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Modulation of neural stem/progenitor cell proliferation during experimental Herpes Simplex encephalitis is mediated by differential FGF-2 expression in the adult brain

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ABSTRACT

Neural stem cells (NSCs) respond to inflammatory cues induced during brain injury and are thought to be involved in recovery from brain damage. Little is known about NSC response during brain infections. The present study evaluated NSC proliferation during Herpes Simplex Virus-1 brain infection. Total numbers of nestin(+) NSCs increased significantly in infected brains at 6 days post infection (p.i.). However, by 15 days p.i. the nestin(+) population decreased significantly below levels observed in uninfected brains and remained depressed through 30 days p.i. This initial increase in NSC population occurred concurrently with increased brain cell proliferation, which peaked at 3 days p.i. On closer examination, we found that while actively proliferating Sox2(+) NSCs increased in number at 6 days p.i., proliferating DCX(+) neuroblasts contributed to the increased response at 3 days p.i. However, overall proliferation decreased steadily from 15 days p.i. to below control levels. To determine the mechanisms involved in altering NSC proliferation, neurotrophin and growth factor expression profiles were assessed. FGF-2 gene expression increased at 5 days p.i. and was robustly down-regulated at 15 days p.i. (>1000-fold), which was further confirmed by increased FGF-2 immunostaining around the lateral ventricles. Furthermore, supplementing infected animals with recombinant FGF-2, at 15 days p.i., significantly increased the number of proliferating brain cells. These findings demonstrate that the temporal changes in NSC proliferation are mediated through the regulation of FGF-2 and that the NSC niche may benefit from supplementation with FGF-2 during HSV-1 brain infection.

Introduction

Herpes Simplex encephalitis (HSE) is the most common cause of sporadic viral encephalitis in the United States. While acyclovir treatment is a highly effective means to control HSV-1 brain infection (Whitley, 2006); greater than fifty percent of human patients with HSE develop life altering neurological deficits (Kimberlin, 2007; McGrath et al., 1997). These neurological deficits are the result of severe brain damage manifesting as reactive astrogliosis and necrosis (Webb et al., 1989) which in human infections manifests with significant vascular changes with neuronal loss during early stages of the disease and the development of astroglial scaring later in the infection (Whitley, 2006). The volume of glial scarring correlates to the level of neurological impairment, although the origins of HSV-1-mediated neurological damage and scar formation remain unclear.

In the murine model of experimental HSE, which closely resembles the human disease, immune responses to HSV-1 brain infection begins with infiltration of neutrophils and macrophages between 3 and 5 days p.i. T cells infiltrate between 6 and 8 days p.i. and peak between 14 and 16 days p.i. with significant persistence beyond 30 days p.i. (Armien et al., 2010; Marques et al., 2008). However, viral antigen is undetectable at 15 days p.i. The role of persistent T lymphocytes at this stage of disease is unknown; however the presence of activated immune cells is indicative of ongoing inflammation that facilitates development of damage and resultant neurological deficits (Armien et al., 2010). Similar studies in the murine model of Japanese encephalitis and experimental autoimmune encephalitis (EAE) have found that CD8(+) T lymphocyte mediated inflammation may be responsible for brain damage and development of long-term sequellae (Steinman, 2001; Wang et al., 2003).

A small population of endogenous brain cells called neural stem/progenitor cells (NSCs) play a significant role in adult neurogenesis

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(Lichtenwalner and Parent, 2005; Parent et al., 2006). NSCs are maintained within distinct germinal niches, which support their multipotent phenotype and self-renewal properties. Adult neurogenesis is composed of highly complex and temporally non-linear progression of cell differentiation that begins with proliferation and ends with integration into existing brain circuitry. Three NSC subpopulations have been shown to proliferate during physiological adult neurogenesis: neural stem cells (B cells), transit-amplifying cells (C cells), and migratory neuroblasts (A cells) (Brown et al., 2003; Doetsch et al., 1997) Previous studies have shown that B/C cells (neural stem/progenitor cells) are immunoreactive for nestin, Sox2, mushashi, and oct4 although levels of expression of these markers may depend on cell progression through differentiation (Kempermann, 2011). Doublecortin (DCX) is the primary cell marker expressed in A cells (Brown et al., 2003; Doetsch et al., 1997; Kempermann, 2011). Brain injury and its resultant inflammation initiate neurogenesis by altering the niche microenvironment to stimulate proliferation and NSC migration (Merkle et al., 2004). NSCs express numerous receptors that respond to immune signals which influence proliferation, direct migration to sites of damage and determine the fate of these pluripotent cells in the injured brain (Chu et al., 2004; Matsuoka et al., 2003).

Inflammation plays a complex role in the post-injury recovery of the CNS. It has been shown that T cells specific to CNS antigens promote recovery from brain injury, provided their activity is controlled (Hauben et al., 2000; Hofstetter et al., 2003; Yoles et al., 2001). However, during EAE, persistent neuroinflammation has been shown to abrogate proliferation of endogenous NSCs and neuroblast migration (Pluchino et al., 2008). In fact, neurogenesis in several brain damage models, including acute ischemic injury (Arvidsson et al., 2002), neurotoxins (Faiz et al., 2005; Tattersfield et al., 2004), and degenerative diseases like Alzheimer's and Parkinson's disease, have varying temporal neurogenic profiles (Capone et al., 2007), potentially directed by relatively unique inflammatory environments. The mechanisms instructing NSCs to maintain an undifferentiated state or differentiate within an inflamed tissue are poorly understood, particularly following viral encephalitis.

The objective of the present study was to evaluate NSC proliferation in the context of HSV-1 brain infection and its associated inflammation. Given the chronic inflammatory response observed during HSE, we hypothesized that NSC proliferation may be impaired during HSE which ultimately may contribute to the development of neurological deficits.

Materials and methods

Virus and infection

HSV-1 brain infection that manifests as acute encephalitis in mice was performed as previously described (Marques et al., 2008). Briefly, HSV-1 strain Syn17+, a neurovirulent strain of HSV, was used in all experiments. The virus was propagated in rabbit skin fibroblasts (CCL68; American Type Culture Collection) and titrated using standard plaque assay. Eight to ten week-old female BALB/c mice (Charles River Laboratories, Wilmington, MA) were infected with 2.5×10^5 plaque forming units (PFU) by intranasal instillation in anesthetized animals as previously described (Marques et al., 2006).

Transplantation of luciferase(+) NSC

Embryonic NSCs were isolated from the cortices of E14.5 BALB/c mice expressing luciferase (kindly provided by Dr. James Lokensgard, University of Minnesota). The luciferase(+) Balb/c transgenic mice were generated by backcrossing FVB mice expressing luciferase under the control of a CAGGs-CMV promoter into the Balb/c background for ten generations. Luciferase positive (luc(+)) NSCs (≥90% nestin(+) by flow cytometry) were expanded in culture and transplanted by stereotaxic implantation into the subventricular zone (SVZ), within the striatum, of MHC-matched, non-transgenic BALB/c mice using previously described methods (Ni et al., 2004). Briefly, Luc(+) NSCs (10^6 cells in 3 μL serum free medium) or 3 μL serum-free medium control was transplanted into the right SVZ using specific stereotaxic coordinates: rostrocaudal vector [AP] = 0 mm, interaural vector [ML] = +1.2 mm and the dorsoventral vector [DV] = −3.0 mm; delivered over a period of 7–10 min. The number of luc(+) NSCs to be transplanted was determined by measuring the minimum number of luc(+) cells necessary to provide consistent measurable baseline bioluminescent signal 7 days post transplantation, when recipient mice were infected by intranasal instillation of 2.5×10^5 PFU HSV-1 (Syn17+). By 7 days post-transplant, surgery-induced neuroinflammation had subsided and did not affect establishment of and mortality from HSV-1 brain infection. Bioimaging for luciferase expression in transplanted cells was performed using an *in vivo* imaging system, IVIS50 (Xenogen/Caliper Life Sciences, Alameda, CA) equipped with a charge-coupled camera device, as previously described (Marques et al., 2008). Briefly, 150 μg of D-luciferin (Gold Biotechnology) was administered to anesthetized mice by i.p. injection. Animals were imaged 5–10 min after D-luciferin administration and data were acquired using a 5-min exposure window. Signal intensity of luciferase expression, as measured by the total amount of transmitted light, was quantified as a photons/s/cm^2 using LivingImage (Caliper Life Sciences, Alameda CA) and Igor (Wavemetrics, Portland, OR) image analysis software. Change in bioluminescence was used as a measure of the numbers of luc(+) NSCs at indicated time points.

Flow cytometric quantification of endogenous neural stem cells

Mouse brain regions from −1 to +3 mm Bregma, which includes the neurogenic regions in the brain, were isolated using a coronal brain matrix (Braintree, Braintree, MA), and a papain-based neural tissue dissociation kit (Miltenyi Biotec, Auburn, CA) was used to generate a single-cell suspension. Myelin was depleted using myelin depletion beads (Miltenyi, CA). Live cells were enumerated, and 5×10^5 cells were immunostained for CD45 and nestin (BD Biosciences, San Jose, CA), Ki-67 (Abcam, Cambridge, MA) or SRY-related HMG box-gene (Sox)-2 (eBioscience, San Diego, CA) expression. For absolute quantification of immunostained cells expressing these markers, 50 μL blank AccuCount particles (Spherotech, Lake Forest, IL) were added to samples immediately before analysis on the flow cytometer (BD FACSCanto). Absolute numbers of each cell population was calculated per the manufacturer's instruction, as a ratio of CD45(−) nestin(+), CD45(−)Ki-67(+), or CD45(−)Ki-67(+)Sox2(+) events to number of AccuCount particles counted.

Immunohistochemistry

Mice were deeply anesthetized using a mixture of ketamine and xylazine and perfused intracardially with 4% paraformaldehyde. Brains were post fixed in 4% paraformaldehyde for 24 h and equilibrated in 30% sucrose. Fixed equilibrated tissue was frozen in OCT under liquid nitrogen vapor and sectioned at 30 μm thickness onto gelatin-coated slides. Coronal sections thus obtained were quenched in a 0.3% peroxide solution for ten minutes and blocked with goat serum (5%) in PBS with 0.5% Triton-X for one hour at 25 °C. Primary antibodies (Abs) were incubated in the blocking solution overnight at 4 °C. Primary antibodies used were rabbit anti-doublecortin Ab (1:1000; Pierce Biotechnologies, Rockford, IL, and PerkinElmer Tyramide Signal Amplification PLUS Flurorescein, PerkinElmer, Waltham, MA), rabbit or mouse anti-PCNA Ab (1:50; Abcam, Cambridge, MA), mouse anti-Sox2 or rabbit anti-FGF-2 Abs (1:200, Abcam), rat anti-Ki-67 (1:50, eBioscience), and goat anti-HSV-1 (1:100, ViroStat, Portland, MA). This HSV-1 polyclonal antibody is reactive to both immediate early and late structural antigens. Immunostaining using the Tyramide Signal Amplification kit utilized