DNGR-1+ Dendritic Cells are Located in Meningeal Membrane and Choroid Plexus of the Noninjured Brain

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The role and different origin of brain myeloid cells in the brain is central to understanding how the central nervous system (CNS) responds to injury. C-type lectin receptor family 9, member A (DNGR-1/CLEC9A) is a marker of specific DC subsets that share functional similarities, such as CD8α+ DCs in lymphoid tissues and CD103+CD11b+DCs in peripheral tissues. Here, we analyzed the presence of DNGR-1 in DCs present in the mouse brain (bDCs). Dngr-1/Clec9a mRNA is expressed mainly in the meningeal membranes and choroid plexus (m/Ch), and its expression is enhanced by fms-like tyrosine kinase 3 ligand (Flt3L), a cytokine involved in DC homeostasis. Using Clec9aegfp/egfp mice, we show that Flt3L induces accumulation of DNGR-1-EGFP+ cells in the brain m/Ch. Most of these cells also express major histocompatibility complex class II (MHCII) molecules. We also observed an increase in specific markers of cDC CD8α+ cells such as Batf-3 and Irf-8, but not of costimulatory molecules such as Cd80 and Cd86, indicating an immature phenotype for these bDCs in the noninjured brain. The presence of DNGR-1 in the brain provides a potential marker for the study of this specific brain cell subset. Knowledge and targeting of brain antigen presenting cells (APCs) has implications for the fight against brain diseases such as neuroinflammation-based neurodegenerative diseases, microbe-induced encephalitis, and brain tumors such as gliomas.

Key words: C-type lectin receptors, myeloid cells, CD11c, Flt3L, neuroinflammation, Batf3

Introduction

The immune-privileged state of the brain has received renewed attention in recent years (Galea et al., 2007). Immune cells are today believed to contribute to the physiology of the nondiseased brain, and there is increasing evidence for the existence of distinct cells of myeloid origin in the brain with functions that are still not fully understood (Prinz et al., 2014 and references therein). Brain myeloid cells comprise microglia cells, perivascular, meningeal, and choroid plexus macrophages, periphery-derived monocytes, and brain dendritic cells (bDC). Parenchymal microglia is clearly implicated in the steady-state brain. Microglia cells in the healthy brain have a small soma with extensive radial ramifications that actively survey the microenvironment (Nimmerjahn et al., 2005). They have a myeloid origin; they derive from embryonic yolk sac progenitors (Ginhoux et al., 2010) and are sustained by local progenitors (Ajami et al., 2007). A further focus of interest is the trafficking of leukocytes and monocytes through the meningeal and choroid plexus (m/Ch) barrier to patrol the healthy brain or contribute to repair after sterile or nonsterile CNS injury (Ajami et al., 2007; Ransohoff and Cardona, 2010; Shechter et al., 2013).

The existence of dendritic cells in the brain has been described before in noninjured rat and mouse m/Ch...
(McMenamin, 1999; Matyszak and Perry, 1996; McMenamin et al., 2003), in the EAE (experimental autoimmune encephalomyelitis) mouse model (Serapino et al. 2000; Fischer and Reichmann, 2001; McMahon et al., 2005), and in multiple sclerosis (MS) in the human brain (Prodinger et al., 2011). Recent data obtained with transgenic mouse lines confirm the existence of resident bDCs in the nondiseased mouse brain; for example, bDCs from transgenic CD11c-EYFP mice identified by EYFP expression colocalize with other immune markers commonly associated with DCs and microglia/macrophages (Axtell and Steinman, 2009; Bulloch et al., 2008; D’Agostino et al., 2012). Furthermore, bDC CD11c+ cells have been identified in CD11c-GFP mice (Jung et al., 2002; Prodinger et al., 2011). In the periphery, DCs are the antigen presenting cells (APC) that mediate T-cell immunity and tolerance. The DC network is complex and comprises several DC subsets, and DC development has been shown to be dependent on fms-like receptor tyrosine kinase 3 ligand (Flt3L) (Heath and Carbone, 2009; Watowich and Liu, 2010). The m/Ch barrier contains a population of Flt3L-dependent DCs akin to the CD8α+ subset of spleen conventional DCs (cDC) (Anandasabapathy et al., 2011). However, further work is needed to precisely characterize the DC subsets in the noninjured brain.

DNGR-1 (CLEC9A) is a C-type lectin receptor that specifically marks a functional subset of DCs defined by dependence on BATF-3, IRF-8, and Id2. Differentiated DCs expressing DNGR-1 include CD8α+ cDCs and CD103+ DCs in peripheral tissues (Huysamen et al., 2008; Iborra et al., 2012; Poulin et al., 2010; Poulin et al., 2012; Sancho et al., 2008; Schraml et al. 2013). DNGR-1 is involved in recognizing death cell antigens (Sancho et al., 2009), and DNGR-1+ DCs could play a role in presentation of antigen to CD4+ T cells and cross-presentation to CD8+ T cells. Dngr-1/Clec9a mRNA has been examined in total human and mouse tissues, with the highest expression detected in human brain, thymus, and spleen (Huysamen and Brown, 2009), but the specific location of this expression in the brain has so far not been reported. In mouse tissues, different Dngr-1 isoforms are detected in spleen DCs (Sancho et al., 2008). Here, we analyzed the distribution of brain myeloid cells and the expansion of a DNGR-1+ DC subset after treatment with Flt3L. DNGR-1+ DCs localize in meningeal membranes and choroid plexus (m/Ch) but are scarce in brain parenchyma (BP), and we show that DNGR-1 expression in the brain is increased in response to Flt3L. The tracking of DNGR-1+ DCs will facilitate future studies into the role of this DC subset in the brain.

### Materials and Methods

**Cells:** B16 murine Flt3L-secreting tumor cells were grown in DMEM supplemented with 10% FBS (Iborra et al., 2012).

**Animals**

The transgenic mouse line c-fms-EGFP (Csf1r-EGFP) (Sasmono et al., 2003; macgreen), mice expressing EGFP under the promoter of c-fms (CSF1R) are characterized by the expression of enhanced green fluorescence in brain myeloid cells. Clec9a<sup>egfp/egfp</sup> mice (DNGR-1-deficient) (Sancho et al., 2009) on the C57BL/6 background were backcrossed more than 10 times to C57BL/6J-Crl. Batf3<sup>−/−</sup> mice (Hildner et al., 2008) were backcrossed more than 10 times to the C57BL/6 background and further backcrossed with C57BL/6 mice at the CNIC to establish WT and Batf3<sup>−/−</sup> colonies from the heterozygotes. Animal studies were approved by the local ethics committee. Mice were bred and maintained in animal facilities at the Instituto de Salud Carlos III. All animal procedures conformed to EU Directive 2010/63EU and Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced in Spanish law under Real Decreto 1201/2005.

### Tissue Processing

For experiments involving immunochemistry, mice were deeply anesthetized by i.p. injection of a mixture of ketamine and xylazine and transcardially perfused with 25–30 mL of saline solution for 5 min, followed by 4% PFA (Sigma Aldrich, St Louis, MO), pH 7.4, in 0.1 M phosphate buffer (PB, Sigma). After perfusion with the fixative, brains were dissected out and postfixed with 4% PFA for 18–20 h at 4°C. After fixation, brains were rinsed in 0.1 M PB and placed in 15% glucose at 4°C until they sank, and then in 30% sucrose in PB at 4°C for 72 h. Finally, brains were embedded in tissue freezing medium (Tissue-Tek O.C.T.™, Sakura), frozen immediately in dry-ice-cooled 2-methylbutane (Sigma-Aldrich), and stored...
Coronal sections (30 μm) were cut using a CM1950 cryostat (Leica Microsystems) and stored at -20°C until use. For the CD11c staining, mice were perfused for 1 min with saline followed by 4% ice-cold PFA and postfixation for not more than 8 h. The 3D reconstruction in Fig. 1 and the meningeal membrane studies were performed with 80 μm vibratome horizontal sections (Leica VT1200s).

**Immunohistochemistry**

Immunohistochemistry was performed on frozen brain sections by standard indirect staining (primary antibody followed by a fluorescent secondary antibody) as described in (Serrano-Perez et al., 2011) but using phosphate as buffer and 10% FBS for blocking. Antibodies were diluted in 0.1 M PB containing 1% FBS (Hyclone), 0.06% Triton-X100 (Sigma), and 150 mM glycine (Merck). Rabbit anti-Iba1 (1:100, Wako) was used to detect expression of microglia, Rat anti-mouse I-A/I-E (1:100, clone 2G9, B.D. Pharmingen) was used to detect antigen-presenting cells, Rabbit anti-pan-laminin (L9393 Sigma) was used to detect basal membrane, Rat anti-mouse CD31 (clone MEC13.3 BD Pharmigen) to detect endothelial cells, and biotin-anti-mouse CD11c (clone N418, Biolegend) to detect DCs. Samples stained with CD11c biotinylated antibodies were treated with the Biotin Blocking System (Vector Laboratories) before endogenous peroxidase activity was quenched with hydrogen peroxide. Anti-CD11c staining was detected with horseradish peroxidase-conjugated streptavidin/Cy3-tyramide using the tyramide signal-amplification system (Perkin Elmer, Boston, MA) according to the manufacturer's instructions. The secondary antibodies used were...
labeled with Cy3, 488, and 647 fluorochromes (Jackson Immunoresearch). Controls were conducted by omitting the primary antibody, MHC-II controls incorporate the isotype Rat IgG2aK, and CD11c controls incorporate biotin-control isotype IgG. Slides were finally counterstained with DAPI (5 μg/mL, Life technologies) and mounted in a mixture of Mowiol/50% DABCO (Sigma). Antibodies are described in Supporting Information, Table 1.

Confocal Microscopy and Analysis
Images were acquired on a Leica TCS SP5 inverted confocal laser scanning microscope (Leica Microsystems). Brain maps in Fig. 1 were imaged using a 20× objective and a 1.7× digital zoom (4 μm step size, 500 × 500 pixel resolution). Other images were acquired using a 40× objective (1 μm step size, 1024 × 1024 pixel resolution) for standard images and a 63× objective with a 3× digital zoom (0.5 μm step size, 1024 × 1024 pixel resolution) for detailed images. Images are presented as average projections of z-stacks and keeping parameters constant using negative control slides stained with primary antibody to identify potential non-specific, background fluorescence. Exceptions are mentioned in figure legends. Acquired z-stacks were background-subtracted with Leica LAS AF 2.6.3 software and secondary processed and analyzed using Adobe Photoshop CS3 (Adobe Systems) and ImageJ (National Institute of Health, http://rsb.info.nih.gov/ij) for ROI quantification and cell counting. For 3D reconstructions, the plugging 3D viewer for ImageJ was used.

Cell Preparation
Mice with an age of 6–12 weeks with different genetic background as specified were left noninjected or injected subcutaneously (s.c.) in the flank with 5 × 10⁶ B16 murine Flt3L-secreting tumor cells. Between days 12 and 16 after injection, when tumors measured approximately 1 cm², mice were sacrificed, and intracardiac perfusion was performed using phosphate buffered saline (PBS) with observed blanching of the spleen during 5 min at a speed of 5 mL/min. Complete brains and spleens were dissected and for most experiments meningeal (piamater) membranes and choroid plexus (m/Ch) were carefully removed with fine tweezers. Brain tissue was finely minced into small pieces and treated with a specific protease mix depending on the tissue. For brain without m/Ch, cells were prepared as in Sierra et al. (2007) with modifications. Each brain, without the m/Ch and cerebellum, was digested in 5 mL of enzyme solution (20 units/mL papain (Worthington) and 0.025 units/mL DNase (Sigma) in buffer containing 116 mM NaCl, 5.4 mM KCl, 26 mM NaHCO₃, 1 mM NaH₂PO₄, 1.5 mM CaCl₂, 1 mM MgSO₄, 0.5 mM EDTA, 25 mM glucose, and 1 mM L-cysteine, pH 7.5) for 30 min at room temperature (RT) with agitation. The brain homogenate was washed and filtered once through a 70-μm cell strainer. All subsequent steps were performed at 4°C in PBS, 2% FBS.

Flow Cytometry
Single-cell suspensions were prepared as above and resuspended in staining buffer (2.5% PBS in Dulbecco’s PBS; Biowhittaker, Lonza Group). Nonspecific binding to Fc receptors was blocked with 10 μg/mL of 2.4G2 mAb (Fc block) (BD Biosciences). Staining followed standard protocols, and antibodies and reagents are listed in Supporting Information, Table 1. Fluorochrome-labeled antibodies specific for mouse CD45, CD11b, CD11c, and MHCII were from BD Biosciences or Biolegend. Mouse cell suspensions were incubated with Fc block and were then stained on ice-cold PBS supplemented with 2 mM EDTA, 2% FBS, and 0.02% sodium azide. Cells were analyzed on a FACSCANTO cytometer using FlowJo version 6.3.4 (Tree Star, Ashland, OR).

RNA Isolation, Reverse Transcription, and Real-Time PCR
Total mRNA was isolated with TRIzol Isolation Reagent (Roche Applied Sciences, Spain). Total mRNA was quantified and reverse transcribed to cDNA with MMLV Transcriptase Reverse (Invitrogen), following the manufacturer’s instructions except for the use of 300 ng of random primers and 4 units of RNase OUT (Invitrogen). Semiquantitative PCR of brain and spleen tissues was performed with the AmpliTaq Gold® kit (Applied Biosystems), using 5 ng of total cDNA and 1 μM primers. Spleen samples were loaded at a dilution of 1/30 relative to brain samples to avoid image saturation. The actin primers were forward, GTGGGCCGCTCTAGGCACCACAT, and reverse, CTCTTTGATGTCAAGCAGATTTTC. Clec9a/Dngr-1 primers were forward, AGACTGCTTACCACTCACA, and reverse, CTTGGGCAATAFGGACAAGGT (Sancho et al., 2008).

Relative amounts of cDNA were analyzed by real-time PCR (RT-PCR) using the FAST SYBR-green system (Applied Biosystems). Each 15 μL reaction volume included 5 ng total cDNA and 0.5 μM of each primer. Mouse 36B4 was used as an internal control to normalize for variation in input RNA. Primers used are as described in Supporting Information, Table 2. The amount of target mRNA in samples was estimated by the 2-ΔΔCT relative quantification method (Livak and Schmittgen, 2001). Ratios were calculated between the amounts of mRNA from the different tissues.
Statistical Analysis
Statistical analyses were performed using GraphPad Prism 4.0 software. The \( p \) values were calculated with the two-tailed Student’s \( t \) test (\( \* p \leq 0.05, \* \* p \leq 0.01, \* \* \* p \leq 0.001 \)). Data are presented as the means ± SEM of a number of experiments (\( n \)) indicated in each case.

Results
Myeloid Cells in the Noninjured Brain
We first analyzed the myeloid compartment present in the intact brain by confocal microscopy of coronal brain sections from mice expressing the c-fms-EGFP reporter construct. The c-fms gene encodes the receptor for mouse macrophage colony-stimulating factor (CSF1R/CD115), which in brain is expressed only in cells of myeloid origin (Sasmono et al. 2003). From hereon, we refer to this line as Csf1r-EGFP mice. CSF1R-EGFP+ cells were present throughout the brain in two distinct localizations, the parenchyma itself (BP) and in the perivascular spaces, as shown in (Fig. 1A, panels i and ii). CSF1R-EGFP+ cells were found in choroid plexus (Fig. 1B, panels i and ii) and in the piamater meningeal membrane (Fig. 1C, panels i and ii). Interestingly, CSF1R-EGFP+ cells in these localizations have different morphologies: CSF1R-EGFP+ cells in the BP proper have a small soma with extensive radial ramifications and are very evenly distributed throughout the noninjured brain; in contrast, CSF1R-EGFP+ cells in the perivascular areas from the brain parenchyma have a more amoeboid morphology with fewer ramifications and were situated in close contact with the basement membrane of brain vessels (Fig. 1A, panel ii). CSF1R-EGFP+ cells from choroid plexus were as well brighter, amoeboid in shape, and they were situated in close contact with CD31+ blood vessels from the plexus as shown in Fig. 1B, panel ii. We analyzed the piamater or pial leptomeninges (meningeal) sliced from the surface of the cerebral hemispheres from the Csf1r-EGFP mouse brains, and observed a dense network of CSF1R-EGFP+ cells that resemble the CD11b+ (OX-42) cells previously described in rat pial leptomeningeal membrane (McMenamin, 1999; McMenamin et al., 2003). To assess the situation of these CSF1R-EGFP+ cells, and to determine if they were situated on top of the piamater or in the BP, we counterstained with an antibody that recognizes the glial fibrillary acidic protein (GFAP). This antibody stains the glial limitans membrane. In Supporting Information, Fig. 1s, we show that the CSF1R-EGFP+ cells constitute a barrier of cells situated in the meningeal membrane above the GFAP+ area. A widely used marker of brain microglia is the ionized calcium binding adapter molecule 1 (Iba1), which recognizes a microglia/macrophage-specific calcium-binding protein (Ito et al., 1998). Analysis of Iba1 expression in uninjured Csf1r-EGFP brains revealed co-expression of Iba1 in 99 ± 0.7% of CSF1R-EGFP+ cells in brain (Supporting Information, Fig. 2s, panel A). Iba1low/+ CSF1R-EGFP+ cells were very rare in the brain itself, and the few that we found were next to blood vessels (data not shown). Analysis of m/Ch detected a population of Iba1low/+ CSF1R-EGFP+ cells, accounting for 7 ± 2% of the total CSF1R-EGFP+ cells in the choroid plexus (Supporting Information, Fig. 2s, panel B). We could not observe Iba1+ cells that were CSF1R-EGFP+. To analyze Iba1+ and CSF1R-EGFP+ in the meningeal (m) membranes, we used 80 μm horizontal vibratome sections, choosing the very first slide from the brains. In this brain localization, we observed CSF1R-EGFP+ cells that were clearly Iba1low/+ (Supp. Info., Fig. 2s, panel C); in fact, most of the very bright CSF1R-EGFP+ located in the pia leptomeningeal membrane were Iba1low−, and we could only see Iba1+ CSF1R-EGFP+ cells in the proper brain tissue.

To further analyze brain myeloid cells, we isolated CD45+ cells from the brain without (w/o) m/Ch preparations and m/Ch of Csf1r-EGFP mice; the gating strategy used to exclude dead cells and doublings is presented in Supporting Information, Fig. 3s. Cells of ectodermal origin, such as astrocytes, oligodendrocytes, and neurons, which together with endothelial cells constitute the majority of cells in the brain, are not labeled with CD45 antibodies. Therefore, due to the lack of specific markers for microglia itself, we used CD45 expression to characterize the proper microglia, which have a medium expression of CD45 (CD45m), and myeloid cells from the periphery as CD45 high (CD45h) (Carson et al., 1998; Ford et al., 1995). CD45h cells accounted for 30–40% of total live cells in brain w/o m/Ch isolates (Fig. 2A, panels i–iv), and 20–30% of cells obtained from m/Ch preparations (Fig. 2B, panels i–iv). The percentage of CD45h cells obtained was similar in wild type (WT) and Csf1r-EGFP mice. In isolates from Csf1r-EGFP mice, ≥96% of cells were CSF1R-EGFP+ CD45h (Fig. 2A,B). We found few CD45+ EGFP− cells in the brain w/o m/Ch cell isolates (Fig. 2A, panel v) and in the m/Ch preparations (Fig. 2B, panel v). To include this small CSF1R-EGFP− CD45+ subset in our study, we used CD45 labeling instead of EGFP expression to study brain myeloid cells for subsequent analysis.

Ftx3L-Dependent Accumulation of Cd45+ Cells in Brain
We are interested in the different myeloid cell subsets present in the steady-state brain. In this context, an increase in bDC numbers has been reported after treatment of mice with Ftx3L (Greter et al., 2005; Anandasabapathy et al., 2011). The latter authors observed an increase in a CD45+CD11c+MHCII+ cell population that was expanded after treatment of mice with Ftx3L. We isolated brain myeloid
cells from untreated (−Flt3L) and Flt3L-treated (+Flt3L) WT animals and analyzed CD45+ cell numbers. In brain w/o mCh preparations, we distinguished two CD45+ populations according to fluorescence intensity in response to FLT3L, and in accordance with published data (Carson et al., 1998), we refer to these as CD45m (medium intensity) and CD45h (high intensity) (Fig. 3). Flt3L treatment did not increase the percentage of total CD45+ cells isolated (Fig. 3A, panel I). However, Flt3L treatment did increase the frequency and numbers of cells in the CD45h population in isolated cells from brain w/o mCh (Fig. 3A, panels II and 3C). The Flt3L-induced increase in the brain CD45 h subpopulation prompted us to analyze if these cells were of myeloid origin. For this analysis, we detected the marker CD11b, also known as Integrin alpha M chain (ITGAM), in an approach similar to that used in rat by Ford et al. (1995). Three subpopulations were detected in brain w/o mCh preparations. The largest (69 ± 7% of CD45+ cells) was CD45mCD11b+ (Fig. 3B); these cells correspond to brain microglia based on their medium staining with the CD45 marker and their staining with the CD11b marker. The other two subpopulations subdivide the CD45h population according to CD11b expression: CD45hCD11b+ (15 ± 4%) and CD45hCD11blow (15 ± 5%), and correspond to blood-borne cells as described by using irradiation chimeras (Ford et al., 1995).

Similar analyses were performed in mCh cell preparations (Fig. 4). The percentage of CD45+ cells was 32 ± 6% in the absence of Flt3L, increasing to 44 ± 6% upon Flt3L treatment in these brain localizations. The numbers of isolated CD45+ cells varied between the untreated and Flt3L-treated groups (Fig. 4A, panel I). CD45 and CD11b marker expression distinguished only two subpopulations: CD45hCD11b+ (66 ± 2%) and CD45hCD11blow (26 ± 1%) (Fig 4B). The percentage and numbers of total CD45+ cells obtained from mCh was not significantly affected by Flt3L treatment, but the relative frequency and numbers of

FIGURE 2: Identification of CD45-positive brain cells. Flow cytometry dot plots of live cells isolated from (A) brain except cerebellum, meningeal membranes, and choroid plexus (B) meningeal membranes and choroid plexus (m/Ch). Cells were isolated from wild type (WT) and Csf1r-EGFP mice, and stained with control isotype antibody (APC rat IgG2b; panel i) or with APC rat anti-mouse CD45 (panels ii and iii). Panels iv show the arithmetic mean ± SEM (n = 5) of the percentage of CD45+ (black) and CD45- (white) cells isolated. Panel v represents the arithmetic mean ± SEM (n = 5) of percentage of EGFP+ cells in the Csf1r-EGFP cell isolates. The gating strategy was as presented in Supporting Information, Fig. 3s.
CD45^hCD11blow cells was increased (Fig. 4A, panels II and 4C). The number of CD45^hCD11blow cells was 82 ± 15 cells/10^3 CD45^1 cells, increasing approximately threefold to 261 ± 11 cells/10^3 CD45^1 cells in the presence of Flt3L (Fig. 4C). bDCs have been identified mainly in the m/Ch (see the section titled “Introduction”). Flt3L-responsive m/Ch cells in the brain have been reported to have a CD45^hCD11c^1MHCII^1 phenotype, detected mainly with the use of the CD11c-EYFP transgenic mouse (Anandasabapathy et al., 2011). Using our m/Ch brain isolation methodology, we were unable to detect CD11c^1 cells in flow cytometry (FC) analyses, although the antibody was able to recognize the presence of CD11c^+ cells in the spleen (Supp. Info., Fig. 4s and the section titled “Discussion”). Since bDCs should express major histocompatibility complex class II (MHCII) antigens in their membrane, we first analyzed the expression of MHCII by immunofluorescence (IF). MHCII^1 cells were rarely found in the BP, but when found, they were situated always in close proximity with blood vessels (Fig. 5A) and probably correspond to perivascular macrophages (see the section titled “Discussion”). MHCII^+ cells in the Flt3L-treated mice were mainly situated in the plexus (Fig. 5B). Most of the cells analyzed showed co-localization of both markers, although we noted that stronger staining of
the MHCII antigen corresponded to a less bright CSF1R-EGFP fluorescence by IF (not quantified) (Fig. 5B, panel ii). In the pia leptomeningeal membrane, most CSF1R-EGFP cells were MHCII (Fig. 5C, panel i), and when MHCII cells were found, they were situated close to vessels and in clusters (Fig 5C, panel ii inset). These MHCII cells in meningeal membrane showed a diminished EGFP fluorescence (Fig. 5C, panel ii), and were seen only in Flt3L-treated mice.

We wanted to analyze the presence of CD11c in the noninjured mice without using transgenic mice such as the CD11c-YFP (Bulloch et al., 2008) or CD11c-GFP (Pro-
from the brain w/o m/Ch preparations of untreated control animals (~Flt3L) had a CD45<sup>+</sup> phenotype, and FC analysis showed that these cells were MHCII<sup>−</sup>. Flt3L treatment induced MHCII expression in <1% of cells in this CD45<sup>+</sup> subpopulation (Fig. 6A, panel I). The CD45<sup>+</sup>CD11b<sup>+</sup> population contained 36 ± 5% MHCII<sup>−</sup> cells, and this percentage...
did not vary significantly in the presence of Flt3L (41 ± 7\%) (Fig. 6A, panel II). The amount of CD45\(^h\)CD11blow cells obtained in the noninjured brain varied between different brain preparations. The majority of these cells (79 ± 7\%) expressed MHCII in their membranes (Fig. 6A, panel III). Since we did not observe MHCII\(^1\) cells in the parenchyma proper by IF analysis, we believe that these MHCII\(^1\) cells might reflect contamination by m/Ch cells or perivascular myeloid cells. Further analysis is required to assess the localization and origin of these MHCII\(^1\) cells in the brain parenchyma.

When m/Ch preparations were analyzed, we observed a threefold increase in the number of CD45\(^h\)CD11blow cells, as shown before (Fig. 4). Of the CD45\(^h\)CD11blow cells obtained from m/Ch, 70 ± 3\% were MHCII\(^1\) (Fig. 6A, panel III). In conclusion, Flt3L treatment preferentially expands a CD45\(^h\)CD11blow subset of MHCII\(^1\) cells in the m/Ch. The fact that these cells are increased in the presence of Flt3L and that they have a CD45\(^h\)CD11blowMHCII\(^1\) phenotype leads us to think that they are the bDCs previously described in m/Ch (Anandasabapathy et al., 2011; McMenamin, 1999; McMenamin et al., 2003; Prodinger et al., 2011), and that we detected localized mainly in the choroid plexus (Fig. 5).

**Expression of Dngr-1 Transcripts in Mouse Brain**

The gene expression profile of Flt3L-responsive m/Ch myeloid cells bears strong similarities to that of the CD8\(\alpha\)^+ DC subpopulation in the spleen (Anandasabapathy et al., 2011). The high expression of DNGR-1 (CLEC9A) is an excellent marker of Batf3-IRF8-Id2-dependent DCs of the CD8\(\alpha\)^+ family (Caminschi et al., 2009; Huysamen et al., 2008; Sancho et al., 2008); reviewed in Shortman and Heath, 2010. We expanded DNGR-1\(^1\) DCs by treatment with Flt3L (Iborra et al. 2012) and analyzed Dngr-1 mRNA expression in spleen (positive control) and in an array of brain structures: olfactory bulb (Ob); cerebellum (Cbl); brain cortex (Ctx); striatum (Str); meningeal membranes and choroid plexus (m/Ch); subventricular zone (SVZ) isolated as in Ferron et al. (2007) and Guo et al. (2012); hippocampal formation (Hpp); and thalamus (Th). Only the m/Ch samples showed expression of all Dngr-1 isoforms (Fig. 7A,B), similarly to the defined isoforms in the mouse spleen (Sancho et al., 2008). As a control of specificity, we used spleen and brain tissue from untreated and Flt3L-treated Clec9a\(^{egfp/egfp}\) mice, which lack DNGR-1, confirming no amplification of the specific Dngr-1 transcript in these samples (data not shown).
Selective expression of Dngr-1 in m/Ch of Flt3L-treated animals was corroborated by real-time PCR. The higher sensitivity of this technique allowed detection of Dngr-1 transcripts in the brain w/o m/Ch preparations, but in lower amounts than in m/Ch. Flt3L strongly augmented the amount of Dngr-1 transcript in splenocytes and m/Ch preparations, with the expression of Dngr-1 in Flt3L-treated spleenocytes around 5 times higher than in m/Ch cell preparations (Fig. 7C). This Flt3L-triggered increase in the expression of Dngr-1 is consistent with the increase in the percentage of CD45h cells induced by the same cytokine (Figs. 3 and 4). Together, these results show that Flt3L induces Dngr-1 expression preferentially in the m/Ch.

The procedures for isolating cell populations from the adult brain expose cells to enzymes and mechanical forces that can damage membranes or membrane molecules. Some of these enzymatic procedures could cleave specific membrane cellular markers of interest such as DNGR-1, leading to false-negative results in the FC analysis. To circumvent this problem, we used Clec9aegfp/egfp mice (Sancho et al., 2009), which express a farnesylated form of EGFP in frame with the first two aminoacids of DNGR-1. Thus, expression of EGFP marks DNGR-1-expressing cells, and is restricted in the spleen to CD8α1cDC cells (Sancho et al., 2009). Accordingly, Flt3L treatment induced a 10-fold expansion of EGFP1DCs in the spleen (Supp. Info., Fig. 3s). Cytometry analysis of brain w/o m/Ch and m/Ch cells isolated from ±Flt3L-treated WT and Clec9aegfp/egfp mice revealed EGFP expression in CD45h cells in both cell preparations. In brain w/o m/Ch preparations, there was a modest but significant increase in the numbers of CD45h EGFP+ cells (Fig. 8A), whereas in m/Ch preparations, the CD45h EGFP+ cells were increased 10-fold by Flt3L treatment (Fig. 8B).

We next analyzed the expression of MHCII antigen in EGFP+ cells. As a positive control, we analyzed the Flt3L-induced increase in EGFP+ splenocytes, finding that all
CD45\(^+\) DNGR-1-EGFP\(^+\) cells in the spleen were MHCII\(^+\) (Supp. Info., Fig. 4s, panel B). Analysis of brain w/o m/Ch and m/Ch isolates showed that DNGR-1-EGFP\(^+\) cells were also MHCII\(^+\) in the brain by FC analysis (Fig. 9A,B). EGFP\(^+\) cells have low expression of CD11b, and correspond to the CD45\(^+\)CD11b\(^{low}\) subpopulation described in Figs. 3 and 4. Since MHCII\(^+\) cells in Flt3L-treated animals are situated mainly in the choroid plexus (Fig. 5), we analyzed if the DNGR-1-EGFP\(^+\) cells were positive for CD11c and MHCII, both known DC markers (see the section titled “Introduction”). DNGR-1-EGFP\(^+\) cells were localized mainly in the plexus and were detected by a weak direct fluorescence of EGFP protein. These DNGR-1-EGFP\(^+\) cells co-expressed the MHCII and CD11c markers (Fig. 9C). These findings thus establish the existence of a subset of CD11c\(^+\) MHCII\(^+\) cells that express DNGR-1 in the brain. Our conclusion is that DNGR-1 expression allows for further discrimination of DC subsets in the brain.

The surface protein and mRNA expression analyses performed here indicate that the majority of Flt3L-dependent cells in the brain are located in the m/Ch. To confirm the DC-nature of the Flt3L-dependent cell population in the

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**FIGURE 8: DNGR-1-EGFP\(^+\) cells in brain cell isolates.** Myeloid cells were analyzed from the brain as before (A) and m/Ch (B) of Cle9a\(^{-/}\) wt and Cle9a\(^{egfp/egfp}\) littermates that were untreated (−Flt3L) or treated with Flt3L (+Flt3L) for 14 days. Flow cytometry dot plots show staining with APC rat anti-mouse CD45 antibody from a representative experiment of 5 performed. Gates are shown corresponding to total CD45\(^+\) cells (dotted line) and CD45\(^+\) cells with high intensity (CD45\(^{hi}\); dashed line). The oval regions demark the population of CD45\(^{hi}\) DNGR-1-EGFP\(^+\) cells. Bar charts in A and B show numbers of CD45\(^{hi}\) DNGR-1-EGFP\(^+\) cells per 10\(^3\) CD45\(^+\) cells from control (−Flt3L) and treated (+Flt3L) animals. Data are mean ± SEM of 5 independent experiments. To obtain sufficient cell numbers, m/Ch isolates from two brains were pooled for each condition.
m/Ch, we analyzed the mRNA expression of DC molecular markers typical of DNGR-1<sup>+</sup> DCs (Poulin et al., 2010; Poulin et al., 2012) by quantitative RT-PCR. Choroid plexus from Flt3L-treated mice showed increased mRNA expression of the transcription factors Irf-8 (interferon regulatory factor 8) and Batf-3 (basic leucine zipper transcription factor ATF-like 3) (Fig. 9E). We also detected increased expression of Cd103 (integrin alpha E (ITGAE)), a molecule expressed in dendritic cells from nonlymphoid tissues (reviewed in del Rio et al., 2010). In contrast, mRNAs encoding the co-stimulatory molecules Cd40 (data not shown), Cd80, and Cd86 were not increased after Flt3L treatment, consistent with nonactivated state of these DCs in the normal brain. These results thus indicate that Flt3L-treated m/Ch show increases in the expression of genes that specifically mark the splenic CD8α<sup>+</sup> DC and peripheral CD103<sup>+</sup> DC subsets, and therefore, the bDCs described here share similarities with this specific DC subset.
Deletion of the transcription factor Batf3 has been shown to ablate development of CD8α+ DCs in the periphery (Hildner et al., 2008). We therefore predicted that the m/Ch of mice lacking this transcription factor (Batf3−/−) would be defective for Flt3L accumulation. Flt3L-treated Batf3−/− animals did not show increases in Dngr-1 mRNA when compared with similarly-treated wild-type littermates (Fig 9E). This implies that Flt3L-induced DNGR-1-EGFP+ CD11c+ MHCIi+ cells in the brain are very similar and have the same requirements as their peripheral CD8α+ DC counterparts. The exact role of these cells in the intact and injured brain remains to be defined.

**Discussion**

In this study, we have shown Flt3L-dependent accumulation of a population of DNGR-1(CLEC9A)-EGFP+ CD45b+ CD11b+ MHCIi+ myeloid cells in the brain. These cells accumulate mainly in the m/Ch that surrounds the brain. We call these cells m/Ch DCs, as has been proposed before (Anandasabapathy et al., 2011). These cells have been shown to share functional and developmental characteristics with CD8α+ cDCs from spleen, and here we show that m/Ch DCs express the C-type lectin receptor DNGR-1/CLEC9A.

The entry and activation of T cells in the brain parenchyma requires a pre-existing interaction of these cells with APCs. Most cells in the body can present antigen to CD8α+ T cells via MHC class I molecules, and thus act as APCs. However, the term APC is often reserved for specialized cells that can prime CD8+ (cytotoxic) T cells through MHC class I molecules and also stimulate CD4+ (helper) T cells through MHC class II. APCs that express MHC class II molecules are often called professional APCs, and mainly consist of macrophages and dendritic cells. To isolate brain myeloid cells which express MHCIi in their membranes, and therefore can function as professional APCs in the brain, we made use of Csf1r-EGFP mice (Figs. 1 and 2), which express EGFP protein under the control of the Csf1r/CD115/fms promoter (Sasmono et al., 2003). Csf1r plays an important role in the development and maintenance of brain microglia (Elmore et al., 2014; Erblitch et al., 2011), and our data show the existence of morphologically distinct Csf1r-EGFP+ cells in BP and m/Ch of Csf1r-EGFP mice (Fig. 1). We are aware that brain parenchymal cells should refer only to the proper microglial cells (CD45med), Therefore, we have tried to avoid using the term BP referring to myeloid cells isolated from brain without m/Ch, which contains perivascular macrophages with expected different functions. The presence of differences in the Csf1r-EGFP+ cells within the distinct brain areas are in line with previously described results showing myeloid cells from noninjured rat and mouse brains, identified with an array of different antibodies and methods (Buloch et al., 2008; Matyszak and Perry, 1996; McMenamin, 1999; McMenamin et al., 2003; Prodinger et al., 2011). The use of a well-known marker for brain microglia cells such as Iba-1 showed that in the brain tissue, this cellular marker colocalizes with Csf1r-EGFP+ in the IF analyses (Supp. Info., Fig. 2s). These results show that Csf1r-EGFP transgenic mice reproduce the morphological difference between the distinct myeloid cells existing in the brain. We could detect two different myeloid cells in the brain: the microglia proper, with small soma and long prolongations as described in the literature (see the section titled “Introduction”), and more ameboid cells in close proximity to the vessels, which might correspond to perivascular macrophages, as observed before (Prodinger et al., 2011). Although these authors reported a population of CD11c-GFP+ cells in this location, we could not observe CD11c+ cells by IF analysis. Most of the Csf1r-EGFP+ cells isolated from noninjured brain w/o m/Ch showed moderate expression of CD45 (CD45™), with very few CD45high expressing cells, a finding in accord with the low frequency of infiltration by peripheral leukocytes in the healthy brain (Ginhoux et al., 2009; Mildner et al., 2007). Our isolation methods are based on those described by other authors (Carson et al., 1998; Ford et al., 1995), and in agreement with their data, our isolation procedure with long perfusion of the brains before isolation eliminates most of the CD45high cells from the brain preparations once we have dissected out the m/Ch. It is still possible that this isolation method does not efficiently extract these perivascular cells, and that we are therefore not evaluating the exact number of these CD45high cells in the FC analysis. Further work will be required to clarify the number of CD45high cells residing in the perivascular space and determine to what extent they are induced by Flt3L.

In cells obtained from the m/Ch, 4 ± 3% of isolated CD45+ cells were Csf1r-EGFP− by FC. These cells are of myeloid origin, since they were labeled with anti-CD11b antibody. The use of the Csf1r-EGFP mice together with IF with other well-known myeloid markers reveals the complexity of the myeloid cells in the brain. We observed a variation in the intensity of Csf1r-EGFP+ staining, and since 7 ± 2% of the Csf1r-EGFP+ cells we detected were Iba1low/− in the m/Ch preparations, this suggests that different myeloid cells coexist in the normal brain. As yet we do not know the exact origin of these cells, or if they differ functionally from the Csf1r-EGFP+ Iba1+ cells. Csf1r-EGFP+ Iba1− cells were very prominent in the meningeal membrane, and future studies will need to analyze the choroid plexus and meningeal membrane separately. We are currently studying how these cell populations change in response to neuroinflammatory challenges. So far, our data suggest that more than one myeloid-specific marker might be required to give a
clear picture of the number and complexity of myeloid cells in the brain, particularly in the brain membranes and choroid plexus, and that analyses by cell suspension FC and IF will be required.

Flt3L treatment did not increase the numbers of CSF1R-EGFP\(^+\) cells in brain isolates or m/Ch compartments (data not shown). This finding is in accordance with previous data showing that Flt3L-responsive m/Ch DCs express the Flt3L receptor but not the CSF1R/CD115 receptor on their cell surface (Anandasabapathy et al., 2011). Our data show that, while the Csf1r-EGFP mouse is a good model for visualizing microglia cells in the brain, there is a subset of myeloid cells that we might be overlooking. For this reason, we carried out most of our brain myeloid analysis on CD45\(^+\) cells, distinguishing the CD45\(^a\) resident microglia cells in the BP from CD45\(^b\) myeloid cells of peripheral origin. Although we did not confirm this observation in mouse-chimera experiments, this distinction between resident and peripheral-origin CD45\(^c\) cells in the brain has been observed by many studies of microglia and peripheral infiltrating brain macrophages (Anandasabapathy et al., 2011; Ford et al., 1995).

These results raise the question of whether these Flt3L-dependent cells are true dendritic cells. The existence of brain parenchymal dendritic cells in the normal brain is still debated, although cells with DC markers have been found in various CNS locations (Matyszak et al., 1992; Matyszak and Perry, 1996; McMenamin, 1999; McMenamin et al., 2003). More recently, the presence of CD11c\(^+\) cells have been described through the use of transgenic mice such as the CD11c-enhanced yellow fluorescent protein (EYFP) model, which was constructed to identify dendritic cells in the periphery (Lindquist et al., 2004), and in CD11c-GFP mice (Jung et al., 2002). The analysis of these transgenic mice showed CD11c-fluorescent cells residing mainly in the m/Ch, the perivascular spaces and juxtavascular parenchyma, with the entire population of CD11c-EYFP\(^+\) cells co-expressing Iba1 and MHCII in the steady-state brain parenchyma (Anandasabapathy et al., 2011; Bulloch et al., 2008; Prodinger, et al., 2011). Previous work has shown the presence of MHCII\(^+\) cells in the m/Ch but not in BP from noninjured rat and mice brain by IF analysis (Matyszak and Perry, 1996), in line with our results. Many more studies have described an increase in these MHCII\(^+\) cells under neuroinflammatory conditions (Lv et al., 2011; Prodinger et al., 2011; Greter et al., 2005). Our analysis of the noninjured brain shows clear expression of MHCII\(^+\) cells in m/Ch, and we expected that these cells would express CD11c.

In their study, Bulloch et al. discuss the difficulty in identifying CD11c protein in young, steady-state brains with the antibodies available, which were found to be unreliable and give inconsistent results in confocal microscopy analyses. These results are in line with previous reports (Fischer and Reichmann, 2001; Greter et al., 2005; Karman et al., 2006; Matyszak and Perry, 1996; McMenamin, 1999) in which DCs were detected with the same antibodies in the setting of neuroinflammation but very rarely in the noninjured brain (reviewed in (Pashenkov et al., 2003; Ransohoff and Engelhardt, 2012). The use of transgenic mice such as CD11c-YFP and CD11c-EGFP has been very useful to visualize CD11c\(^+\) cells in noninjured mice, although the use of DT in CD11c-DTR-GFP mice did not exclusively lead to depletion of DCs, but also targeted macrophages in various tissues (Probst et al., 2005; van Rijt et al., 2005). Here, we used a tyramide-based method to enhance positive staining with the CD11c antibody. CD11c\(^+\) cells in the noninjured brain were studied in Flt3L-treated mice, and were found mainly in the choroid plexus. Using these methods, we clearly show the presence of CD11c\(^+\) cells in the undamaged brain, although the combined use of other molecular markers such as MHCII might provide a way to detect more accurately the presence of myeloid cells with a potential role as APCs. As we show in the nondiseased brain (Fig. 5), MHCII\(^+\) cells are present mainly in the brain choroid plexus in our brain preparations, although Flt3L induced the presence of MHCII\(^+\) in the meningeal membrane close to blood vessels (Fig. 5C). In accordance with previous published work in rat brain (Matyszak et al., 1992; McMenamin, 1999; McMenamin et al., 2003), MHCII\(^+\) cells are very prominent in the brain membranes, mainly in the choroid plexus, but very rare in the BP. We detected some but not many MHCII\(^+\) cells near the vessels, in close proximity to the basement membrane (Fig. 5A and Supp. Info., Fig. 5s), but were unable to detect CD11c\(^+\) cells in the perivascular or juxtavascular area as shown in Prodinger et al., 2011. High background around bigger vessels in the brain parenchyma made it difficult to visualize CD11c\(^+\) cells in this brain localization (data not shown). We did not observe this background around vessels in m/Ch preparations (Fig. 5).

As we mentioned before, we have thoroughly tried to analyze the presence of this marker in our preparation by FC analyses, without success. Our failure to detect CD11c by FC analysis might be explained in part by the susceptibility of membrane markers to digestion with the proteases used in the cell preparation protocol. Very recently (Immig et al., 2015), a very weak CD11c staining of brain myeloid cells has been shown by FC; these authors obtained their brain preparation without proteases, and this technique should be taken into account in future FC analysis of CD11c\(^+\) cells in the uninjured brain.

The results presented here were generated in the noninjured mouse brain, with no neuroinflammatory trigger. In line with the literature (Carson et al., 1998), our data indicate that brain w/o m/Ch preparations contain a low percentage of CD45\(^h\) cells in this condition. Nevertheless, we...
consistently observed that Flt3L treatment increased the number of CD45<sup>b</sup> cells in these preparations (Fig. 3), which contains few MHCII<sup>c</sup> cells (Fig. 5). Our IF analysis with anti-MHCII antibodies barely detected any MHCII<sup>c</sup> cells in the brain parenchyma itself (Fig. 5), and those that were present were situated in contact with blood vessels (Fig. 5A). In this context, Iming et al., 2015 show a lack of MHCII staining in their CD11c<sup>+</sup>-positive microglia cells. Our CD11c analyses by IF did not show any CD11c<sup>+</sup> cell in the parenchyma when the CD11c<sup>+</sup> cells in the m/Ch were nearly observed. It is still possible that since the mean of fluorescence (MFI) in microglia is lower that might well be below the detection threshold of the technique used in these studies.

At present, we cannot discard the possibility that the Flt3L-enhanced presence of CD45<sup>b</sup> MHCII<sup>c</sup> brain cells preparations in the FC analysis might reflect contamination by m/Ch membrane cells. In m/Ch, we observed a Flt3L-triggered increase in the percentage of CD45<sup>b</sup> CD11b<sup>low</sup> MHCII<sup>c</sup> cells, which corresponded to the number of MHCII<sup>c</sup> cells observed by IF (Figs 5 and 6). Our findings are thus in accordance with previous data indicating the absence of MHCII<sup>c</sup> cells in the BP proper of the non-diseased young brain and their localization in the m/Ch (Greter et al., 2005; Lv et al., 2011; Prodinger et al., 2011; Puntener et al., 2012). Further combined analysis by FC and IF with an array of different cellular markers will shed new light on the phenotype, activation stage and role of brain myeloid cells in the response to diverse neuroinflammatory stimuli.

There is an emerging role for DNGR-1<sup>+</sup> cells in the induction of T cytotoxic cell responses to cancer, viruses, and other pathogenic infections. In the brain, Piva et al. reported that depletion of DNGR-1-positive cells decreases the number of CD8<sup>+</sup> T cells in the brain after induction of experimental cerebral malaria (Piva et al., 2012). Here, we have shown a clear accumulation of DNGR-1-EGFP<sup>+</sup> cells in the Flt3L-treated m/Ch (Fig. 8). Moreover, these cells are enriched in Dngr-1 mRNA, (Fig. 7) and there is a parallel increase in the numbers of MHCII<sup>c</sup> cells (Figs. 6 and 8). We also observed a very dull fluorescence of DNGR-1-EGFP<sup>+</sup> cells in the m/Ch, but not in brain w/o m/Ch preparations (data not shown). In fact, we could not clarify whether DNGR-1<sup>+</sup> cells are present in the BP itself. On one hand, RT-PCR analysis detected a weak but significant increase in Dngr-1 mRNA expression in response to Flt3L (data not shown) and an increase in the number of Flt3L-dependent DNGR-1-EGFP<sup>+</sup> cells in the BP (Fig. 8A). On the other hand, our immunofluorescence analysis did not detect MHCII<sup>c</sup> cells in the BP. Therefore, it seems likely that the DNGR-1-EGFP<sup>+</sup> and MHCII<sup>c</sup> cells detected by FC in brain w/o m/Ch preparations reflect contamination by membrane structures during dissection.

The relationship between mouse CD8α<sup>+</sup> DCs from lymphoid tissues and mouse CD103<sup>+</sup>CD11b<sup>low</sup>DCs from nonlymphoid tissues has been established (Ginhoux et al., 2009). These cells and their human counterparts all express the DNGR-1 receptor (Poulin et al., 2012). CD8α<sup>+</sup> peripheral DCs have been implicated in the homeostatic maintenance of immune tolerance and the prevention of autoimmune disease. Furthermore, in spleen, CD8α<sup>+</sup> DNGR-1/CLEC9A DCs share with other DCs the ability to process and present antigens in the context of MHC class II, but are more efficient in the phagocytic uptake of dead cells (Inaba et al., 1998; Iyoda and Inaba, 2002; Schulz and Reis e Sousa, 2002). Indeed, DNGR-1 has been identified as a receptor able to recognize cell-death antigens and is a dedicated receptor for cross-presentation of cell-associated antigens (Pooley et al., 2001; Zelenay et al., 2012). Characterizing the exact role of these cells accumulating in the brain in the presence of Flt3L will be an interesting subject to pursue in the coming years.

Our results support a dendritic-like nature for Flt3L-dependent cells in the brain. First, they are responsive to Flt3L, a cytokine that has been shown to increase the dendritic repertoire. Second, they express DNGR-1, indicated by examination of Cle9a<sup>−/−</sup> mice and the expression of Dngr-1 mRNA. Finally, they express the transcription factors Batf-3 and Irf-8, both of which are required for the maturation of CD8α<sup>+</sup> classical DCs (Murphy, 2013). Although the presence of MHCII on the membrane is a requisite for antigen presentation, it is not sufficient to obtain a full T cell activation. CD80 and CD86 are costimulatory molecules that have an important role for antigen presentation to T-cells. In presence of Flt3L, we have not observed an increase in the mRNA levels of these molecules in the mCh despite the Flt3L-dependent increase in MHCII<sup>c</sup> cells (Fig 9B). Therefore, our data so far do not clarify whether these cells are efficient in their role as antigen-presenting cells or if extra signals are required to achieve a mature DC phenotype. Since we have not observed an increase in the costimulatory molecules, we hypothesize that these cells do not efficiently activate T cells, and that an extra neuroinflammatory signal is required. We are currently analyzing what signals are required to obtain a full immune response in these cells.

The existence, regulation, and role of APCs in the brain DCs has implications for the development of strategies to target cargoes to these m/Ch brain DCs using receptor-specific antibodies. The expression of the highly specific receptor DNGR-1 in these cells makes such strategies especially attractive. The similarity of these cells to lymphoid CD8α<sup>+</sup>DNGR-1<sup>+</sup> cells suggests that they might be responsible for producing efficient CTL responses (Caminschi et al., 2008; Sancho et al., 2008), and therefore could be an important target in brain disorders.
such as neuroinflammation-based neurodegenerative diseases, microbe-induced encephalitis, and brain tumors such as gliomas.

Acknowledgment

Grant sponsor: Fondo de Investigaciones Sanitarias (FIS) Spain; Grant numbers: PI09/0218 and PI12/0238; Grant sponsor: Red Temática Investigación Cooperativa en Cáncer (RTICC); Grant number: RD12/0036/0027

We thank David Hume (The Roslin Institute, University of Edinburgh) for providing the c-fms/Csf1r EGFP mice. Batf-3−/− was kindly provided by Dr. Kenneth M. Murphy, Washington University, St. Louis, MO, USA. We thank Amanda Sierra (Universidad del Pais Vasco) for her scientific discussions. Simon Bartlett (CNIC) provided English editing.

Competing Interests

The authors declare that they have no competing interests.

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