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CUTTING EDGE: DOCK8 regulates a subset of DC which is critical for the development of EAE

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Abstract

Dedicator of cytokinesis (DOCK) 8 is a guanine nucleotide exchange factor with essential role in cytoskeletal rearrangement, cell migration and survival of various immune cells. Interestingly, DOCK8 deficient mice are resistant to the development of experimental autoimmune encephalomyelitis (EAE). To understand if EAE resistance in these mice results from an alteration in dendritic cell (DC) functions, we generated mice with conditional deletion of DOCK8 in DCs and observed attenuated EAE in these mice compared to control mice. Additionally, we demonstrated that DOCK8 is important for the existence of splenic conventional DC2 and lymph node migratory DCs, and further established that migratory DC, rather than resident DC, are essential for the generation and proliferation of pathogenic T cell populations upon immunization with myelin antigen in adjuvant. Therefore, our data suggests that limiting migratory DCs through DOCK8 deletion and possibly other mechanisms could limit the development of central nervous system (CNS) autoimmunity.

Introduction

Multiple sclerosis (MS) is an inflammatory and autoimmune disease of the central nervous system (CNS) characterized by axonal loss and demyelination (1). Several aspects of MS pathogenesis are recapitulated in the animal model, Experimental autoimmune encephalomyelitis (EAE) initiated by myelin reactive CD4+ T cells (2). Dendritic cells (DCs) are critical players in the initiation and development of the immune response. They are an heterogeneous population of cells which comprise classical/conventional DCs (cDCs), and plasmacytoid DCs (pDCs) (3). cDC and pDC originate from a common DC progenitor which colonize both the secondary lymphoid organs and tissues, giving rise to two different types of DCs. In the spleen and lymph nodes, these progenitors differentiates and give rise to resident DC (3). In tissues and under the influence of local cues, the DC progenitors differentiate into migratory DC, which express high levels of MHCII and CCR7, and have the capacity to recirculate via lymphatics to secondary lymph nodes (4, 5). In addition to

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Conflict of interest
The authors have no conflicts of interest.

T cells, DCs have also been shown to play a key role in EAE pathogenesis, especially for the initiation and reactivation of the inflammatory T cell response (5-8). In fact, three recent publications establish the requirement of MHCII on dendritic cells but not on monocytes, macrophages and CNS resident cells for the development of EAE (9-11). However, the nature of the dendritic cells that drive the activation of myelin reactive T cells during EAE remain elusive. cDCs have been further subdivided into type I (cDC1) and type 2 (cDC2) cDCs based on cell marker and transcription factor expression, cytokine production, and anatomic location at steady state (12).While resident cDC2 have been proposed to prime CD4+ T cells, migratory DCs are cDCs that survey the tissue for damage or infection and upon such detection migrate to the lymph nodes (LN) in a CCR7 dependent manner to activate naïve T cells (13).

Dedicator of cytokinesis 8 (DOCK8) is a guanine nucleotide exchange factors (GEFs) that regulates the activity of Rho GTPases, such as Cdc42 and Rac1 (14, 15) and whose expression is upregulated in PBMC of MS patients (16). Interestingly elimination of DOCK8 (Dock8$^{pri/pri}$ mice) render mice resistant to the development of EAE (authors' own observations and (16)), a resistance which has been attributed to the inhibition of DOCK mediated migration of CD4^{+} T cells (16). The aim of our study here was to determine whether DOCK8 impacted the development of EAE through its effect on dendritic cells.

Materials and Methods

Mice

All mice are on the C57Bl/6 background and both male and females were used in experiments. C57Bl/6, B6.SJL, B6/B6.SJL, CD11cCre and 2D2 transgenic mice were purchased from The Jackson Laboratory. DOCK8$^{fl/fl}$ (17) were provided by Dr. Mohamed Oukka (University of Washington). "WT control (Ctrl)" mice were either C57Bl/6J, CD11cCre or DOCK8$^{fl/fl}$ mice. All animals were bred and maintained under specific pathogen-free conditions at the Benaroya Research Institute (Seattle, WA) and all experiments were performed in accordance with the guidelines of the Benaroya Research Institute Animal Care and Use Committee.

EAE induction

EAE was induced by s.c immunization with an emulsion of 150 μg of myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide in CFA supplemented with 4mg/ml of *Mycobacterium tuberculosis* extract H37Ra (Difco). Additionally, the mice received 200ng of pertussis toxin (List Biological Laboratories) i.p. on days 0 and 2 after immunization. Mice were monitored daily for EAE using the following scoring system: 0, normal; 1, flaccid tail; 2, impaired righting reflex and/or gait; 3, partial hind limb paralysis; 4, total hind limb paralysis; 5, hind limb paralysis with partial forelimb paralysis; 6, moribund.

Cell isolation and flow cytometry

Spleen and LNs were digested with Collagenase D (Roche). Skin was isolated from mice earpieces which were digested with collagenase D and DNase I (Roche). Afterwards, single cell suspensions were obtained through mechanical dissociation through a 70um

strainer. Spleen cells were lysed with ACK lysing buffer prior to phenotyping. For isolation of mononuclear cells from CNS, mice were perfused with 1X PBS. Brain and spinal cords were isolated, digested with Collagenase D. Mononuclear cells were isolated over a 37%/70% Percoll gradient (VWR). Prior to staining, a 10 minutes CD16/32 blocking step with 2.4G2 supernatant was performed to prevent non-specific antibody binding. All antibodies were obtained either from ebioscience or Biolegend. Cells were acquired on an LSRII (BD Biosciences) and data were analyzed using FlowJo software (Tree Star). In Figures 2A-B and 3A-B, $CD11c^{+}IA/IE^{+}$ cells are pre-gated on live, lineage negative ($CD19^{-}DX5^{-}CD3^{-}$) cells and migratory DCs further gated on $IA/IE^{high}CD11c^{+}$ cells (Fig. 2C-D). In figures 2F-G, $CD11c^{+}I\text{-}A/I\text{-}E^{High}$ migratory cells were pre-gated on $CD45^{+}Lin^{-}$ ($CD19^{-}CD3^{-}NK1.1^{-}Ly6G^{-}Ly6C^{-}CD64^{-}$) cells and further divided into Langerhans cells (LC: $CD24^{+}CD11b^{+}$), and different subsets of DC ($CD24^{-}CD11b^{-}$, $CD24^{+}CD11b^{-}$, $CD24^{-}CD11b^{+}$) as previously described (18). CNS cell suspensions were separated into two fractions for staining. $CD45^{High}$ cells were gated on live cells (Supplemental Fig. 1A). In one staining, $CD45^{+}$ were further gated on Lin- ($CD3^{-}CD19^{-}Ly6G^{-}NK1.1^{-}$) cells to identify different myeloid cells (Supplemental Fig. 1B) as previously described (19). In the other fraction, cells were stimulated with PMA/ionomycin and stained intracellularly. Cytokine producing $CD4^{+}$ T cells were identified after gating on $CD4^{+}TCR\beta^{+}$ cells (Fig. 1B).

T cell proliferation

Mice were immunized s.c. with 150μg $MOG_{35\text{-}55}$ emulsified in CFA without pertussis toxin. The next day, approximately $3x10^{6}$ $CD4^{+}$ T cells were isolated from 2D2 transgenic mice using the negative CD4+ isolation kit (StemCell Technologies), labelled with Cell Trace Violet (CTV) (Invitrogen) and injected i.v. into the immunized mice. Three days later, in vivo proliferation of $CD4^{+}$ 2D2 T ($V\beta11^{+}CD45.1^{+}$) cells was analyzed from the draining LNs and spleen of immunized mice, by assessing CTV dilution (Fig. 1C-D). In vitro T cell proliferation was assessed by isolating sorted dendritic cells from the spleen of naïve mice (Supplemental Fig. 1C), or the spleens (Fig. 4G) or LNs (Fig. 4H) of MOG immunized mice and culturing them at a 1:1 ratio with CTV labeled 2D2 transgenic CD4+ T cells and MOG peptide or protein for 4 days.

Skin DC Migration

Skin-derived DC migration was measured by applying 100μl of 0.5% FITC dilution in 1:1 acetone and dibutyl phthalate (DBP) to the shaved abdomen of mice. After 16hrs, skin draining LNs were removed and single cell suspensions were prepared as described above.

Bone marrow DC generation and in vitro DC migration

Bone marrow (BM) cells were obtained from mice tibia and femurs and grown for ~9 days in petri dishes containing culture medium supplemented with 10ng/ml GM-CSF. BMDCs were harvested, enriched in $CD11c^{+}$ cells using Miltenyi beads and $0.5x10^{5}$ cells were loaded in 100μl of culture media in the upper chamber of a Transwell plate (5um pore size, Corning). Six hundred μl of culture media with or without CCL21 (100ng/ml) was added in the lower chamber. After 2 hrs at 37°C, migrated cells were harvested from the lower chamber and counted by flow cytometry. The transmigration index was calculated by

dividing the number of DCs in the lower chamber with CCL21 over the number of DCs in the lower chamber without CCL21.

Generation of mixed BM chimeras

Mixed BM chimeras were generated by injecting i.v. a 1:1 mixture ($1x10^7$ total) of BM cells from untreated CD45.1$^+$ B6.SJL and CD45.2$^+$ CD11c^{Cre}DOCK8$^{fl/fl}$ mice into B6/B6.SJL (CD45.1+CD45.2+) hosts that were given 2 doses of 500 rads γ irradiation 3-4 hours apart. Mixed BM chimeras were analyzed after two months.

Results and Discussion

To understand whether the resistance of DOCK8 deficient mice to the development of EAE (16, 20) could result from an alteration in dendritic cell functions, we generated mice with preferential deletion of DOCK8 in dendritic cells (CD11c^{Cre}DOCK8$^{fl/fl}$). When immunized with MOG_{35-55}, CD11c^{Cre}DOCK8$^{fl/fl}$ mice developed less severe EAE than control WT mice (Fig. 1A). Mean max clinical score ± SEM in CD11c^{Cre}DOCK8$^{fl/fl}$ mice (1.96±1.75) was significantly lower compared to control mice (3.07±1.98). Consistent with reduced disease severity, we observed fewer CD45^{hi} cells, which include CNS infiltrating innate and adaptive cells, in CD11c^{Cre}DOCK8$^{fl/fl}$ mice compared to control mice (Supplemental Fig. 1A). While CD11c is mainly expressed on dendritic cells at steady state, other myeloid cells including inflammatory monocytes and moDC (monocyte derived DC) can also express CD11c during CNS inflammation (19). Therefore, we enumerated these cells in the CNS of mice during EAE. However, there was no difference in the number of inflammatory monocytes, moDCs nor DCs present in the CNS of CD11c^{Cre}DOCK8$^{fl/fl}$ and control mice at the peak of EAE (Supplemental Fig. 1B). Active MOG35-55-induced EAE is predominantly mediated by CD4+ T cells that produce IFN-γ, IL-17 and GM-CSF (2) while IL-4 production has been known to attenuate EAE (21). We observed an overall decrease in the number of CNS infiltrating CD4+ T cells secreting proinflammatory cytokines (IFN-γ$^+$, IL-17$^+$ and GM-CSF$^+$) in CD11c^{Cre}DOCK8$^{fl/fl}$ mice compared to control mice during EAE (Fig. 1B). However, we did not detect IL-4$^+$CD4$^+$ Th2 like cells in the CNS of CD11c^{Cre}DOCK8$^{fl/fl}$ and control mice during EAE and we detected similar proportion of cytokine positive cells (IL-17+, IFN-γ+, GM-CSF+ and IL-4+) in in the lymph nodes of CD11c^{Cre}DOCK8$^{fl/fl}$ and control mice ten days after immunization (data not shown). Together, these data show that DOCK8 deficiency in dendritic cells does not promote a shift of the Th cell response responsible for EAE resistance but it might limit T cell priming and proliferation. First, we accessed the capacity of DC to present antigen in vitro. Splenic dendritic cells from wild type or DOCK8 deficient mice were isolated, pulsed with MOG35-55 peptide or MOG protein and cultured with CTV labeled MOG specific 2D2 T cells. Dendritic cells from both groups could efficiently and similarly present antigen to MOG specific T cells and promote their proliferation as shown by equivalent CTV dilution (Supplemental Fig. 1C). Next, to address whether DC specific deletion of DOCK8 impacted T cell proliferation in vivo, we transferred CTV labeled MOG-specific 2D2 CD4$^+$ T cells in CD11c^{Cre}DOCK8$^{fl/fl}$ and control mice previously immunized with MOG_{35-55} (Fig. 1C-D). There, proliferation was significantly reduced in 2D2 MOG specific T cells isolated from dLNs of CD11c^{Cre}DOCK8$^{fl/fl}$ mice compared to those of control mice (Fig. 1C-D).

The type of dendritic cells that drives the priming of autoreactive and pathogenic T cell subsets is not fully defined. However, several studies suggest that cDC1 are important for the priming of CD8+ T cells whereas cDC2 are important for the priming of CD4+T cells (3, 12). Since T cells from the lymph nodes of $CD11c^{Cre}DOCK8^{fl/fl}$ mice did not proliferate efficiently in response to MOG, we hypothesized that DOCK8 might be altering the function or frequency of DCs responsible for the priming of MOG specific $CD4^+$ T cells in the lymph nodes. There, dendritic cells have been subdivided into two different subsets based on their expression of MHCII and/or CCR7. Migratory DC express high levels of MHCII molecules and express CCR7 which allows them to migrate from tissues to lymph nodes (4). Resident DC, on the other hand, express lower level of MHCII and no CCR7 (4). Four days after immunization, while the percentage of $CD11c^+IA/IE^{mid}$ resident DCs were similar between $CD11c^{Cre}DOCK8^{fl/fl}$ mice and control mice, the frequency of $CD11c^+IA/IE^{hi}$ or $CD11c^+CCR7^+$ migratory DCs were diminished in $CD11c^{Cre}DOCK8^{fl/fl}$ mice compared to control mice (Fig. 2A, B). Since CCR7 is important for the migration of DC (4, 5, 22), we evaluated its expression on DC after immunization with MOG35-55. However, the expression level of CCR7 on these migratory DC was comparable between $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice (Fig. 2C, D). Furthermore, DCs from $CD11c^{Cre}DOCK8^{fl/fl}$ could migrate efficiently and similarly to WT DCs toward the CCR7 ligand CCL21 in a trans-well migration assay (Fig. 2E). We further examined the capacity of DC from $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice to migrate from the skin to draining lymph nodes in vivo when FITC was applied to the skin. There were negligeable $FITC^+$ cells in the IA/IE^{mid} resident population. When enumerated, the percentage of $CD11c^+IA/IE^{high}FITC^+$ in the LN were similar between $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice (Fig. 2F-G). When $CD11c^+IA/IE^{high}$ cells were subdivided into migratory DC subsets and Langerhans cells based on their expression of CD24 and CD11b (18), similar proportions of $FITC^+$ cells were detected among each subset (Supplemental Fig. 1D-E). This suggests that the decrease in lymph node migratory DC in $CD11c^{Cre}DOCK8^{fl/fl}$ mice compared to controls may be independent of their migratory proprieties.

To determine whether DOCK8 controls the maintenance of migratory DCs in the dLNs independently of inflammation, we also assessed the distribution of DC populations at steady state. Consistent with immunized mice, naïve $CD11c^{Cre}DOCK8^{fl/fl}$ also has reduced frequencies of migratory DCs but unchanged frequencies of resident DC in the dLNs compared to controls (Fig. 3A-B). The reduced frequencies of migratory DCs in the lymph nodes was not associated with a compensatory increase of these cells in the skin (Supplemental Fig. 1F-G). In naïve mice, we further assessed the distribution of DC subsets in the spleen where cDC1, cDC2 and pDC can be identified. The frequencies of total splenic dendritic cells were similar between $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice (data not shown). We further phenotype splenic DCs based on the expression of CD4 and CD8 which selectively identified cDC2 and cDC1. In the spleen, we observed a marked decreased in the percentage of cDC2 and an increase in cDC1 population in $CD11c^{Cre}DOCK8^{fl/fl}$ compared to control mice (Fig. 3C-D). While perturbation of some DC subsets during antigen challenge in DOCK8 deficient animals has previously been described (23), to our knowledge, our data represent the first demonstration that DOCK8 is important for the

presence of two major populations of dendritic cells: splenic cDC2 and migratory DC at steady state.

To assess whether the reduction in migratory DCs in the LNs and of cDC2 in the spleen were cell intrinsic, we created bone marrow (BM) chimeras, by injecting an equal number of bone marrow cells from CD11c^{Cre}DOCK8$^{fl/fl}$ (CD45.2^{+}) and B6.SJL (CD45.1^{+}) mice into lethally irradiated B6/B6.SJL (CD45.1^{+}CD45.2^{+}) hosts. After hematopoietic reconstitution, we assessed the distribution of wild type and DOCK8 deficient DCs in the spleen and lymph nodes of these mice. In agreement with our data in CD11c^{Cre}DOCK8$^{fl/fl}$ mice (Fig. 3C-D), mixed BM chimeras had fewer cDC2 and more cDC1 in their spleen originating from CD11c^{Cre}DOCK8$^{fl/fl}$ BM than those coming from WT BM (Fig. 4A, B). In the inguinal and auxiliary LNs of these mice, migratory DCs were composed primarily (at 70.04 ± 4.56 %) by cells originating from WT BM and only 29.96 ± 4.56 % by cells from CD11c^{Cre}DOCK8$^{fl/fl}$ BM indicating that the latter had a competitive disadvantage when they were differentiated in the presence of WT cells (Fig. 4C-D). Resident DC in the lymph nodes, on the other hand were more equally divided between CD11c^{Cre}DOCK8$^{fl/fl}$ and B6.SJL (Fig. 4C-D). Using CD103 and CD11b to define DC subsets in the migratory population, we observed that both cDC1 (CD103+) and cDC2 (CD11b+) migratory DC subsets originating from CD11c^{Cre}DOCK8$^{fl/fl}$ mice represented 32.86 ± 5.74% and 37.56 ± 4.63% of their respective population compared to 67.14 ± 5.74 % and 62.44 ± 4.63% for WT control cells (Fig. 4F). The fact that both DOCK8 deficient migratory cDC1 and cDC2 are diminished at steady state in bone marrow chimeras demonstrates that DOCK8 is required for the emergence of the migratory DC population as a whole in the lymph nodes. Furthermore, our results establish that DOCK8 controls the generation of resident cDC2 in the spleen during homeostasis. This raises the question of which of these two populations modulated by DOCK8 deficiency can efficiently stimulate MOG specific T cells to drive the development of EAE?

In order to determine whether migratory or resident DCs induce T cell proliferation, control mice were immunized with MOG35-55 in CFA. IA/IE^{high} migratory DCs and IA/IE^{mid} resident DCs from the draining lymph nodes, or splenic resident cDC1 (CD8+) and cDC2 (CD4+) were sorted and cultured with MOG specific 2D2 T cells labeled with CTV. Proliferation of MOG specific T cells was access by CTV dilution. Splenic resident DCs, regardless of their subset (cDC1 or cDC2) could not induce significant proliferation of MOG specific T cells, as shown by the absence of CTV dilution (Fig. 4G). Among DCs isolated from the lymph nodes, resident DCs failed to promote T cell division and only migratory DCs could induce significant and robust MOG specific T cell proliferation (Fig. 4H). Therefore, migratory DCs are primarily responsible in mice immunized subcutaneously with myelin antigen in adjuvant for inducing CD4+ T cell priming and proliferation.

In summary, we observed fewer migratory DC in the lymph nodes of CD11c^{Cre}DOCK8$^{fl/fl}$ mice upon immunization with MOG leading to attenuated EAE development. Interestingly, however, DOCK8 deficiency did not impact DC expression of CCR7, their capacity to migrate to CCL21 and their ability to migrate to the lymph nodes upon FITC painting. This contrast with data showing fewer FITC+ DC in DOCK8 KO mice (24) and therefore suggest that other DOCK8 deficient cells might impact the migration of DC in lymph nodes

in DOCK8 KO mice. In contrast, our data demonstrate that the reduction of migratory DC in the lymph nodes is cell intrinsic, occurs at steady state and is likely developmental, an observation further supported by our bone marrow chimeras. Another report has recently described a selective defect in migratory cDC2 cells in the mediastinal LNs of $CD11c^{Cre}DOCK8^{fl/fl}$ mice intranasally challenged with ovalbumin (23). After subcutaneous antigen challenge, here we found however that both migratory cDC1 and cDC2 were significantly diminished in the inguinal draining lymph nodes. This difference could be due to the route of antigen delivery and/or preferential migration of migratory cDC1 over cDC2 in mediastinal LNs compared to inguinal LNs. Nevertheless, the study by Krishnaswamy et al. (23) and ours converge on the importance of DOCK8 for migratory DCs (subsets) to prime respectively Tfh in the mediastinal LN and autoreactive effector T cells in the inguinal lymph nodes.

While failure of dendritic cells to efficiently prime T cells is often been attributed to their inadequate localization and migration, or inability to process and present antigen to T cells (23, 24), we found that DOCK8 deficient DCs could appropriately migrate in vitro to CCL21 and in vivo to inguinal lymph nodes and could efficiently process MOG protein and present MOG35-55 peptide to stimulate 2D2 specific T cells. In contrast, we demonstrated that DOCK8 is important for the homeostasis of splenic cDC2 and migratory DC at steady state. The requirement of DOCK8 for cDC2 and migratory DC is reminiscent, to some extend of the one from transcriptional factor IRF4 which promote the activity of migratory DCs (25) and the survival of cDC2s (25). Therefore, it would be interesting to determine in future studies whether IRF4 expression is dysregulated in the absence of DOCK8 in DCs.

In addition, GM-CSF and Flt3 ligand (Flt3L) are two cytokines which control the DC lineage. Flt3L drives differentiation of cDCs from hematopoietic stem cells, while GM-CSF can act as a survival factor for some DC populations in non-lymphoid organs (3, 26). Since Flt3L and GM-CSF preferentially signal via STAT3 and STAT5 respectively, and we (26, 27) and others (28, 29) have shown that DOCK8 regulates Stat5 and Stat3 mediated signaling, it is possible that DOCK8 shapes the homeostasis/ survival of different subsets of DCs through modulation of signaling downstream of cytokines. Importantly, while we have established that $CD11c^{Cre}DOCK8^{fl/fl}$ mice had a deficiency in both splenic resident cDC2 and migratory DCs, we have demonstrated that only migratory DC, but not splenic resident DC could stimulate 2D2 MOG specific T cells (Fig. 4H). Although splenic resident DC are important for the control of systemic infections (30), our data showing the importance of migratory DCs for the priming of CD4+ T cells are consistent with earlier report that migratory radioresistant APCs rescue the T cell proliferative defect observed in $CD11c/A_{\beta}{}^{b}$ mice (31). Interestingly, the phenotype of migratory DC ($MHCII^{hi}CCR7^{+}$) is reminiscent of the phenotype of mature DC observed in human lymph nodes (32). In the context of central nervous system autoimmunity, CCR7 has been shown to regulate the recirculation of DC from the CNS during EAE (5). Furthermore, two recent papers demonstrate the importance of cDC for antigen presentation and the development of EAE (10, 11). Interestingly, both CD26+ cDC and meningeal DCs or choroid plexus DCs express high level of CCR7 (9). These data, together with the identification of lymphatic vessels in the CNS (33), suggest that the aforementioned CNS DCs might be migratory DCs. Therefore, building on recent papers showing the importance of DC in EAE progression (5, 9-11), our data suggest that

targeting of DOCK8 in DCs could offer perspective to limit pathogenic T cell responses in CNS autoimmunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Key points

- Deficiency of DOCK8 in dendritic cells limits the development of EAE
- DOCK8 controls the presence of splenic cDC2 and migratory DC in the lymph nodes
- Migratory DC prime myelin specific $CD4^+$ T cells

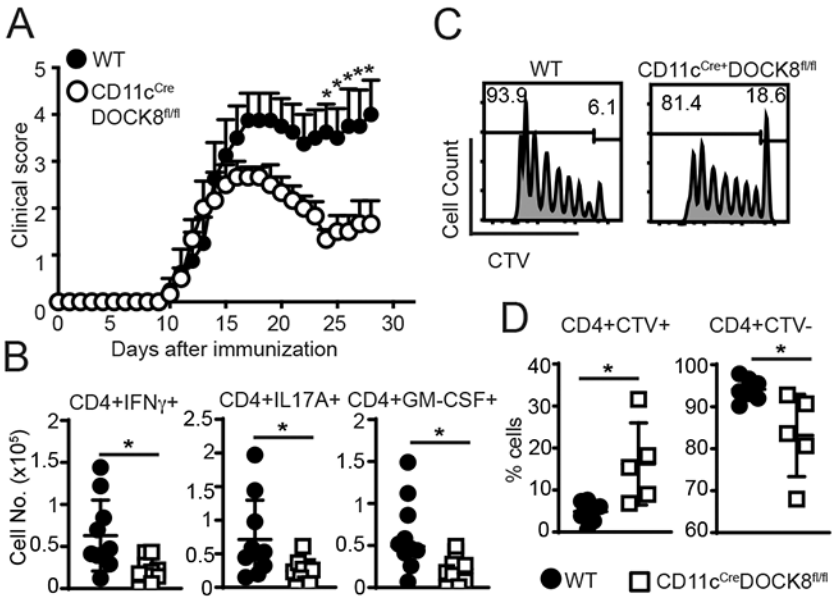

Figure 1. DOCK8 deficiency in CD11c+ cells limits EAE severity and T cell proliferation in vivo.

A) Clinical scores of Ctrl and $CD11c^{Cre}DOCK8^{fl/fl}$ mice over time (one experiment with n=6-8 mice/group, representative of 4 experiments). **B)** Number (± SEM) of $CD4^{+}IFN\text{-}\gamma^{+}$, $CD4^{+}IL\text{-}17^{+}$, and $CD4^{+}GM\text{-}CSF^{+}$ T cells in the CNS of $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice at the peak of EAE (One experiment, n=10 mice/group). **C-D**) In vivo proliferation of MOG specific 2D2 T cells in the dLNs of $CD11c^{Cre}DOCK8^{fl/fl}$ and control mice previously immunized with MOG35-5 peptide. **C)** Representative flow cytometry plot of CTV dilution. **D)** Scatter plots representing the percentage proliferated (CTV^{-}) and unproliferated (CTV^{+}) 2D2 cells in the dLNs (two experiments, n=5-7 mice/group). For the clinical score, two-way mixed effects analysis ANOVA was conducted. For the scatter plots, a two-tailed Mann Whitney test was conducted, *$p<0.05$ or $p<0.005$.

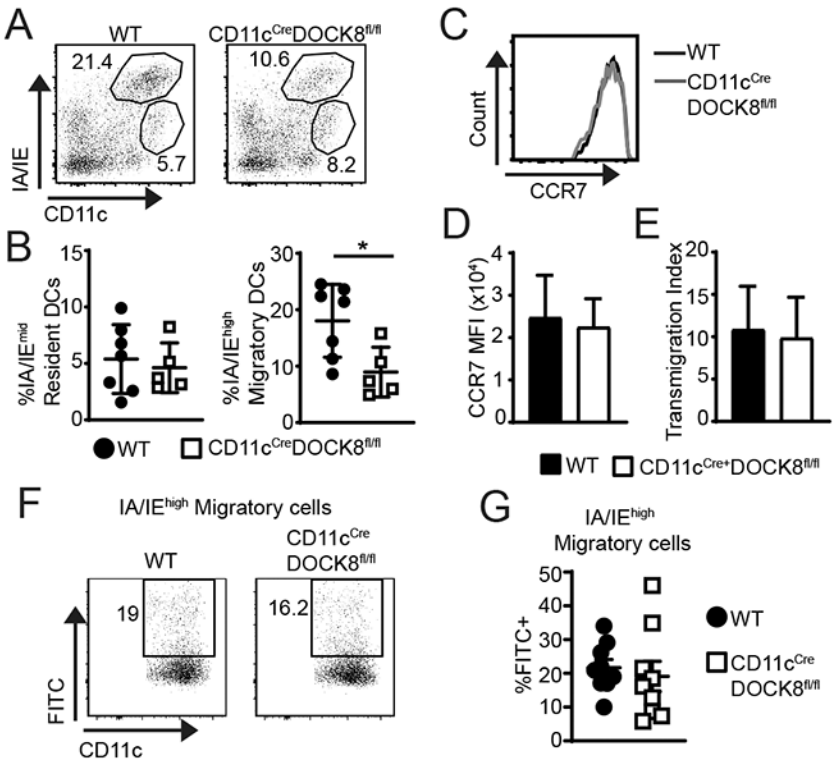

Figure 2. DOCK8 deficient DC can migrate efficiently.

Representative flow plots **(A)** and frequency ± SEM **(B)** of IA/IE^{mid} resident and IA/IE^{high} migratory DCs in the dLNs 4 days after immunization (2 experiments, n=5-7 mice/group). **C)** Expression and **D)** mean fluorescence intensity (± SD) of CCR7 within IA/IE^{high} migratory DCs 5-7 days after immunization with MOG35-55/CFA (2 experiments, n=5 mice/group). **E)** CCR7 dependent migration of BMDC toward CCL21. The transmigration index (± SD) was calculated as described in the Materials and Methods (one representative experiment of 2, n=5 mice/group). Representative FACS plot **(F)** and bar graph **(G)** showing the frequency of FITC+ within CD11c^{+}IA/IE^{high} migratory cells after FITC painting (3 experiments, n=9 mice/group). For statistical analysis, two-tailed Mann Whitney test was conducted, *p<0.05.

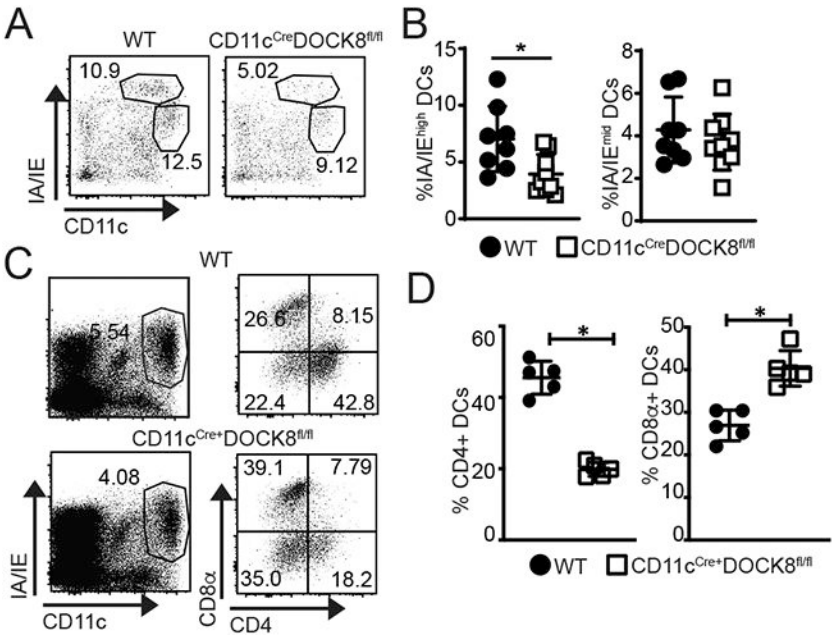

Figure 3. The deletion of DOCK8 in CD11c+ cells limits the frequency of migratory DCs and cDC2 in the spleen.

Representative flow plots **(A)** and frequency (± SEM) **(B)** of IA/IE^{mid} resident and IA/IE^{high} migratory DCs in the skin draining lymph nodes of naïve mice (4 experiments, n=8-9 mice/group). **C)** Representative flow plots of CD11c^{+}IA/IE^{high} cells (left) and CD4^{+} cDC2 and CD8α^{+} cDC subsets (right) and their frequency **(D)** in the spleen of naïve mice (2 experiments, n=5 mice/group). For statistical analysis, two-tailed Mann Whitney test was conducted, *p<0.05 or p<0.005.

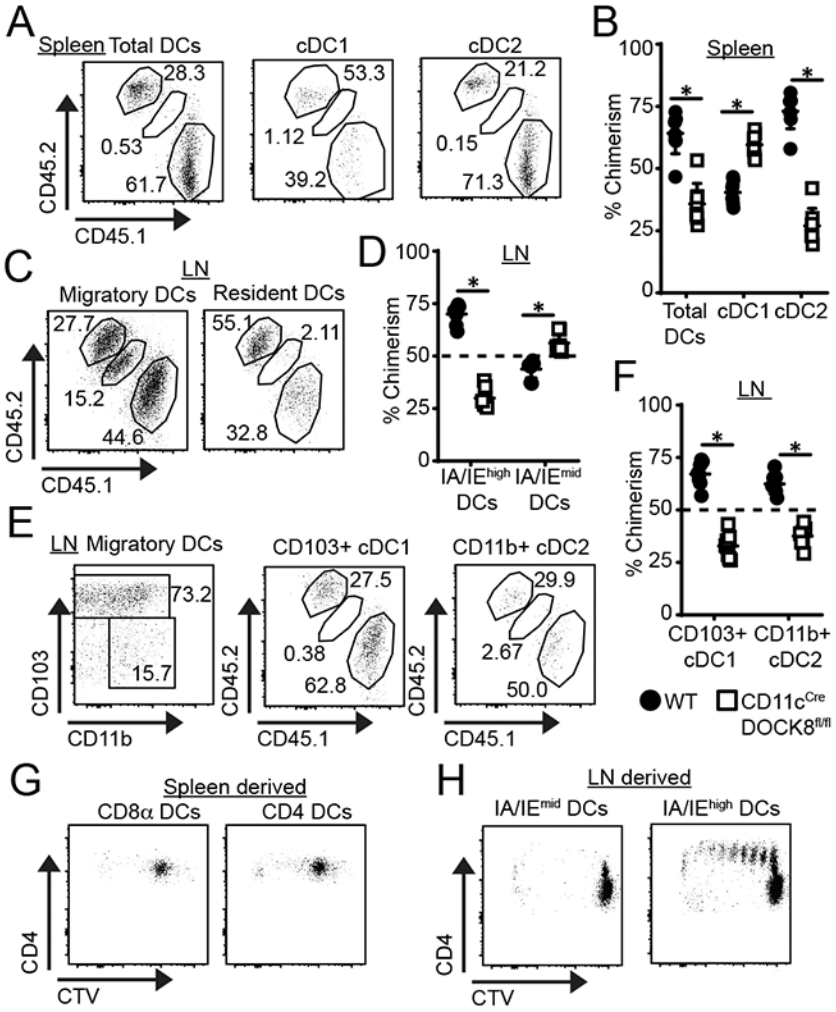

Figure 4. Migratory DC reduction caused by DOCK8 deficiency is cell-intrinsic and IA/IE^{high} migratory DCs are the primary cells necessary for T cell proliferation.

A) Representative flow cytometry plots showing the contribution of CD45.1$^+$ and CD45.2$^+$ cells in total splenic DCs and within CD4$^+$ and CD8α$^+$ DCs subsets. **B)** Percentage chimerism within total splenic DCs and CD4$^+$ and CD8α$^+$ DC subsets. Flow plots representing the proportion **(C)** and percentage chimerism **(D)** of CD45.1$^+$ versus CD45.2$^+$ cells within IA/IE^{mid} resident and IA/IE^{high} migratory DCs in the skin draining lymph nodes. **E)** Representative plots showing the contribution from CD45.1$^+$ and CD45.2$^+$ cells and **(F)** the percentage chimerism of these cells among IA/IE^{high} migratory CD103+ cDC1 and CD11b+ cDC2 cells in the lymph nodes (one experiment, n=8 mice/group). For statistical analysis, two-way ANOVA using Sidak's multiple comparisons test was conducted *p<0.05 or p<0.005. **G)** Representative flow plots of CTV dilution showing the proliferation of 2D2 cells co-cultured with sorted spleen resident CD4+ and CD8+ DCs subsets, and **(H)** CD11c$^+$IA/IE^{high} migratory and CD11c$^+$IA/IE^{mid} resident DCs isolated from lymph nodes of WT mice that had been immunized with MOG35-55 peptide. Representative of 2 experiments (cells pooled from up to 5 mice).