Dedicator of cytokinesis 8–deficient patients have a breakdown in peripheral B-cell tolerance and defective regulatory T cells

Erin Janssen, MD, PhD,[a,b]* Henner Morbach, MD,[c]* Sumana Ullas, MS,[a] Jason M. Bannock, MA,[c] Christopher Massad, BS,[c] Laurence Menard, PhD,[c] Isil Barlan, MD,[d] Gerard Lefranc, PhD,[e] Helen Su, MD, PhD,[f] Majed Dasouki, MD,[g] Waleed Al-Herz, MD,[h] Sevgi Keles, MD,[a,i] Talal Chatila, MD,[a,b] Raif S. Geha, MD,[a,b]‡ and Eric Meffre, PhD[c]‡ *Boston, Mass, New Haven, Conn, Istanbul and Konya, Turkey, Montpellier, France, Bethesda, Md, Kansas City, Kan, and Kuwait City, Kuwait*

Background: Dedicator of cytokinesis 8 (DOCK8) deficiency is typified by recurrent infections, increased serum IgE levels, eosinophilia, and a high incidence of allergic and autoimmune manifestations.
Objective: We sought to determine the role of DOCK8 in the establishment and maintenance of human B-cell tolerance.
Methods: Autoantibodies were measured in the plasma of DOCK8-deficient patients. The antibody-coding genes from new emigrant/transitional and mature naive B cells were cloned and assessed for their ability to bind self-antigens. Regulatory T (Treg) cells in the blood were analyzed by means of flow cytometry, and their function was tested by examining their capacity to inhibit the proliferation of $CD4^+CD25^-$ effector T cells.
Results: DOCK8-deficient patients had increased levels of autoantibodies in their plasma. We determined that central B-cell tolerance did not require DOCK8, as evidenced by the normally low frequency of polyreactive new emigrant/transitional B cells in DOCK8-deficient patients. In contrast, autoreactive B cells were enriched in the mature naive B-cell compartment, revealing a defective peripheral B-cell tolerance checkpoint. In addition, we found that Treg cells were decreased and exhibited impaired suppressive activity in DOCK8-deficient patients.
Conclusions: Our data support a critical role for DOCK8 in Treg cell homeostasis and function and the enforcement of peripheral B-cell tolerance. (J Allergy Clin Immunol 2014;134:1365-74.)

Key words: *Dedicator of cytokinesis 8, autoimmunity, B-cell tolerance, regulatory T cells*

Dedicator of cytokinesis 8 *(DOCK8)* has been identified as the major causative gene in patients with autosomal recessive hyper-IgE syndromes.[1,2] DOCK8 deficiency is associated with atopic dermatitis, asthma, food allergies, an unusual susceptibility to viral mucocutaneous infections, T-cell lymphopenia, reduced proliferative T-cell responses, and impaired antibody responses.[1,2] In addition, DOCK8-deficient patients are prone to autoimmune disease, including autoimmune hemolytic anemia, vasculitis, colitis, and hypothyroidism.[2-6]

B-cell autoimmunity has been linked to defects in the central and/or peripheral B-cell tolerance checkpoints involved in the elimination of autoreactive B cells.[7] The central B-cell tolerance checkpoint occurs in the bone marrow, where autoreactive immature B cells are silenced by receptor editing, anergy, or deletion,[8-10] and relies on signaling through the B-cell receptor (BCR)[11,12] and Toll-like receptors (TLRs).[13] Defects in central B-cell tolerance have been identified in patients with Bruton tyrosine kinase deficiency, which impairs BCR signaling,[11] as well as IL-1 receptor–associated kinase 4, myeloid differentiation response gene 88, and transmembrane activator deficiencies, which abrogate the function of most TLRs.[13,14] B-cell autoreactivity in the periphery is controlled by regulatory T (Treg) cells.[15] This is illustrated by the abundance of autoreactive mature naive B cells in patients with mutations in the Treg cell master transcription factor forkhead box P3 (FOXP3)[16] and in patients with CD40 ligand and class II major histocompatibility deficiency who display low Treg cell numbers.[17]

Here we show that DOCK8 deficiency is associated with increased production of autoantibodies, a defective peripheral B-cell tolerance checkpoint, and quantitative and qualitative deficiencies in Treg cells.

From [a]the Division of Immunology, Boston Children's Hospital; [b]the Department of Pediatrics, Harvard Medical School, Boston; [c]the Department of Immunobiology, Yale University School of Medicine, New Haven; [d]Marmara University, Istanbul; [e]IMGT, University Montpellier, and CNRS Institute of Human Genetics, Montpellier; [f]the Human Immunological Diseases Unit, National Institutes of Health, Bethesda; [g]the Department of Pediatrics and Department of Internal Medicine, Division of Genetics, Endocrinology & Metabolism, University of Kansas Medical Center, Kansas City; [h]the Department of Pediatrics, Faculty of Medicine, Kuwait University; and [i]the Division of Pediatric Immunology and Allergy, Meram Medical Faculty, Necmettin Erbakan University, Konya.

*These authors contributed equally to this work.

‡These authors are equal senior authors.

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Corresponding authors: Raif S. Geha, MD, One Blackfan St, Boston Children's Hospital, Boston, MA, 02115. E-mail: raif.geha@childrens.harvard.edu. Or: Eric Meffre, PhD, Yale University School of Medicine, 300 George St, New Haven, CT 06511. E-mail: eric.meffre@yale.edu.

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Abbreviations used
ANA: Anti-nuclear antibody
BAFF: B-cell activating factor
BCR: B-cell receptor
DOCK8: Dedicator of cytokinesis 8
dsDNA: Double-stranded DNA
FOXP3: Forkhead box P3
HD: Healthy donor
HSCT: Hematopoietic stem cell transplantation
IL-7Rα: IL-7 receptor α
Teff: Effector T
TLR: Toll-like receptor
Treg: Regulatory T

METHODS

Patients and control subjects

Twenty-two DOCK8-deficient patients were enrolled in this study. The patients' sex, age, and homozygous *DOCK8* mutations are shown in Table I. All patients lacked detectable DOCK8 expression, as determined by means of immunoblotting. Blood was obtained either during evaluation at Boston Children's Hospital or received within 48 hours of collection. Healthy donors (HDs) included 8 shipping control subjects. Study participants were recruited by using written informed consent approved by the local institutional review boards.

TABLE I. Homozygous mutations in DOCK8-deficient patients

Patient no.	*DOCK8* mutation*	Sex	Age (y)
1	c.[1-?_2891+?del]+[1-?_2891+?del]	F	12
2	c.[1-?_528+?del]+[1-?_528+?del]	F	6.5
3	c.[1-?_528+?del]+[1-?_528+?del]	M	0.5
4	c.[1-?_528+?del]+[1-?_528+?del]	M	6
5	c.[1-?_528+?del]+[1-?_528+?del]	F	1.5
6	c.[1-?_528+?del]+[1-?_528+?del]	F	3.5
7	c.[4181T>A]+c.[4181T>A] Ser1357X	M	14
8	c.[1-?_528+?del]+[1-?_528+?del]	F	4.5
9	c.[4999-?_5191+?del]+[4999-?_5191+?del]	F	16
10	c.[1-?_268+?del]+[1-?_268+?del]	F	10
11	c.[3503-?_3642+?del]+[3503-?_3642+?del]	M	6
12	c.[1398-?_1534+?del]+[1398-?_1534+?del]	F	5
13	c.[3503-?_6412*del]+[3503-?_6412*del]	F	4
14	c.[1-?_528+?del]+[1-?_528+?del]	F	12
15	c.[2360G>T]+c.[2360G>T] Glu750X	M	3
16	c.[5244C>G]+c.[5244C>G] Ser1711X	F	4
17	c.[1641_1642del2]+c.[1641_1642del2]	F	7
18	c.[1641_1642del2]+c.[1641_1642del2]	M	9
19	c.[2318-3C>G]+c.[2318C>G]	F	5
20	c.[1-?_2222+?del]+[1-?_2222+?del]	M	7
21	c.[1641_1642del2]+c.[1641_1642del2]	M	3.5
22	c.[2360G>T]+c.[2360G>T] Glu750X	F	10

F, Female; *M*, male.
*The reference coding sequence is accession number AB191037.1.

Autoantibody and cytokine analysis

PBMCs were isolated by using a Ficoll gradient. Plasma was analyzed for autoantibodies with the University of Texas Southwestern microarray of 84 autoantigens.[18] Data were normalized to a fold increase over HDs. Heat maps were generated by using the Multiple Experiment Viewer (version 4.9.0).[19] Anti-nuclear antibodies (ANAs; Genway Biotech, San Diego, Calif), double-stranded DNA (dsDNA) antibodies (Alpha Diagnostics, San Antonio, Tex), and B-cell activating factor (BAFF) concentrations (R&D Systems, Minneapolis, Minn) were measured according to the manufacturers' directions. Plasma was diluted at 1:40, and HEp-2 cell slides were stained according to the manufacturer's directions (Antibodies, Davis, Calif), and nuclei were stained with 4′-6-diamidino-2-phenylindole dihydrochloride (Life Technologies, Grand Island, NY).

Cell sorting, RT-PCR, antibody production, and ELISA

B cells were purified from PBMCs by means of positive selection with CD20 magnetic beads (Miltenyi Biotec, Cambridge, Mass). Single $CD19^+CD10^+IgM^{hi}CD21^{lo}CD27^-$ new emigrant/transitional and $CD19^+CD10^-IgM^+CD21^+CD27^-$ mature naive B cells were sorted on a FACSAria flow cytometer (BD Biosciences, San Jose, Calif) into 96-well PCR plates. Reverse transcription of cDNA, RT-PCR reactions, primer sequences, cloning strategy, expression vectors, antibody expression, antibody purification, and recombinant antibody reactivity determination were performed, as previously described.[7] A highly polyreactive antibody (ED38)[7,20] was used as a positive control in HEp-2 reactivity and polyreactivity ELISAs. Antibodies were considered polyreactive when they recognized all 3 analyzed antigens: dsDNA, insulin, and LPS. The reactivity of purified recombinant antibodies was also tested on HEp-2 cell–coated slides (Bion Enterprise, Des Plaines, Ill) by using indirect immunofluorescence.

B-cell staining and stimulation

$CD20^+$ purified B cells were stained for surface markers by using fluorochrome-labeled antibodies against CD19, CD27, CD10, CD69, CD86, CD25 (BioLegend, San Diego, Calif), and CD21 (BD Biosciences). In stimulation experiments B cells were plated at 150,000 to 200,000 cells per well in a 96-well plate in RPMI with 10% FBS and 2 μg/mL polyclonal $F(ab)'_2$ rabbit anti-human IgM (Jackson Immunoresearch, West Grove, Pa), 2 μg/mL Gardiquimod (TLR7 agonist; InvivoGen, San Diego, Calif), or 0.5 μg/mL CpG (TLR9 agonist, InvivoGen) for 48 hours.

Treg cell staining

PBMCs were stained for the surface markers CD3, CD4, CD25, CD127, and CD45RO by using fluorochrome-labeled antibodies (eBioscience, San Diego, Calif, and BioLegend) and then permeabilized with a FOXP3 permeabilizaton kit according to the manufacturer's instructions (eBioscience) and stained with antibodies against FOXP3 (eBioscience), Ki-67 (BioLegend), or the appropriate isotype control (eBioscience).

Treg cell suppression assays

$CD4^+$ T cells were isolated by means of negative selection with magnetic beads (Miltenyi Biotec). For isolation of $CD4^+CD25^-$ effector T (Teff) cells, $CD25^+$ cells were depleted with anti-CD25 beads (Miltenyi Biotec). Treg cells were isolated by sorting for $CD4^+CD25^+CD127^-$ cells. Teff cells were labeled with CellTrace Violet or carboxyfluorescein succinimidyl ester (Life Technologies) and stimulated with anti-CD3–, anti-CD28–, and anti-CD2–coated beads (Treg Suppression Inspector, human; Miltenyi Biotec), and autologous or allogeneic Treg cells were added at a ratio of 1:1; cell divisions were evaluated by using flow cytometry.

Statistical analysis

Comparisons between DOCK8-deficient patients and HD control subjects were analyzed for statistical significance with unpaired Student *t* tests (GraphPad Prism; GraphPad Software, La Jolla, Calif).

RESULTS

Increased autoantibody production in DOCK8-deficient patients

DOCK8-deficient patients (n = 12) had significantly higher levels of IgG antibodies against 14 of 84 autoantigens tested

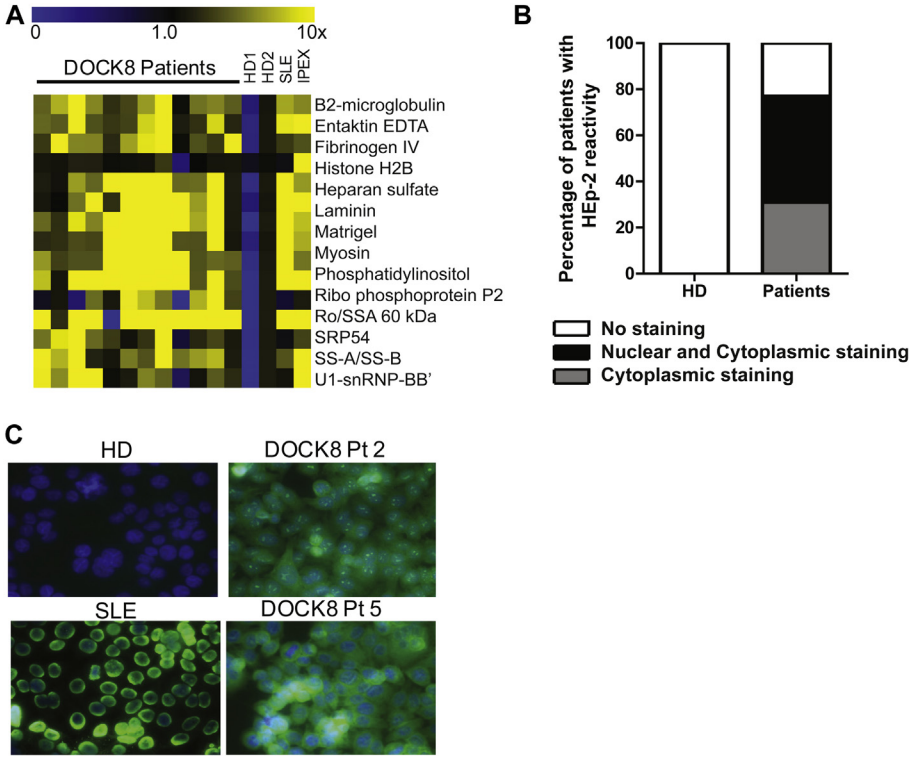

FIG 1. Autoantibodies are present in DOCK8-deficient patients. **A,** Heat map of the reactivity of IgG antibodies against self-antigens in 12 DOCK8-deficient patients, 2 HD control subjects, a patient with systemic lupus erythematosus *(SLE)*, and a patient with Foxp3 deficiency (immunodysregulation–polyendocrinopathy–enteropathy–X-linked syndrome *[IPEX]*). Only the 14 autoantigens for which binding was significantly higher ($P < .05$) in the 12 DOCK8-deficient patients compared with the HDs are shown. The colors represent the fold increase relative to HD control subjects. **B,** HEp-2 cell reactivity of plasma from 14 DOCK8-deficient patients and 7 HDs. **C,** Representative photomicrographs of HEp-2 cells incubated with plasma from 2 DOCK8-deficient patients, an HD, and a patient with systemic lupus erythematosus. *Green* represents bound IgG antibodies, and *blue* 4′-6-diamidino-2-phenylindole dihydrochloride staining shows the nucleus.

compared with HD control subjects (Fig 1, *A*) and a high frequency of reactivity against many of the other autoantigens in the panel (see Fig E1 in this article's Online Repository at www.jacionline.org). Antibodies binding to cytoplasmic and extracellular matrix antigens predominated over antibodies binding to nuclear antigens. Plasma from 11 of 14 DOCK8-deficient patients but none of 7 HDs reacted with HEp-2 cells (Fig 1, *B*). Reactivity was directed against cytoplasmic proteins, although weak nuclear reactivity was present in some patients (Fig 1, *B* and *C*). The levels of ANAs and dsDNA antibodies were not increased in the patients' sera (see Fig E2 in this article's Online Repository at www.jacionline.org). Thus autoantibody production to cytoplasmic antigens is characteristic of DOCK8 deficiency.

DOCK8-deficient patients have an increase in activated B-cell numbers

$CD19^+CD27^-CD10^-CD21^+$ mature naive B cells represent the vast majority of B cells in DOCK8-deficient patients.[21,22] Mature naive B cells from the patients expressed significantly higher levels of the activation marker CD69 in comparison with HD control subjects (Fig 2, *A* and *B*), whereas CD86 and CD25 expression was comparable (Fig 2, *A* and *B*). The patients displayed an expansion of $CD19^+CD27^-CD10^-CD21^{-/lo}$ B cells, which are enriched in patients with autoimmunity and express autoreactive BCR (Fig 2, *C* and *D*).[23-25] Hence DOCK8-deficient patients harbor partially activated B cells and have an expansion of B cells known to contain autoreactive clones.[23-25]

Central B-cell tolerance is intact in DOCK8-deficient patients

DOCK8-deficient B cells responded normally to BCR, TLR7, and TLR9 stimulation by upregulating CD69 and CD86 expression, suggesting that pathways sensing self-antigens might be effective in the absence of functional DOCK8 (see Fig E3 in this article's Online Repository at www.jacionline.org). To assess the integrity of the central B-cell tolerance checkpoint in patients with DOCK8 deficiency, we analyzed the reactivity of antibodies expressed by single $CD19^+CD10^{++}CD21^{lo}IgM^{hi}CD27^-$ new emigrant/transitional B cells isolated from the PBMCs of 3 DOCK8-deficient patients and compared them with those from 11 HD control subjects. Antibody-coding genes were cloned from the sorted B cells, expressed *in vitro*, and tested for polyreactivity against dsDNA, insulin, and LPS, as previously reported.[7] The frequencies of polyreactive and ANA reactive clones in new emigrant/transitional B cells from DOCK8-deficient patients were low and similar to those in control subjects (Fig 3).[7,20] Antibody sequences from new emigrant/transitional B cells from patients and control subjects did not reveal any statistical differences in variable heavy CDR3 length, the

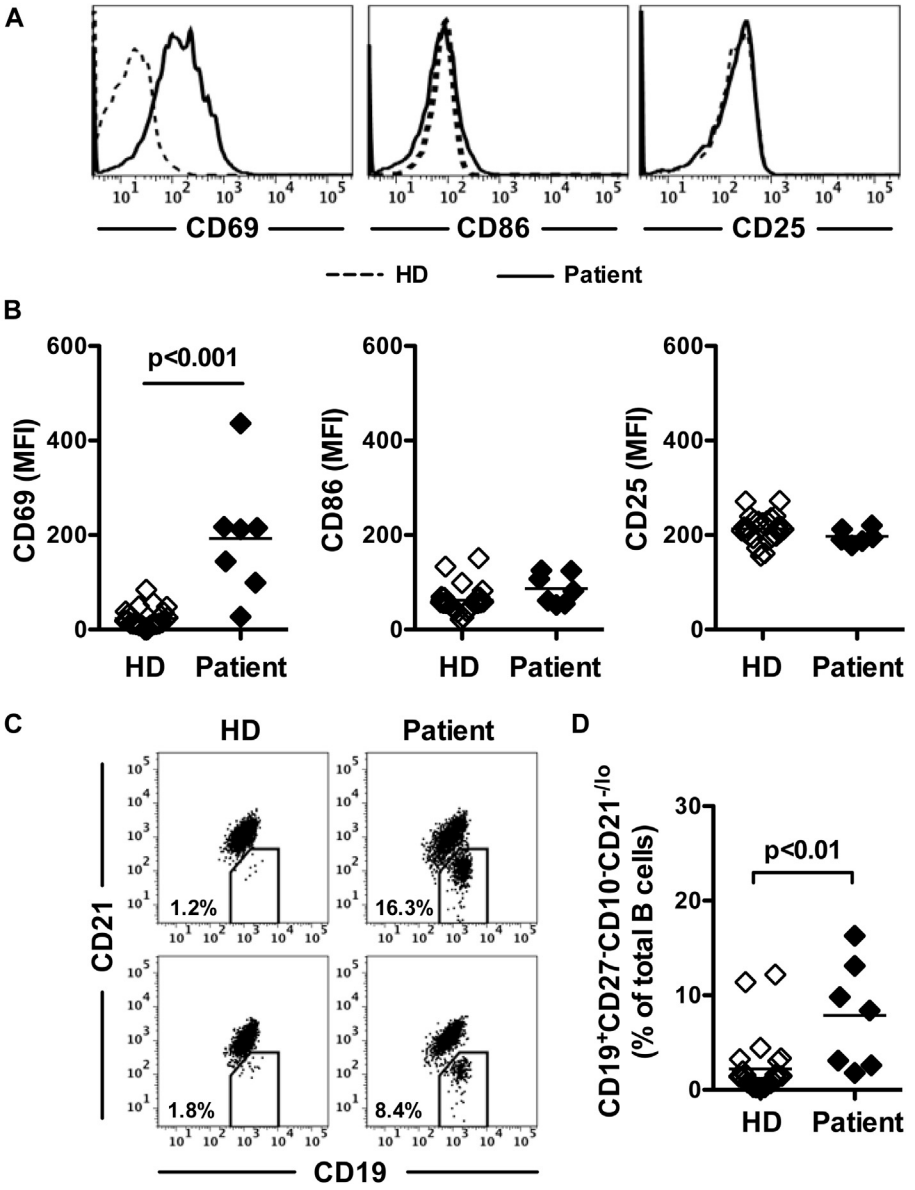

FIG 2. Mature naive B cells from DOCK8-deficient patients have an activated phenotype. **A,** Representative CD69, CD86, and CD25 expression profiles of $CD19^+CD27^-CD21^+$ mature naive B cells from a DOCK8-deficient patient and a representative control subject. **B,** Mean fluorescence intensities *(MFI)* of CD69, CD86, and CD25 expression on mature naive B cells from 7 DOCK8-deficient patients and 25 HD control subjects. **C,** Representative flow cytometric plots for $CD19^+CD21^{-/lo}$ B cells for 2 DOCK8-deficient patients and 2 HDs. **D,** $CD19^+CD27^-CD10^-CD21^{-/lo}$ B cells as a percentage of total B cells in 7 DOCK8-deficient patients and 25 HD control subjects.

frequency of positively charged amino acids, or use of the self-reactive *IGHV4-34* gene (see Tables E1-E3 and Fig E4, *A*, in this article's Online Repository at www.jacionline.org and data not shown), 3 features often associated with abnormal central B-cell tolerance.[17,26] Thus central B-cell tolerance is functional in DOCK8-deficient patients.

DOCK8-deficient patients display a defect in peripheral B-cell tolerance

To test the peripheral B-cell tolerance checkpoint, we examined the reactivity of recombinant antibodies from fluorescence-activated cell sorting–sorted single $CD19^+CD10^-CD21^+IgM^+CD27^-$ mature naive B cells against HEp-2 cell lysates and the sequences of their antibody-coding genes.[7] The frequency of mature naive B cells that expressed HEp-2–reactive antibodies was significantly increased in the DOCK8-deficient patients (35.3% to 40%) compared with that seen in the HDs (17% to 26%, $P < .0001$; Fig 4, *A* and *B*). Immunofluorescent staining of HEp-2 cells with recombinant antibodies from these B cells showed predominantly cytoplasmic staining (Fig 4, *C*). DOCK8-deficient patients did not have an increased frequency of ANA-reactive mature naive B cells but did have a significant increase in polyreactive mature naive B-cell numbers compared with those seen in control subjects ($P < .0001$; Fig 4, *D-F*, and see Fig E5 in this article's Online Repository at www.jacionline.org). This is consistent with our

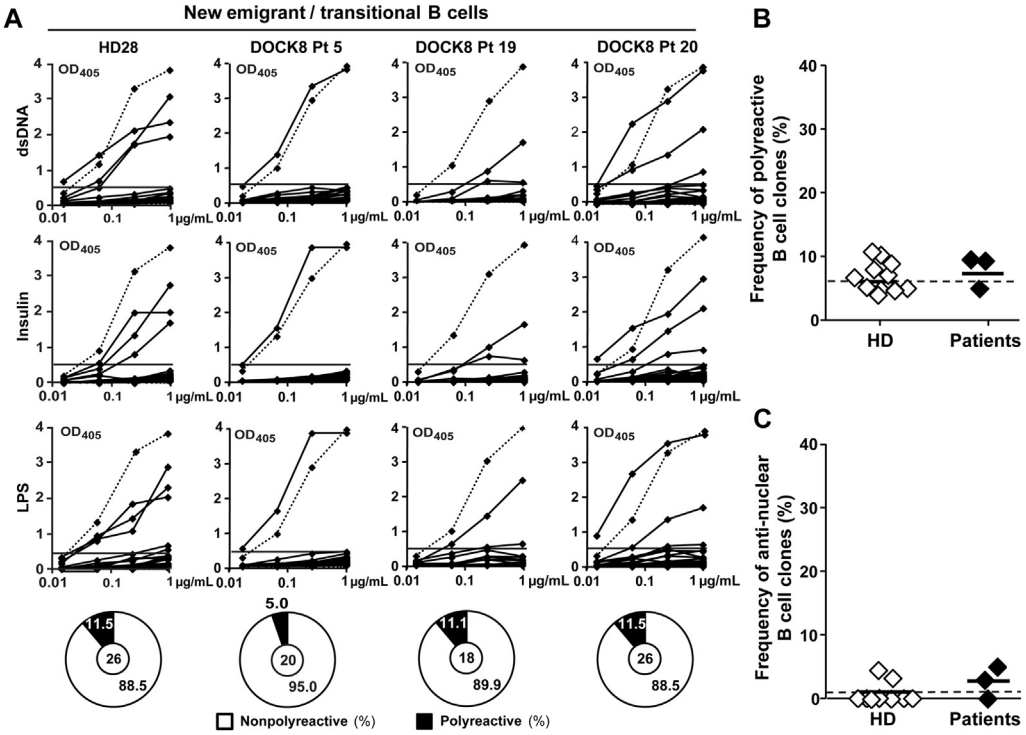

FIG 3. The central B-cell tolerance checkpoint is intact in DOCK8-deficient patients. **A,** Antibodies cloned from new emigrant/transitional B cells from a representative HD control subject (HD28) and 3 DOCK8-deficient patients (Table I; patients 5, 19, and 20) were tested by means of ELISA for reactivity against dsDNA, insulin, and LPS. *Dotted lines* show the ED38$^+$ control,[7,20] and *solid lines* show binding for each cloned recombinant antibody. *Horizontal lines* define the cutoff for positive reactivity. For each subject, the frequency of polyreactive *(solid area)* and nonpolyreactive *(open area)* clones is summarized in a pie chart, with the total number of clones tested indicated in each center. **B,** Frequency of polyreactive new emigrant/transitional B cells in 3 DOCK8-deficient patients and 11 HD control subjects. **C,** Normal low frequency of anti-nuclear antigen–reactive new emigrant/transitional B cells in 3 DOCK8-deficient patients and 9 HD control subjects. The *solid line* represents the mean for each group, and the *dotted line* is the mean for HD control subjects in Fig 3, *B* and *C*.

previous observation that low and nonspecific dsDNA reactivity detected in the polyreactivity assay is rarely associated with ANA reactivity.[27] Analysis of the antibody repertoire revealed increased use of the self-reactive *IGHV4-34* gene (see Tables E4-E6 and Fig E4, *B*, in this article's Online Repository at www.jacionline.org).[17,26] These findings demonstrate a defective peripheral B-cell tolerance checkpoint in the absence of DOCK8.

Treg cells are decreased in DOCK8-deficient patients

Increased serum concentrations of the B-cell survival factor BAFF[28,29] have been observed in patients with autoimmune conditions.[30,31] BAFF plasma concentrations were not significantly increased in DOCK8-deficient patients (see Fig E6 in this article's Online Repository at www.jacionline.org). In contrast, the proportion of CD3$^+$CD4$^+$CD25^{hi}CD127^{lo}FOXP3$^+$ Treg cells among CD4$^+$ cells was significantly decreased in DOCK8-deficient patients compared with that seen in control subjects (Fig 5, *A* and *B*). An altered Treg cell phenotype was further evidenced by significantly decreased expression of CD127 and a reduced percentage of CD45RO$^+$ cells (Fig 5, *C*, *E*, and *F*). Non-Treg cells from DOCK8-deficient patients also exhibited decreased expression of CD127/IL-7 receptor α (IL-7Rα) and CD45RO$^+$ memory T cells (Fig 5, *C*, *E*, and *F*).

CD45RO$^+$ Treg cells that coexpress Ki67 might represent cells that have been previously activated, express proinflammatory cytokines, and display reduced suppressive capabilities.[16,32-34] A higher proportion of CD45RO$^+$ Treg cells coexpressed Ki67 in DOCK8-deficient patients than in HDs (see Fig E7 in this article's Online Repository at www.jacionline.org). Ki67 expression was also significantly higher in CD4$^+$ non-Treg cells from DOCK8-deficient patients indicative of increased Teff cell activation (see Fig E7). These findings suggest that Treg cell function might be impaired in the absence of functional DOCK8.

Treg cell suppressive activity is defective in DOCK8-deficient patients

We examined Treg cell function by testing the ability of CD4$^+$CD25^{hi}CD127^{lo} T cells to suppress the proliferation of CD4$^+$CD25$^-$ Teff cells when stimulated with beads coated with CD3, CD28, and CD2 mAbs. DOCK8-deficient Treg cells had a significantly decreased ability to suppress Teff cell proliferation compared with Treg cells from HD control subjects (Fig 6, *A* and *B*). Teff cells from DOCK8-deficient patients and HDs were similarly suppressed by normal Treg cells, indicating that DOCK8-deficient Teff cells are not resistant to suppression (Fig 6, *C*). Thus Treg cells are quantitatively and qualitatively defective in DOCK8-deficient patients.

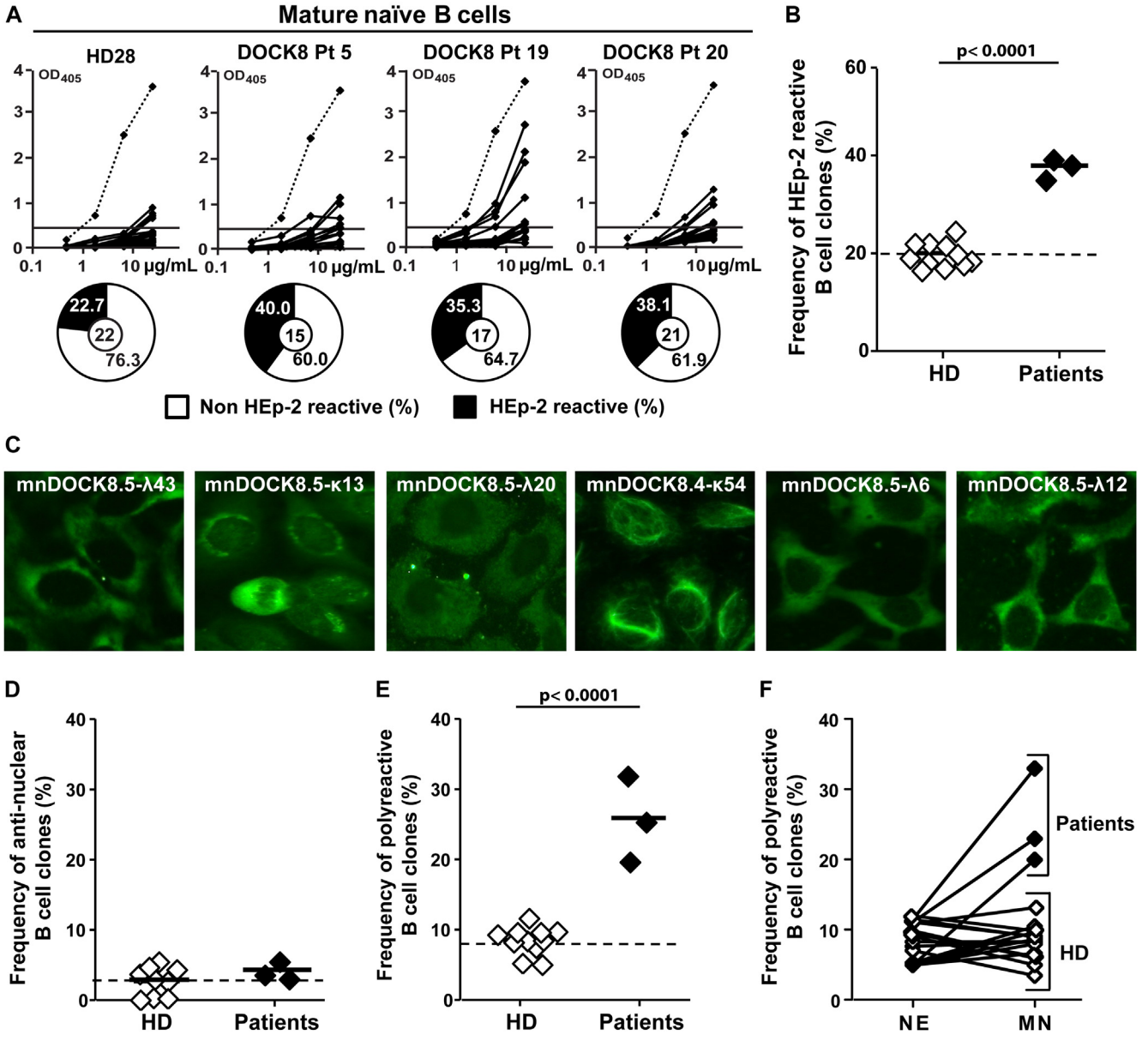

FIG 4. The peripheral B-cell tolerance checkpoint is defective in DOCK8-deficient patients. **A,** Antibodies from mature naive B cells from a representative HD and 3 DOCK8-deficient patients (Table I; patients 5, 19, and 20) were tested by means of ELISA for anti–HEp-2 cell reactivity. *Dotted lines* show ED38$^+$ control,[7,20] and *solid lines* show binding for each cloned recombinant antibody. *Horizontal lines* define cutoffs for positive reactivity. For each subject, the frequency of HEp-2–reactive *(solid area)* and non–HEp-2–reactive *(open area)* clones is summarized in pie charts, with the total number of clones tested indicated in the centers. **B,** Frequencies of HEp-2–reactive mature naive B cells in 3 DOCK8-deficient patients and 11 HD control subjects. **C,** Patterns of cytoplasmic HEp-2 staining by autoreactive antibodies from mature naive B-cell clones from DOCK8-deficient patients. **D,** Normal low frequencies of anti-nuclear mature naive B cells in 9 HD control subjects and 3 DOCK8-deficient patients. **E,** Frequencies of polyreactive mature naive B cells in 11 HD control subjects and 3 DOCK8-deficient patients. **F,** The evolution of polyreactivity between the new emigrant/transitional *(NE)* and mature naive *(MN)* B-cell compartments is represented. The *solid line* represents the mean for each group, and the *dotted line* is the mean for HD control subjects in Fig 4, *B*, *D*, and *E*.

DISCUSSION

Here we report that DOCK8-deficient patients produce autoreactive antibodies and experience defective peripheral B-cell tolerance associated with decreased Treg cell numbers and function.

Despite their young age, all 12 of the DOCK8-deficient patients studied had circulating autoantibodies, directed mostly against cytoplasmic proteins. However, we were unable to detect increased titers of dsDNA and nuclear autoantibodies in their plasma. The secretion of dsDNA autoantibodies relies on the activation of B cells by TLR9, which bind dsDNA.[35,36] Although some TLR9 functions are preserved in the absence of DOCK8, as illustrated by the normal upregulation of activation markers, disruption of a TLR9–DOCK8–PYK2–signal transducer and

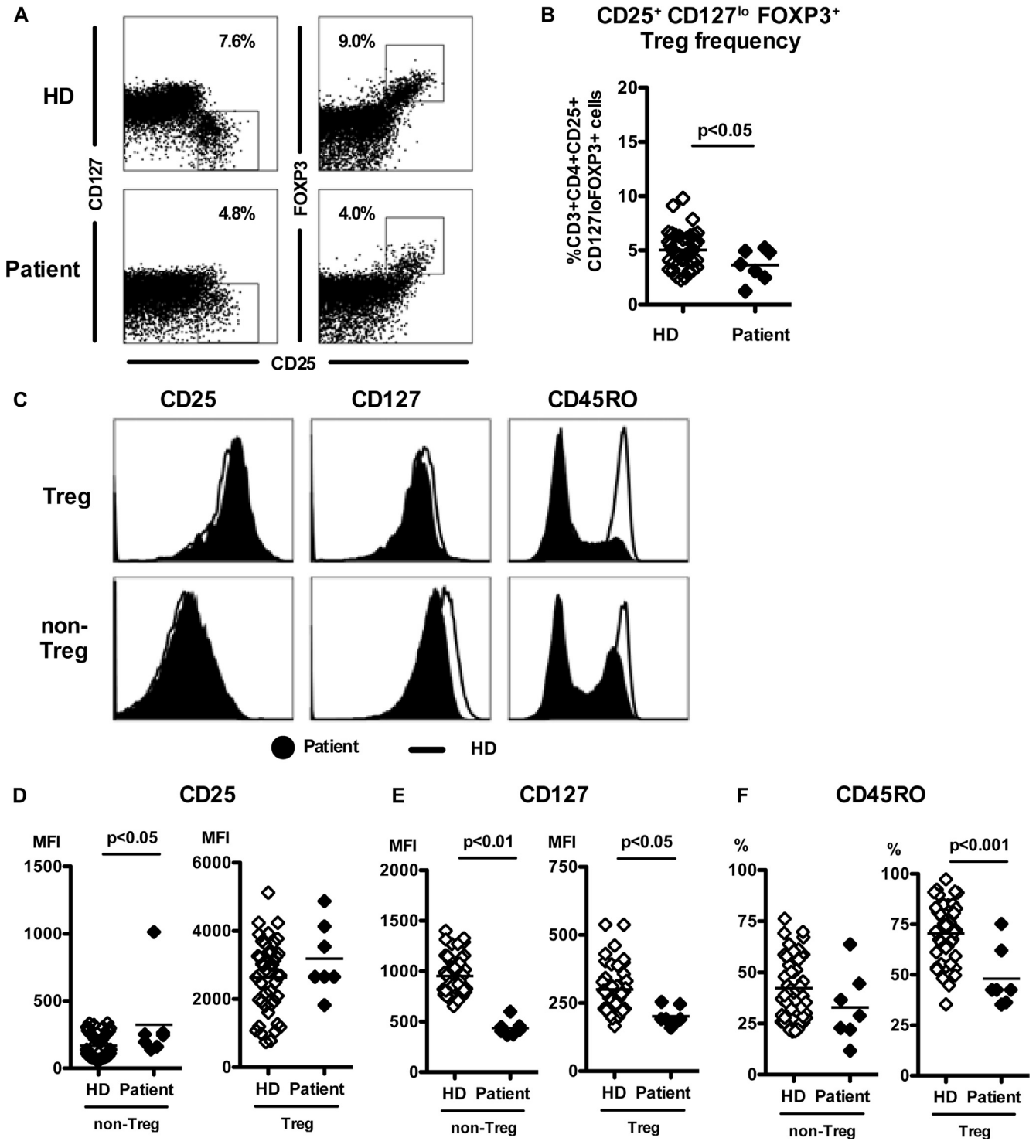

FIG 5. DOCK8-deficient patients have decreased Treg cell numbers. **A,** Dot plots showing the analysis of gated $CD25^{hi}CD127^{lo}$ *(left)* and $CD25^{hi}FOXP3^{+}$ *(right)* $CD4^{+}$ T cells of a representative HD control subject and a DOCK8-deficient patient. **B,** Decreased frequency of $CD4^{+}CD25^{hi}CD127^{lo}FOXP3^{+}$ Treg cells in 7 DOCK8-deficient patients compared with 45 HD control subjects. Each *symbol* represents a subject, and *horizontal bars* display means. **C,** Expression levels of CD25, CD127, and CD45RO on $CD4^{+}$ Treg cells from a representative DOCK8-deficient patient *(solid black)* and HD *(bold).* The mean fluorescence intensities *(MFI)* of CD25 **(D)** appear normal, whereas CD127 expression **(E)** and the frequency of $CD45RO^{+}$ cells **(F)** are decreased in both non-Treg and Treg cells from DOCK8-deficient patients.

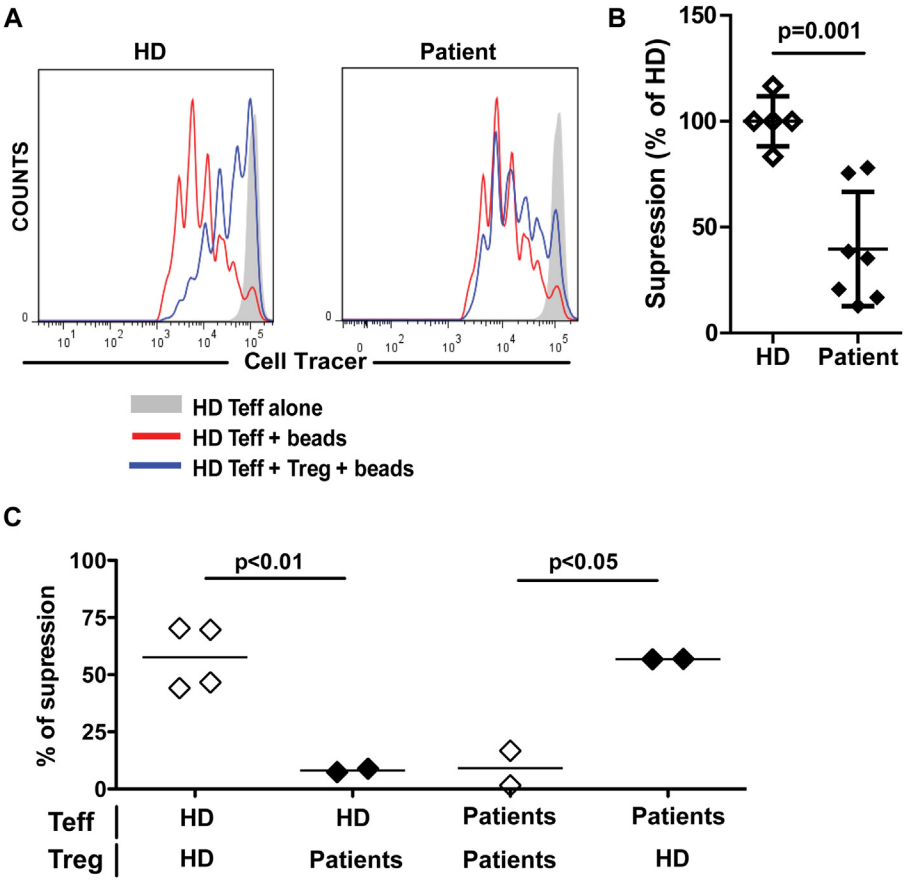

FIG 6. DOCK8-deficient Treg cells have reduced suppressive activity. **A,** $CD4^+CD25^-$ Teff cells from an HD were loaded with CellTrace Violet and cocultured with $CD4^+CD25^+CD127^{lo}$ Treg cells from an HD *(left panel)* and a DOCK8-deficient patient *(right panel)*. Cells were stimulated with anti-CD3, CD28, and CD2 beads. On day 4, proliferative profiles of the labeled Teff cells were analyzed by means of flow cytometry. **B,** Suppressive activity of Treg cells from 7 DOCK8-deficient patients relative to that of Treg cells from 5 HD shipping control subjects. **C,** Proliferation of Teff cells from DOCK8-deficient patients can be suppressed by HD Treg cells. Teff cells ($CD4^+CD25^-$) from 2 DOCK8-deficient patients were loaded with carboxyfluorescein succinimidyl ester and cocultured with either autologous $CD4^+CD25^+CD127^-$ Treg cells or Treg cells from a shipped HD control subject.

activator of transcription 3 signaling pathway important for B-cell proliferation and immunoglobulin production[21] might partially explain the absence of DNA antibodies in DOCK8-deficient patients.

DOCK8-deficient patients had intact central B-cell tolerance, as evidenced by a normal frequency of autoreactive emigrant/transitional B cells. In contrast, DOCK8-deficient patients had an increase in autoreactive B-cell numbers in the mature naive B-cell compartment, indicating a breakdown in peripheral B-cell tolerance. This was associated with increased surface expression of CD69, but not CD25 or CD86, on mature naive B cells.[16] The increase in numbers of $CD69^+$ activated B cells could be secondary to impaired Treg cell function, might be associated with chronic microbial stimulation, or both. DOCK8-deficient patients also showed an expansion of $CD19^+CD27^-CD10^-CD21^{-/lo}$ autoreactive B cells, a finding reported in some patients with common variable immunodeficiency displaying an abnormal peripheral B-cell tolerance checkpoint.[23] The breakdown of the peripheral B-cell tolerance checkpoint in DOCK8 deficiency was not associated with increased plasma BAFF concentrations but was connected with a decrease in the percentage of $CD25^+CD127^{lo}FOXP3^+$ Treg cells among circulating $CD4^+$ cells.

Treg cells, as well as other non-Treg T cells, in DOCK8-deficient patients displayed decreased memory $CD45RO^+$ expression associated with downregulated CD127/IL-7Rα expression. It is unclear how mutations in DOCK8 affect CD127/IL-7Rα expression, but the IL-7 signaling pathway is an important regulator of T-cell homeostasis and is essential for the development and maintenance of memory T cells.[37] There was increased expression of Ki67 in the patients' $CD45RO^+$ Treg cells, a feature indicative of Treg cell activation associated with proinflammatory cytokine production and reduced suppressive capabilities.[16,32-34] On a cell-per-cell basis, the suppressive activity of purified $CD4^+CD25^{hi}CD127^{lo}$ Treg cells was significantly impaired in DOCK8-deficient patients. These findings strongly suggest that the defective peripheral B-cell tolerance checkpoint in these patients is likely explained by quantitative and qualitative defects in Treg cells. Whether the same mechanism impairs Treg cell and T_H17 cell

function[38] in DOCK8-deficient patients requires further investigation.

Numeric and functional deficiencies in Treg cells in DOCK8-deficient patients likely lead to the abrogation of peripheral B-cell tolerance and autoantibody production at an early age. The development of overt autoimmune disease is likely regulated by modifier genes, triggered by recurrent infections, or both. The standard of care for the treatment of DOCK8-deficient patients is hematopoietic stem cell transplantation (HSCT).[39-45] HSCT is expected to restore the Treg cell compartment; however, autoantibody-producing plasma cells might persist for a long time after HSCT because they are relatively resistant to the immunosuppressive regimens used in the conditioning of the recipients.[46,47] Our findings should prompt the evaluation of DOCK8-deficient patients for autoantibodies and the possible emergence of autoimmunity and end-organ damage, even after HSCT.

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Clinical implications: DOCK8-deficient patients should be evaluated for autoantibodies, the possible emergence of autoimmunity, and end-organ damage.

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