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Mapping the accumulation of co-infiltrating CNS dendritic cells and encephalitogenic T cells during EAE

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ABSTRACT

Evidence from experimental autoimmune encephalomyelitis (EAE) suggests that CNS-infiltrating dendritic cells (DCs) are crucial for restimulation of coinfiltrating T cells. Here we systematically quantified and visualized the distribution and interaction of CNS DCs and T cells during EAE. We report marked periventricular accumulation of DCs and myelin-specific T cells during EAE disease onset prior to accumulation in the spinal cord, indicating that the choroid plexus-CSF axis is a CNS entry portal. Moreover, despite emphasis on spinal cord inflammation in EAE and in correspondence with MS pathology, inflammatory lesions containing interacting DCs and T cells are present in specific brain regions.

1. Introduction

Dendritic cells (DCs) are migratory antigen (Ag)-presenting cells (APCs) that are capable of presenting major histocompatibility complex (MHC)-restricted Ag to CD4 + T cells and CD8 + T cells (cross presentation) and thus play central roles in priming adaptive immune responses. DCs are found in most organ tissues where, under steady-state conditions, they continually collect tissue Ag, migrate through lymphatic ducts to regional lymph nodes, and interact with naive T cells in order to maintain peripheral tolerance. In contrast to most tissues, brain and spinal cord are considered "immune privileged," although this term has come under scrutiny in recent years. Nevertheless, several features of the central nervous system (CNS) have been shown to regulate immune responses generated in these tissues. For example, brain and spinal cord lack conventional lymphatics for Ag drainage and exist behind the blood–brain barrier which limits immune cell ingress. Additionally, very few MHCII + DCs can be identified within the healthy CNS parenchyma—though this may partly because of the lack of widely recognized distinguishing surface markers for DCs (reviewed in Clarkson et al., 2012; Zozulya et al., 2010).

Recently, Dr. Michel Nussenzweig's group developed several fluorescent transgene-expressing reporter mouse lines for more readily identifying and tracking DCs in vivo. Using transgenic mice expressing enhanced yellow fluorescent protein (eYFP) downstream of the DC-associated CD11c promoter (CD11c-eYFP), Bulloch et al. described a discrete network of DCs within the healthy adult, developing, and aged mouse brain as well as during disease states including seizures, stroke, viral encephalitis, and in response to intracerebral (i.c.) cytokine injection (Bulloch et al., 2008; Gottfried-Blackmore et al., 2009; Felger et al., 2010; D'Agostino et al., 2012; Kaunzner et al., 2012). DCs are also thought to accumulate in the CNS during experimental autoimmune encephalomyelitis (EAE, a mouse model of multiple sclerosis), and we have shown that increasing the number of CNS DCs by i.c. injection prior to EAE induction accelerates disease onset (Zozulya et al., 2009). This suggests that CNS DCs may promote neuroinflammation and that DC number in the CNS may be a rate limiting factor in EAE disease progression.

After crossing the CNS vasculature, encephalitogenic CD4 + T cells must reencounter their cognate Ag in the context of MHCII molecules in order to carry out effector functions, such as cytokine secretion. Since CNS myelinating oligodendrocytes do not constitutively present self-Ag on surface MHCII nor do they express costimulatory molecules, other tissue APCs are thought to be required to restimulate CNS-infiltrating T cells. Reciprocal bone marrow (BM) chimera experiments

Abbreviations: DC, Dendritic cell; EAE, Experimental autoimmune encephalomyelitis; MS, Multiple sclerosis; RMS, Rostral migratory stream; Ag, Antigen; BM, Bone marrow; MHC, Majorhistocompatibility complex; MOG, Myelin oligodendrocyte glycoprotein; i.c., Intracerebral.

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with MHCII deficient (−/−) mice have revealed that MHCII expression by BM derived cells and not radio-resistant cells (such as microglia) are required for disease induction. This signifies that co-infiltrating APCs contribute to CNS T cell restimulation. Furthermore, restricting MHCII expression (and thus Ag presentation to CD4+ T cells) to cells expressing the DC-associated marker CD11c was shown to be sufficient for EAE disease induction and progression (Greter et al., 2005). These studies suggest that circulating BM-derived DCs are crucial APCs that accumulate in the CNS during EAE and are indispensable for disease onset. However, DC–T cell accumulation and interaction within the CNS are still poorly characterized. Thus, we sought to track and visualize the distribution and interaction of co-infiltrating CNS DCs and T cells during EAE using CD11c-eYFP reporter mice and purified T cells from 2D2.Dsred T cell receptor transgenic mice with MOG_{35-55}-$H2^b$-restricted CD4+ T cells.

2. Materials and methods

2.1. Mice

C57BL/6 (H2b) wild type (WT, stock #000664), and B6.Cg-Tg(CAG-Dsred*MST)1Nagy/J (Dsred, stock #006051) transgenic mice were obtained from The Jackson Laboratory (Bar Harbor, ME). B6.Cg-Tg(Itgax-Venus)1Mnz/J (CD11c-eYFP) transgenic mice on the C57BL/6 background were a generous gift from Dr. Michel C. Nussenzweig (Rockefeller University, NY). C57BL/6-Tg (Tcra2D2, Tcrb2D2)1Kuch/J (2D2) T cell receptor-transgenic mice with MOG_{35-55}-H2b-restricted CD4+ T cells were a gift from Dr. Vijay Kuchroo (BWH, Harvard Medical School, Boston, MA). 2D2 mice were crossed with congenic homozygous Dsred mice to generate 2D2.Dsred mice. All F1 offspring used in experiments were screened for TCR-(Vα3Vβ11) and Dsred-transgene expression by flow cytometry on immune cells isolated from blood. Standard PCR screening was used for CD11c-eYFP mice (tgc tgg ttg ttg tgc tgt ctc atc, ggg ggt gtt ctg ctg gta gtg gtc). All animal procedures used in this study were conducted in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Wisconsin Center for Health Sciences Research Animal Care Committee.

2.2. Induction of EAE

EAE was induced in mice by MOG immunization as previously described (Lee et al., 2009). Briefly, recombinant mouse MOG_{35-55} (MEVGWYRSPFSRVVHLYRNGK, 2 mg/ml, 100 μg per mouse) was mixed with an equal volume of incomplete Freund adjuvant (IFA), completed by supplementing with M. tuberculosis H37Ra (5 mg/ml, Difco, Detroit, MI). MOG–CFA mixture was emulsified by sonication using an ultrasonic homogenizer (Model 300VT equipped with a titanium cup tip, Biologics Inc., Monassas, VA) and injected subcutaneously between the shoulder blades of each mouse. Pertussis toxin (200 ng/mouse, i.p.) was injected on the day of immunization and 2 days after. Mice were monitored daily for the development of clinical signs beginning at day 7 post immunization. Clinical scores were recorded as follows: 0, no clinical disease; 1, flaccid tail; 2, gait disturbance or hind limb weakness; 3, hind limb paralysis and no weight bearing on hind limbs; 4, hind limb paralysis with forelimb paresis and reduced ability to move around the cage; and 5, moribund or dead. The mean daily clinical score and standard error of the mean were calculated for each group. The significance of differences was calculated by Student's t and Wilcox tests as described elsewhere (Fleming et al., 2005).

2.3. Fluorescent immunohistochemistry

For preparation of fixed frozen tissues, mice were perfused with PBS followed by 3–4% formalin in PBS. Tissues were removed and post-fixed in 3% formalin/25% sucrose in PBS for >4 h. Tissues were embedded in optimal cutting temperature (O.C.T.) compound (Tissue-Tek Sakura, Torrance, CA), frozen on dry ice, and stored at −80 °C until used. Using a cryostat, sections (5–30 μm) were cut from O.C.T.-embedded CNS and PLO tissue samples and affixed to TES-treated slides. Before staining sections were again fixed for 20 min in ice-cold acetone and rehydrated in PBS for 30 min. Sections were mounted using ProLong Gold anti-fade reagent (Invitrogen, Carlsbad, CA) with 4′, 6-diamidion-2phenylindole (DAPI). Fluorescent images were acquired at 40–400× with Picture Frame software (Optronics Inc.) using an Olympus BX41 microscope (Leeds Precision Instruments) equipped with a camera (Optronics Inc., Goleta, CA). For serial sagittal brain section arrays, images were acquired using Vectra automated multispectral imaging system in collaboration with the Translational Research in Pathology (TRIP) core facility. Digital images were processed and analyzed using Photoshop CS4 software (Adobe Systems). Color balance, brightness, and contrast settings were manipulated to generate final images. All changes were applied equally to entire image.

2.4. Mononuclear cell isolation

For isolation of immune cells from CNS tissues, whole tissues were extracted from saline-perfused mice. Brain hemispheres were separated and cerebellum, brain stem (pons, medulla, and rostral spinal cord), and olfactory bulb were dissected with micro-dissecting scissors. The caudal surface of the remaining brain hemispheres were lightly chilled on pre-chilled petri-dishes (1–2 min) and then inverted so that the midbrain and subsequently the hippocampus could be separated by making caudo-rostral incisions. After gross dissection of CNS tissues, immune cells were isolated as previously described (Karman et al., 2004; Lee et al., 2009; Zozulya et al., 2009). Briefly, tissues were weighed, finely minced, homogenized by trituration with 18 gauge needle and incubated with collagenase Type IV (1 mg/ml) and DNase (28 U/ml) at 37 °C for 45 min under continuous inversion. Samples were further triturated by passage through 20–23 gauge needles and then filtered through a 70 μm cell strainers. Immune cells were separated from tissues by gradient centrifugation. After washing, CNS homogenates were resuspended in 70% Percoll (Pharmacia, Piscataway, NY) and carefully overlaid with 30% Percoll. The gradients were centrifuged at 2500 rpm (625 × *g*) for 30 min at 4 °C with brake left off. Immune cells were collected from the interface and washed with saline for further analysis.

Fig. 1. Bone marrow CD11c-eYFP + cells accumulate within CNS during EAE. A) Standard PCR screening of Itgax-Venus (CD11c-eYFP) mice. UV transilluminated image of eYFP PCR product (visualized with ethidium bromide) separated by size using gel electrophoresis showing eYFP amplicons (550 bp) in samples from Itgax-Venus (lanes 2–5) but not congenic wild-type mice (lane 1) relative to 100 bp DNA ladder. Endogenous reference gene is present for all samples (200 bp). B) Representative 100× images of DAPI stained fixed frozen tissue sections of cervical lymph node and spleen from CD11c-eYFP mice, showing CD11c-eyfp+ transgene expression (green) and DAPI stained cell nuclei (blue). C–F) Representative DAPI stained sagittal brain sections (merged from multiple 40× images) showing CD11c-eYFP transgene expression (green) in CD11c-eYFP mice in healthy mice (C) and 12 (D), 16 (E), or 20 (F) days after EAE induction. Cell nuclei are shown in blue. High magnification insets (100×) show regions of CD11c-eYFP+ cell accumulation (boxes on left). choroid plexus (CP), ventricle (V), fimbria of hippocampus (fH), cerebellum (CB), CA3 are of hippocampus (CA3), dentate gyrus (DG), piamater (P), superior colliculus (SC), superficial gray layer (sgL), olfactory bulb (OB), olfactory ventricle (oV), olfactory tubercle (oT), ventral taenia tecta (vTT), glomerular layer (GL) and external plexiform layer (epL). Images are representative of two independent experiments with n = 3–4 mice. G) Histograms show frequency of CD11c-eYFP+ cells among total CD45+ bone marrow cells 0–11 days after MOG immunization. Mean values ± s.e.m. are plotted below. Data are representative of three independent experiments with n = 3–5 mice. H) Dot plots show frequency of CD11c-eYFP+ bone marrow cells 5 days after mice were treated as indicated. Mean values ± s.e.m. are plotted below. Data are representative of two independent experiments with n = 3 mice. *$p < 0.05$, Student's *t* test.