Experimental Neurology 250 (2013) 348–352

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Short Communication

Mobilization of progenitors in the subventricular zone to undergo oligodendrogenesis in the Theiler's virus model of multiple sclerosis: Implications for remyelination at lesions sites

M. Mecha, A. Feliú, F.J. Carrillo-Salinas, L. Mestre, C. Guaza *

Department of Functional and Systems Neurobiology, Neuroimmunology Group, Cajal Institute, CSIC, Madrid, Spain

ARTICLE INFO

Article history:
Received 18 June 2013
Revised 8 October 2013
Accepted 14 October 2013
Available online 19 October 2013

Keywords:
Subventricular zone
Oligodendrogenesis
Multiple sclerosis
Remyelination
Theiler's murine encephalomyelitis virus

ABSTRACT

Remyelination involves the generation of new myelin sheaths around axons, as occurs spontaneously in many multiple sclerosis (MS) lesions and other demyelinating diseases. When considering repairing a diseased brain, the adult mouse subventricular zone (SVZ) is of particular interest since the stem cells in this area can migrate and differentiate into the three major cell types in the central nervous system (CNS). In Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), we assessed the relative contribution of the SVZ to the remyelination in the corpus callosum at preclinical stages in this MS model. CNPase, MBP and Luxol Fast Blue staining revealed prominent demyelination 35 days post-infection (dpi), concomitant with a strong staining in $GFAP^+$ type B astrocytes in the SVZ and the increased proliferation in this area. The migration of oligodendrocyte progenitors from the SVZ contributed to the remyelination observed at 60 dpi, evident through the number of $APC^+/BrdU^+$ mature oligodendrocytes in the corpus callosum of infected animals. These data suggest that the inflammation induced by the Theiler's virus not only provokes strong preclinical demyelination but also, it is correlated with oligodendrocyte generation in the adult SVZ, cells that along with resident progenitor cells contribute to the prompt remyelination observed in the corpus callosum.

The pathological loss of myelin in diseases like multiple sclerosis (MS) is usually followed by a phenomenon of remyelination, in which oligodendrocytes synthesize new myelin sheaths to cover the exposed axons in the adult central nervous system (CNS; Chari, 2007). This remyelination is not only important to restore saltatory conduction but it also protects axons from different insults, thereby limiting the clinical disability associated to demyelinating diseases (Chandran et al., 2008). In such pathological situations, much attention has been paid to the mammalian subventricular zone (SVZ) as a potential source of cells that can replace those that are lost following insult or injury. The stem cells in this niche support long-distance migration (Kim and Szleze, 2008), and they can be activated in MS patients to promote gliogenesis (Nait-Oumesmar et al., 2007). Although $NG2^+$ precursor cells are the first to react to demyelination, displaying the highest rate of proliferation (Watanabe et al., 2002), the relative contribution of the SVZ in the response to inflammatory demyelination induced by Theiler's virus infection and oligodendrogenesis has yet to be addressed. In the present study, we investigated the behavior of the SVZ in Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). We show that demyelination occurs in the corpus callosum during the preclinical phase of the disease in this viral model of MS, which is accompanied by a mobilization of progenitors in the SVZ to undergo oligodendrogenesis. We observe an increase in the proliferative rate in this area, with no activation of $NG2^+$ precursors but strong $GFAP^+$ type B astrocytes staining close to the lateral brain ventricles. Finally, we show for the first time that Theiler's infection enhances the mobilization of SVZ progenitor cells to the surrounding demyelinated corpus callosum, generating mature APC^+ oligodendrocytes.

Materials and methods

Animals and Theiler's virus infection

TMEV-IDD-susceptible female SJL/J mice from our in-house colony (Cajal Institute, Madrid) were maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Four-week-old mice were inoculated intracerebrally into the right hemisphere with 2×10^6 plaque forming units (pfu) of the Daniels (DA) strain of TMEV kindly provided by Dr. Moses Rodriguez, in 30 μl of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), as described

Abbreviations: EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; LPC, lysophosphatidyl-choline; MS, multiple sclerosis; SVZ, subventricular zone; TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease.

* Corresponding author at: Department of Functional and Systems Neurobiology, Neuroimmunology Group, Cajal Institute, CSIC, Avda Dr Arce 37, 28002 Madrid. Fax: +34 915854754.
E-mail address: cgjb@cajal.csic.es (C. Guaza).

0014-4886
http://dx.doi.org/10.1016/j.expneurol.2013.10.011

previously (Lledo et al., 1999). Sham uninfected mice were administered 30 μl of DMEM + 10% FCS alone. Animals were handled in accordance with the European Union animal care guidelines (2010/63/EU).

Proliferation and cellular migration protocols

To study proliferation, Sham and TMEV mice received two intraperitoneal (i.p.) injections of BrdU (5 mg/kg: Sigma) at 2 h intervals on day 35 post-infection (dpi), and they were sacrificed 2 h after the last injection. To study SVZ cell migration, Sham and TMEV mice received four i.p. injections of BrdU (5 mg/kg) at 2 h intervals on 2 days at 35 dpi. The mice were maintained in our animal house for 15 days and they were sacrificed on day 45 pi. This tracing protocol has been described previously to label cells restricted to the SVZ and the rostral migratory stream (Picard-Riera et al., 2002). To study myelin proteins in the corpus callosum, animals were maintained until day 60 pi based on previous studies of the laboratory (Mecha et al., 2013).

Tissue processing

Mice were anesthetized with pentobarbital (50 mg/kg body weight, i.p.) and perfused with saline. The animal's brain were fixed overnight in 4% paraformaldehyde prepared in 0.1 M phosphate buffer (PB), and cryoprotected in 15% sucrose solution in 0.1 M PB, and then in a 30% sucrose solution. Coronal cryostat sections (30 μm thick) were obtained and processed for immunohistochemistry.

Immunohistochemistry

Free-floating brain sections were washed three times for 10 min with 0.1 M PB and after inhibiting the endogenous peroxidase, they were blocked for 1 h at room temperature (RT) in blocking buffer [0.2% Triton X-100 and 5% normal goat serum (NGS); Vector Laboratories, CA, USA]. The sections were then incubated overnight at 4 °C in blocking buffer containing the antibody diluted 1:500 against GFAP (generated in mouse, Sigma–Aldrich, MO, USA), MBP (generated in mouse, Millipore, MA, USA) or CNPase (generated in mouse, Sigma–Aldrich, MO, USA). The following day, the sections were rinsed three times for 10 min with PB + 0.2% Triton X-100 and they were then incubated for 1 h with a biotinylated goat-anti mouse antibody (Vector Laboratories, CA, USA). For immunostaining with DAB, the sections were incubated for 1 h with an avidin–biotin–peroxidase (ABC) complex (Vector Laboratories, CA, USA) and finally with the chromogen 3,3′ diaminobenzidine tetrahydrochloride (DAB; Sigma–Aldrich, MO, USA). For immunofluorescence, sections were incubated with a fluorescent secondary antibody conjugated with Alexa® fluorophore (1:500; Molecular Probes, OR, USA) in blocking buffer, washed and mounted. After staining, the sections were dehydrated, cleared with xylene and coverslipped. In all cases, the specificity of the staining was confirmed by omitting the primary antibody.

Immunofluorescence for proliferative and recruited cells

Sections were first incubated in HCl 2 N for 2 h at RT and they were then washed four times in PB and blocked for 1 h at RT in blocking buffer. The sections were then incubated for four days at 4 °C in blocking buffer containing the antibody against BrdU (1:200; generated in rat, Abcam, Cambridge, UK) and NG2 (1:200, generated in rabbit, Millipore, MA, USA) or APC (1:500 generated in mouse; Sigma-Aldrich, MO, USA). Subsequently, the sections were rinsed four times in PB and then incubated with the fluorescent secondary antibody conjugated with Alexa® fluorophore (1:500; Molecular Probes, OR, USA) in blocking buffer. Finally, the secondary antibody was washed with PB and the slides were mounted with PB:glycerol.

Analysis and counting of proliferative and recruited cells

The mean number of total $BrdU^+$ cells, $NG2^+/BrdU^+$, and $APC^+/BrdU^+$ labeled cells was determined by manual counting positive cells with the cell counter of Image J software (designed by National Institutes of Health) at the level of the SVZ in the lateral ventricles (for proliferative cells) or the corpus callosum (for proliferative $NG2^+$ cells and for APC^+ recruited cells) of eight consecutive sections. For each brain structure, the data are expressed as the number of cells/mm^2 as the mean of at least 4 mice per experimental group.

Microscopy and image analysis of MBP staining

Immunofluorescent images were acquired on a Leica TCS SP5 confocal microscope and a Zeiss Axiocam high resolution digital color camera was used to record the immunohistochemistry images. Individual images of 8 sections acquired from at least 4 animals per group were analyzed quantifying the intensity of MBP staining in the corpus callosum using ImageJ software as detailed as follows: first, we defined the regions of interest (corpus callosum) with the freehand tool; second, we split the channels of the selected area, obtaining one image per channel; third, we established a constant threshold of intensity in Sham animals to apply it to all the experimental groups; and four, we measured the staining intensity of all images within the experiments. The threshold intensity was maintained constant during the comparison and measuring all experimental and control images.

Data analysis

All the data are expressed as the mean ± SEM. One-way ANOVA followed by the Bonferroni post-hoc test, or Kruskal–Wallis ANOVA followed by Mann–Whitney U test was used to determine the statistical significance. The level of significance was set at $p \leq 0.05$.

Results

Demyelination and remyelination in the corpus callosum of TMEV-infected mice

We previously described presumptive remyelination in the motor cortex and brainstem of TMEV-IDD mice (Mecha et al., 2013). Here, we examined typical myelin markers in the corpus callosum, such as CNPase (non-compact myelin marker), MBP (compact myelin marker), and Luxol Fast Blue (LFB) staining, which revealed strong demyelination in this structure 35 dpi with TMEV (Fig. 1A). Notably, the loss of these markers was followed by an increase in their expression at 60 dpi, as measured with the quantification of MBP staining in this zone at 35 dpi (5.6 ± 2.19% staining intensity, $p \leq 0.001$ vs Sham) and 60 dpi (53.66 ± 11.47% staining intensity, $p \leq 0.01$ vs TMEV 35 dpi) suggesting a remyelination process in the damaged brain of infected animals long before any motor symptoms are evident.

$GFAP^+$ type B astrocytes in the lateral ventricles: SVZ mobilization

We used GFAP as a marker for astrocytes and stem cells to assess the relative contribution of the SVZ of TMEV-IDD mice (Alvarez-Buylla and Garcia-Verdugo, 2002). At 35 dpi we found GFAP staining of cells that were in contact with the ventricular surface of this region, which had a large soma and a long apical process running parallel to the ependymal layer (Supplemental Fig. 1A). These cells were type B astrocytes, previously described SVZ stem cells (Supplemental Fig. 1B) that can generate $NG2^+$ progenitor cells that express oligodendrocyte lineage transcription factor 2 (Olig2), and can be the origin of mature myelinating oligodendrocytes (Menn et al., 2006).

Besides, the strong staining of $GFAP^+$ cells with a type B astrocyte morphology close to the wall of the lateral ventricles in infected mice