Experimental Neurology 297 (2017) 129–137

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Monocyte depletion early after stroke promotes neurogenesis from endogenous neural stem cells in adult brain

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ARTICLE INFO

Keywords:
Neurogenesis
Monocyte depletion
Reactive gliosis

ABSTRACT

Ischemic stroke, caused by middle cerebral artery occlusion, leads to long-lasting formation of new striatal neurons from neural stem/progenitor cells (NSPCs) in the subventricular zone (SVZ) of adult rodents. Concomitantly with this neurogenic response, SVZ exhibits activation of resident microglia and infiltrating monocytes. Here we show that depletion of circulating monocytes, using the anti-CCR2 antibody MC-21 during the first week after stroke, enhances striatal neurogenesis at one week post-insult, most likely by increasing short-term survival of the newly formed neuroblasts in the SVZ and adjacent striatum. Blocking monocyte recruitment did not alter the volume of the ischemic lesion but gave rise to reduced astrocyte activation in SVZ and adjacent striatum, which could contribute to the improved neuroblast survival. A similar decrease of astrocyte activation was found in and around human induced pluripotent stem cell (iPSC)-derived NSPCs transplanted into striatum at one week after stroke in monocyte-depleted mice. However, there was no effect on neurogenesis in the graft as determined 8 weeks after implantation. Our findings demonstrate, for the first time, that a specific cellular component of the early inflammatory reaction in SVZ and adjacent striatum following stroke, i.e., infiltrating monocytes, compromises the short-term neurogenic response neurogenesis from endogenous NSPCs.

1. Introduction

Ischemic stroke caused by middle cerebral artery occlusion (MCAO) in adult rodents leads to transient increase of neural stem/progenitor cell (NSPC) proliferation in the subventricular zone (SVZ) (Arvidsson et al., 2002; Jin et al., 2001) and continuous production of new neuroblasts for several months (Thored et al., 2006). The newly generated neuroblasts migrate into the injured striatum, where they differentiate to mature neurons (Arvidsson et al., 2002; Parent et al., 2002), become integrated (Yamashita et al., 2006), project to substantia nigra (Sun et al., 2012), and seem to be functional (Hou et al., 2008). Striatal neurogenesis occurs in adult humans (Ernst et al., 2014), and there is evidence for enhanced SVZ cell proliferation and neuroblast formation also in stroke patients (Macas et al., 2006; Marti-Fabregas et al., 2010; Minger et al., 2007).

Concomitantly with the continuous neuroblast formation, there is a long-lasting accumulation of activated, resident microglia and monocyte-derived macrophages (MDMs) in the ipsilateral SVZ (Thored et al., 2009). The infiltration of MDMs into the ischemic hemisphere occurs mainly over the first week and peaks at 3 days after the insult both in a transient (Wattananit et al., 2016) and a permanent (Miro-Mur et al., 2016) ischemia model in mice. A bulk of experimental data indicates that factors released from microglia/macrophages take part in the regulation of striatal neurogenesis after stroke. For example, IGF-1 (Thored et al., 2009; Yan et al., 2006), IL-15 (Gomez-Nicola et al., 2011) and TGF-α (Choi et al., 2017) have been reported to stimulate NSPC proliferation in SVZ whereas TNF-α signaling, mediated through TNF-R1, gives rise to suppression (Iosif et al., 2008). Moreover, activated microglia/macrophages direct the neuroblasts formed after the ischemic insult towards the damaged area by secretion of CXCL12 (Robin et al., 2006), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) (Yan et al., 2007) and osteopontin (OPN; Yan et al., 2009). We recently showed that inflammation without neuronal death, caused by injection of LPS in the striatum, leads to the formation of new striatal neurons comparable to that after a stroke (Chapman et al., 2015). This finding raises the possibility that the inflammation

Abbreviations: GFAP, glial fibrillary acidic protein; iPSCs, induced pluripotent stem cells; MCAO, middle cerebral artery occlusion; MCP-1, monocyte chemoattractant protein-1; MDMs, monocyte-derived macrophages; NSPCs, neural stem/progenitor cells; OB, olfactory bulb; OPN, osteopontin; SVZ, subventricular zone

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http://dx.doi.org/10.1016/j.expneurol.2017.07.012
Received 26 April 2017; Received in revised form 23 June 2017; Accepted 20 July 2017
Available online 23 July 2017
0014-4886/

accompanying an ischemic insult and not the injury per se is the major inducer of striatal neurogenesis after stroke.

The distinct roles of resident microglia and MDMs, respectively, in the regulation of stroke-induced neurogenesis are currently unknown. Here we have addressed this by blocking monocyte recruitment to the brain during the period of maximum entry, i.e. the first week after stroke, in mice using the anti-CCR2 antibody MC-21. We show that the depletion of circulating monocytes promotes neurogenesis after the ischemic insult, most likely through increased short-term survival of the newly formed neuroblasts in the SVZ and adjacent striatum. Our finding provides the first experimental evidence indicating that blood borne monocytes, which enter the brain early after stroke and give rise to MDMs, suppress the neurogenic response.

2. Materials and methods

2.1. Animals and experimental design

All procedures were carried out in accordance with the guidelines set by the Malmö-Lund Ethical Committee for the use of laboratory animals, and were conducted in accordance with the European Union directive on the subject of animal rights. We used male C57BL/6J mice (25–30 g, Charles River, Germany), all of which were subjected to 30 min MCAO followed by injection of MC21 antibody or saline from day 0 to day 3. In the first experiment, animals were given daily intraperitoneal (i.p.) injections of BrdU (50 mg/kg, Sigma-Aldrich) from day 4 to 6, killed after one week and analyzed for effects of monocyte depletion on striatal neurogenesis. In the second experiment, animals were transplanted intrastriatally with green fluorescent protein (GFP)-labeled human induced pluripotent stem cell (iPSC)-derived NSPCs at 1 week after MCAO. Mice were sacrificed 8 weeks later and we evaluated the effects of monocyte depletion on neurogenesis from grafted NSPCs. All animals were kept in 12 h light/12 h dark cycles with ad libitum access to food and water.

2.2. Middle cerebral artery occlusion

Ischemic stroke was induced by MCAO as previously described (Wattananit et al., 2016; Hara et al., 1996; Andsberg et al., 2001). Briefly, the right common carotid artery and external carotid artery were ligated, and internal carotid artery was temporarily occluded using a metal micro-vessel clip. A nylon monofilament was introduced into the internal carotid artery until resistance was felt (approx. 9 mm distance) and removed after 30 min. External carotid artery was ligated permanently, and the surgical wound was closed. If required during the first week after MCAO, animals were provided with high calorie gel diet (DietGel™ Boost, Clear H_2O) and injected subcutaneously with Ringer's solution in case of dehydration. Animals were randomly allocated to the different experimental groups.

2.3. MC-21 injection

The anti-CCR2 monoclonal antibody MC-21 was produced as previously described (Mack et al., 2001). Each batch was tested to assure the complete depletion of $CD115^+/CD11b^+/Ly6C^+$ monocytes from the peripheral blood. We injected MC-21 i.p. immediately after MCAO and on the following 3 days (i.e., on d0, d1, d2 and d3 post-injury) according to the protocol used by Wattananit et al. (2016). In that study, we found that neither the isotype control for MC-21 antibody (Rat IgG2b; BD Pharingen) nor saline injections had any effect on the number of circulating monocytes. There were no differences between the control antibody- and saline-injected groups and, therefore, we used saline injection as treatment control in both experiments of the present study.

2.4. Transplantation

Intrastriatal implantation of human iPSC-derived NSPCs, which had been previously transduced with lentivirus carrying GFP (Koch et al., 2009; Tornero et al., 2013; Tornero et al., 2017), was performed stereotaxically at 7 days after MCAO. On the day of surgery, human iPSC-derived NSPCs were resuspended to a final concentration of 100,000 cells/μl. A volume of 1 μl was injected at the following coordinates (from bregma and brain surface): anterior/posterior: + 0.5 mm; medial/lateral: + 2.2 mm; dorsal/ventral: − 2.5 mm. Tooth-bar was set at − 3.3 mm. Mice were injected subcutaneously with 10 mg/kg Cyclosporine A every day during the first month after transplantation and every other day during the second month.

2.5. Immunohistochemistry

Mice were deeply anaesthetized with an overdose of pentobarbital and transcardially perfused with cold saline and then 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA and incubated in 20% sucrose for 24 h before being cut in 30 μm thick coronal sections on a microtome. Sections were preincubated in blocking solution (5% normal serum and 0.25% Triton X-100 in 0.1 M potassium-phosphate buffered solution). Primary antibodies were diluted in the blocking solution and incubated overnight at 4 °C (Suppl. Table 1). Fluorophore-conjugated secondary antibodies (Molecular Probes or Jackson Laboratories) were diluted in blocking solution and applied for 2 h. Nuclei were stained with Hoechst (Molecular Probes or Jackson Laboratories) for 10 min and sections were mounted with Dabco mounting medium. Images were obtained using epifluorescence (Olympus, Germany) and confocal (Zeiss, Germany) microscopes.

Single labeling for NeuN was performed followed by biotin-avidin amplification. Briefly, after incubation with the primary rabbit anti-NeuN antibody, the samples were incubated with biotinylated secondary horse anti-rabbit antibody and the staining visualized with avidin-biotin-peroxidase complex (Elite ABC kit, Vector Laboratories), followed by peroxidase-catalyzed diaminobenzidine (DAB) reaction.

2.6. Quantifications and statistical analysis

All quantifications and statistical analyses were performed by researchers blinded to the experimental groups. Lesion volume was assessed in NeuN-DAB-stained sections. Light microscopic images were first digitalized. Intact areas, identified by $NeuN^+$ cells in the ipsilateral and contralateral hemispheres, were delineated and then measured using C.A.S.T. software (Visiopharm, Denmark). The lesion area was calculated by subtracting the non-lesioned (stained) area in the injured hemisphere from the corresponding area in the contralateral hemisphere. Lesion volume was then obtained by multiplying the lesion area by the distance between the sections (240 μm).

To evaluate the magnitude of glial reaction we analyzed glial fibrillary acidic protein (GFAP) immunoreactivity after stroke in three different areas: SVZ, striatum adjacent to the SVZ (i.e., 500 μm from the SVZ in lateral direction, as defined in Thored et al., 2007), and whole striatum. One picture per section was acquired at 4 × magnification in the epifluorescence microscope, and a total of 3 sections per mouse were analyzed. The area of GFAP-immunoreactivity was determined by image analysis using CellSens Dimension 2010 software (Olympus, Germany). In each section, areas of immunoreactivity were identified using defined threshold for specific signal. Using these defined parameters, the images of each region were analyzed by the software, which calculated the total area covered by pixels/specific immunopositive signal. The values corresponding to total fluorescence areas were averaged and expressed as the percentage of area covered by GFAP per animal. The same procedure was used to measure OPN immunoreactivity.

Numbers of $ED1^+$, $Iba1^+$, DCX^+, $BrdU^+$, $Ki67^+$ cells were