Neuroscience 311 (2015) 56–66

BILATERAL RETINAL MICROGLIAL RESPONSE TO UNILATERAL OPTIC NERVE TRANSECTION IN RATS

L. P. CEN,[a] M. HAN,[a] L. ZHOU,[a] L. TAN,[a] J. J. LIANG,[a] C. P. PANG[a,b] AND M. ZHANG[a]*

[a] *Joint Shantou International Eye Center, Shantou University and The Chinese University of Hong Kong, Shantou, China*

[b] *Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China*

Abstract—When retinal ganglion cells undergo apoptosis after optic nerve (ON) injury, microglial cells proliferate and promptly clear the degenerated debris in the ipsilateral retina. However, microglial changes in the contralateral retina have not been fully elucidated. This study characterized the long-term bilateral retinal microglial responses after unilateral ON transection. We analyzed the time course of proliferation and morphology changes of microglial cells, between 3 days and 12 weeks post ON transection, of undisturbed and reactive microglia in bilateral retinas of adult Fischer rats with unilateral ON transection. Microglia in retinas without ON transection were distributed homogeneously and possessed a highly ramified morphology, as judged by immunohistochemistry for ionized calcium-binding adapter molecule 1 (Iba1). After ON transection, microglia density in the ipsilateral retina increased gradually from 3 days to 2 weeks, and decreased from 3 weeks to 12 weeks, along with dramatic inverted alteration of process branch points of microglia in the ganglion cell layer (GCL). Transformation of ramified microglia into ameboid-like macrophages with few branching processes was observed in the ipsilateral retina from 1 week to 3 weeks. Though an increase in microglial density was weak in the contralateral retina and could only be statistically detected in the central retina, the morphological alteration over time was obvious and similar to that of the ipsilateral retina. In the inner plexiform layer (IPL), cell density and morphological changes of microglia in both the ipsilateral and contralateral retina were not prominent. These findings indicates that, though proliferation of microglial cells is weak in the contralateral retina after unilateral ON transection, conspicuous alterations in microglial morphology occur bilaterally. These suggest that using the contralateral retina as a control in studies of retinal degeneration should be considered with caution.
© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: retina, microglia, bilateral, optic nerve transection.

INTRODUCTION

Under normal circumstances, most mature retinal ganglion cells (RGCs) undergo apoptosis during the several weeks following optic nerve (ON) injury. Although a few RGCs can survive the injury, almost no axonal regeneration from the injured RGCs can be seen (Berkelaar et al., 1994). In addition to investigating the degenerating neurons themselves, glial cells adjacent to the RGCs have been vigorously studied in recent years. Microglia are considered to be among the most important glial cells that not only participate in the progress of retinal degeneration (Wohl et al., 2010), but also play crucial roles in maintaining a stable environment for neural activities.

Microglia are uniquely specialized glial cells in the central nervous system (CNS), and were first distinguished from other glial cell types by del Rio-Hortega by silver carbonate staining. Under normal physiological conditions, being resident innate immune cells, microglia display ramified morphologies with highly motile processes to survey the surrounding environment (Nimmerjahn et al., 2005; Damani et al., 2011). Once exposed to various stimuli, microglia can respond rapidly, transforming from a ramified to ameboid-like macrophage phenotype with abilities for migration and proliferation (Tambuyzer et al., 2009). For example, when the retina is infected or when retinal neuronal cells die, microglia transform into macrophages to clear infected or dead cells (Thanos, 1991a). Activation of microglia is often observed in neurodegenerative disease to produce neurotoxic substances. In Alzheimer's disease, for example, amyloid-β protein can activate microglia to release neurotoxic factors, such as nitric oxide (NO), tumor necrosis factor alpha (TNFα) and superoxide (Qin et al., 2002; Dheen et al., 2005). However, microglia also have neuroprotective and neurotrophic roles by eliminating neurotoxins and producing neurotrophic molecules in spinal cord injury (Dougherty et al., 2000).

Because of its well-characterized anatomical structure and easy accessibility, the visual system has been widely used for investigation of neural diseases. The retina and ON contralateral to an injured eye are often used as a control in ON transection and glaucoma studies (Levkovitch-Verbin et al., 2013). However, this may not be appropriate, as recent studies indicate that unilateral

*Corresponding author. Tel: +86-754-88393561; fax: +86-754-88393575.

E-mail address: zmz@jsiec.org (M. Zhang).

Abbreviations: BrdU, bromodeoxyuridine; GCL, ganglion cell layer; Iba1, ionized calcium-binding adapter molecule 1; IPL, inner plexiform layer; MHC-II, histocompatibility complex class II; ON, optic nerve; OPL, outer plexiform layer; PBS, phosphate-buffered saline; RGC, retinal ganglion cell.

http://dx.doi.org/10.1016/j.neuroscience.2015.09.067
0306-4522/© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

ON crush injury not only induces microglial activation and proliferation in the ipsilateral retina, but also stimulates responses in the contralateral side (Bodeutsch et al., 1999; Panagis et al., 2005). Although specific mechanisms of bilateral responses after unilateral injury remain to be illuminated, several studies have been conducted to explore the glial changes in the contralateral retina or ON after unilateral injury. The number of microglia has been observed to increase in the contralateral ON within 6 days after ON crush injury (Bodeutsch et al., 1999). Bromodeoxyuridine (BrdU) and major histocompatibility complex class II (MHC-II)-positive microglia could also be seen in the contralateral retina (Gallego et al., 2012). Yet, long-term comparative evaluation of bilateral changes of retinal microglia after unilateral ON injury have not been systematically described. In the present study, we addressed the long-term bilateral retina microglial responses up to 12 weeks after unilateral ON transection. In addition to characterization of changes in cell number, morphological transformation of microglia was also investigated by using a "branch point" concept, which has previously been applied to describe the extent of dendritic branching of human RGCs (Peterson and Dacey, 2000).

EXPERIMENTAL PROCEDURES

Animals

A total of 31 young adult (8–12 weeks old) female Fischer 344 rats (F344) (Vital River, Beijing, China) were used in this study. Rats were housed under standard conditions with a 12-h light/dark cycle and fed food and water *ad libitum*. All animal surgical procedures were approved by the local institution animal ethics committees and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All surgery was performed with rats under anesthesia of a 1:1 mixture (1.5 ml/kg) of ketamine (100 mg/ml) and xylazine (20 mg/ml), and topical anesthetic drops applied to the eye.

ON transection

ON transection was performed intraorbitally according to procedures that are standard and regularly used in our laboratory. Briefly, under anesthesia, a small incision was made in the temporal conjunctiva of the right eye. Under an operating microscope, the left ON was exposed intra-orbitally after separating the extraocular muscles, and was transected 1.5 mm behind the nerve head. Caution was taken to avoid injury to the ophthalmic artery under the ON. Each surgical eye was inspected by ophthalmoscopy to ensure the integrity of blood flow to the eye, followed by antibiotic ointment application topically to avoid infection after the surgical procedure.

Experimental groups

To evaluate the long-term responses of retinal microglia cells, animals were assigned to six groups according to the different time points after ON transection, which included 3 days ($n = 4$), 1 week ($n = 5$), 2 weeks ($n = 5$), 3 weeks ($n = 5$), 6 weeks ($n = 4$) and 12 weeks ($n = 4$) following ON transection. In addition, animals without ON transection served as the naive group ($n = 4$). In order to reduce the influence of age, rats at the same age, in weeks, were randomly assigned to every group, so that every group contained animals at different ages (range from 8 to 12 weeks).

Tissue processing and immunohistochemistry

After euthanasia with an overdose of ketamine and xylazine, rats were perfused with cold saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Retinas from both eyes were then dissected out from the eyecups and postfixed in 4% paraformaldehyde for 1 h. After thorough washing with PBS, retinas were blocked with 10% normal goat serum and 0.2% Triton X-100 (Sigma) for 1 h, and then incubated in the same medium with Iba1 antibody (1:1000, Cat.# 019-19741, Wako Pure Chemical Industries, Osaka, Japan), for 1 day at 4 °C, to label microglia. After additional washes, retinas were incubated with cyanine 3-conjugated goat anti-mouse immunoglobulin G (IgG) (1:400; Cat.# 111-165-003, Jackson ImmunoResearch Laboratories, Inc, Baltimore Pike, West Grove, PA, USA) secondary antibody overnight at 4 °C. After making relieving slits, retinas were flat-mounted on slides, mounted in anti-fade mounting medium (Dako Corporation, Carpinteria, CA, USA), and examined under a fluorescence microscope (Nikon Eclipse 800i, Japan).

Quantitative and morphological evaluation of microglia

Though diverse descriptions of layer separation have been shown by other groups with different investigative methods (Santos et al., 2008; Bosco et al., 2011; Kezic et al., 2013; Choe et al., 2014), microglia were found, in the present study, to be mainly located in three separate layers by epifluorescence microscopy on retinal flat mounts: the ganglion cell layer (GCL, including the nerve fiber layer), inner plexiform layer (IPL), and outer plexiform layer (OPL). Studies have shown that the most obvious microglial proliferation is detected in the GCL after ON transection, whereas microglia in the OPL remain undisturbed (Garcia-Valenzuela et al., 2005). Therefore, for the bilateral investigation of microglia in this study, we mainly focused on the GCL and IPL. Retinal areas used for cell density measurements and morphological analysis were located at three different eccentricities from the optic disk: central (0.5 mm), middle (2 mm), and peripheral (4 mm), in four quadrants of the retina. Eight fields, with two located in each quadrant, were chosen for analysis for each eccentric area (schematic shown in Fig. 1D). There were in total 24 fields sampled from each retina. Cell density measurements were conducted under a 400x fluorescence microscope (Nikon Eclipse 800i, Japan) by counting Iba1-labeled microglia in $0.1 \times 0.1\ mm^2$ microscopic fields. Microglia located in the GCL and IPL were both counted separately by changing the focusing depth. The average density of microglial

Download English Version:

https://daneshyari.com/en/article/6271670

Download Persian Version:

https://daneshyari.com/article/6271670

Daneshyari.com