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BRAIN RESEARCH

Research Report

Cytokinetics of adult rat SVZ after EAE

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ABSTRACT

Cytokinetics regulating cell cycle division can be modulated by several endogenous factors. EAE (experimental autoimmune encephalomyelitis) increases proliferation of progenitor cells in the subventricular zone (SVZ). Using cumulative and single S phase labeling with 5-bromo-2-deoxyuridine, we examined cell cycle kinetics of neural progenitor cells in the SVZ after EAE. 20% of the SVZ cell population was proliferating in adjuvant control rats. However, EAE significantly increased them up to 27% and these cells had a cell cycle length (TC) of 15.6 h, significantly ($P<0.05$) shorter than the 19 h TC in non EAE SVZ cells. Few TUNEL (+) cells were detected in the SVZ cells of adjuvant controls. EAE increased ($P<0.05$) TUNEL (+) nuclei in SVZ suggesting early stage progenitor cell death. Cell cycle phase analysis revealed that EAE substantially shortened the length of the G1 phase (9.6 h) compared with the G1 phase of 12.25 h in adjuvant control SVZ cells ($P<0.05$). This reduction in G1 contributes to EAE-induced reduction of TC because no significant changes were detected on the length of S, G2 and M phases between the two groups. Our results show a surge in proliferating progenitor cells in the SVZ with concomitant increase in apoptotic cell death after EAE. Furthermore, increase in the SVZ proliferation contributes to EAE-induced neurogenesis and this increase is regulated by shortening the G1 phase. Our investigation suggests the activation of quiescent cells in SVZ to generate actively proliferating progenitors. Moreover, the increase in the cell death in proliferating population may contribute towards negative regulation of proliferative cell number and hence diminished regenerative capacity of CNS following EAE.

1. Introduction

EAE (experimental autoimmune encephalomyelitis) is an autoimmune neuroinflammatory model which can be induced using several myelin antigens in a variety of laboratory animals. EAE has led us to valuable insights into the basic mechanisms of neuroinflammation in human multiple sclerosis. Autoantibodies, T-lymphocytes are the leading causes of the tissue destruction in EAE (Friese and Fugger, 2009). Demyelination is mediated by either phagocytosis of myelin or the death of oligodendrocytes (Sajad et al., 2010). Apart from demyelination, acute neuronal loss has been noticed in the lesions of EAE (Meyer et al., 2001).

Neuronal progenitor cell proliferation has been reported earlier in EAE leading to the generation of committed precursors of glial lineage. Neocortical development involves

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the aligned migration and differentiation of neuronal precursors that arise from proliferating ventricular zone (VZ) (Takahashi et al., 1993; Caviness et al., 2003; Noctor et al., 2004). In adult rodents, VZ is replaced by the ependymal layer, while the subventricular zone (SVZ) still persists (Morshead et al., 1998). The adult SVZ consists of actively proliferating progenitor cells and relatively quiescent stem cells, which generate neurons and glia throughout adulthood (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Morshead et al., 1998). SVZ cells can be recruited to proliferate more extensively and differentiate into astrocytes in response to injury (Holmin et al., 1997; Weinstein et al., 1996). This proliferation can even replace specific neuronal populations after neuronal loss (Fallon et al., 2000). Furthermore, these cells have been demonstrated to proliferate, migrate, and differentiate into astrocytes and oligodendrocytes in response to lysolecithin-induced demyelination of the white matter (Nait-Oumesmar et al., 1999), suggesting that they could serve as an additional source of oligodendrocyte precursors for CNS remyelination. The inflammatory nature of EAE may upregulate several neurotrophins like brain derived neutrophic factor (BDNF) (Kerschensteiner et al., 1999) since its receptors are expressed by astrocytes (Stadelmann et al., 2002) to counter ongoing cellular pathology. Moreover, EAE increases neurogenesis in the SVZ and newly generated proliferative cells migrate towards carpus callosum, cortex and olfactory bulb where they undergo oligogenesis (Picard-Riera et al., 2002).

Proliferation and differentiation of neural progenitor cells is strictly regulated by cell cycle kinetics (Takahashi et al., 1993; Caviness et al., 2003). Cellular signaling can impact neocortical neurogenesis by changing several parameters of cytokinetics e.g. growth fraction (GF; proportion of proliferating cells) and the length of cell cycle (TC) (Nowakowski et al., 1989; Takahashi et al., 1993; Caviness et al., 2003). Studies in neonatal and postnatal rats show that the cell cycle length of the SVZ ranges from 14 to 18.6 h (Schultze and Korr, 1981; Smith and Luskin, 1998). Increase in the proliferating cell population in the SVZ after EAE could result from increase of GF and/or shortening of TC of progenitor cells in the SVZ. However, the influence of EAE on cell cycle kinetics in the adult brain has not been investigated. Accordingly, using cumulative and single 5-bromo-2-deoxyuridine (BrdU) labeling protocols developed by Nowakowski et al. (1989), we investigated the sequence of cell cycle parameters including TC (total cell cycle length) and the length of four cell cycle phases G1, S, G2 and M, and the rate of the cells produced in the SVZ of adult rats after EAE.

2. Results

2.1. *EAE leads to demyelination, disruption of blood-brain barrier (BBB) and loss of CNP*

EAE was induced after a single injection of 50 μg/kg MOG subcutaneously and the signs of the disease were evident after 1 week post immunization (p.i.) which included loss of tail tone. Mean neurological deficits were calculated groupwise which represents average score of an animal in a particular group. The disease reached its maximum neurological deficit after 14±1 days p.i. with hind limb paralysis. The first acute period lasted for about 18–20 days p.i. (Fig. 1). Disruption of BBB was evidenced by increased absorbance of Evans blue in EAE rats (Fig. 2A) and increment in the fluorescence intensity of Evans blue–albumin dye complex in EAE rats when compared to adjuvant controls (Fig. 2C). EAE rats displayed pronounced demyelination (Fig. 3B) which was not limited to periventricular white matter in comparison to controls (Fig. 3A). Some of the lesions could be also seen in grey matter. Moreover, there was a steep decrease ($P<0.001$) in the brain CNP activity (Fig. 3C) suggesting oligodendrocyte loss after the first acute episode.

2.2. *Expansion of the subventricular zone after EAE*

The SVZ of the lateral ventricular wall in adjuvant control rats was approximately 21.11 μm (21.11±4.2 μm) wide on the coronal sections being analyzed (Fig. 4A), whereas the width of the SVZ at 20 days after induction expanded to nearly 78.08 μm (78.08±12.22 μm) from the lateral ventricle to the striatum (Fig. 4B). Expansion of the SVZ was associated with increase in the number of BrdU+ cells in EAE rats in comparison with the number in adjuvant control SVZ. These data indicate that BrdU+ cells in the SVZ represent proliferating cells.

2.3. *Increased cell death in proliferative population after EAE*

TUNEL assay labels nuclei undergoing apoptosis. Analysis revealed few TUNEL (+) cells in the adjuvant controls (Fig. 5A) where as EAE increased their number significantly ($P<0.05$) in the SVZ (Fig. 5B). Data suggests role of apoptosis in development of SVZ. The increased number of TUNEL (+) (Fig. 5C) cells in EAE suggests early stage cell death in SVZ after MOG induced EAE.

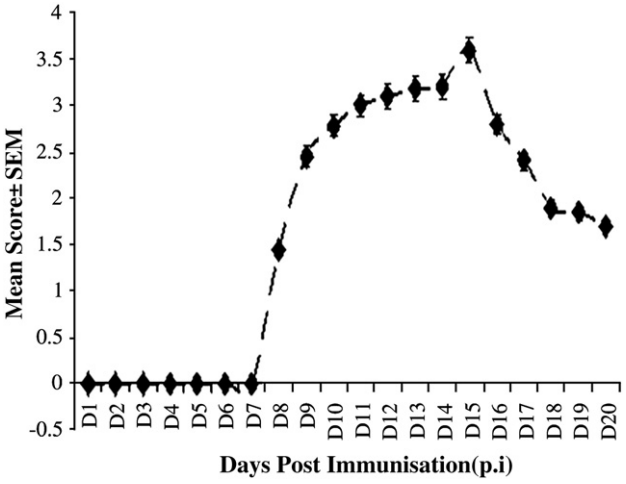

Fig. 1 – Induction of active EAE in Wistar rats. Scatter plot of mean neurological deficits obtained in each group after single subcutaneous injection of MOG (50 μg/kg) in rats. The first episode lasted for 18–20 days post induction (p.i.).