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Clinical, Immunologic and Genetic Profile of DOCK8-Deficient Patients in Kuwait

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Abstract

Deficiency of dedicator of cytokinesis 8 (DOCK8) is a newly described combined primary immunodeficiency disease. It was found to account for 15% of combined immune deficiency cases in the National Primary Immunodeficiency Disorders Registry in Kuwait, a country with high prevalence of consanguinity. We present the clinical, immunologic and molecular characteristics of 9 Kuwaiti patients with DOCK8 deficiency and discuss differences that distinguish DOCK8 deficiency from atopic dermatitis. Clinical immunologists in areas with high incidence of consanguinity should have a high index of suspicion of DOCK8 deficiency in children with recalcitrant eczema, recurrent non-cutaneous infections and lymphopenia.

Keywords

DOCK8 deficiency; combined immunodeficiency; IgE; eczema; consanguinity; Kuwait

1.0 INTRODUCTION

Deficiency of dedicator of cytokinesis 8 (DOCK8) is a newly described combined primary immunodeficiency disease (PID) characterized by susceptibility to viral infections, recurrent sinopulmonary infections, atopy, early onset malignancies and autoimmunity [1-4]. Reported immunologic abnormalities include T-cell lymphopenia, defective T-cell proliferation, decreased IgM, elevated IgE, eosinophilia and a variable IgG antibody response [5]. The disease is caused by biallelic loss-of-function mutations in the DOCK8 gene, most of which lead to absent or trace amounts of expressed DOCK8 protein [6].

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Autosomal recessive primary immunodeficiency diseases are more prevalent in Kuwait compared to other populations, because of a high rate of consanguinity [7]. The present study presents the clinical, immunologic and molecular characteristics of 9 Kuwaiti patients who presented with DOCK8 deficiency between the years 2004 and 2011.

2.0 METHODS

2.1 Patients data

The patients data were retrieved from the Kuwait National Primary Immunodeficiency Disorders Registry (KNPIDR), which began recruiting patients in 2004. The project was approved by the Human Subjects Research and Ethics Committee of the Ministry of Health, Kuwait.

2.2 Mutation analysis of the DOCK8 gene

Genomic DNA was extracted from whole blood using QIAamp DNA Blood Kit (Qiagen, Valencia, CA). For some patients and family members, total RNA was prepared from blood using QIAamp RNA Blood Kit (Qiagen), and cDNA was synthesized as previously described (1). Exons and flanking intron/exon boundaries from DOCK8 gene were amplified from genomic DNA by PCR according to standard protocols with Taq polymerase. Primer sequences are available upon request. The PCR products were purified by agarose gel electrophoresis, extracted and sequenced using the ABI PRISM BigDye Terminator kit V3.1 (Applied Biosystems, Foster City, CA) and the 3130xl Applied Biosystems Genetic Analyzer. The results were analyzed with Sequencer (version 5.0, Gene Codes Corp, Ann Arbor, MI).

2.3 Western blot analysis

Peripheral blood mononuclear cells (PBMC) or Epstein–Barr virus (EBV)-transformed B cell lines (10^6 cells/condition) were lysed in a buffer solution containing 1% Triton X-100, 100 mMTris-Cl (pH 7.5), and 50 mMNaCl. The lysates were denatured by boiling in sample buffer, separated on 8% acrylamide gels, and subjected to western blot analysis using anti-DOCK8 (Sigma-Aldrich, St. Louis, MO) or anti-actin antibodies (Chemicon, Billerica, MA).

2.4 Lymphocyte markers

Peripheral venous blood was drawn using tubes containing EDTA. Blood samples were processed within two hours of collection. Test tubes were prepared with 100 μl of blood, and 10 μl of the CYTO-STAT tetra CHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5, CYTO-STST tetra CHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 murine monoclonal antibody mixture, anti-CD4/CD45RA or CD4/CD45RO, anti-HLA class I molecules, or anti-HLA class II molecules (Beckman Coulter, USA) was added. These antibody mixtures allow for the simultaneous identification and quantification of total $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$ and $CD3^-/CD56^+$ lymphocyte subpopulations, the expression of CD45RA or CD45RO on $CD4^+$ T cells, and the expression of MHC class I and class II molecules. The samples were incubated in the dark at room temperature for 10 min. After incubation, stabilization and fixation of the stained cells were performed by adding Immunoprep kit reagents (Beckman Coulter, USA). The analysis of the lymphocyte subsets was performed with an EPICS XL-MCL flow cytometer (15 mW) (Beckman Coulter Electronics, FL) equipped with an argon ion laser that was tuned to a wavelength of 488 nm. The values of the lymphocyte subpopulations were determined as a percentage of mononuclear cells. Absolute values of the lymphocyte subsets (counts per μl) were determined via the addition of flow count fluorospheres (Beckman Coulter, USA). We performed a fluorescence gating strategy, using $CD45^+$ vs. side scatter. Internal quality

assurance was performed using optical alignment beads, which are compensation reagents that are used to eliminate bleed through fluorescence, and Immunotrol control cells. Data analysis was performed with the Coulter tetraONE SYSTEM software and System II software.

2.5 T-lymphocyte proliferative responses

Peripheral blood was obtained from the subjects by venipuncture, and PBMC were separated by Ficoll-paque (Pharmacia Biotech, Sweden) density gradient centrifugation. The PBMC were suspended in RPMI medium (GIBCO, USA) containing 10% fetal calf serum (GIBCO, USA). The PBMC were aliquoted into 96 well tissue culture plates at a density of 10^5 cells per well and stimulated with one of the following agents: phytohemagglutinin (PHA) (5 μg/ml, Sigma-Aldrich, USA), anti-CD3 antibody (OKT3, 10 μg/ml, Bender MedSystems), candida antigen (2 μg/ml, Greer Laboratories, USA) or purified protein derivative (PPD, 10 μg/ml, CSL Limited, Australia). The supernatants were harvested at 24 and 96 h for the measurement of the cytokine levels. Cultures were pulsed at 96 hrs with [^{3}H]thymidine (1 μCi per well) to assess mitogen/antigen-induced proliferation, and the thymidine uptake into DNA was determined 18 hrs later.

2.6 Cytokine production by PBMC

Culture supernatants were tested by ELISA for their levels of IL-2, IL-4, TNFα (Beckman-Coulter, France) and IFN-γ (Immunotech, France) as per the manufacturer's instructions. The samples were tested in triplicate at 24 h for IL-4 and IL-2 and at 96 h for TNFα and IFN-γ. The sensitivities of cytokine detection were 3 pg/ml for IFN-γ, 10 pg/ml for TNFα, 5 pg/ml for IL-2 and 5 pg/ml for IL-4.

2.7 Serum immunoglobulin and antibodies levels

The quantitation of immunoglobulins (IgM, IgG, IgA) in serum was performed by rate nephelometry using the Beckman specific protein analyzer (Beckman Instruments Inc., CA). Allergen-specific and total IgE concentrations were measured by the HY.TEC enzyme immunoassay (EIA) (Hycor Biomedical, Inc., Garden Grove, CA).

Serum levels of IgG antibodies against tetanus toxoid (TT), diphtheria toxoid (DT), *pneumococcal capsular polysaccharide* (PCP), and Haemophilus influenza type b capsular polysaccharide (Hib) were measured using a commercial (ELISA) kit (The Binding site, USA). The antibody concentrations were derived from a standard calibration curve and reported in IU/ml for anti-TT and anti-DT and in mg/L for anti-PCP and anti-Hib.

2.8 Statistical analysis

The data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA 2007). A *P*-value ≤ 0.05 was used as the cutoff level for statistical significance. The nonparametric Mann-Whitney U test was used to compare the distributions of two non-normal quantitative variables.

3.0 RESULTS

3.1 Patient characteristics and clinical presentations

A total of 9 DOCK8-deficient patients (3 males and 6 females) from 4 families are presented in this report. They represent 3.73% of all patients with PID and 15% of the patients with combined T- and B-cell immunodeficiencies registered in the KNPIDR. All of the patients were born to consanguineous parents. The details of the clinical presentations are shown in Table I. One patient (A49) was screened and diagnosed early in life because of family

history of the disease. All of the patients developed allergic/atopic manifestations, including atopic eczema and food allergy. Eight of the 9 patients developed viral and recurrent sinopulmonary infections and 6 developed skin abscesses. One patient developed fungal infections, chorioretinitis and uveitis suspected to be autoimmune in origin, and widespread vascular aneurysms, including in the coronary arteries. Two patients developed hyperpigmented lesions caused by non-specific hypermelanosis that affected the oral mucosa (Fig. 1). All patients except one received the BCG vaccine, and none of the patients developed complications. The DOCK8 deletions and mutation identified in the patients are listed in Table II. The DOCK8 protein was not detected in the PBMC or EBV-transformed B-cell lines of 7 of the 7 tested patients.

3.2 Immunologic evaluation

The details of the immunologic evaluations are shown in Table III. All of the patients had eosinophilia and elevated IgE levels. In one patient (A49) IgE level was initially normal at the age of 4 months, but was elevated when retested at the age of 11 months. Four patients had $CD3^+$ T cell lymphopenia. Three patients had selective $CD4^+$ T cell lymphopenia at their initial presentation, and 2 of them (i.e., A38 and A52) progressed to $CD3^+$ T cell lymphopenia. B cell count was low in one patient, elevated in another and normal in seven. PBMC from the patients had significantly lower T-lymphocyte proliferation in response to both PHA and anti-CD3 compared to healthy controls, with *P*-values of 0.003 and 0.008, respectively (Fig. 2). In contrast, their proliferation to PPD and candida antigens was comparable to that of controls. Three patients had IgM deficiency, two had elevated serum IgG levels, five had elevated serum IgA levels and one had IgA deficiency. Eight patients were tested for antibody responses against previous vaccines, and all eight had good responses.

3.3 Cytokine levels in the culture supernatants

PBMC from the patients secreted significantly lower amounts of IL-2, TNF-α and IFN-γ than those from healthy controls in response to stimulation with PHA (Fig. 3). In contrast, they secreted comparable amounts of IL-4. We have calculated the means of the secreted cytokines then calculated the ratios of the mean secreted IL-4 to all other three cytokines tested. DOCK8-deficient patients had higher ratios than controls. The IL-4:IL-2 ratio was 0.19 in patients vs.0.086 in controls, IL-4:IFN-γ ratio was 107.2 in patients vs.3.2 in control and the IL-4:TNF-α ratio was 0.187 in patients vs.0.069 in controls, indicating a selective Th2 bias in DOCK8 deficiency.

3.4 Management and outcome

All of the patients were treated with intravenous immunoglobulin replacement and prophylactic antibiotics (trimethoprim-sulfamethoxazole). One patient (A44) died due to cytomegalovirus (CMV) sepsis and pneumonia at age of 16 years. Two of the patients received a fully matched hematopoietic stem cell transplant (HSCT) and achieved good immune reconstitution. One of the two received a liver transplant from her mother for *Cryptosporidium*-associated liver failure prior to HSCT. Both transplanted patients continue to have high IgE levels and clinical evidence of food allergy. However, the eczema resolved few months after the transplant in both patients and the frequency and distribution of the infections significantly improved, including disappearance of molluscum contagiosum lesions in one of them. Five patients are awaiting HSCT. The parents of one patient have declined treatment with HSCT.

5.0 DISCUSSION

Combined immunodeficiency due to DOCK8 deficiency is an autosomal recessive disease, which appears to be common in populations where the frequency of consanguineous marriages is high. In Kuwait, where the incidence of consanguinity is 54% [11], DOCK8 deficiency accounted for a significant proportion (15%) of patients who suffer from combined T- and B-cell immunodeficiencies.

The clinical phenotype in the present group of patients is consistent with that in the two initial reports of DOCK8 deficiency [1,2]. The finding of hypermelanosis of oral mucosa in 2 patients has not been previously described and could not be readily explained. The *Cryptosporidium* associated liver disease in one of our patients has not been reported to date in DOCK8 deficiency, but is a well-known complication in a number of combined immunodeficiencies [12,13].

We noted progressive T cell lymphopenia in 2 of our patients as observed in prior studies on DOCK8 deficient patients [6]. The T-cell lymphopenia and the poor T cell proliferation to mitogens observed in the present study could be due to defects in T cell survival *in vitro*, which have been previously documented [14]. Our study is the first one we are aware of that has examined T cell proliferation to antigens. The preserved T-cell proliferation to antigens in our patients in the face of an impaired T cell proliferation to mitogens suggests that DOCK8-deficient T cells may be more susceptible to cell death in response to intense TCR stimulation. In contrast to previous findings of impaired antibody responses in DOCK8-deficient humans and mice [2,15], antibody responses to vaccines were found to be in the normal range. The antibody response of DOCK8-deficient mice is normal early after immunization, but fails to be sustained. This may reflect defective generation of memory B cells, as suggested by studies in DOCK8-mutant mice [15] and by our own observations of virtual lack of circulating $CD19^{+}CD27^{+}$ cells in DOCK8-deficient patients [16]. It is possible that the antibody responses in our patients may wane as more time elapses after vaccination.

The abnormalities in cytokine secretion in the present patients with severely impaired production of IL-2, IFN-γ and TNF-α are in agreement with previous findings [2,14]. The resulting strong Th2 bias in DOCK8 deficient patients may help explain their susceptibility to atopy. It will be interesting to determine whether patients treated with HSCT will outgrow their eczema and food allergy. To date our experience in four DOCK8 patients who underwent successful HSCT, including the two presented in this study, indicates that the eczema resolves within 6-12 months post-transplant.

Clinical immunologists, particularly in areas with high incidence of consanguinity should have a high index of suspicion of DOCK8 deficiency in children with recalcitrant eczema and recurrent infections. Recurrent cutaneous and deep-seated infections, T cell lymphopenia and virtual absence of $CD27^{+}$ memory B cells in DOCK8 deficiency are clinical and laboratory features that help differentiate DOCK8 deficiency from atopic dermatitis.

Acknowledgments

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HIGHLIGHTS

- DOCK8 deficiency should be suspected in children with recalcitrant eczema and recurrent non-cutaneous infections.
- DOCK8-deficient patients have selective TH2 bias which may explain several features of the disease phenotype.
- DOCK8 deficiency is characterized by progressive lymphopenia and defective T-lymphocyte stimulation in response to mitogens.
- Hypermelanosis of oral mucosa is a new finding in DOCK8-deficient patients.

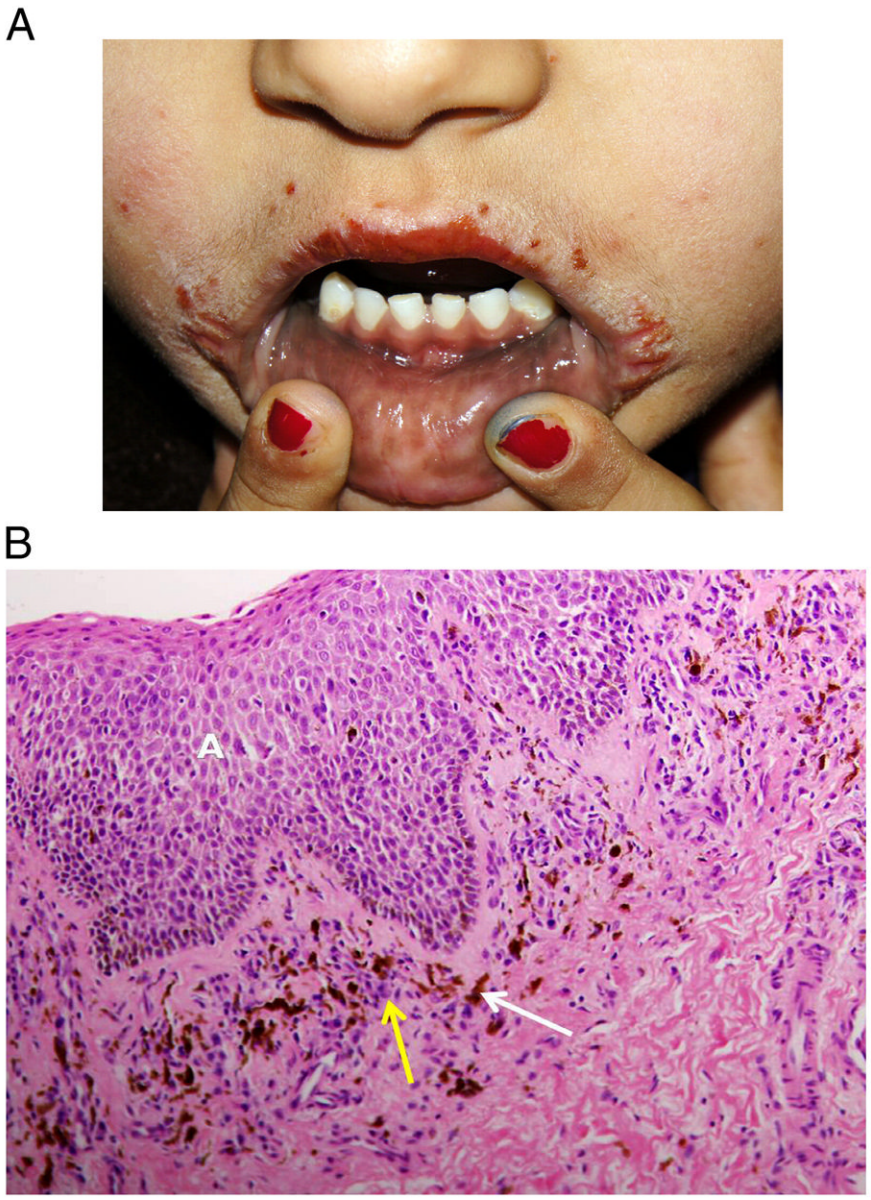

Figure 1.

A. Patient A54 with perioral dermatitis and diffuse hypermelanosis of oral mucosa

B. Oral mucosal biopsy shows acanthotic epithelium (A). In the subepithelium there are large number of melanophages (white arrow) admixed with chronic mixed inflammatory cell infiltrate (yellow arrow) (H.E. × 20).

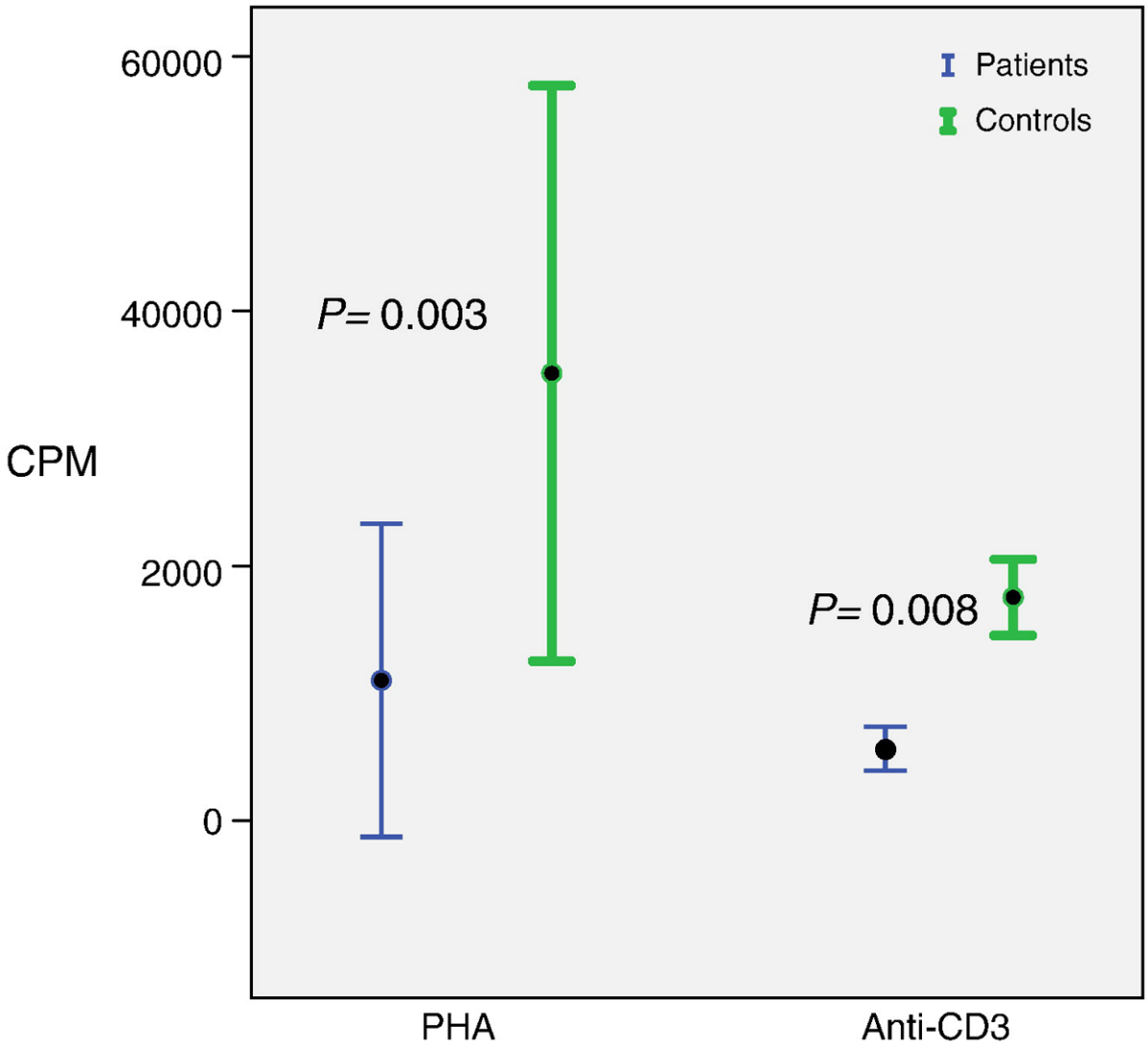

Figure 2.
DOCK8 deficiency impairs T cell activation.
T-lymphocyte proliferative responses using both PHA and anti-CD-3 in patients and controls (n = 5/group).

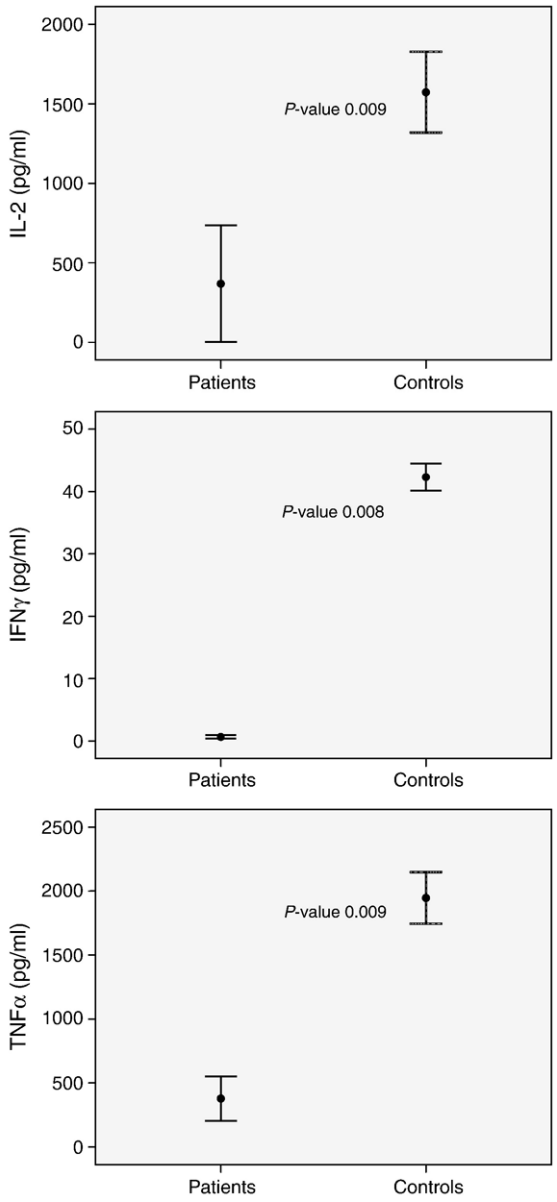

Figure 3.

Cytokine levels in the culture supernatants.

PBMC from the patients secreted significantly lower amounts of IL-2, TNF-α and IFN-γ than those from healthy controls in response to stimulation with PHA (Mean & 95% Confidence Intervals) (n = 5/group)

Table I

Clinical features of 9 patients with DOCK8 deficiency

	Patient A35 ‡	Patient A38	Patient A44	Patient A49 ‡	Patient A52	Patient A53 ‡	Patient A54 ‡	Patient A59 ‡	Patient A60 ‡
Age at diagnosis (months)	56	84	16	4	60	32	54	14	70
Sex	M	M	F	M	F	F	F	F	F
Recurrent sino-pulmonary infections	+	+	+	-	+	+	+	+	+
Bronchiectasis	-	+	+	-	-	-	-	-	-
Skin abscesses	+	-	+	-	+	+	+	-	+
Fungal infections									
Candida infection	-	-	+	-	-	-	-	-	-
Aspergillus infection	-	-	+	-	-	-	-	-	-
Viral infections*									
HSV1	-	-	-	-	+	+	-	+	-
VZV	-	+	-	-	-	-	-	+	-
EBV	-	-	+	-	-	-	-	-	-
Molluscum	+	-	-	+	-	-	-	-	-
HPV	-	-	-	-	+	-	-	-	-
CMV	-	-	+	-	+	-	-	-	-
Allergies									
Eczema	+	+	+	+	+	+	+	+	+
Asthma	-	-	-	-	+	+	+	-	+
Food allergy	+	+	-	-	+	+	+	-	-
Malignancy	-	-	-	-	-	-	-	-	-
Autoimmunity	-	-	Chorioretinitis Uveitis	-	-	-	-	-	-
Vascular findings	-	-	Aneurysms¶	-	-	-	-	-	-
Failure to thrive	-	-	+	-	+	-	-	-	-
Outcome #	HSCT A & W	A	D	A & W	HSCT Liver transplant A & W	A	A	A	A
Others					Cryptosporidium liver disease		Hyper-pigmented oral lesions		Hyper-pigmented oral lesions

‡Members of the same family

*HSV1: Herpes simplex virus 1; VZV: Varicella zoster virus; EBV: Epstein-Barr virus; CMV: Cytomegalovirus; HPV: Human papilloma virus

¶Coronaries, carotids and aorta

#A: Alive; W: Well; D: Deceased; HSCT: Hematopoietic stem cell transplant

Table II

DOCK8 gene mutations in the patients

Patients	Details of mutations
A35, A49, A53, A54, A59, A60 ‡	c.[1-?_404+?del]+[1-?_404+?del] (includes deletion of exons 1 to 5)
A38	c.[4070C>A]+[4070C>A]; p.[S1357*]+[S1357*] (mutation in exon 33)
A44	c.[54-?_1285+?del]+[54-?_1285+?del] (includes deletion of exons 2 to 12)
A52	c.[1-?_2778+?del]+[1-?_2778+?del] (includes deletion of exons 1 to 23)

‡Members of the same family

Table III

Immunologic parameters in 9 patients with DOCK8 mutations

	Patient A35 ‡	Patient A38	Patient A44	Patient A49 ‡	Patient A52	Patient A53 ‡	Patient A54 ‡	Patient A59 ‡	Patient A60 ‡
Eosinophil s (cells/μL)#	21940 (100-1000)	1270 (100-1000)	7530 (100-1000)	1165 (100-1000)	4510 (100-1000)	2650 (100-1000)	1725 (100-1000)	3060 (100-1000)	3800 (100-1000)
Lymphocytes (cells/μL)*									
$CD3^+$	791 (1400-3700)	611 (1200-2600)	532 (1000-2200)	2550 (1900-5900)	764 (1200-2600)	1496 (1400-3700)	1825 (1400-3700)	2375 (2100-6200)	2014 (1200-2600)
$CD4^+$	472 (700-2200)	241 (650-1500)	162 (530-1300)	2240 (1400-4300)	247 (650-1500)	686 (700-2200)	991 (700-2200)	773 (1300-3400)	456 (650-1500)
$CD8^+$	252 (490-1300)	456 (370-1100)	347 (330-920)	310 (500-1700)	397 (370-1100)	787 (490-1300)	763 (490-1300)	1465 (620-2000)	1089 (370-1100)
$CD19^+$	631 (390-1400)	291 (270-860)	306 (110-570)	2040 (610-2600)	127 (270-860)	386 (390-1400)	2248 (390-1400)	1611 (720-2600)	463 (270-860)
$CD16^+$	44 (130-720)	329 (100-480)	224 (70-480)	10 (160-950)	130 (100-480)	163 (130-720)	226 (130-720)	194 (180-920)	298 (100-480)
Serum immunoglobulins¶									
IgG (mg/dL)	1100 (490-1610)	2800 (540-1610)	1280 (310-1380)	500 (240-880)	1530 (490-1610)	652 (490-1610)	991 (490-1610)	1150 (310-1380)	1880 (490-1610)
IgA (mg/dL)	394 (40-200)	300 (50-240)	315 (30-120)	238 (10-50)	448 (40-200)	77.2 (40-200)	113 (40-200)	56.2 (30-120)	6 (40-200)
IgM (mg/dL)	48 (50-200)	16 (50-180)	20.4 (50-220)	72.5 (20-100)	55.1 (50-200)	46.8 (50-200)	37 (50-200)	48.5 (50-220)	38.1 (50-200)
IgE (IU/ml)	6985	801	>2500	2221	9888	>2000	>2000	331.9	288.2

‡Same family

#Normal range of values for age in parenthesis [9]

*Normal range of values for age in parenthesis [10]

¶Prior to IVIG replacement, normal range of values for age in parenthesis [11]