Published in final edited form as:
J Allergy Clin Immunol. 2014 December ; 134(6): 1365–1374. doi:10.1016/j.jaci.2014.07.042.

DOCK8 deficient patients have a breakdown in peripheral B cell tolerance and defective regulatory T cells

Erin Janssen, MD, PhD[1,2,†], **Henner Morbach, MD**[3,†], **Sumana Ullas, MS**[1], **Jason M. Bannock, MA**[3], **Christopher Massad, BS**[3], **Laurence Menard, PhD**[3], **Isil Barlan, MD**[4], **Gerard Lefranc, PhD**[5], **Helen Su, MD, PhD**[6], **Majed Dasouki, MD**[7], **Waleed Al-Herz, MD**[8], **Sevgi Keles, MD**[1,9], **Talal Chatila, MD**[1,2], **Raif S. Geha, MD**[1,2,#], and **Eric Meffre, PhD**[3,#]

[1]Division of Immunology, Boston Children's Hospital, Boston, MA [2]Department of Pediatrics, Harvard Medical School, Boston, MA [3]Department of Immunobiology, Yale University School of Medicine, New Haven, CT [4]Marmara University, Istanbul, Turkey [5]IMGT, University Montpellier and CNRS Institute of Human Genetics, Montpellier, France [6]Human Immunological Diseases Unit, National Institute of Health, Bethesda, Maryland [7]Department of Pediatrics and Department of Internal Medicine, Division of Genetics, Endocrinology & Metabolism, University of Kansas Medical Center, Kansas City, KS [8]Department of Pediatrics, Faculty of Medicine, Kuwait University, Kuwait [9]Division of Pediatric Immunology and Allergy, Meram Medical Faculty, Necmettin Erbakan University, Konya, Turkey

Abstract

Background—Dedicator of Cytokinesis 8 (DOCK8) deficiency is typified by recurrent infections, elevated 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 flow cytometry, and their function was tested by examining their capacity to inhibit the proliferation of $CD4^{+}CD25^{-}$ T effector (Teff) 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 normal low

Correspondence: Raif S. Geha, One Blackfan Street, Boston Children's Hospital, Boston, MA, 02115, USA, Tel: 617-919-2482; Fax: 617-730-0528, raif.geha@childrens.harvard.edu. Eric Meffre, Yale University School of Medicine, 300 George Street, New Haven, CT 06511, USA, Tel: +1-203-737-4535; Fax: +1-203-785-7903, eric.meffre@yale.edu.
[†]EJ and HM contributed equally to this work.
[#]RSG and EM are equal senior authors.

frequency of polyreactive new emigrant/transitional B cells in DOCK8 deficient patients. In contrast, autoreactive B cells were enriched in the mature naïve 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.

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

Keywords

Dedicator of Cytokinesis 8; autoimmunity; B cell tolerance; regulatory T cells

INTRODUCTION

DOCK8 has been identified as the major causative gene in 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 develop 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 (BM) 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 BTK deficiency, which impairs BcR signaling [11], as well as IRAK4, MyD88, and TACI 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 naïve B cells in patients who have mutations in the Treg cell master transcription factor forkhead box P3 (FOXP3) [16], and in patients with CD40L 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.

METHODS

Patients and controls

Twenty two DOCK8 deficient patients were enrolled in this study. The patients' gender, age, and homozygous *DOCK8* mutations are shown in Table I. All patients lacked detectable DOCK8 expression by immunoblotting. Blood was obtained either during evaluation at Boston Children's Hospital or received within 48 hours of collection. Healthy donors (HD)

included 8 shipping controls. Study participants were recruited using written informed consent approved by the local Institutional Review Boards.

Autoantibody and cytokine analysis

Peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll gradient. Plasma was analyzed for autoantibodies using the University of Texas Southwestern microarray of 84 autoantigens [18]. Data was normalized to fold increase over HD. Heat maps were generated using Multiple Experiment Viewer (version 4.9.0) [19]. Anti-nuclear antibodies (ANAs) (Genway Biotech, San Diego, CA), dsDNA (double stranded DNA) antibodies (Alpha Diagnostics, San Antonio, TX), and B-cell activating factor (BAFF) concentrations (R&D Systems, Minneapolis, MN) 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, Inc., Davis, CA); nuclei were stained with DAPI (Life Technologies, Grand Island, NY).

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

B cells were purified from PBMCs by positive selection using CD20 magnetic beads (Miltenyi Biotec, Cambridge, MA). 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, CA) 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, which included dsDNA, insulin, and lipopolysaccharide (LPS). The reactivity of purified recombinant antibodies was also tested on HEp-2 cell coated slides (Bion Enterprise, LTD, Des Plaines, IL) by indirect immunofluorescence.

B cell staining and stimulation

$CD20^{+}$ purified B cells were stained for surface markers using fluorochrome labeled antibodies against CD19, CD27, CD10, CD69, CD86, CD25 (Biolegend, San Diego, CA), and CD21 (BD Biosciences). In stimulation experiments, B cells were plated at 150,000–200,000 cells per well in a 96 well plate in RPMI with 10% fetal bovine serum 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, CA), 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 using fluorochrome labeled antibodies (eBioscience, San Diego, CA and BioLegend), then permeabilized using 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 negative selection using magnetic beads (Miltenyi Biotec). For isolation of $CD4^+CD25^-$ effector T (Teff) cells, $CD25^+$ cells were depleted using anti-CD25 beads (Miltenyi Biotec). Treg cells were isolated by sorting for $CD4^+CD25^+CD127^-$ cells. Teff cells were labeled with CellTrace Violet or CFSE (Life Technologies) and stimulated with anti-CD3, CD28, and 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 flow cytometry.

Statistical Analysis

Comparisons between DOCK8 deficient patients and HD controls were analyzed for statistical significance using unpaired Student *t* tests (GraphPad Prism).

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 compared to HD controls (Fig 1, A), and a high frequency of reactivity against many of the other autoantigens in the panel (see Fig E1 in the online repository). 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 ANA and dsDNA antibodies were not increased in the patients' serum (see Fig E2 in the online repository). Thus, autoantibody production to cytoplasmic antigens is characteristic of DOCK8 deficiency.

DOCK8 deficient patients have an increase in activated B cells

$CD19^+CD27^-$ $CD10^-CD21^+$ mature naïve B cells, represent the vast majority of B cells in DOCK8 deficient patients [21, 22]. Mature naïve B cells from the patients expressed significantly higher levels of the activation marker CD69 in comparison to HD controls (Fig 2, A and B), while CD86 and CD25 expression was comparable (Fig 2, A and B). The patients displayed an expansion of $CD19^+CD27^-CD10^-CD21^{-/lo}$ B cells, that are enriched in patients with autoimmunity and express autoreactive BcR [23–25] (Fig 2, C and D). 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 may be effective in the absence of functional DOCK8 (see Fig E3 in the online repository). To assess the integrity of the central B cell tolerance checkpoint in 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 three DOCK8 deficient

patients and compared them to those from 11 HD controls. 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 controls (Fig 3, AC). Antibody sequences from new emigrant/transitional B cells from patients and controls did not reveal any statistical differences in variable heavy (VH) CDR3 length, the frequency of positively charged amino acids, or usage of the self-reactive IGHV4-34 gene (see Tables E1–E3 and Fig E4, A in the online repository and data not shown), three 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 FACS sorted single $CD19^+CD10^-CD21^+IgM^+CD27^-$ mature naïve 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 (35.3–40%) compared to HDs (17–26%, $p<0.0001$) (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 naïve B cells, but did have a significant increase in polyreactive mature naive B cells compared to controls ($p<0.0001$) (Fig 4, D–F and see Fig E5 in the online repository). This is consistent with our previous observation that low and non-specific dsDNA reactivity detected in the polyreactivity assay is rarely associated with ANA reactivity [27]. Analysis of the antibody repertoire revealed increased usage of the self-reactive IGHV4-34 gene[17, 26] (see Tables E4–E6 and Fig E4, B in the online repository). 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 autoimmune conditions [30, 31]. BAFF plasma concentrations were not significantly increased in DOCK8 deficient patients (see Fig E6 in the online repository). 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 to controls (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 and D). Non-Treg cells from DOCK8 deficient patients also exhibited decreased expression of CD127/IL-7Rα and of $CD45RO^+$ memory T cells (Fig 5, C and D).

$CD45RO^+$ Treg cells that co-express Ki67 may represent cells that have been previously activated, express pro-inflammatory cytokines, and display reduced suppressive capabilities [16, 32–34]. A higher proportion of $CD45RO^+$ Treg cells co-expressed Ki67 in DOCK8 deficient patients than in HDs (see Fig E7, A and B in the online repository). Ki67 expression was also significantly higher in $CD4^+$ non-Treg cells from DOCK8 deficient

patients indicative of increased Teff cell activation (see Fig E7, A and B in the online repository). These findings suggest that Treg cell function may 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 monoclonal antibodies. DOCK8 deficient Treg cells had a significantly decreased ability to suppress Teff cell proliferation compared to Treg cells from HD controls (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.

DISCUSSION

Here, we report that DOCK8 deficient patients produce autoreactive antibodies and suffer from defective peripheral B cell tolerance associated with decreased Treg cell number 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 elevated 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-STAT3 signaling pathway important for B cell proliferation and immunoglobulin production [21] may partially explain the absence of DNA antibodies in DOCK8 deficient patients.

DOCK8 deficient patients had intact central B cell tolerance, evidenced by a normal frequency of autoreactive emigrant/transitional B cells. In contrast, DOCK8 deficient patients had an increase in autoreactive B cells in the mature naïve 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 naïve B cells [16]. The increase in $CD69^+$ activated B cells could be secondary to impaired Treg cell function and/or may be associated with chronic microbial stimulation. 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 activation associated with pro-inflammatory 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 Th17 cell function [38] in DOCK8 deficient patients requires further investigation.

Numerical 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 or/and triggered by recurrent infections. The standard of care for treatment of DOCK8 deficient patients is hematopoietic stem cell transplant (HSCT) [39–45]. HSCT is expected to restore the Treg cell compartment; however, autoantibody producing plasma cells may persist for a long time after HSCT as 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by supported by National Institute of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) grants AI061093, AI071087, AI082713, AI095848 (to EM), AI100315 and HL059561 (to RSG) and 5K 12HD052896 (to EJ), and by grants from the Manton Foundation (to EJ), German Research Foundation grant DFG MO2160/2-1 (to HM), Dubai-Harvard Foundation for Medical Research and the Jeffrey Model Foundation (to RSG), and the Kuwait Foundation for Advancement of Sciences 2010-1302-05 (to WA).

We thank Dr. L. Devine and C. Wang for cell sorting, also the Intramural Research Program of the NIH, NIAID (H.S).

ABBREVIATIONS

ANA	anti-nuclear antibodies
BAFF	B-cell activating factor
BcR	B cell receptor
BM	bone marrow
DOCK8	Dedicator of Cytokinesis 8
dsDNA	double stranded DNA

FOXP3	forkhead box P3
HD	healthy donor
HSCT	hematopoietic stem cell transplant
LPS	lipopolysaccharide
MFI	mean fluorescence intensity
PBMC	peripheral blood mononuclear cell
Teff	T effector
Treg	T regulatory
SLE	Systemic lupus erythematosus
TLR	Toll-like receptor
VH	variable heavy

References

1. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med. 2009; 361:2046–55. [PubMed: 19776401]
2. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol. 2009; 124:1289–302. e4. [PubMed: 20004785]
3. Al-Herz W, Ragupathy R, Massaad MJ, Al-Attiyah R, Nanda A, Engelhardt KR, et al. Clinical, immunologic and genetic profiles of DOCK8-deficient patients in Kuwait. Clin Immunol. 2012; 143:266–72. [PubMed: 22534316]
4. Alsum Z, Hawwari A, Alsmadi O, Al-Hissi S, Borrero E, Abu-Staiteh A, et al. Clinical, immunological and molecular characterization of DOCK8 and DOCK8-like deficient patients: single center experience of twenty-five patients. J Clin Immunol. 2013; 33:55–67. [PubMed: 22968740]
5. Sanal O, Jing H, Ozgur T, Ayvaz D, Strauss-Albee DM, Ersoy-Evans S, et al. Additional diverse findings expand the clinical presentation of DOCK8 deficiency. J Clin Immunol. 2012; 32:698–708. [PubMed: 22476911]
6. Su HC. Dedicator of cytokinesis 8 (DOCK8) deficiency. Curr Opin Allergy Clin Immunol. 2010; 10:515–20. [PubMed: 20864884]
7. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003; 301:1374–7. [PubMed: 12920303]
8. Halverson R, Torres RM, Pelanda R. Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. Nat Immunol. 2004; 5:645–50. [PubMed: 15156139]
9. Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 1988; 334:676–82. [PubMed: 3261841]
10. Nemazee DA, Burki K. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature. 1989; 337:562–6. [PubMed: 2783762]
11. Ng YS, Wardemann H, Chelnis J, Cunningham-Rundles C, Meffre E. Bruton's tyrosine kinase is essential for human B cell tolerance. J Exp Med. 2004; 200:927–34. [PubMed: 15466623]

12. Menard L, Saadoun D, Isnardi I, Ng YS, Meyers G, Massad C, et al. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. J Clin Invest. 2011; 121:3635–44. [PubMed: 21804190]
13. Isnardi I, Ng YS, Srdanovic I, Motaghedi R, Rudchenko S, von Bernuth H, et al. IRAK-4- and MyD88-dependent pathways are essential for the removal of developing autoreactive B cells in humans. Immunity. 2008; 29:746–57. [PubMed: 19006693]
14. Romberg N, Chamberlain N, Saadoun D, Gentile M, Kinnunen T, Ng YS, et al. CVID-associated TACI mutations affect autoreactive B cell selection and activation. J Clin Invest. 2013; 123:4283–93. [PubMed: 24051380]
15. Gu H, Tarlinton D, Muller W, Rajewsky K, Forster I. Most peripheral B cells in mice are ligand selected. J Exp Med. 1991; 173:1357–71. [PubMed: 1903427]
16. Kinnunen T, Chamberlain N, Morbach H, Choi J, Kim S, Craft J, et al. Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. Blood. 2013; 121:1595–603. [PubMed: 23223361]
17. Herve M, Isnardi I, Ng YS, Bussel JB, Ochs HD, Cunningham-Rundles C, et al. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. J Exp Med. 2007; 204:1583–93. [PubMed: 17562816]
18. Li QZ, Zhou J, Wandstrat AE, Carr-Johnson F, Branch V, Karp DR, et al. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol. 2007; 147:60–70. [PubMed: 17177964]
19. Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, et al. TM4: a free, open-source system for microarray data management and analysis. Biotechniques. 2003; 34:374–8. [PubMed: 12613259]
20. Meffre E, Schaefer A, Wardemann H, Wilson P, Davis E, Nussenzweig MC. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. J Exp Med. 2004; 199:145–50. [PubMed: 14699083]
21. Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A, et al. DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. Nat Immunol. 2012; 13:612–20. [PubMed: 22581261]
22. Janssen E, Tsitsikov E, Al-Herz W, Lefranc G, Megarbane A, Dasouki M, et al. Flow cytometry biomarkers distinguish DOCK8 deficiency from severe atopic dermatitis. Clin Immunol. 2014; 150:220–4. [PubMed: 24440647]
23. Isnardi I, Ng YS, Menard L, Meyers G, Saadoun D, Srdanovic I, et al. Complement receptor 2/CD21− human naive B cells contain mostly autoreactive unresponsive clones. Blood. 2010; 115:5026–36. [PubMed: 20231422]
24. Saadoun D, Terrier B, Bannock J, Vazquez T, Massad C, Kang I, et al. Expansion of autoreactive unresponsive CD21−/low B cells in Sjogren's syndrome-associated lymphoproliferation. Arthritis Rheum. 2013; 65:1085–96. [PubMed: 23279883]
25. Terrier B, Joly F, Vazquez T, Benech P, Rosenzwajg M, Carpentier W, et al. Expansion of functionally anergic CD21−/low marginal zone-like B cell clones in hepatitis C virus infection-related autoimmunity. J Immunol. 2011; 187:6550–63. [PubMed: 22084433]
26. Meyers G, Ng YS, Bannock JM, Lavoie A, Walter JE, Notarangelo LD, et al. Activation-induced cytidine deaminase (AID) is required for B-cell tolerance in humans. Proc Natl Acad Sci U S A. 2011; 108:11554–9. [PubMed: 21700883]
27. Meffre E. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. Ann N Y Acad Sci. 2011; 1246:1–10. [PubMed: 22236425]
28. Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J Exp Med. 1999; 189:1747–56. [PubMed: 10359578]
29. Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol. 2009; 9:491–502. [PubMed: 19521398]
30. Kinnunen T, Chamberlain N, Morbach H, Cantaert T, Lynch M, Preston-Hurlburt P, et al. Specific peripheral B cell tolerance defects in patients with multiple sclerosis. J Clin Invest. 2013; 123:2737–41. [PubMed: 23676463]

31. Scholz JL, Oropallo MA, Sindhava V, Goenka R, Cancro MP. The role of B lymphocyte stimulator in B cell biology: implications for the treatment of lupus. Lupus. 2013; 22:350–60. [PubMed: 23553778]
32. Booth NJ, McQuaid AJ, Sobande T, Kissane S, Agius E, Jackson SE, et al. Different proliferative potential and migratory characteristics of human CD4+ regulatory T cells that express either CD45RA or CD45RO. J Immunol. 2010; 184:4317–26. [PubMed: 20231690]
33. d'Hennezel E, Yurchenko E, Sgouroudis E, Hay V, Piccirillo CA. Single-cell analysis of the human T regulatory population uncovers functional heterogeneity and instability within FOXP3+ cells. J Immunol. 2011; 186:6788–97. [PubMed: 21576508]
34. Walter GJ, Evans HG, Menon B, Gullick NJ, Kirkham BW, Cope AP, et al. Interaction with activated monocytes enhances cytokine expression and suppressive activity of human CD4+CD45ro+CD25+CD127(low) regulatory T cells. Arthritis Rheum. 2013; 65:627–38. [PubMed: 23280063]
35. Christensen SR, Kashgarian M, Alexopoulou L, Flavell RA, Akira S, Shlomchik MJ. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med. 2005; 202:321–31. [PubMed: 16027240]
36. Jackson SW, Scharping NE, Kolhatkar NS, Khim S, Schwartz MA, Li QZ, et al. Opposing Impact of B Cell-Intrinsic TLR7 and TLR9 Signals on Autoantibody Repertoire and Systemic Inflammation. J Immunol. 2014; 192:4525–32. [PubMed: 24711620]
37. Bradley LM, Haynes L, Swain SL. IL-7: maintaining T-cell memory and achieving homeostasis. Trends Immunol. 2005; 26:172–6. [PubMed: 15745860]
38. Al Khatib S, Keles S, Garcia-Lloret M, Karakoc-Aydiner E, Reisli I, Artac H, et al. Defects along the T(H)17 differentiation pathway underlie genetically distinct forms of the hyper IgE syndrome. J Allergy Clin Immunol. 2009; 124:342–8. 8e1–5. [PubMed: 19577286]
39. Barlogis V, Galambrun C, Chambost H, Lamoureux-Toth S, Petit P, Stephan JL, et al. Successful allogeneic hematopoietic stem cell transplantation for DOCK8 deficiency. J Allergy Clin Immunol. 2011; 128:420–22. e2. [PubMed: 21546070]
40. Bittner TC, Pannicke U, Renner ED, Notheis G, Hoffmann F, Belohradsky BH, et al. Successful long-term correction of autosomal recessive hyper-IgE syndrome due to DOCK8 deficiency by hematopoietic stem cell transplantation. Klin Padiatr. 2010; 222:351–5. [PubMed: 21058221]
41. Boztug H, Karitnig-Weiss C, Ausserer B, Renner ED, Albert MH, Sawalle-Belohradsky J, et al. Clinical and immunological correction of DOCK8 deficiency by allogeneic hematopoietic stem cell transplantation following a reduced toxicity conditioning regimen. Pediatr Hematol Oncol. 2012; 29:585–94. [PubMed: 22897717]
42. Gatz SA, Benninghoff U, Schutz C, Schulz A, Honig M, Pannicke U, et al. Curative treatment of autosomal-recessive hyper-IgE syndrome by hematopoietic cell transplantation. Bone Marrow Transplant. 2011; 46:552–6. [PubMed: 20622910]
43. Ghosh S, Schuster FR, Fuchs I, Laws HJ, Borkhardt A, Meisel R. Treosulfan-based conditioning in DOCK8 deficiency: complete lympho-hematopoietic reconstitution with minimal toxicity. Clin Immunol. 2012; 145:259–61. [PubMed: 23128504]
44. McDonald DR, Massaad MJ, Johnston A, Keles S, Chatila T, Geha RS, et al. Successful engraftment of donor marrow after allogeneic hematopoietic cell transplantation in autosomal-recessive hyper-IgE syndrome caused by dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol. 2010; 126:1304–5. e3. [PubMed: 20810158]
45. Metin A, Tavil B, Azik F, Azkur D, Ok-Bozkaya I, Kocabas C, et al. Successful bone marrow transplantation for DOCK8 deficient hyper IgE syndrome. Pediatr Transplant. 2012; 16:398–9. [PubMed: 22248482]
46. Hoyer BF, Moser K, Hauser AE, Peddinghaus A, Voigt C, Eilat D, et al. Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. J Exp Med. 2004; 199:1577–84. [PubMed: 15173206]
47. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol. 2006; 6:741–50. [PubMed: 16977339]

Highlights

- DOCK8 is not required for the removal of autoreactive B cells in the bone marrow.
- DOCK8 patients have abrogation of peripheral B cell tolerance with autoantibody production.
- DOCK8 deficient patients have quantitative and qualitative deficiencies in regulatory T cells.

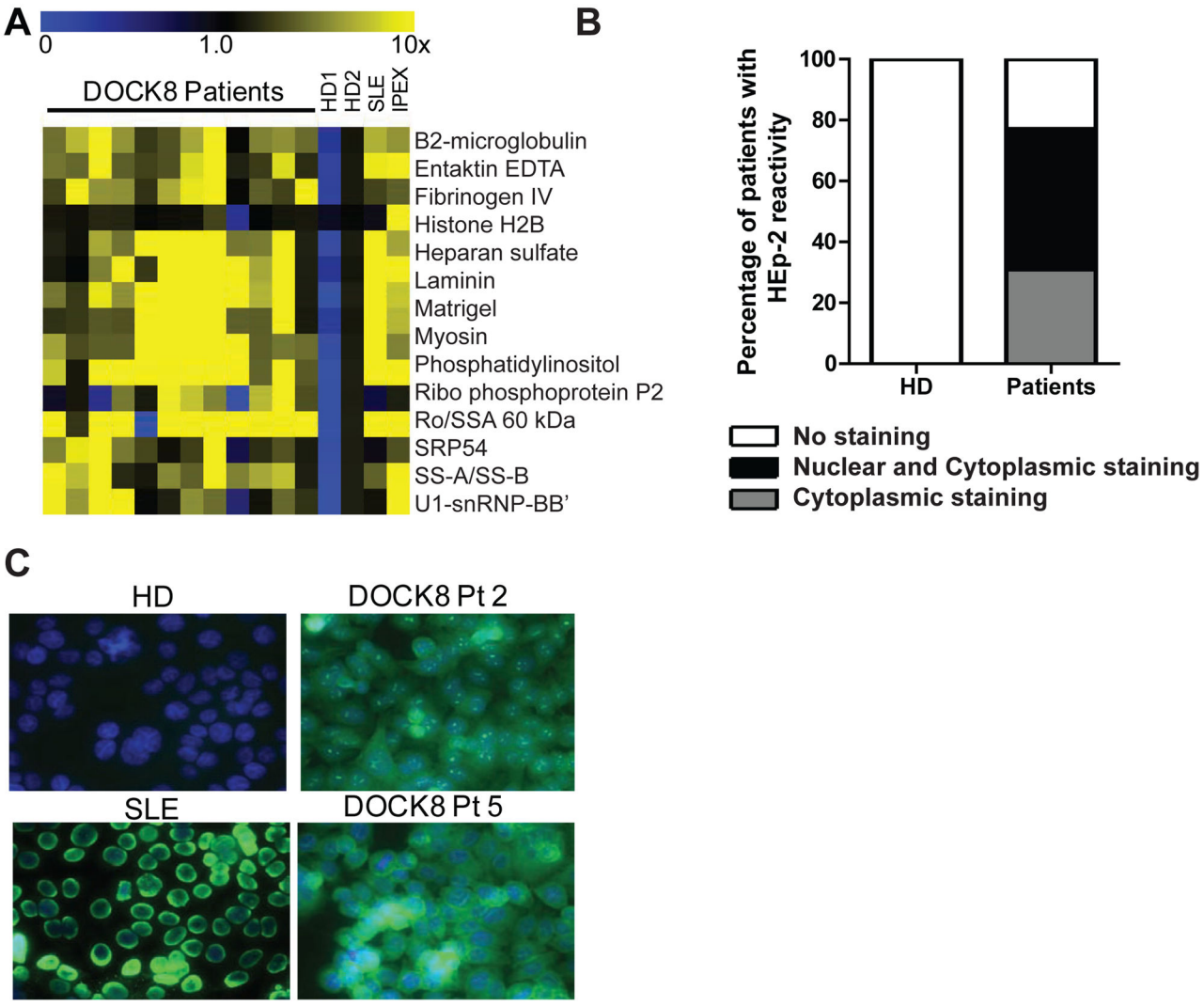

FIG. 1. Autoantibodies are present in DOCK8 deficient patients

A, A heat map of the reactivity of IgG antibodies against self-antigens in 12 DOCK8 deficient patients, 2 HD controls, a patient with systemic lupus erythematosus (SLE), and a patient with Foxp3 deficiency (IPEX). Only the 14 autoantigens for which binding was significantly higher ($p<0.05$) in the 12 DOCK8 deficient patients compared with the HD are shown. The colors represent the fold increase relative to HD controls. **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, a HD, and an SLE patient. Green represents bound IgG antibodies bound, and blue DAPI staining shows the nucleus.

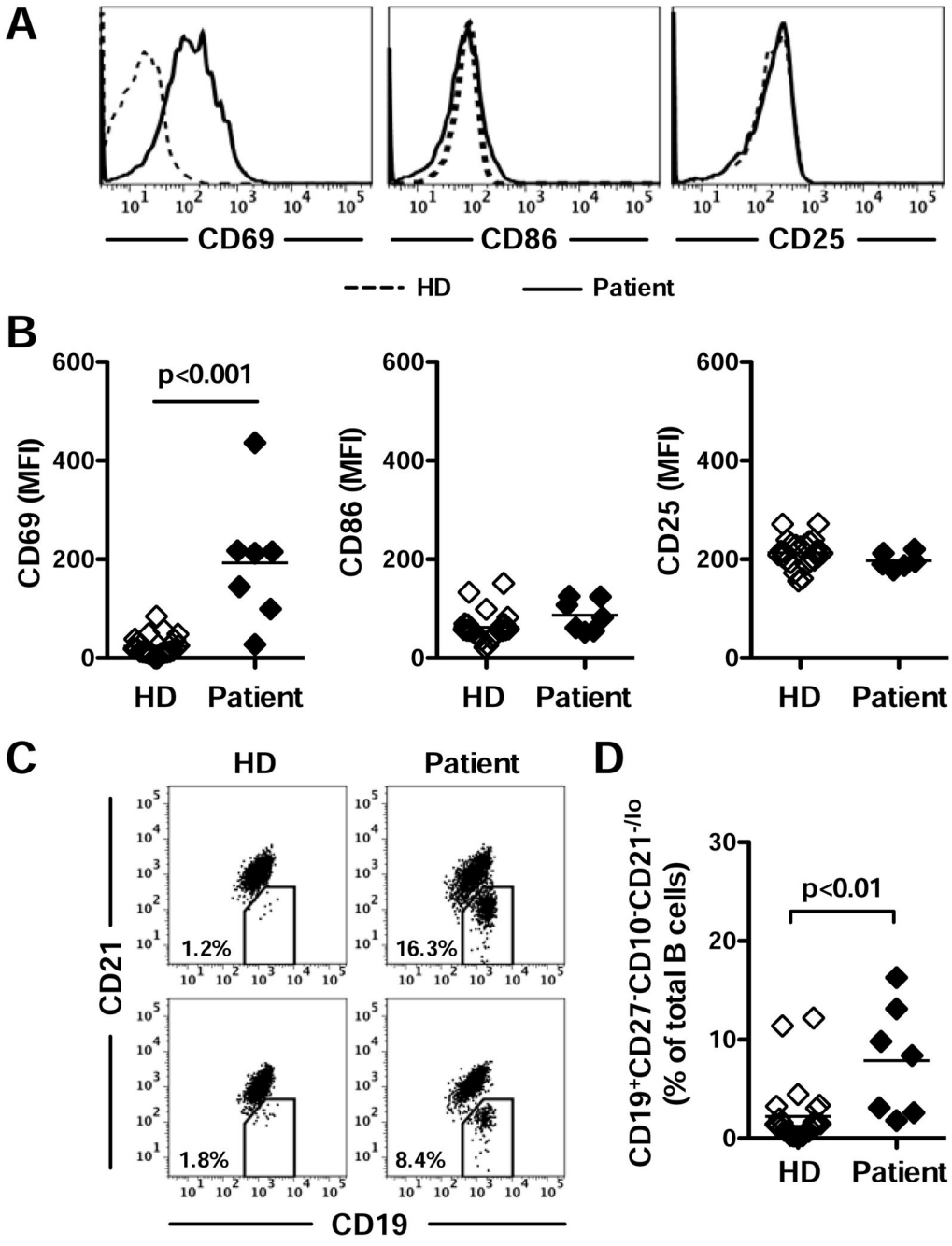

FIG. 2. Mature naïve B cells from DOCK8 deficient patients have an activated phenotype

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. **B,** Mean fluorescence intensities (MFI) of CD69, CD86, and CD25 expression on mature naive B cells from 7 DOCK8 deficient patients and 25 HD controls. **C,** Representative flow cytometry plots for $CD19^{+}CD21^{-/lo}$ B cells for two DOCK8 deficient patients and two HD. **D,** $CD19^{+}CD27^{-}CD10^{-}CD21^{-/lo}$ B cells as a percentage of total B cells in 7 DOCK8 deficient patients and 25 HD controls.

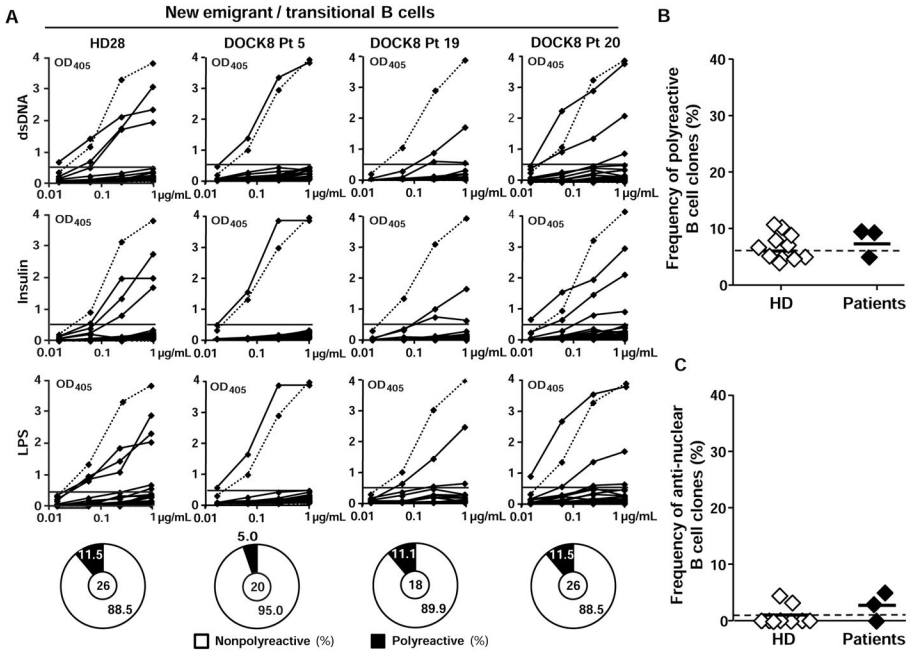

FIG. 3. The central B cell tolerance checkpoint is intact in DOCK8 deficient patients

A, Antibodies cloned from new emigrant/transitional B cells from a representative HD control (HD28) and three DOCK8 deficient patients (Table I, patients 5, 19, and 20) were tested by ELISA for reactivity against dsDNA, insulin, and LPS. Dotted lines show the ED38-positive control [7, 20] and solid lines show binding for each cloned recombinant antibody. Horizontal lines define the cutoff for positive reactivity. For each individual, the frequency of polyreactive (filled 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 controls. **C**, Normal low frequency of antinuclear antigen reactive new emigrant/transitional B cells in 3 DOCK8 deficient patients and 9 HD controls. The solid line represents the mean for each group and the dotted line is the mean for HD controls in B and C.

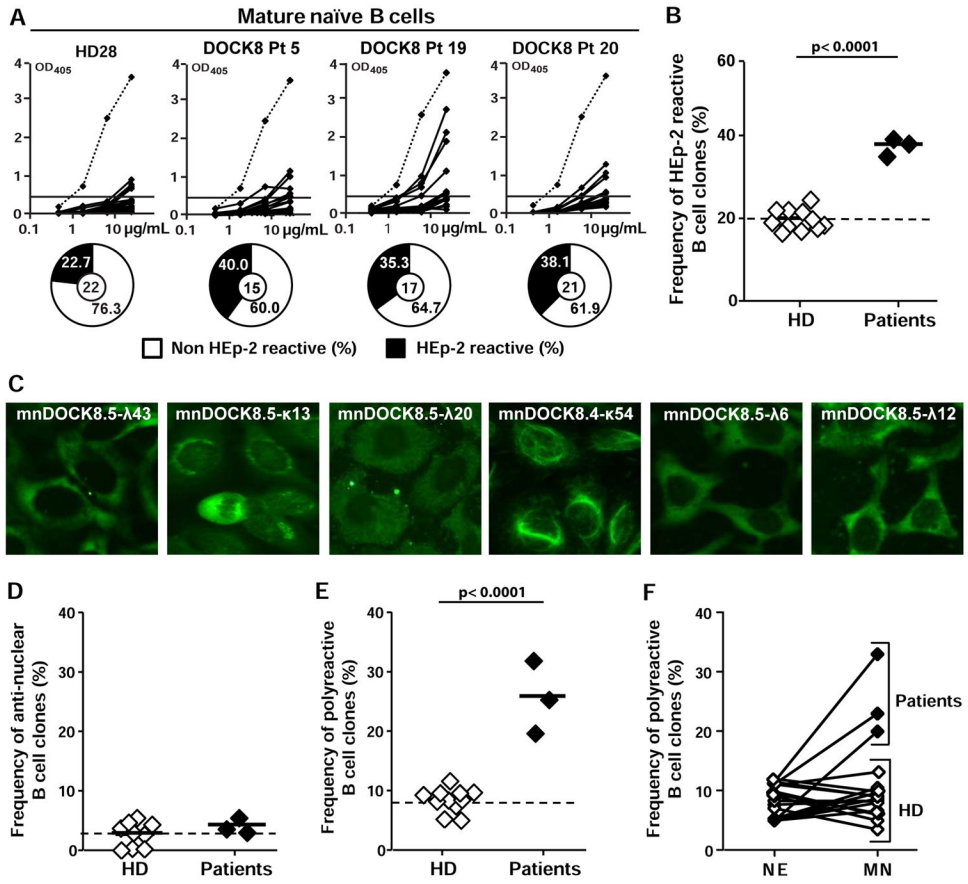

FIG. 4. The peripheral B cell tolerance checkpoint is defective in DOCK8 deficient patients

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 ELISA for anti-HEp-2 cell reactivity. Dotted lines show ED38-positive control [7, 20] and solid lines show binding for each cloned recombinant antibody. Horizontal lines define cutoff for positive reactivity. For each individual, the frequency of HEp-2–reactive (filled 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 controls. **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 controls and 3 DOCK8 deficient patients. **E**, Frequencies of polyreactive mature naive B cells in 11 HD controls and 3 DOCK8 deficient patients. **F,** The evolution of polyreactivity between the new emigrant/ transitional and mature naive B cell compartments is represented. The solid line represents the mean for each group and the dotted line is the mean for HD controls in B, D, and E.

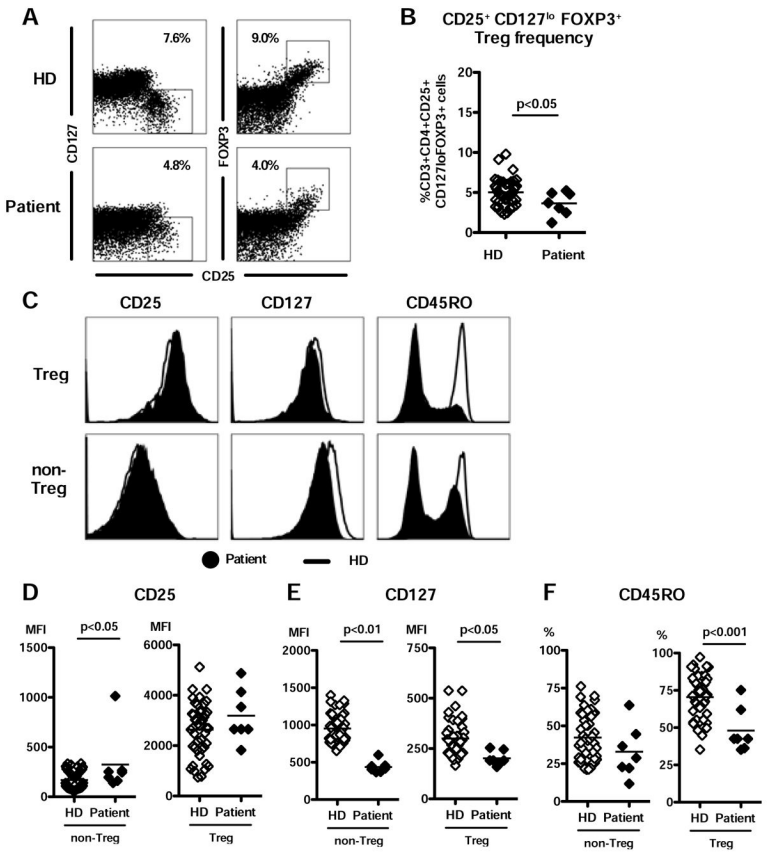

FIG. 5. DOCK8 deficient patients have decreased Treg cells

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 and a DOCK8 deficient patient. **B**, Decreased frequency of $CD4^{+}CD25^{hi}CD127^{lo}FOXP3^{+}$ Treg cells in 7 DOCK8 deficient patients compared to 45 HD controls. Each symbol represents an individual, 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). **D,** The mean fluorescence intensities (MFI) of CD25 appear normal, whereas CD127 expression and the frequency of $CD45RO^{+}$ cells are decreased in both non-Treg and Treg cells from DOCK8 deficient patients.

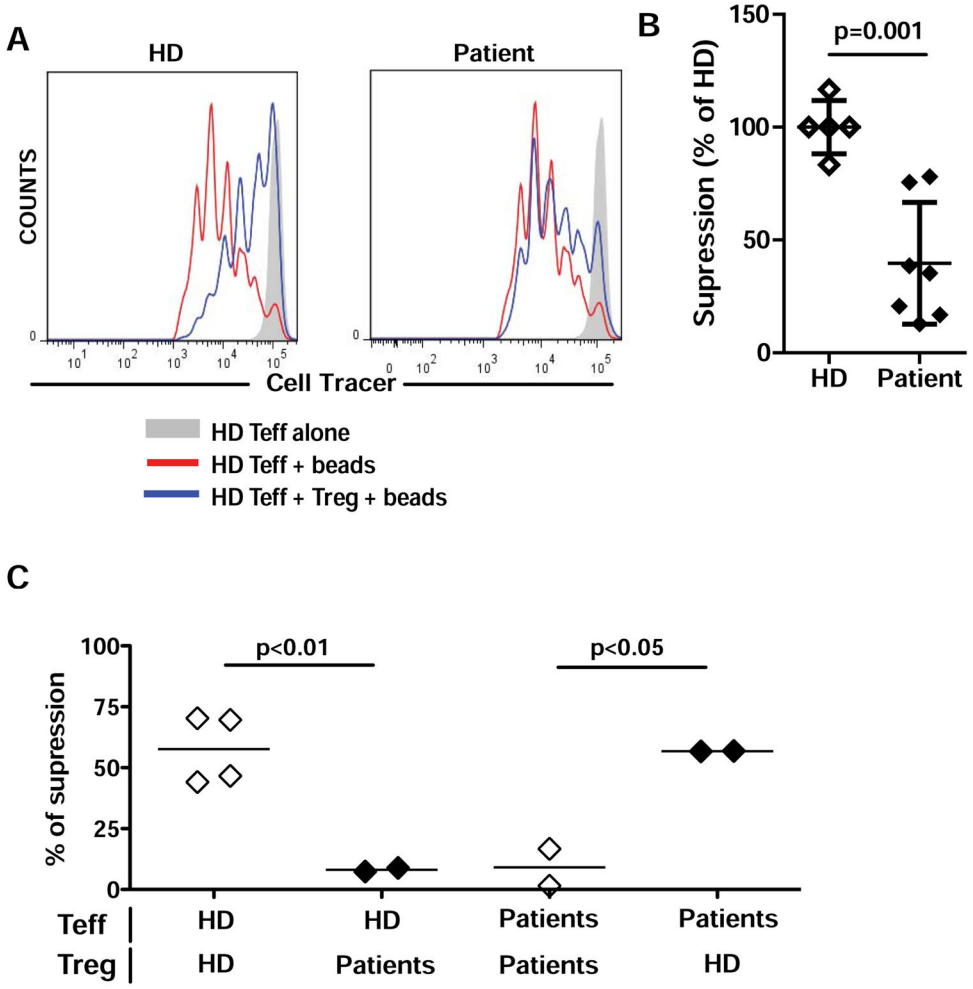

FIG. 6. DOCK8 deficient Treg cells have reduced suppressive activity

A, $CD4^+CD25^-$ Teff cells from a HD were loaded with CellTrace Violet and co-cultured with $CD4^+CD25^+CD127^{lo}$ Treg cells from a HD (left panel) and 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 flow cytometry. **B**, Suppressive activity of Treg cells from 7 DOCK8 deficient patients relative to that of Treg cells from 5 HD shipping controls. **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 CFSE and co-cultured with either autologous $CD4^+CD25^+CD127^-$ Treg cells or Treg cells from a shipped HD control.

TABLE I

Homozygous mutations in DOCK8 deficient patients.

Patient	*DOCK8* Mutation*	Gender	Age (years)
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

* The reference coding sequence is accession number AB191037.1.