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DOCK2 and a Recessive Immunodeficiency with Early-Onset Invasive Infections

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Abstract

Background—Combined immunodeficiencies (CIDs) denote inborn errors of T-cell immunity with T cells present but quantitatively or functionally deficient. Impaired humoral immunity, either due to a primary B cell defect or secondary to the T-cell defect, is also frequent. Consequently, patients with CID display severe infections and/or autoimmunity. The specific molecular, cellular, and clinical features of many types of CID remain unknown.

Methods—We performed genetic and cellular immunological studies in five unrelated children who shared a history of early-onset invasive bacterial and viral infections, with lymphopenia and defective T-, B-, and NK-cell responses. Two patients died early in childhood, whereas the other three underwent allogeneic hematopoietic stem cell transplantation with normalization of T cell function and clinical improvement.

Results—We identified bi-allelic mutations in the *Dedicator Of Cytokinesis 2 (DOCK2)* gene in these five patients. RAC1 activation was impaired in T cells. Chemokine-induced migration and actin polymerization were defective in T, B, and NK cells. NK-cell degranulation was also affected. The production of interferon (IFN)- α and - λ by peripheral blood mononuclear cells (PBMCs) was diminished following virus infection. Moreover, in DOCK2-deficient fibroblasts, virus replication was increased and there was enhanced virus-induced cell death, which could be normalized by treatment with IFN- α 2 β or upon expression of wild-type DOCK2.

Conclusions—Autosomal recessive DOCK2 deficiency is a Mendelian disorder with pleiotropic defects of hematopoietic and non-hematopoietic immunity. Children with clinical features of CID, especially in the presence of early-onset, invasive infections may have this condition.

Combined immunodeficiencies (CIDs) comprise a heterogeneous group of inherited defects of the immune system characterized by quantitative and/or qualitative defects of T lymphocytes, which are associated with primary or secondary defects of B lymphocytes¹. In patients with CID, impairment of adaptive immunity causes increased susceptibility to early-onset, severe infections by a variety of viruses, bacteria, fungi, and parasites^{1,2}. Furthermore, autoimmune manifestations, allergy, and malignancies can also occur².

Identification of CID-causing gene defects has not only considerably helped patients but also provided novel and important insights into mechanisms governing T-cell development and function in humans², and has helped understand the molecular and cellular basis of more common conditions, including autoimmunity, allergy, inflammation, and cancer. However, the specific molecular, cellular, and clinical features of many CIDs remain poorly defined.

Next generation sequencing has revolutionized studies of human genetic diseases, enabling the identification of novel causative genetic variations in an increasing number of patients with PIDs³⁻⁷. We herein report human DOCK2 deficiency as a CID in five unrelated patients of different ethnic origin who presented with a distinctive clinical phenotype of early-onset, invasive infections, associated with a broad spectrum of defects of hematopoietic and non-hematopoietic immunity.

METHODS

Study oversight

The study was approved by the Institutional Review Boards of Kuwait Ministry of Health, the INSERM Institute, the Rockefeller University, the Medical University of Vienna, Ankara University Medical School, Boston Children's Hospital, and the Duke University Medical School. Informed consent was obtained from the patients' guardians.

Study subjects

Pedigrees of the five unrelated index patients are reported in Fig.1A and Fig.S1. Clinical and laboratory data are summarized in Table1.

Patient P1, a boy born to consanguineous Lebanese parents, presented at 3 months with respiratory syncytial virus (RSV) bronchiolitis, followed by recurrent episodes of pneumonia. At 5 months of age, severe T-cell lymphopenia and markedly reduced *in vitro* T-cell proliferation were observed (Table1). At 9 months of age, he received T-cell-depleted haploidentical hematopoietic stem cell transplantation (HSCT) from his father after myeloablative conditioning with busulfan and fludarabine. He is alive and well, and off-intravenous immunoglobulins (IVIG), 13 months after HSCT.

Patient P2, a girl born to non-consanguineous Finnish parents, suffered from recurrent otitis media, pneumonia, diarrhea and three episodes of thrombocytopenia in the first two years of

life that resolved spontaneously. At 2.5 years of age, she developed vaccine strain-related varicella, with liver and lung involvement and multiple pulmonary infiltrates, requiring ventilatory support (Fig.1B). Several months later, a chest CT showed a new pulmonary infiltrate (Fig.S2A). A lung biopsy revealed granulomatous inflammation (Fig.S2B) with acid-fast bacilli. *Mycobacterium avium* was cultured from the biopsy, and human herpes virus-6 DNA was detected. Immunological investigations revealed T- and B-cell lymphopenia, defective *in vitro* T-cell proliferation and lack of specific antibody responses (Table1), consistent with CID. At the age of 3.8 years, she received matched unrelated donor HSCT with reduced intensity conditioning using treosulfan, fludarabine, and alemtuzumab. She is alive and well, 8 months after HSCT.

Patient P3, a boy born to consanguineous Turkish parents, suffered from recurrent respiratory tract infections from the age of 3 months. At 6 years of age, he developed two episodes of meningoencephalitis presumed to be due to mumps virus infection, based on cerebrospinal fluid examination (1,000 cells/mm³, 74% lymphocytes), demonstration of high serum amylase levels (762U/l) and detection of anti-mumps IgM, concurrent with an outbreak of mumps at school. At the age of 6.3 years, the patient developed severe chickenpox (Fig.1B) with alveolar infiltrates, rapidly progressing to multiorgan failure and death. Laboratory studies during hospitalization demonstrated severe T-cell lymphopenia, impaired T-cell activation, and lack of antibody responses to VZV (Table1). Post-mortem examination of liver and lungs revealed coagulation necrosis, apoptosis, inflammatory infiltrates with neutrophils and monocytes, and nuclear inclusion bodies within pneumocytes, consistent with viral pneumonitis (Fig.S2C,D).

Patient P4, a boy born to consanguineous Turkish parents, suffered from neonatal-onset chronic mucous diarrhea and recurrent episodes of fever and oral moniliasis. A liver biopsy, performed at 3 months of age because of persistently elevated transaminases, disclosed macrovesicular steatosis, non-necrotic eosinophilic granuloma-like lesions and lobular inflammation (Fig.S2E). During admission at 1 year of age, growth failure (body weight: 4.5 kg, 3.5kg below third percentile; length: 64 cm, 9cm below third percentile), nodular erythematous lesion at the site of BCG vaccination, and hepatomegaly were detected. In addition, colon histopathology revealed focal active colitis (Fig.1B), associated with paucity of B and plasma cells and to a lesser extent of T cells in the lamina propria of the gut. Immunological investigations (Table1) revealed T-cell lymphopenia and defective T-cell activation in response to PHA. Subsequently, the patient developed multiple pneumonias due to parainfluenza virus type 3 and adenovirus, several episodes of CMV reactivation, and ultimately died at the age of 20 months due to *Klebsiella pneumoniae* sepsis.

Patient 5 (P5), a Hispanic boy born to non-consanguineous parents from Honduras and Nicaragua, presented at the age of 4 months with interstitial pneumonia that responded to high-dose trimethoprim/sulfamethoxazole. Immunological investigations were consistent with CID (Table1). At 2 years of age, he developed rectal fistula. At 3 years of age, he received HSCT from his HLA-identical brother with myeloablative doses of busulfan and cyclophosphamide. This patient is alive and well, and off-IVIG at 17.5 years after transplant.

In all five patients, T-cell lymphopenia and impaired *in vitro* T-cell activation in response to PHA were documented (Table1). Maternal T-cell engraftment was excluded in all patients. More detailed immunological analyses performed in P1, P2, and P5 revealed markedly reduced proportion of naïve (CD45RA⁺CCR7⁺) CD4⁺ and CD8⁺ lymphocytes, associated with an increased proportion of effector memory (CD45RA⁻CCR7⁻) CD4⁺ T lymphocytes and of either effector memory or of CD45RA⁺CCR7⁻ T_{EMRA} CD8⁺ T (Table 1). B-lymphocyte counts were reduced in P2 and P4 (Table1). Despite normal IgG and IgM serum levels, P2, P4 and P5 showed defective antibody production to T-dependent immunization antigens (Table1). Finally, levels of T-cell receptor excision circles (TRECs), a marker of active thymopoiesis, were markedly reduced in dried blood spots collected at birth in P2 and P4 (Table 1).

Genetic, immunological and biochemical analyses

Methods for genomic, immunological and biochemical studies are detailed in the Supplementary Appendix.

RESULTS

Identification of biallelic deleterious mutations in the *DOCK2* gene

Whole-exome sequencing (WES) was performed to elucidate the genetic basis of the patients' CID. Linkage analysis or homozygosity mapping were also performed in P3 and P4, who were born to consanguineous parents. We selected genes harboring previously unreported homozygous variants in P1–P5, and genes bearing two or more variants in P2 and P5 (for whom no parental consanguinity was known) (Table S1). Bi-allelic mutations in *DOCK2* were identified and confirmed by Sanger sequencing in all five patients (Fig.1A). No other gene harbored bi-allelic mutations in two or more patients. P1 and P4 were homozygous for *DOCK2* dinucleotide insertions leading to frameshift and premature termination, P3 was homozygous for a missense mutation, while P2 and P5 were compound heterozygous for different missense and nonsense *DOCK2* mutations (Fig.1A). Multiple sequence alignment illustrated that all of the three missense mutations affect evolutionarily conserved residues (Fig.S3). Intra-familial segregation was consistent with autosomal recessive inheritance with complete penetrance (Fig.S1). Collectively, we identified seven distinct *DOCK2* rare mutations in five patients of different ethnic origin, four of which lead to premature termination, and three of which were predicted deleterious missense mutations affecting conserved residues of *DOCK2* (Fig.1C, Table S2 and Fig.S3).

DOCK2 mutations affect protein expression and T- and B-cell signaling

Immunoblot analysis revealed absent or trace amounts of *DOCK2* protein expression in T cell lines from P1 and P2, respectively, and markedly reduced levels of protein were detected in P3 EBV-B cells (Fig.1D, Fig.S4A). No biological specimens were available from P4 and P5, however, overexpression of Strep-HA-tagged *DOCK2* bearing the p.F744Cfs*27 mutation (present in P4) shows expression of a truncated *DOCK2* protein (Fig.S4B) which lacks the DHR2 domain critical for the *DOCK2* GEF function.

Previous studies in mice have shown that Dock2 is essential for Rac1 activation downstream of the T cell receptor (TCR)^{8,9}. Upon activation of polyclonal human T-cell lines with anti-CD3 mAb, GTP-bound RAC1 was clearly detected in T cells from the healthy control and from P2's mother, but not from P1 and P2 (Fig.2A and Fig.S5).

Chemokine-mediated cell migration is critically important during lymphocyte development, immune surveillance of lymph nodes and recruitment of immune cells to sites of inflammation. Previous experimental data had suggested a role for DOCK2 in actin polymerization and chemotactic responses of T and B lymphocytes upon chemokine stimulation^{9,10}. Indeed, the chemotactic response of T and B lymphocytes from P1 and P2 was profoundly impaired (Fig.2B). Furthermore, CXCL12-induced actin polymerization in T and B lymphocytes from P1 and P2 was impaired and delayed (Fig.2C,D). Interestingly, baseline levels of polymerized actin (F-actin) were also reduced in DOCK2-deficient lymphocytes from P1 and P2 (Fig.2C,D).

NK and NKT defects in patients with DOCK2 deficiency

Invasive viral infections were a prominent clinical feature in P2, P3 and P4. DOCK2-deficient patients had normal numbers of NK cells (Table1), and NK cells from P2 had a normal immunophenotype (Fig.S6). However, NK cells from P1 and P2 showed impaired degranulation upon stimulation with the human erythroleukemia cell line K562 (Fig.3A).

The ability of NK cells to lyse virus-infected and tumor target cells correlates with the functionality of a variety of activating NK receptors interacting with distinct adaptor (DAP10, DAP12) and signaling (CD3 ζ , Fc ϵ RI γ) molecules¹¹⁻¹³. Triggering of activating NK receptors induces actin polymerization, phosphatidylinositol-3-OH kinase activation and phosphorylation of MEK and ERK, ultimately promoting NK-cell cytotoxicity¹⁴. We analyzed NK-cell degranulation upon engagement of CD16, NKp30, NKp46 (all of which use CD3 ζ and Fc ϵ RI γ), or NKG2D (which recruits the DAP10 adaptor), and observed severely impaired degranulation in P1 (Fig.3B), and moderately impaired in P2, likely corresponding to residual amounts of DOCK2 protein in P2's hematopoietic cells (Fig.S4A). Degranulation was also impaired in P2's IL-2-activated polyclonal NK-cells upon engagement of NKp44 (which utilizes DAP12) (Fig.S7). Furthermore, we observed reduced levels of F-actin in P2's NK cells upon CD16 and NKp46 stimulation (Fig.3C) reminiscent of observations in *Dock2*^{-/-} mice¹⁵, possibly reflecting impaired tonal signaling through antigen and chemokine receptors. Reduced phosphorylation of ERK1/2 and MEK, and impaired actin polymerization were also detected in polyclonal NK cells from P2 upon cross-linking of NKp30, NKp44 (Fig.3D), CD16, and NKp46. NK cells are also involved in cytokine production¹⁶. Upon overnight stimulation with IL-12 and IL-18, the proportion of NK cells expressing IFN- γ was markedly reduced in P2 (Fig.3E).

Finally, the number of circulating NKT cells was severely reduced in P1 and P2 (Fig.S8). Altogether, these data demonstrate that DOCK2 serves an essential role in NK and NKT cell biology, consistent with similar observations in mice^{17,18}.

Impaired anti-viral interferon responses in hematopoietic and non-hematopoietic cells from DOCK2-deficient patients

Human interferon (IFN)- α/β and - λ immunity has been suggested to be essential in host defense against viral infections¹⁹. Plasmacytoid dendritic cells (pDCs) represent the major source of IFN- α in human blood in response to enveloped viruses and synthetic TLR7 and TLR9 agonists²⁰. Previous studies in mice have shown that Dock2 serves an essential function in regulating IFN- α production in pDCs, without perturbing pDC development^{15,21}. Although a normal proportion of circulating pDCs was detected in P2 (Fig.S9), the production of IFN- α and IFN- λ in P1-P3's PBMCs upon stimulation with herpes simplex virus type 1 (HSV-1) or vesicular stomatitis virus (VSV) was markedly impaired (Fig.4A, Fig.S10). By contrast, similar amounts of IL-6 were produced by PBMCs from DOCK2-deficient patients and from healthy donors (Fig.4A, Fig.S10). Altogether, these data indicate that in addition to T, NK and NKT cells, pDCs are defective in patients with DOCK2 deficiency, which may also contribute to the patients' viral susceptibility.

Although DOCK2 is preferentially expressed in hematopoietic cells²², low expression levels were detected in fibroblasts from healthy controls, but not from P1 and P2 (Fig.S4A). Only minimal expression was detected in P3's fibroblasts (Fig.S4A). To investigate whether DOCK2 contributes to cell-intrinsic anti-viral responses in non-hematopoietic tissues, we studied SV40-immortalized fibroblasts from patients P1-P3 and healthy controls. Upon infection with VSV or EMCV, we found enhanced levels of viral replication and decreased viability of DOCK2-deficient SV40-fibroblasts (Fig.4B and Fig.S11); similar results were observed in SV40-fibroblasts from patients with TLR3 or STAT1 deficiency (Fig.4B and Fig.S11), affecting production of or response to IFN- α/β and - λ , respectively²³. Both treatment with recombinant IFN- α 2b (Fig.4B) and lentiviral-mediated wild-type DOCK2 expression (Fig.4C) protected DOCK2-deficient fibroblasts from virus-induced cell death (Fig.4D). The *DOCK2* mutations may therefore also impair cell-intrinsic, non-hematopoietic immunity, at least in fibroblasts and in response to some viruses.

DISCUSSION

We herein report bi-allelic mutations in *DOCK2* as the molecular etiology of a distinctive type of CID, characterized by early-onset, invasive bacterial and viral infections, associated with T-cell lymphopenia, impaired T-, B- and NK-cell function, and defective IFN immunity in both hematopoietic and non-hematopoietic cells. Our results also demonstrate that this disease can be detected at birth with newborn screening for SCID (Table1), and can be cured by HSCT (Fig.S12).

The observation that DOCK2 deficiency in humans leads to impaired RAC1 activation, and to defects of actin polymerization, T-cell proliferation, chemokine-induced lymphocyte migration and NK-cell degranulation, confirms and extends similar observations in *Dock2*^{-/-} mice^{9,10,17,24}, and underlines the essential role of regulated actin dynamics for immune cell function, as illustrated also by other CIDs with defective actin polymerization such as Wiskott-Aldrich syndrome²⁵, and deficiency of WIP²⁶, DOCK8²⁷, RHOH²⁸, and MST1^{29,30} proteins.

Occurrence of invasive viral infections, including disseminated vaccine-strain varicella, was a prominent feature in patients with DOCK2 deficiency. Human antiviral immunity is critically dependent on intact T-cell function as well as innate immune responses, as indicated by observations in patients with genetic defects affecting T-, NK-, NKT- and dendritic-cell development and/or function^{1,31}, or with mutations affecting production of (or response to) IFN- α ,- β , and - λ in both hematopoietic and non-hematopoietic cells³². We observed defective IFN- α/β and IFN- λ responses in both hematopoietic and non-hematopoietic cells from DOCK2-deficient patients. pDCs are the most potent IFN- α $\pi\rho\delta\upsilon\chi\iota\nu\gamma$ $\chi\varepsilon\lambda\lambda\sigma$ ³³. Severe reduction of splenic and lymph node pDCs and of their capability of IFN- α production were observed in *Dock2*^{-/-} mice^{15,21}. We here show impaired IFN- α and IFN- λ production in DOCK2-deficient PBMCs upon stimulation with HSV-1 or VSV, possibly reflecting a defect of pDCs. Treatment with IFN- α/β might be therefore beneficial in DOCK2-deficient patients with severe viral infections. Furthermore, our study suggests a previously unrecognized role for DOCK2 in antiviral responses also in non-hematopoietic cells, possibly also contributing to the pathogenesis of severe viral infections.

Normalization of immunological abnormalities and resolution of infections was observed in P1, P2 and P5 following HSCT (Fig.S12), implying that correction of hematopoietic cells may suffice to rescue the clinical phenotype, possibly by providing a source of IFN- α/β -producing cells (e.g., pDCs) complementing the defect also in non-hematopoietic tissues.

K. pneumoniae sepsis was the cause of death in P4. Besides impaired antibody production, susceptibility to invasive bacterial infections in DOCK2-deficient patients may also reflect impaired function of neutrophil granulocytes. Defective neutrophil chemotaxis has been reported in *Dock2*^{-/-} mice³⁴; we could not test whether a similar defect exists in patients due to lack of primary material.

Mutations in *DOCK8*, another member of the DOCK family of proteins, have been recently identified in other patients with CID^{35,36}. When comparing DOCK2- and DOCK8-deficient patients, similarities but also important differences emerge. Both conditions are characterized by recurrent bacterial and viral infections³⁶, associated with T-cell lymphopenia³⁶, defective NK-cell function³⁷, aberrant NKT-cell survival and function³⁸, and impaired antibody responses³⁹. However, the natural course of DOCK2 deficiency appears to be more severe than that of DOCK8 deficiency. While DOCK8 deficiency is characterized mostly by cutaneous viral infections³⁶, DOCK2-deficient patients suffer from early-onset life-threatening, invasive viral and bacterial infections. Furthermore, severe food allergy, eczema, and autoimmunity are commonly observed in DOCK8 deficiency^{36,40,41} whereas none of the DOCK2-deficient patients had such manifestations.

In summary, we have identified DOCK2 deficiency as a pleiotropic immunodeficiency leading to early-onset, invasive bacterial and viral infections. The broad spectrum of infections observed in DOCK2-deficient patients highlights the impact of DOCK2 function on several aspects of immunity, and raises caution about the application of novel immunosuppressive agents targeting DOCK2⁴². Our observations indicate that HSCT can

provide cure and should be considered soon after diagnosis to prevent life-threatening infections early in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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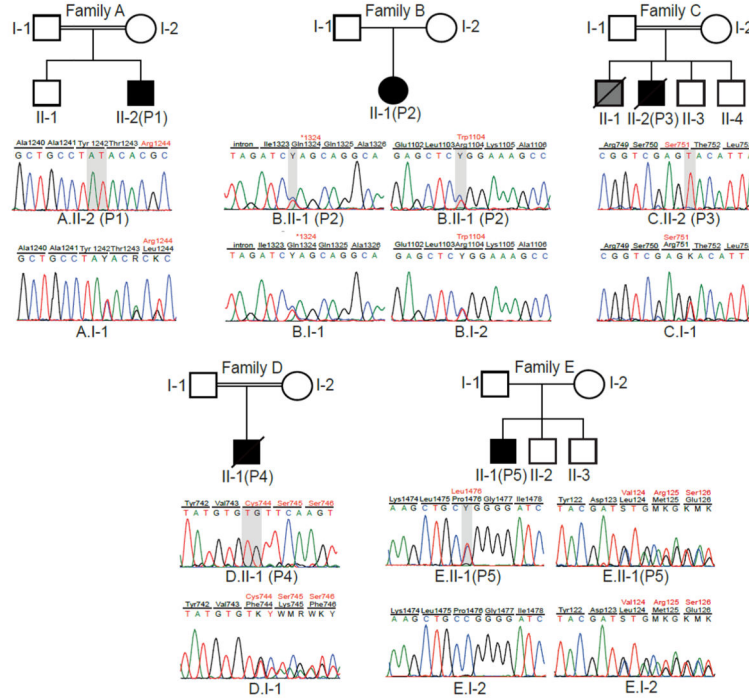
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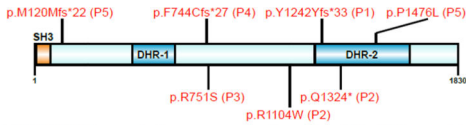
A Family pedigrees and identification of *DOCK2* mutations



B Clinical features in *DOCK2*-deficient patients



C *DOCK2* protein domains and synopsis of *DOCK2* mutations



D *DOCK2* protein expression in P1, P2 and P3

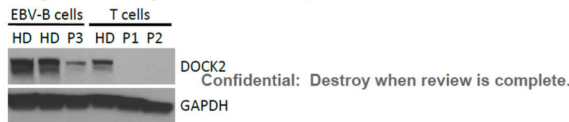


Figure 1. Identification of Mutations in the *Dedicator of Cytokines 2 (DOCK2)* Gene in Patients with Combined Immunodeficiency

Panel A shows the pedigrees of five families with affected individuals indicated by solid symbols and chromatograms corresponding to the identified *DOCK2* mutations in five studied patients (P1–P5) and heterozygous carriers for each family. Panel B illustrates the clinical spectrum of *DOCK2* deficiency (from left to right): episode of pneumonia in P2 requiring intubation; photograph of the skin rash, showing the vesicular lesions due to chickenpox in P3; neutrophil infiltrate in colonic lamina propria and crypt epithelium

consistent with focal active colitis in P4 (hematoxylin eosin staining, magnification $\times 100$). Panel C shows the distribution of the identified mutations relative to the DOCK2 protein structure depicting the SRC homology 3 (SH3) domain, the DOCK homology region (DHR)-1 domain and the DHR-2 domain. Panel D shows the immunoblot analysis of protein lysates obtained from EBV-transformed B-cell lines derived from P3 and two healthy donors (HD) and protein lysates obtained from T-cell lines derived from P1, P2, and a healthy donor (HD). For panels D, GAPDH served as protein loading control.

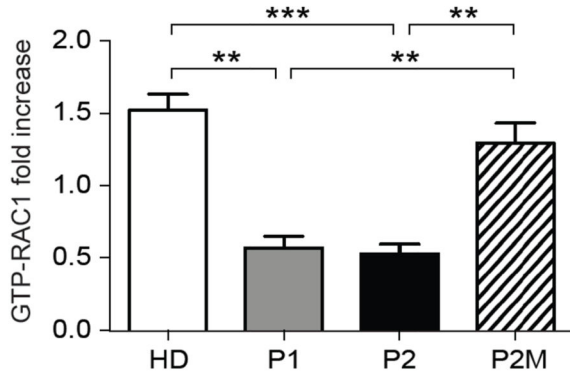
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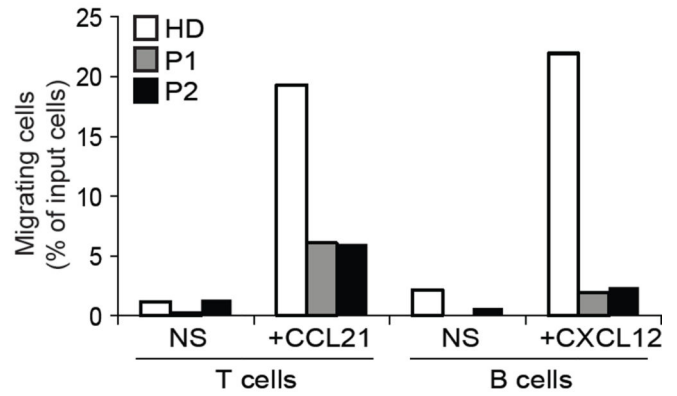
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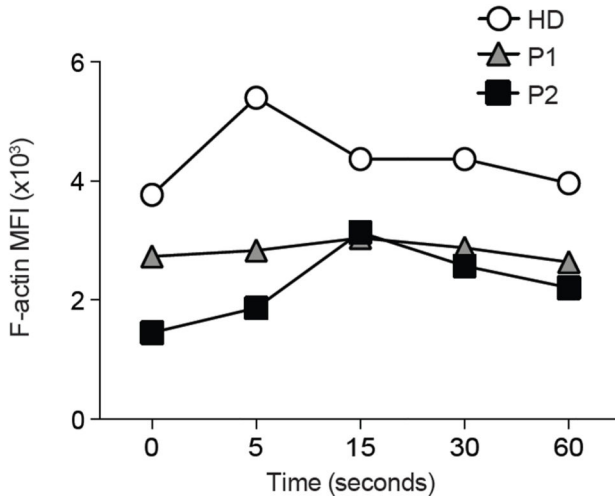
A RAC1 activation



B Chemotaxis in T and B cells



C Actin polymerization in T cells



D Actin polymerization in B cells

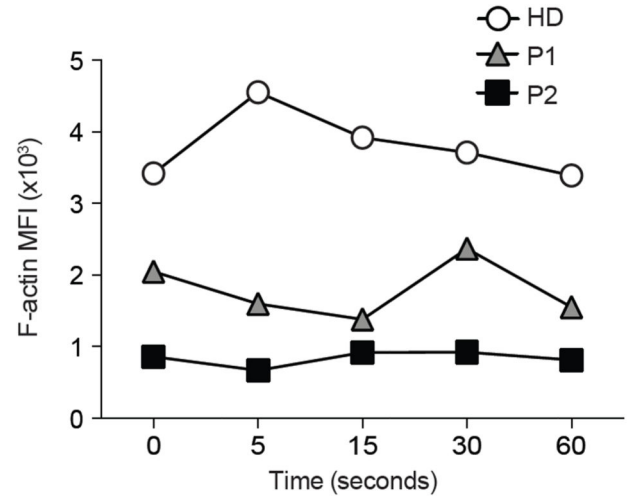
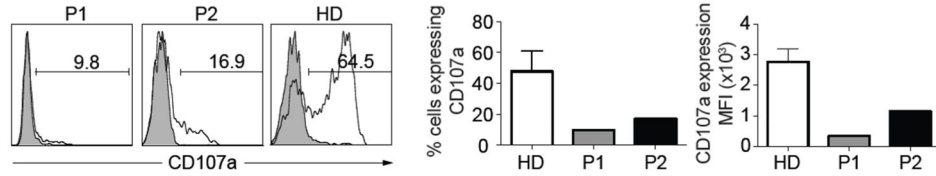


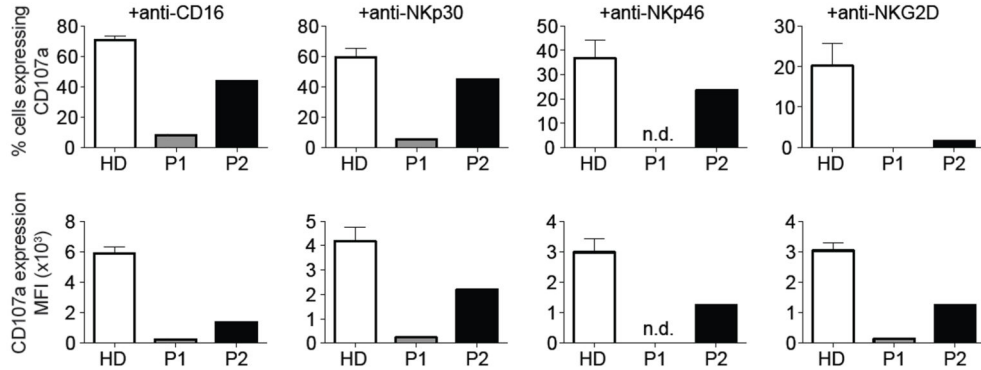
Figure 2. Defective RAC1 Activation, Lymphocyte Migration and Actin Polymerization in DOCK2-deficient Patients

Panel A shows impaired RAC1 activation in T-cell lines from P1 and P2 as compared to a healthy donor (HD) and P2’s mother (P2M) upon stimulation of the T-cell receptor using anti-CD3 monoclonal antibodies, comparison done with one-way ANOVA. ***, $p < 0.001$; **, $p < 0.01$; horizontal bars represent means; error bars represent means \pm s.d. of three independent experiments. Panel B shows reduced T- and B-cell migration in response to CCL21 or CXCL12 in P1 (grey bar) and P2 (black bar) as compared to a healthy control (white bar). NS, not stimulated. Panels C and D show reduced levels of polymerized, filamentous actin (F-actin) in P1 and P2 as revealed by phalloidin staining in T and B cells, respectively. MFI – mean fluorescence intensity.

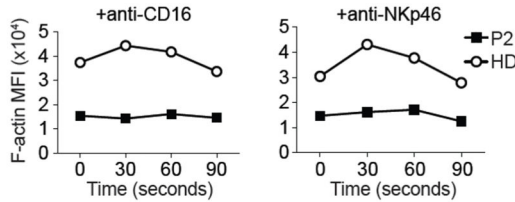
A NK-cell degranulation upon stimulation with K562 cells



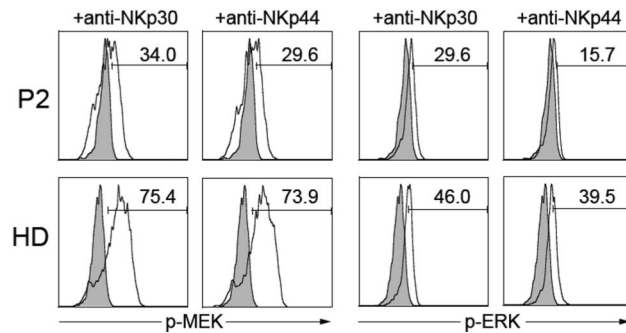
B NK-cell degranulation upon triggering activating NK-cell receptors



C Actin polymerization upon triggering activating NK-cell receptors



D MEK/ERK phosphorylation



E IFN-γ expression upon IL-12/IL-18 stimulation

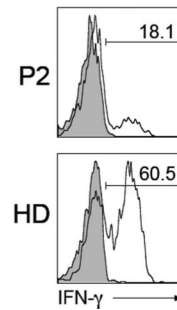


Figure 3. NK Cell Cytotoxicity and Signaling Defects in DOCK2-deficient Patients

Panel A shows impaired NK-cell degranulation (monitored by CD107a surface expression) upon stimulation with K562 cells in P1 and P2 as compared to healthy donors (HD, n=10), horizontal bars represent means; error bars represent means ± s.d. Panel B shows defective degranulation upon triggering activating NK-cell receptors in P1 and P2 as compared to six healthy donors, horizontal bars represent means; error bars represent means ± s.d. n.d., not done. Panel C shows impaired actin polymerization upon triggering activating NK-cell receptors CD16 (left) and NKp46 (right). Panel D shows impaired MEK/ERK

phosphorylation in P2's NK cells upon triggering activating receptors, as detected by flow cytometry. Panel E shows decreased IFN- γ production upon P2's NK cell stimulation with IL-12 and IL-18.

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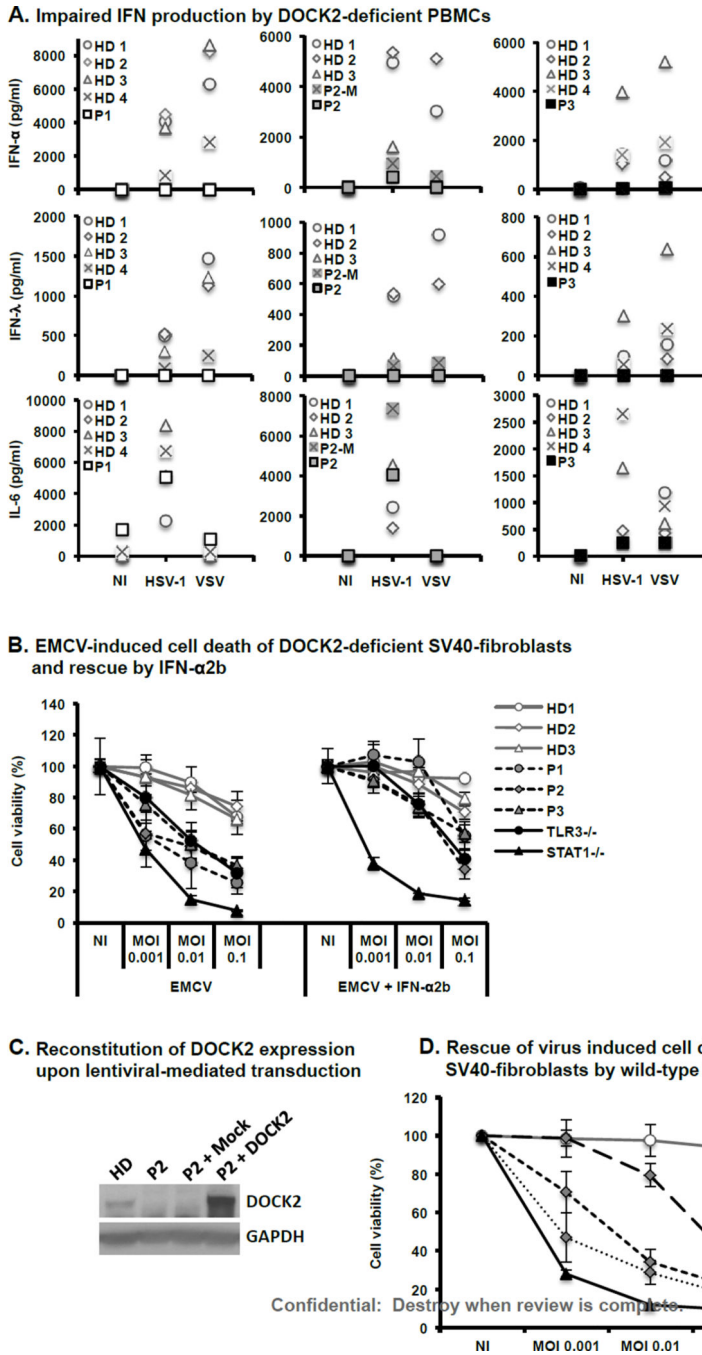


Figure 4. Impaired IFN Responses to Viruses in DOCK2-deficient Leukocytes and Fibroblasts
 Panel A shows impaired IFN- α and IFN- β production by DOCK2-deficient peripheral blood mononuclear cells (PBMC) upon exposure to either herpes simplex virus 1 (HSV-1) or vesicular stomatitis virus (VSV) for 24 hours each. By contrast, production of IL-6 by the patients' PBMCs was comparable to the healthy donor (HD) PBMCs, and served as assay control. NI, not infected. Panel B shows increased EMCV-induced cell death of DOCK2-deficient SV40-fibroblasts (left panel) and rescue by the addition of IFN- α 2b (right panel). TLR3- and STAT1-deficient SV40-immortalized fibroblast cell lines served as examples of

defective IFN-dependent anti-viral immunity. Panel C shows immunoblot analysis of protein lysates obtained from SV40-fibroblasts derived from a healthy donor (HD) and P2, as well as from P2's mock-transduced and DOCK2-transduced fibroblasts. Panel D shows rescue of EMCV-induced cell death of P2 SV40-fibroblasts by exogenous expression of wild-type *DOCK2*. STAT1-deficient fibroblasts were used as negative control. The data in panels B and D depict one representative out of three independent experiments with technical triplicates carried out in each experiment. MOI, multiplicity of infection.

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Table 1

Immunological data of DOCK2-deficient patients

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at evaluation	5 mo	2.5 y	6.3 y	1 y	4 mo
ALC (cells/mm ³ × 10 ⁻³)	1.22 [3.9–9.0]	1.24 [2.3–5.4]	1.1 [1.9–3.7]	2.3 [3.6–8.9]	4.4 [3.9–9.0]
CD3 ⁺ T cells/mm ³	114 [2500–5600]	548 [1400–3700]	341 [1200–2600]	1173 [2100–6200]	830 [2500–5600]
CD4 ⁺ T cells/mm ³	57 [1800–4000]	305 [700–2200]	176 [650–1500]	129 [1300–3400]	511 [1800–4000]
CD45RA ⁺ CCR7 ⁺	4.0% [76.7–91.4]	0.4% [65.2–84.8]	n.d.	n.d.	4.5% [76.7–91.4]
CD45RA ⁻ CCR7 ⁺	16.0% [6.7–15.6]	19.2% [10.5–23.2]	n.d.	n.d.	33.8% [6.7–15.6]
CD45RA ⁻ CCR7 ⁻	78.0% [1.1–5.3]	79.8% [2.9–9.8]	n.d.	n.d.	44.5% [1.1–5.3]
CD45RA ⁺ CCR7 ⁻	12.0% [0.1–1.9]	0.6% [0.2–3.0]	n.d.	n.d.	17.2% [0.1–1.9]
CD8 ⁺ T cells/mm ³	24 [590–1600]	133 [490–1300]	187 [370–1100]	763 [620–2000]	319 [590–1600]
CD45RA ⁺ CCR7 ⁺	8.5% [62.1–94.0]	1.6% [39.0–89.0]	n.d.	n.d.	22% [62.1–94.0]
CD45RA ⁻ CCR7 ⁺	14.7% [0.9–5.6]	5.2% [0.9–5.7]	n.d.	n.d.	26.3% [0.9–5.6]
CD45RA ⁻ CCR7 ⁻	72.1% [1.3–19.5]	74.0% [3.4–28.2]	n.d.	n.d.	9.8% [1.3–19.5]
CD45RA ⁺ CCR7 ⁻	4.7% [1.5–22.7]	19.2% [4.8–30.0]	n.d.	n.d.	41.9% [1.5–22.7]
CD19 ⁺ B cells/mm ³	646 [430–3000]	146 [390–1400]	473 [270–860]	299 [720–2600]	1857 [430–3000]
CD16 ⁺ 56 ⁺ cells/mm ³	191 [170–830]	489 [130–720]	187 [100–480]	138 [180–920]	1539 [170–830]
IgG (mg/dL)	350 [172–814]	788 [424–1051]	559 [633–1280]	821 ^a [217–904]	413 [164–558]
IgA (mg/dL)	77 [8.1–84]	422 [14–123]	131 [33–202]	192 ^a [11–90]	50 [4.4–73]
IgM (mg/dL)	24 [33–108]	37 [48–168]	82 [48–207]	31 ^a [34–126]	170 [27–101]
IgE (IU/ml)	n.d.	2704 [0.3–29.5]	41 [1–161.3]	5.28 ^a [0.8–7.3]	26 ^a [0.4–3.8]
Ab responses	n.d.	non protective to TT, PRP, <i>S. pneumoniae</i>	no Ab response to VZV	undetectable to HBV	undetectable to KLH
TRECs (at birth)	n.d.	<252 copies/uL ^b	n.d.	<252 copies/uL ^b	n.d.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Response to PHA	2.6 [38.4] ^c	0.1 [278.2] ^c	9 [46–89] ^d	18 [52–94] ^d	6.04 [289.49] ^c

^a measured at 9 months of life;

^b cut-off value for newborn screening for SCID in Massachusetts. Normal amplification of the RNaseP reference gene was detected in dried blood spots from both P2 and P4;

^c measured as SI;

^d measured as % of CD25⁺ cells; n.d. not done; PHA, phytohemagglutinin; TRECs: T-cell receptor excision circles.

Serum samples from patients were tested for antibodies against tetanus toxoid (TT), poliovirus type 1 (PV1), varicella zoster virus (VZV) and Hepatitis B Virus (HBV), keyhole limpet hemocyanin (KLH) and pneumococcal vaccine PCV10 (Synflorix®).