Defective actin accumulation impairs human natural killer cell function in patients with dedicator of cytokinesis 8 deficiency

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Background: Dedicator of cytokinesis 8 (DOCK8) mutations are responsible for a rare primary combined immunodeficiency syndrome associated with severe cutaneous viral infections, increased IgE levels, autoimmunity, and malignancy. Natural killer (NK) cells are essential for tumor surveillance and defense against virally infected cells. NK cell function relies on Wiskott-Aldrich syndrome protein for filamentous actin (F-actin) accumulation at the lytic NK cell immunologic synapse. DOCK8 activates cell division cycle 42, which, together with Wiskott-Aldrich syndrome protein, coordinates F-actin reorganization. Although abnormalities in T- and B-cell function have been described in DOCK8-deficient patients, the role of NK cells in this disease is unclear.

Objectives: We sought to understand the role of DOCK8 in NK cell function to determine whether NK cell abnormalities explain the pathogenesis of the clinical syndrome of DOCK8 deficiency.

Methods: A cohort of DOCK8-deficient patients was assembled, and patients' NK cells, as well as NK cell lines with stably reduced DOCK8 expression, were studied. NK cell cytotoxicity, F-actin content, and lytic immunologic synapse formation were measured.

Results: DOCK8-deficient patients' NK cells and DOCK8 knockdown cell lines all had decreased NK cell cytotoxicity, which could not be restored after IL-2 stimulation. Importantly, DOCK8 deficiency impaired F-actin accumulation at the lytic immunologic synapse without affecting overall NK cell F-actin content.

Conclusions: DOCK8 deficiency results in severely impaired NK cell function because of an inability to form a mature lytic immunologic synapse through targeted synaptic F-actin accumulation. This defect might underlie and explain important attributes of the DOCK8 deficiency clinical syndrome, including the unusual susceptibility to viral infection and malignancy. (J Allergy Clin Immunol 2013;131:840-8.)

Key words: *DOCK8 deficiency, natural killer cells, actin, cytotoxicity, immunologic synapse*

Patients with autosomal recessive hyper-IgE syndrome (HIES) can be affected by recalcitrant cutaneous herpes virus and papillomavirus infections, as well as immune dysregulation.[1,2] The major genetic abnormality found in these patients is deletion or loss-of-function mutations in the gene encoding dedicator of cytokinesis 8 (DOCK8).[3,4] Mutagenized mice with an autoimmune phenotype were also identified as possessing DOCK8 mutations.[5] Both DOCK8-deficient patients and mice are documented to have combined immunodeficiency of the B- and T-cell compartments.[5-8] However, the high incidence of recurrent cutaneous viral infections distinguishes them from other patients with combined immunodeficiencies, and a mechanistic explanation for this phenotype is lacking. Currently, the only definitive therapy for patients with DOCK8 deficiency is hematopoietic stem cell transplantation.[9-11]

One immunologic characteristic found in patients with susceptibility to herpesviruses and papillomaviruses is abnormalities

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Abbreviations used
Cdc42: Cell division cycle 42
DOCK8: Dedicator of cytokinesis 8
F-actin: Filamentous actin
HIES: Hyper-IgE syndrome
IS: Immunologic synapse
MTOC: Microtuble organizing center
NK: Natural killer
shRNA: Short-hairpin RNA
STAT3: Signal transducer and activator of transcription 3
WAS: Wiskott-Aldrich syndrome
WASp: Wiskott-Aldrich syndrome protein

of natural killer (NK) cells.[12,13] This is illustrated by a number of human NK cell deficiency states, NK cell–depleted murine models, and some of the specific aspects of virology.[13-15] NK cells are lymphocytes of the innate immune system that can inherently recognize many virally infected or malignantly transformed cells, especially those that escape the adaptive T-cell response caused by downregulated MHC (as in herpesvirus infections). NK cells have not been previously evaluated in patients with DOCK8 deficiency other than absolute numbers, which were normal.[1,3,4] Specifically, functional assessments of NK cells in patients with DOCK8 deficiency have not been reported.

DOCK8 is a member of the DOCK180 superfamily of atypical guanine-nucleotide exchange factors[16,17] and activates cell division cycle 42 (Cdc42), a Rho GTPase critical for reorganization of the filamentous actin (F-actin) cytoskeleton in NK and dendritic cells.[18,19] Cdc42 functions along with Wiskott-Aldrich syndrome (WAS) protein (WASp) to regulate NK cell function.[20] Defects in WASp are associated with the well-known primary immunodeficiency WAS, and these patients exhibit several characteristics shared with patients with DOCK8 deficiency, including susceptibility to cutaneous viral infections and malignancy. NK cells isolated from patients with WAS are functionally deficient.[20-22] Given the clinical phenotype of patients with DOCK8 and its integral role in Cdc42 activation, we hypothesized that there was a substantive defect in NK cell function in patients with DOCK8 deficiency.

METHODS

Patients and human subjects

Ten patients with DOCK8 mutations were given diagnoses based on either targeted gene sequence or genomic approaches. Patients DOCK8-4, DOCK8-5, DOCK8-8, and DOCK8-9 have previously been reported.[4,23,24] There were 2 sibling pairs, patients DOCK8-4 and DOCK8-5 and patients DOCK8-3 and DOCK8-6, respectively. Three patients with signal transducer and activator of transcription 3 (STAT3) deficiency were included as disease control subjects. Blood samples from patients and healthy donors were obtained after patient-provided or parental informed consent under the approval of the local Institutional Review Board for the Protection of Human Subjects of the Children's Hospital of Philadelphia, Baylor College of Medicine, or Ludwig-Maximilians-Universität, Munich, Germany.

NK cells and cell lines

PBMCs or *ex vivo* NK cells were isolated from whole blood by means of centrifugation through Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ) with or without negative selection by using the RosetteSep human NK cell enrichment cocktail (STEMCELL Technologies, Vancouver, British Columbia, Canada), as previously described.[25,26] *Ex vivo* NK cells and PBMCs were used immediately after preparation or cryopreserved for later use, and purity was determined by means of flow cytometry. The human NK cell line YTS was used to establish the *in vitro* DOCK8 knockdown model. Briefly, HEK293T cells were cotransfected with lentiviral packaging plasmids (pPACK; System Biosciences, Mountain View, Calif) and pLKO.1 lentiviral vector with puromycin resistance and either DOCK8 or scrambled DOCK8 sequence short-hairpin RNA (shRNA; MissionRNAi; Sigma-Aldrich, St Louis, Mo) to produce replication-incompetent viral particles, which were used to infect YTS cells. 721.221 B-lymphoblastoid, K562 erythroleukemia, and K562 cells stably expressing CD86 (KT86)[27] were used as target cells and maintained as previously described.

Cytotoxicity

Standard ^{51}Cr-release assays were used to measure the cytolytic activity of PBMCs isolated from human samples and NK cell lines, as previously described.[21] Patients DOCK8-1 and DOCK8-4 were assessed 3 distinct times. Controls included both shipping and local donor control blood samples. Higher effector/target cell ratios were used for PBMCs than for YTS cell lines. Lytic units were calculated, as previously described.[21]

Flow cytometry

NK cells among PBMCs were quantified by means of flow cytometry, as previously described,[21] with fluorophore-conjugated mAbs (BD Biosciences, San Jose, Calif) for CD56 and CD3. NK cell F-actin content was also measured by means of flow cytometry using our published methods.[22,27] Patients DOCK8-1 and DOCK8-4 were assessed 3 distinct times.

Microscopy and image analysis

NK cells from patients and control donors, as well as YTS cells, were prepared for evaluation of fixed effector/target cell conjugates by using immunofluorescence microscopy, as previously described,[22,27,28] and images were acquired with a Zeiss-Z1 microscope outfitted with a Yokogawa CSU10 spinning disc and 63× 1.45 NA objective. Images were acquired and quantified by using Volocity software (PerkinElmer, Waltham, Mas), and data were exported to GraphPad Prism software (GraphPad Software, La Jolla, Calif). Previously published quantitative algorithms were applied to measure F-actin and CD18 accumulation[28] and pericentrin distance from the immunologic synapse (IS).[27]

Quantitative PCR

RNA was harvested with the Qiagen RNeasy Mini kit (Qiagen, Hilden, Germany) and reverse transcribed into cDNA by using the TaqMan Reverse Transcription kit (Applied Biosystems, Foster City, Calif). DOCK8 expression was analyzed by using quantitative PCR (7900HT Analyzer, Applied Biosystems) with forward (5′-ACGCGCCGTGTAACTGTGAA-3′) and reverse (5′-CCCCGAGCTCCTGGGCAA-3′) primers, as previously reported,[3] with each assay performed in triplicate. Expression of glyceraldehyde-3-phosphate dehydrogenase (forward 5′-CTCATTTCCTGGTATGACAA CG-3', reverse 5′-TTACTCCTTGGAGGCCATGT-3′) was used as a control for normalization.

Statistics

Data were compared by using unpaired Student 2-tailed *t* tests or exact Wilcoxon-Mann-Whitney *U* tests, with significance defined as a *P* value of less than .05.

RESULTS

DOCK8-deficient patients have impaired NK cell cytotoxicity that is not rescued by IL-2 stimulation

We collected an international cohort of 10 DOCK8-deficient patients to examine NK cell function. Our patients ranged from