cells per microliter) before and after HSCT for DOCK8 deficiency

Patient	Before HSCT	After HSCT (months after HSCT)
1	840	10 (36.2)
3	2080	140 (28.4)
4	2100	720 (33.5)
6	19540	450 (27.2)
8	21940	440 (36.0)
9	2790	80 (37.7)
10	2700	170 (22.2)
11	430	140 (29.7)

TABLE E2. Lymphocyte subsets before and after HSCT for DOCK8 deficiency

Subset	Patient	Pre-HSCT	Post-HSCT (months post-HSCT)
CD3 ⁺ (cells/ μ L)	1	485	1566 (36.2)
	3	685	2253 (28.4)
	4	764	1862 (33.5)
	5	784	1819 (13.4)
	6	980	2165 (27.2)
	8	2760	1570 (23.4)
	9	1350	2459 (37.7)
	10	2550	3110 (22.0)
	11	2015	2182 (29.7)
	1	295	870 (36.2)
	3	205	1065 (28.4)
CD4 ⁺ (cells/ μ L)	4	247	489 (33.5)
	5	591	1300 (13.4)
	6	433	923 (27.2)
	8	1010	890 (23.4)
	9	1045	1310 (37.7)
	10	2240	1510 (22.0)
	11	889	988 (29.7)
	1	186	648 (36.2)
	3	405	1072 (28.4)
	4	397	1025 (33.5)
	5	159	451 (13.4)
CD8 ⁺ (cells/ μ L)	6	449	1022 (27.2)
	8	1650	570 (23.4)
	9	295	1049 (37.7)
	10	310	1360 (22.0)
	11	1061	1098 (29.7)
	1	1.59	1.34 (36.2)
	3	0.51	0.99 (28.4)
	4	0.62	0.48 (33.5)
	5	3.72	2.88 (13.4)
	6	0.96	0.89 (27.2)
	8	0.61	1.56 (23.4)
CD4/CD8	9	3.54	1.25 (37.7)
	10	7.23	1.11 (22.0)
	11	0.84	0.90 (29.7)
	1	1426	385 (36.2)
	3	272	1209 (28.4)
	4	127	640 (33.5)
	5	495	758 (13.4)
	6	208	944 (27.2)
	8	1750	660 (23.4)
	9	518	686 (37.7)
	10	2040	1166 (22.2)
	11	2306	317 (29.7)
CD16/56 ⁺ (cells/ μ L)	1	161	135 (36.2)
	3	37	132 (28.4)
	4	130	350 (33.5)
	5	126	79 (13.4)
	6	349	168 (27.2)
	8	580	170 (23.4)
	9	214	187 (37.7)
	10	100	535 (22.2)
	11	151	80 (29.7)
	1	48.8	56.5 (36.2)
	4	0.2	45.5 (33.5)
CD4 ⁺ CD45RA ⁺ (%)	5	73.1	55 (13.4)
	6	56.3	44.4 (27.2)
	8	59	51.3 (23.4)
	10	78.2	36 (22.0)

(Continued)

TABLE E2. (Continued)

Subset	Patient	Pre-HSCT	Post-HSCT (months post-HSCT)
CD8 ⁺ CD45RA ⁺ (%)	11	56.3	56.1 (29.7)
	1	6.1	78.4 (36.2)
	4	0.9	76.1 (33.5)
	6	6.8	80.2 (27.2)
	8	86	50 (23.4)
	10	85.8	47 (22.0)
CD19 ⁺ CD27 ⁺ IgD ⁻ (%)	11	3.5	47.1 (29.7)
	1	0.6	3 (36.2)
	3	0.8	2.5 (28.4)
	6	2.4	9.2 (27.2)
	11	0.6	13.7 (29.7)
	1	2.1	4.2 (36.2)
CD19 ⁺ CD27 ⁺ IgD ⁺ (%)	3	1	3.6 (28.4)
	6	3.4	7 (27.2)
	11	2.4	13.8 (29.7)

Values in boldface are abnormally low for age.