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Differential vulnerability of adult neurogenesis by adult and prenatal inflammation: Role of TGF-β1

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ABSTRACT

Peripheral inflammation, both during the prenatal period and in adulthood, impairs adult neurogenesis. We hypothesized that, similar to other programming effects of prenatal treatments, only prenatal inflammation causes long-term consequences in adult neurogenesis and its neurogenic niche. To test this, pregnant Wistar rats were subcutaneously injected with lipopolysaccharide (LPS; 0.5 mg/kg) or saline solution every other day from gestational/embryonic day (GD) 14–20. In addition adult animals were injected with a single intraperitoneal saline or LPS injection (1 mg/kg) and the effects on neurogenesis were assessed 7 days later. Alternatively, to evaluate long-term consequences of adult LPS injections, LPS (1 mg/kg) was administered peripherally to adult rats four times every other day, and the effects on neurogenesis were assessed 60 days later.

Prenatal and adult LPS treatments reduced adult neurogenesis and provoked specific microglial (but not astroglial) activation in the dentate gyrus (DG). However, only prenatal inflammation-mediated effects were long-lasting (at least 60 days). Moreover, these effects were specific to the DG since the Subventricular Zone (SVZ) and the Rostral Migratory Stream (RMS) were not affected. In addition, these stimuli caused differential effects on the molecular components of the neurogenic niche; only prenatal LPS treatment reduced the local levels of TGF-β1 mRNA in the DG. Finally, TGF-β1 exerted its pro-neurogenic effects via the Smad2/3 pathway in a neural stem cell culture.

Taken together, these data add evidence to the duration, regional specificity and dramatic consequences of prenatal immune programming on CNS physiology, compared with the limited response observed in the adult brain.

1. Introduction

Neurogenesis from adult neural stem/progenitor cells (NSC) occurs in the subgranular zone (SGZ) of the dentate gyrus (DG) and in the subventricular zone of the lateral ventricle (SVZ). Several studies have shown that pro- and anti-inflammatory stimuli occurring in the CNS or in the periphery modulate adult neurogenesis (Ekdahl et al., 2003; Graciarena et al., 2010; Mathieu et al., 2010b; Monje et al., 2003; Ormerod et al., 2013, reviewed in Mathieu et al., 2010a).

NSCs reside within an environment or niche which modulates NSC proliferation and differentiation. The cellular and molecular factors that modulate these effects need to be further characterized. Microglial cells are components of this niche and are capable of altering adult neurogenesis via net changes in the pattern of secreted factors, including immune cytokines (Ekdahl et al., 2009; Mathieu et al., 2010a,b; Perry et al., 2002; Simard and Rivest, 2004; Whitney et al., 2009; Ziv et al., 2006). Cytokines are part of the molecular components of the neurogenic niche in normal conditions and have shown to play a central role in processes affecting NSCs biology (Gage, 2000; Mathieu et al., 2010a; Perry et al., 2002). Among them, pro-inflammatory Interleukin (IL)-6 has shown to exert anti-neurogenic effects in the adult DG (Monje et al., 2003; Vallieres et al., 2002). Conversely, the anti-inflammatory cytokine Transforming Growth Factor beta-1 (TGF-β1) has proven to be pro-neurogenic *in vitro* and *in vivo* (Battista et al., 2006; Graciarena et al., 2010; Mathieu et al., 2010a,b). Nevertheless, the role of local cytokine expression on adult neurogenesis, the regional and temporal specificity of these effects, as well as the intracellular pathways involved in this modulation awaits further characterization.

Despite the particular regions that retain neurogenic potential, the adult CNS is mainly characterized by its limited plasticity. On the contrary, the developing CNS is characterized by a series of temporal windows where key proliferation, differentiation and

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migration events occur, which will in turn determine many of the CNS functions in the adult. These critical developmental steps are prone to be altered as a consequence of external influences (de Graaf-Peters and Hadders-Algra, 2006) which can be reflected on diverse spectra of CNS dysfunctions in the adult. The concept of programming has been built considering the long term effects that these early external influences have on the physiology and behavior of adult individuals. Programming includes alterations in adult neuronal CNS functions or immune responses caused by early (pre- or early postnatal) administration of inflammatory stimuli. In this regard, experimental studies have shown that inflammation during the prenatal period can lead to diverse alterations in CNS functions in the adult, including behavioral and cognitive impairments (Bilbo and Schwarz, 2009), alterations of the Hypothalamic–Pituitary–Adrenal axis activity (Reul et al., 1994), white matter damage and even cerebral palsy (Shi et al., 2003). In particular, we and others have shown that adult neurogenesis in the DG is impaired by prenatal inflammation triggered by lipopolysaccharide (LPS), a bacterial endotoxin (Graciarena et al., 2010); or polyriboinosinic–polyribocytidylic acid (PolyI:C), a synthetic analog of double-stranded RNA that mimicks components of a viral infection (Meyer et al., 2006). In particular, prenatal LPS administration may model bacterial vaginosis, a condition during pregnancy that put the developing fetus into contact with LPS (Dammann and Leviton, 1997; Purwar et al., 2001; Romero et al., 1989; Thorsen et al., 1998). Approximately 14% of pregnant women get bacterial vaginosis, which leads to a pro-inflammatory environment and has been shown to lead to white matter damage, cognitive limitations and cerebral palsy (Dammann and Leviton, 1997; Yoon et al., 1997). In addition, epidemiological studies have linked infections during pregnancy to later development of neuropsychiatric disorders in the offspring (Hultman et al., 1999; Mednick et al., 1988). These observations increase the relevance of possible prenatal LPS effects on the human population.

Since inflammation can impair adult neurogenesis both at the prenatal period and in the adulthood, here we aimed to compare the duration and magnitude of this effect when LPS is administered prenatally or in adult animals. Also we studied whether both adult neurogenic regions (DG and SVZ) are affected by LPS treatment. Finally, we explored the cellular and molecular alterations in the neurogenic niche that could explain the different effects of adult vs pre-natal LPS.

2. Methods

2.1. Animals

Adult Wistar rats (250–300 g) were bred in the animal house at the Leloir Institute. Animals were housed under controlled temperature (22 ± 2 °C) and artificial light under a 12 h cycle, with water and food available *ad libitum*. All dams were naive breeders. All animal procedures were performed according to the rules and standards for the use of laboratory animals of the National Institutes of Health, USA. Animal experiments were approved by the Ethical Committee of the Leloir Institute Foundation.

2.2. LPS treatment and BrdU staining

2.2.1. Prenatal LPS treatment

Pregnant dams were housed individually. Each received a subcutaneous (sc) injection of LPS (0.5 mg/kg; strain 0111:B4, Sigma–Aldrich, MA, USA) or saline on gestational/embryonic days (GD) 14, 16, 18, and 20. The litters were culled to 10 pups, weaned at postnatal day (PD) 21, and housed with no more than five animals per cage until adulthood (PD60). One to two pups from each litter were randomly sampled and assigned to an experimental group to reduce the influence of litter effects on the variables measured, as it has been previously suggested (Zorrilla, 1997). The time of appearance of typical developmental parameters such as eye opening, ear detachment and testis descent was unaffected by prenatal LPS administration, as it was the weight of the pups between birth and weaning. In depth characterization of the prenatal LPS model in terms of maternal behavior and other parameters can be found in (Graciarena et al., 2010).

2.3. Adult LPS treatment and BrdU injections

Adult male rats prenatally treated with saline received a single intraperitoneal (i.p.) injection of either LPS (1 mg/kg; Saline/LPS7 group) or saline (Saline/Saline7 group). Adult male rats prenatally treated with LPS received a single i.p. injection of saline to control for the effect of injection (LPS/Saline7 group). The adult injection was followed immediately by seven daily i.p. injections of 50 mg/kg 5-bromo-20-deoxiuridine (BrdU, Sigma–Aldrich) and perfusion at the 7th day (Fig. 1A) for immunohistochemistry studies as described below. For RNA isolation animals were decapitated on the 7th day.

To study the DG, a total number of 16 animals (Saline/Saline7, $n = 6$; Saline/LPS7, $n = 4$; LPS/Saline7, $n = 6$) were processed for immunostaining procedures and 21 animals (Saline/Saline7, $n = 7$; Saline/LPS7, $n = 6$; LPS/Saline7, $n = 8$) for mRNA quantitation protocols. For the SVZ and RMS, a total number of nine animals ($n = 3$/group) were processed for immunostaining procedures.

Alternatively, adult male rats with no prenatal treatment received four i.p. injections of LPS (1 mg/kg, LPS60 group) or saline (Saline60 group) every other day. After 60 days, rats received seven daily i.p. injections of BrdU (50 mg/kg), were perfused or decapitated at the 7th day (Fig. 2A). A total number of 16 animals (eight for immunohistochemistry and 8 for RNA isolation, four in each group) were processed for this experimental protocol.

2.4. Tissue sections

Animals were deeply anesthetized and transcardially perfused with heparinized saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH = 7.2). Brains were placed in the same fixative overnight at 4 °C, cryoprotected in 30% sucrose in PB 0.1 M, frozen in isopentane. Serial coronal sections throughout the hippocampus and serial sagittal sections throughout the SVZ and RMS were cut in a cryostat. The 40 μm sections were used for free floating immunostaining.

2.5. Immunostaining

Co-localization of BrdU with other markers was performed as previously described (Graciarena et al., 2010). Briefly, every sixth coronal section was incubated for 2 h in 50% formamide at 65 °C, washed once in 2× saline-sodium citrate (SSC; 0.3 M NaCl, 0.03 M sodium citrate), incubated for 30 min at 37 °C in 2 M HCl, rinsed in 0.1 M borate buffer pH 8.5, and thoroughly washed in Tris-buffered saline (TBS; Tris–HCl 50 mM pH 7.4, NaCl 150 mM). Sections were then blocked in 0.1% Triton X-100, 1% donkey serum in TBS (blocking solution) for 45 min at room temperature. Primary antibodies were diluted in blocking solution and sections were incubated for 48 h at 4 °C. After two washes with 0.1 M PB, sections were incubated in secondary antibodies diluted in 0.1 M PB for 2 h at room temperature. Sections were then washed with 0.1 M PB and mounted in Mowiol (Calbiochem, La Jolla, CA, USA).

Primary antibodies used were rat anti-BrdU (1:200; Abcam, Cambridge, UK); mouse anti-polysialic acid neuronal cell adhesion molecule (PSA-NCAM, 1:200, kindly provided by Dr. Seki, Depart-