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Inflammation without neuronal death triggers striatal neurogenesis comparable to stroke

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ABSTRACT

Ischemic stroke triggers neurogenesis from neural stem/progenitor cells (NSPCs) in the subventricular zone (SVZ) and migration of newly formed neuroblasts toward the damaged striatum where they differentiate to mature neurons. Whether it is the injury *per se* or the associated inflammation that gives rise to this endogenous neurogenic response is unknown. Here we showed that inflammation without corresponding neuronal loss caused by intrastriatal lipopolysaccharide (LPS) injection leads to striatal neurogenesis in rats comparable to that after a 30 min middle cerebral artery occlusion, as characterized by striatal DCX+ neuroblast recruitment and mature NeuN+/BrdU+ neuron formation. Using global gene expression analysis, changes in several factors that could potentially regulate striatal neurogenesis were identified in microglia sorted from SVZ and striatum of LPS-injected and stroke-subjected rats. Among the upregulated factors, one chemokine, CXCL13, was found to promote neuroblast migration from neonatal mouse SVZ explants *in vitro*. However, neuroblast migration to the striatum was not affected in constitutive CXCL13 receptor CXCR5$^{-/-}$ mice subjected to stroke. Infarct volume and pro-inflammatory M1 microglia/macrophage density were increased in CXCR5$^{-/-}$ mice, suggesting that microglia-derived CXCL13, acting through CXCR5, might be involved in neuroprotection following stroke. Our findings raise the possibility that the inflammation accompanying an ischemic insult is the major inducer of striatal neurogenesis after stroke.

1. Introduction

Ischemic stroke following cerebral artery occlusion is a leading cause of chronic disability in humans, and effective treatment to promote recovery is lacking. It is well established that neural stem/progenitor cells (NSPCs) in the subventricular zone (SVZ) of adult rodents continuously produce new neuroblasts that migrate into the injured striatum for several months after stroke (Arvidsson et al., 2002; Thored et al., 2006, 2007; Parent et al., 2002). These neuroblasts differentiate to mature neurons, become integrated (Yamashita et al., 2006), project to substantia nigra (Sun et al., 2012), and seem to be functional (Hou et al., 2008). There is also evidence for enhanced SVZ cell proliferation and neuroblast formation after stroke in humans (Macas et al., 2006; Marti-Fabregas et al., 2010; Minger et al., 2007). The interest in this potential self-repair mechanism was further increased by a recent report showing that neuroblasts from SVZ enter striatum and become interneurons in adult humans under normal conditions (Ernst et al., 2014). However, in rats, only a fraction of stroke-induced neurons survive long-term (Arvidsson et al., 2002), and it is unclear whether they contribute to the spontaneous functional recovery after the insult (Lagace, 2012).

Stroke is associated with inflammation, which exerts a complex influence on several steps of striatal neurogenesis (Tobin et al., 2014). Several months following stroke in rats, activated microglia/macrophages continue to be localized in the ipsilateral SVZ concomitant with the continuous production of new neuroblasts migrating into the striatum (Thored et al., 2009). Factors released from activated microglia/macrophages can either stimulate NSPC proliferation in the SVZ, as with IGF-1 (Thored et al., 2009; Yan et al., 2006) and IL-15 (Gomez-Nicola et al., 2011), or in the case of TNF-α signaling through TNF-R1, suppress it (Iosif et al., 2008). Activated microglia/macrophages are also involved in directing neuroblasts to the damaged area by secreting CXCL12 (Robin et al., 2006), MCP-1 (Yan et al., 2007), and osteopontin (Yan et al., 2009). Finally, one study indicates that

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inflammation associated with stroke contributes to the poor survival of the new striatal neurons (Hoehn et al., 2005), similar to what has been described for new hippocampal neurons in other inflammatory environments (Ekdahl et al., 2003; Monje et al., 2003). The number of recruited neuroblasts correlates with the volume of striatal injury after stroke of different severities (Thored et al., 2006), but whether it is the injury *per se* or the associated inflammation that induces striatal neurogenesis is unknown.

We show here that inflammation without neuronal death, evoked by intrastriatal LPS-injection, is sufficient to trigger striatal neurogenesis similar to that after stroke in rats. Using global gene expression analysis on sorted rat microglia, we identified several potential regulators of this response with changes observed both after LPS and middle cerebral artery occlusion (MCAO). One of the upregulated factors, CXCL13, improved migration of mouse SVZ neuroblasts *in vitro*. However, knockout (KO) mice for CXCR5, the receptor for CXCL13, showed no impairment of neuroblast migration after stroke but more extensive injury and pro-inflammatory phenotype of microglia/macrophages.

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. Procedures were carried out on male Wistar rats (250–300 g, Charles River, Germany), male C57BL/6 J mice (25–30 g, Charles River, Germany), and male CXCR5$^{-/-}$ mice (25–30 g, The Jackson Laboratory, http://jaxmice.jax.org/strain/006659.html), housed under 12 h light/12 h dark cycle with *ad libitum* access to food and water.

In order to characterize immunohistochemically the effect of LPS and MCAO, rats were randomly assigned to one of 8 groups (n = 4–8 per group): LPS, vehicle (veh), 30 min MCAO or sham with either a 2 or 6 weeks recovery. Each animal was given intraperitoneal (i.p) injections of BrdU (50 mg/kg, Sigma) in phosphate-buffered saline (PBS) twice daily for 2 weeks beginning on the evening following surgery.

For analysis of the gene profiles in striatal or SVZ microglia, animals were randomly assigned to LPS, MCAO or naïve groups. Due to low cell numbers, notably in SVZ, each 'n' (4) comprised 3 pooled animals.

For examining the role of CXCL13 and its receptor CXCR5 *in vivo*, wild-type and CXCR5$^{-/-}$ mice (n = 6 and 4, respectively) were subjected to 35 min MCAO. Animals were sacrificed at 2 weeks after surgery.

2.2. Surgical procedures

Animals were anesthetized with isoflurane (3.5% induction; 1.5% maintenance) in 70% N_2O/30% O_2. All animals received locally injected lidocaine for pain relief. While under anesthesia and in the early recovery period (2 h), animals were placed on a heat pad maintained at 37 °C.

LPS from *Salmonella enterica*, serotype *abortus equi* (Sigma-Aldrich; 15 μg in 1.5 μl of artificial CSF (aCSF)) or vehicle (aCSF) was stereotaxically injected using a self-made glass microneedle fixed to a gas-tight syringe (Hamilton company) into the right striatum (coordinates: 1.2 mm rostral, 2.5 mm lateral to bregma, 4.5 mm ventral from brain surface, toothbar at −3.3 mm) (Paxinos and Watson, 1998). In a pilot experiment in mice, a dose–response curve was established with 0.01 to 100 μg LPS administered in ten-fold increasing concentrations as above (coordinates: 0.9 mm rostral, 1.6 mm lateral to bregma and 3.5 mm ventral from brain surface, toothbar at 0 mm).

The intraluminal filament technique was used to induce transient MCAO (Koizumi et al., 1986). In rats, the right carotid arteries were isolated and the common and external carotids were proximally ligated. The internal carotid artery (ICA) was temporarily occluded with a microvascular clip. A small incision was made in the common carotid and a heat-blunted nylon microfilament was advanced into the ICA until resistance was felt (approx. 19 mm). Animals recovered from anesthesia during the occlusion. 30 min after occlusion, animals were re-anesthetized and the filament was withdrawn. Temperature was maintained at 37 ± 0.5 °C while animals were under anesthesia. Sham surgeries were carried out in the same way but the filament was only advanced 2 mm inside the ICA. MCAO animals that did not fulfill predefined inclusion criteria for successful 30 min occlusion (>40% striatal damage; No cortical damage; No subarachnoid hemorrhage) were excluded following NeuN staining. In mice, the procedure was modified as follows: Right carotid arteries were isolated, and the common carotid artery and the external carotid artery were ligated. The ICA was temporarily occluded with a microvascular clip, and a silicon-coated microfilament was placed into the external carotid artery *via* a small incision and advanced into the ICA until resistance was felt (approx. 9 mm). Occlusion was maintained for 35 min before the filament was withdrawn.

2.3. Immunohistochemistry

Rats and mice were deeply anesthetized with an overdose of pentobarbital and transcardially perfused with saline followed by 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA, placed in 20% sucrose for 24 h, and then cut into 8 series of 30 μm thick coronal sections on dry ice. For each staining, one full series was used. Fluorescence double-staining was used for visualization of BrdU+/DCX+, BrdU+/NeuN+, Iba1+/NeuN+, Iba1+/Ki67+, Iba1+/ED1+, CD16/32+/Iba1+, DCX+/HuD+, DCX+/PDGFRα+, DCX+/S100B and GFAP+/Nestin+ cells.

All sections for BrdU staining were pre-treated with 1 M HCl for 10 min at 65 °C and 20 min at room temperature. All double stains were carried out according to the following protocol: Free-floating sections were pre-incubated with the appropriate serum and then incubated with primary antibodies overnight at 4 °C. Sections were incubated for 2 h in the dark with secondary antibodies conjugated with Cy3 or Alexa Fluor 488 (1:200, Molecular Probes, Life Technologies), Cy3-conjugated donkey anti-rat/goat anti-rabbit/donkey anti-mouse (all 1:200, Jackson ImmunoResearch), or biotinylated horse anti-mouse/anti-goat (both 1:200, Vector Laboratories). The primary antibodies used are listed in Table 1.

Single labeling for NeuN was performed with biotinylated horse anti-mouse antibody and visualized with avidin–biotin–peroxidase complex (Elite ABC kit, Vector Laboratories), followed by peroxidase-catalyzed diaminobenzidine reaction.

Table 1
Primary antibodies used for immunohistochemical staining.

Antibody	Host species	Concentration	Company
Anti-BrdU	Rat	1:200	Abcam
Anti-NeuN	Mouse	1:100	Merck Millipore
Anti-PDGFRα	Mouse	1:300	Santa Cruz Biotechnology
Anti-DCX	Goat	1:400	Santa Cruz Biotechnology
Anti-Iba1	Rabbit	1:1000	Wako
Anti-ED1	Rat	1:200	AbD Serotec
Anti-ki67	Mouse	1:500	Novocastra, Leica Biosystems
Anti-HuD	Rabbit	1:200	Sigma-Aldrich
Anti-S100B	Rabbit	1:200	Sigma-Aldrich
Anti-GFAP	Rabbit	1:400	Zymed, Life Technologies
Anti-nestin	Mouse	1:200	Merck Millipore
Anti-CD16/32	Rat	1:200	BD Biosciences
Anti-iNOS	Rabbit	1:200	BD Biosciences
Anti-RECA	Mouse	1:400	AbD Serotec