

Letter to the Editor

Plasmacytoid dendritic cell depletion in DOCK8 deficiency: Rescue of severe herpetic infections with IFN- α 2b therapy*To the Editor:*

Dedicator of cytokinesis 8 (DOCK8) deficiency is a primary combined immune deficiency characterized by profound T-cell lymphopenia and impaired humoral antibody responses, increased serum IgE levels, and eosinophilia.^{1,2} DOCK8 encodes a guanine nucleotide exchange factor that coordinates the actin cytoskeleton response to mitogenic and chemokine signals through the reversible activation of small G proteins, including cell division cycle 42.³ Subjects with DOCK8 deficiency are particularly susceptible to viral infections, especially with herpes simplex virus (HSV), varicella-zoster virus, human papilloma-virus, molluscum contagiosum virus, and JC viruses. Overall, these infections represent a major cause of morbidity and at times mortality in DOCK8 deficiency. We here describe 2 patients with DOCK8 deficiency and severe, progressive oral herpes labialis infection refractory to therapy with acyclovir and its analogue valacyclovir who were successfully treated with systemic IFN- α 2b (IFN- α 2b). Importantly, analysis revealed profound deficiency of plasmacytoid dendritic cells (pDCs), the major immune cell population involved in IFN- α production, in DOCK8-deficient subjects.

The demographic and genetic findings of the 2 patients are detailed in Table E1 and the immunologic findings in Table E2 in the Online Repository at www.jacionline.org. The first patient is a Turkish girl with a homozygous splice acceptor mutation in intron 16 of DOCK8 (c.1869-1 G>C) who first presented at age 5 years

with herpes labialis, confirmed by a positive Tzanck smear at age 5 years. Her infection initially resolved with oral acyclovir treatment but later recurred, leading to her placement on oral acyclovir prophylaxis. At the age of 6½ years, and despite prophylaxis, breakthrough disease recurred and progressively worsened with tissue destruction despite high acyclovir and later valacyclovir therapy (1500 mg/m² and 150 mg/kg/d, respectively) (Fig 1, A and B). Pegilated IFN- α 2b was applied topically to little effect. Subcutaneous pegilated IFN- α 2b was then started on a weekly dose of 40 μ g/m², and the dose was increased stepwise over 4 months to 80 μ g/m²/wk. Clinical response was noted after reaching a dose of 60 μ g/m²/wk during the second month of therapy, and complete remission was achieved at 6 months of therapy (Fig 1, C). The patient continues to be maintained on IFN- α 2b at 80 μ g/m²/wk with no recurrence of disease and no complications of therapy.

The second case is a Turkish girl with a homozygous deletion in DOCK8, encompassing exon 26 through the 3' end of the gene, who was hospitalized at age 6 years with a 4-week history of purulent otitis media resistant to oral antibiotics, a left ear ulceration, and oral vesicular lesions that were positive on Tzanck smears, consistent with herpes labialis. She was hospitalized with intravenous antibiotics and acyclovir. Intravenous immunoglobulin prophylaxis was restarted (0.5 g/kg every 3 weeks). Oral herpes labialis persisted with increasing severity and tissue destruction despite acyclovir and valacyclovir therapy (Fig 1, D and E). Subcutaneous pegilated IFN- α 2b was started at 40 μ g/m² and escalated up to 80 μ g/m² followed by weekly maintenance therapy, as detailed for the first patient, with complete and sustained remission of her lesions (Fig 1, F).

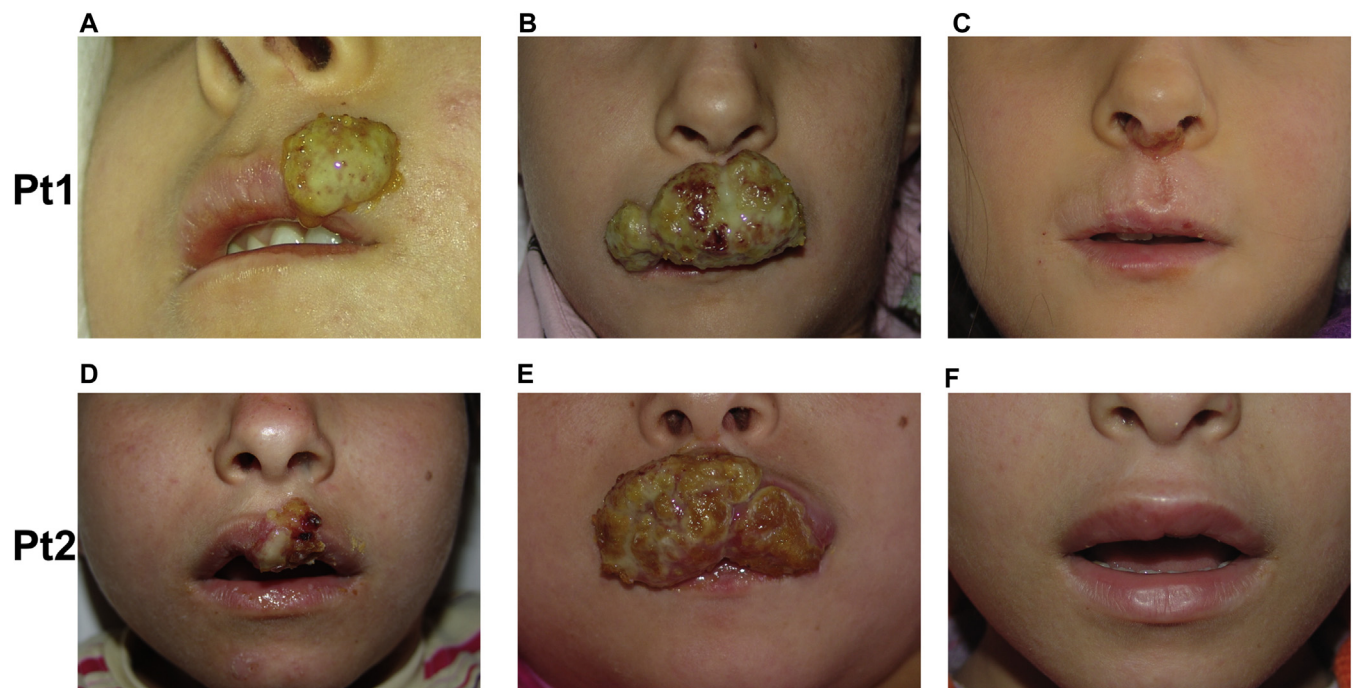


FIG 1. Representative pictures of the lesions in patients (Pt) 1 and 2 early in their infection (A and D), just before IFN- α 2b therapy (B and E) and 6 months into IFN- α 2b therapy (C and F), respectively.

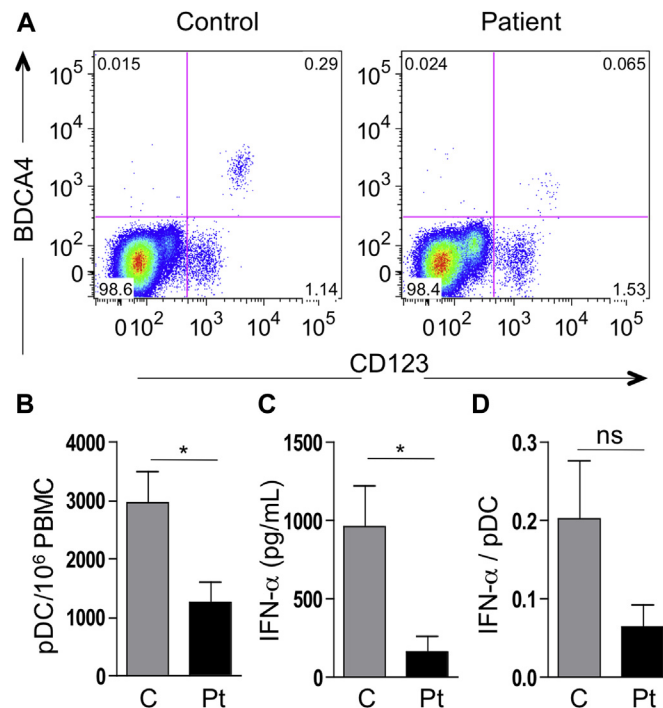


FIG 2. **A**, Representative FACS analysis for BDCA-4⁺ CD123⁺ pDCs in the lymphocyte gate of PBMCs from a control and a DOCK8-deficient subject. **B**, Total number of pDCs/10⁶ cells in controls (C, n = 10) and patients (Pt, n = 9). **C**, IFN-α production in supernatants of CpG-stimulated PBMCs from controls (n = 9) and patients (n = 8). **D**, Production of IFN-α/pDC in controls and patients (n = 7 each). **P* < .05. FACS, Fluorescence-activated cell sorting; ns, nonsignificant.

pDCs play a key role in the innate immune response to viral infections, including HSV, by virtue of their capacity to produce copious amounts of type I IFN on activation.⁴ pDCs sense virus nucleoside-based products derived from DNA viruses including herpes through Toll-like receptor 9 (TLR9) and the TLR adaptor MyD88, a pathway that is profoundly impaired in DOCK8 deficiency, leading us to examine the capacity of patient pDCs to produce IFN-α in response to unmethylated CpG dinucleotides.⁵ To that end, we first analyzed the population of pDCs present in the peripheral blood of a group of DOCK8-deficient as compared with control subjects. Details of the clinical and genetic findings of the DOCK8-deficient subjects we tested are provided in Table E1. Flow cytometric analysis of pDCs, identified by the markers CD123 and BDCA-4, revealed that their severe deficiency in the peripheral blood is a common attribute of DOCK8-deficient subjects (Fig 2, A and B). We further examined the capacity of PBMCs to produce IFN-α *in vitro* in response to CpG-A DNA (ODN 2216) treatment, as measured by ELISA. IFN-α production was profoundly depressed in patients than in control subjects, consistent with the markedly decreased number of pDCs in the former group (Fig 2, C). There was also decreased IFN-α production on per-cell basis down to one third of control values that did not achieve significance (*P* = .1), suggesting that this poor responsiveness to viral DNA may potentially compound pDC deficiency as a cause of decreased IFN-α production in this disease (Fig 2, D).

The mechanisms by which DOCK8 deficiency compromises the pDC compartment are presently unclear, and may include aberrant development, peripheral sequestration, and/or poor survival. DOCK8 regulates interstitial DC migration in response

to chemotactic factors, suggesting that impaired mobilization of pDCs may also be a contributing mechanism.³

IFN-α 2b therapy may act by inhibiting viral replication and also by activating effector lymphocytes, including natural killer (NK) and cytotoxic T cells.⁴ Its utility in DOCK8 deficiency may extend beyond HSV infections to include other viral pathogens. IFN-α has previously been used in a child with Heck's disease, associated with chronic oral and vulvar human papillomavirus infection, later documented to have DOCK8 deficiency due to deletion in exon 38.^{2,6} IFN-α 2b therapy markedly improved the papillomatosis.

Besides circulating pDC deficiency, other mechanisms have been invoked to explain the unusual susceptibility to viral infections in DOCK8-deficient subjects. These include poor CD8⁺ cytotoxic T-cell memory responses, deficiency of NK T cells, impaired NK cell function, and defective DOCK8 and MyD88-dependent TLR signaling.^{5,7-9} These defects may synergistically interact to precipitate severe, persistent viral infections in DOCK8-deficient patients.

In summary, deficiency of circulating pDCs and its attendant decreased IFN-α production are previously undescribed attributes of DOCK8 deficiency that likely contribute to the heightened susceptibility to severe viral infections, especially those affecting the mucocutaneous compartment. Systemic IFN-α therapy may be beneficial in those DOCK8-deficient subjects who prove unresponsive to conventional antiviral chemotherapies.

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METHODS

Flow cytometry

For the detection of pDCs, PBMCs were stained with anti-human CD123 (AC145) and BDCA-4 (AD5-17F6) mAbs (Miltenyi Biotec, San Diego, Calif) with the appropriate isotype controls as described before.^{E1} Standard flow cytometric methods were used for staining. Data collected with a LSRFortessa cell analyzer (BD Biosciences, San Diego, Calif) were analyzed in the lymphocyte gate with FlowJo software (TreeStar, Ashland, Ore).

IFN- α production

Human PBMCs were isolated from heparinized blood obtained from patients and normal donors using Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ). Cells were suspended in RPMI-1640 containing 10%

heat-inactivated FCS (Hyclone), 2 mM L-glutamine, 50 mg/mL streptomycin, and 100 U/mL penicillin. PBMCs (2×10^6 cells/mL) were either unstimulated or stimulated with class A CpG ODN 2216 (0.5 mM, Invivogen, San Diego, Calif). IFN- α in culture supernatants was measured after 24 hours by ELISA (Mabtech, Cincinnati, Ohio) according to the manufacturer instructions.

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TABLE E1. Clinical and genotypic features of DOCK8-deficient subjects

Patient	Clinical findings			Genotyping (reference sequence NM_203447.3)	Reference
	Age (y)	Sex	Viral infections		
1*	5	F	HSV	Homozygous IVS16splice acceptor site mutation: c.[1869 –1 G>C]; [1869 –1 G>C]	This report
2*	4	F	HSV	Homozygous deletion encompassing exons 26-48: c.[3121 –? _7340+?del]; [3121 –? _7340+?del]	This report
3	1	F		Homozygous deletion encompassing exons 1-23: c.[1 –? _2874+? del]; [1 –? _2874+?del]	This report
4	9	F	HPV	Homozygous deletion encompassing exons 26-48: c.[3121 –? _7340+?del]; [3121 –? _7340+?del]	This report
5	4	M	MCV	Homozygous deletion encompassing exon 27: c.[3235 –? _3390+? del]; [3235 –? _3390 +?del]	Jabara et al ⁵
6	6	M	MCV	Homozygous deletion encompassing exons 1 and 2: c.[1 –? _156 +?del]; [1 –? _156+?del]	Jabara et al ⁵
7	8	F	HSV, HPV, MCV	Homozygous deletion encompassing exons 28-35: c.[3391 –? _4473+?del]; [3391 –? _4473+?del]	Jabara et al ⁵
8	10	F	HSV, VZV	Homozygous nonsense mutation: c.[2248G>T]; [2248G>T]	Engelhardt et al ²
9	7	M		Homozygous IVS44 splice donor site mutation: c.[5817 +1 G>A]; [5817 +1 G>A]	Janssen et al ^{1,2}
10†	8	M	HSV	Homozygous IVS44 splice donor site mutation: c.[5817+1 G>A]; [5817 +1 G>A]	This report

Note. Patients' demographic characteristics, viral infections, and DOCK8 genotypes are listed. The genotypes of patients 9 and 10 are described in Janssen et al.^{1,2}

F, Female; HPV, human papillomavirus; M, male; MCV, molluscum contagiosum virus; VZV, varicella-zoster virus.

*Patients treated with IFN- α 2b.

†Deceased.

TABLE E2. Hematologic and immunologic findings in DOCK8-deficient patients treated with IFN- α 2b

Peripheral blood cells	Patient 1 at diagnosis	Patient 1 before IFN- α 2b therapy	Patient 1 2 mo into IFN- α 2b therapy	Patient 2 at diagnosis	Patient 2 before IFN- α 2b therapy	Patient 2 2 mo into IFN- α 2b therapy
White blood cells (mm ³)	11,500	15,400	10,700	17,100	25,000	11,900
Neutrophils (mm ³)	6,040	12,800	8,999	6,480	9,500	8,150
Lymphocytes (mm ³)	2,290	1,810	1,490	2,400	1,999	1,680
Eosinophils (mm ³)	3,500	872	7	8,000	806	1,100
Hemoglobin (g/dL)	9.9	11.3	9.79	10.1	13.1	13.8
Platelets (mm ³)	719,000	435,000	847,000	825,000	374,000	392,000
Serum immunoglobulins						
IgE (IU/mL)	334	1,330		14,000	2,450	
IgA (mg/dL)	94	94.1		176	199	
IgM (mg/dL)	36.5	63.1		84.5	60.9	
IgG (mg/dL)	1,280	994		2,100	1,230	
Lymphocyte subset analysis						
CD3 ⁺ (%)	40	54	54	58	51	53
CD3 ⁺ 4 ⁺ (%)	29	24	28	27	28	33
CD3 ⁺ 8 ⁺ (%)	12	34	33	30	24	19
CD19 ⁺ (%)	47	19	20	15	22	24
CD3 ⁺ CD16 ⁺ CD56 ⁺ (%)	10	24	18	27	24	20