Somatic reversion in dedicator of cytokinesis 8 immunodeficiency modulates disease phenotype

Huie Jing, PhD,[a] Qian Zhang, MD,[a] Yu Zhang, PhD,[a] Brenna J. Hill, PhD,[b] Christopher G. Dove, BS,[a] Erwin W. Gelfand, MD,[c] T. Prescott Atkinson, MD, PhD,[d] Gulbu Uzel, MD,[e] Helen F. Matthews, RN,[f] Peter J. Mustillo, MD,[g] David B. Lewis, MD,[h] Fotini D. Kavadas, MD,[i] I. Celine Hanson, MD,[j] Ashish R. Kumar, MD, PhD,[k] Raif S. Geha, MD,[l] Daniel C. Douek, MD, PhD,[b] Steven M. Holland, MD,[e] Alexandra F. Freeman, MD,[e] and Helen C. Su, MD, PhD[a] *Bethesda, Md, Denver, Colo, Birmingham, Ala, Columbus and Cincinnati, Ohio, Stanford, Calif, Calgary, Alberta, Canada, Houston, Tex, and Boston, Mass*

Background: Autosomal recessive loss-of-function mutations in dedicator of cytokinesis 8 *(DOCK8)* cause a combined immunodeficiency characterized by atopy, recurrent infections, and cancer susceptibility. A genotype-phenotype explanation for the variable disease expression is lacking.
Objective: We investigated whether reversions contributed to the variable disease expression.
Methods: Patients followed at the National Institutes of Health's Clinical Center were studied. We performed detailed genetic analyses and intracellular flow cytometry to detect DOCK8 protein expression within lymphocyte subsets.
Results: We identified 17 of 34 DOCK8-deficient patients who had germline mutations with variable degrees of reversion caused by somatic repair. Somatic repair of the *DOCK8* mutations resulted from second-site mutation, original-site mutation, gene conversion, and intragenic crossover. Higher degrees of reversion were associated with recombination-mediated repair. DOCK8 expression was restored primarily within antigen-experienced T cells or natural killer cells but less so in naive T or B cells. Several patients exhibited multiple different repair events. Patients who had reversions were older and had less severe allergic disease, although infection susceptibility persisted. No patients were cured without hematopoietic cell transplantation.
Conclusions: In patients with DOCK8 deficiency, only certain combinations of germline mutations supported secondary somatic repair. Those patients had an ameliorated disease course with longer survival but still had fatal complications or required hematopoietic cell transplantation. These observations support the concept that some DOCK8-immunodeficient patients have mutable mosaic genomes that can modulate disease phenotype over time. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Dedicator of cytokinesis 8, reversion, somatic repair, recombination, gene conversion, intragenic single crossover, T cell, natural killer cell, allergy, immunodeficiency*

Dedicator of cytokinesis 8 (DOCK8) immunodeficiency is caused by autosomal recessive mutations in the *DOCK8* gene, which encodes an atypical guanine-nucleotide exchange factor for cell division control 42 homolog (CDC42) and Ras-related C3 botulinum toxin substrate activation (RAC).[1,2] Initially described as a hyper-immunoglobulinemia E syndrome, this combined immunodeficiency features atopy, recurrent cutaneous and sinopulmonary infections, and cancer susceptibility.[3] Typically, patients have diffuse eczematous dermatitis with bacterial skin infections early in life, along with respiratory tract infections and severe food allergies accompanied by anaphylaxis, asthma, increased serum IgE levels, and eosinophilia. Intractable viral infections of the skin are caused by herpes simplex virus, molluscum contagiosum virus, varicella-zoster virus, and/or human papillomavirus.[4] Mucocutaneous candidiasis can also occur. Death from infections or cancers usually occurs by late adolescence or early adulthood. However, in some patients the disease course is more aggressive, with severe skin disease and life-threatening infections developing at an earlier age.[5,6] Furthermore, patients have been identified who lack atopic dermatitis, food allergies, increased serum IgE levels, and/or eosinophilia. Because known pathogenic mutations in DOCK8 cause loss of protein expression, a molecular explanation for the phenotypic variability remains lacking.

Loss of DOCK8 expression within T cells, B cells, natural killer (NK) cells, and NKT cells can cause abnormal cytokine production, including T_H2 skewing, as well as defects in

From [a]the Laboratory of Host Defenses and [e]the Laboratory of Clinical Infectious Diseases, and [f]the Laboratory of Immunology, Division of Intramural Research, and [b]the Human Immunology Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda; [c]the Division of Allergy and Immunology, Department of Pediatrics, Division of Cell Biology, National Jewish Health, Denver; [d]the Department of Pediatrics, University of Alabama at Birmingham; [g]the Division of Infectious Diseases and Immunology, Nationwide Children's Hospital, Columbus; [h]the Department of Pediatrics, Division of Immunology, Allergy, and Rheumatology, Stanford University; [i]the Section of Clinical Immunology and Allergy, Department of Pediatrics, Alberta Children's Hospital and University of Calgary; [j]the Section of Allergy and Immunology, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston; [k]the Cancer and Blood Diseases Institute, Division of Bone Marrow Transplantation and Immune Deficiency and Department of Pediatrics, Cincinnati Children's Hospital Medical Center and University of Cincinnati; and [l]the Division of Immunology and Department of Pediatrics, Children's Hospital and Harvard Medical School, Boston.

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Corresponding author: Helen C. Su, MD, PhD, Laboratory of Host Defenses, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg 10CRC, Rm 5W3940, 10CRC Center Dr, MSC 1456, Bethesda, MD 20892-1456. E-mail: hsu@niaid.nih.gov.

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Abbreviations used
DOCK8: Dedicator of cytokinesis 8
HCT: Hematopoietic cell transplantation
NK: Natural killer
SNP: Single nucleotide polymorphism
TCR: T-cell receptor

activation, proliferation, survival, affinity maturation, and cytotoxicity.[1-3,7-12] T cells play a major role in disease pathogenesis because the infection susceptibility is cured by hematopoietic cell transplantation (HCT) when nearly complete donor T-cell chimerism is achieved, even when other leukocyte subsets are of partial donor origin.[13,14] HCT also cures or significantly ameliorates atopic dermatitis, food allergies, increased serum IgE levels, and hypereosinophilia.[13,15-17] However, the minimal level and type of T-cell reconstitution required for cure, as well as the relative contributions of other lymphocytes, are unknown.

Naturally arising somatic reversions of germline mutations have been observed in several primary immunodeficiency disorders, including Wiskott-Aldrich syndrome, severe combined immunodeficiencies, and X-linked lymphoproliferative disease.[18-20] Such cases have provided insights into the relative contributions of loss-of-function mutations in different cell types. Here we sought to determine the circumstances by which reversions occurred in DOCK8 immunodeficiency and whether they could explain phenotypic differences among patients.

METHODS

Patients and their relatives provided written informed consent and were investigated under National Institute of Allergy and Infectious Diseases (NIAID) Institutional Review Board–approved research protocols. Patients 2, 3, 4, 5, 13, 18, and 21 were previously reported as 8-2, 4-1, 4-2, 5-2, 6-1, 2-1, and 1-1, respectively.[1] Patient 1 was reported as ARH011.3.[2] Patients 9, 10, 11, 19, 22, 23, 24, and 27 were also reported elsewhere.[4,11,21] The median ages of patients were calculated from the age of living patients at the most recent evaluation at the National Institutes of Health or when undergoing transplantation or age at the time of death for deceased patients. Disease severity was scored according to the criteria listed in Table E1 in this article's Online Repository at www.jacionline.org. Primers used in this study are listed in Table E2 in this article's Online Repository at www.jacionline.org.

Detailed procedures regarding cell preparation, array comparative hybridization, immunoblotting, flow cytometry, sequencing, and statistical analyses are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Identification of patients who had somatically repaired their germline *DOCK8* mutations

DOCK8 immunodeficiency is caused by autosomal recessive loss-of-function mutations in the *DOCK8* gene.[1,2] We have followed 34 DOCK8-deficient patients from 23 families at the Clinical Center of the National Institutes of Health. Seventeen patients from 11 families formed the core of this study. Clinical diagnoses of DOCK8 immunodeficiency were confirmed by means of mutational analyses showing germline loss-of-function mutations in both *DOCK8* alleles (Table I, columns 3 and 5; Fig 1; and see Figs E1-E8 in this article's Online Repository at www.jacionline.org).

DOCK8-deficient patients normally express no DOCK8 protein in lysates from purified primary T cells (Fig 2, *A*, left panel). As expected, patients also expressed no DOCK8 in B cells (Fig 2, *A*, middle panel) or monocytes (Fig 2, *A*, right panel). However, in some patients normal or near-normal levels of DOCK8 were detected in primary T cells (Fig 2, *A*, middle and right panels). The discrepancy between germline mutations and actual protein expression suggested somatic mosaicism occurring within T cells. The germline mutations had been identified by sequencing genomic DNA from neutrophils. When we compared these against mutational analyses performed on primary T cells and, in some cases, NK cells, we discovered somatic repair in 17 patients (Table I, column 4).

Somatic repair could be categorized into one of 3 groups. In the first group somatic repair resulted from point mutations, which corrected for germline-encoded deleterious single-base substitutions. Patients 1 and 3 had second-site mutations (Fig 1, *A*, left panel, and see Fig E1), whereas patient 4 had an original-site mutation (Fig 1, *A*, right panel). These abolished use of the germline-encoded cryptic splice site or premature stop codon. Patients 2, 5, 6, 7, and 8 were obligate for either a second-site mutation or original-site mutation.

In the second group somatic repair resulted from recombination-mediated gene conversion. For example, in patients 10 and 11, genotyping of single nucleotide polymorphisms (SNPs) throughout the *DOCK8* gene indicated which portions of the *DOCK8* alleles were derived from each parent (Fig 1, *B*, and see Fig E4). In DNA from primary T cells, the paternally inherited large deletion was present, but the maternally inherited indel was absent. Furthermore, maternal SNPs upstream of the deletion were also absent. Thus we inferred that gene conversion repaired the indel on the maternally inherited allele by using the intact undeleted portion of the paternally inherited allele. Gene conversion was likely responsible for somatic repair in T cells from patients 14 and 17 (see Figs E7 and E8).

In the third group somatic repair resulted from recombination-mediated intragenic single crossover. For example, analysis of genomic DNA from primary T cells of patient 9 showed that both maternally and (presumed) paternally inherited mutations and SNPs were present throughout the entire *DOCK8* gene (Fig 1, *C*). However, when sequencing was performed after cloning PCR-amplified regions of cDNA prepared from primary T cells, neither the indel nor the missense mutation was detected. A single wild-type transcript was present with the 5′ portion containing nonmaternal SNPs and the 3′ portion containing maternal SNPs. Thus we inferred that an intragenic single crossover event generated a new allele that lacked both mutations while simultaneously generating a second new allele that contained both mutations and underwent nonsense-mediated decay. Intragenic single crossover was also responsible for somatic repair in T cells from patients 12 (Fig E5) and 16 (Fig E8) and probably patient 13 (Fig E6).

To summarize, 17 DOCK8-immunodeficient patients had somatic mosaicism, which resulted from repair of germline *DOCK8* mutations through compensatory point mutations or recombination. Recombination-mediated gene conversion or intragenic crossover occurred in all patients from our cohort who had a germline mutation on 1 allele plus an intact region corresponding to this mutation on the other allele (Table I, column 2). By contrast, in patients with overlapping deletions on both alleles, repair was not possible and was not observed (see Table E3 in this article's Online Repository at www.jacionline.org and data not shown).