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Bilateral early activation of retinal microglial cells in a mouse model of unilateral laser-induced experimental ocular hypertension

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ABSTRACT

The immune system plays an important role in glaucomatous neurodegeneration. Retinal microglial reactivation associated with ganglion cell loss could reportedly contribute to the glaucoma progression. Recently we have described signs of microglia activation both in contralateral and ocular hypertension (OHT) eyes involving all retinal layers 15 days after OHT laser induction in mice. However, no works available have analyzed the microglial activation at earliest time points after OHT induction (24 h) in this experimental model. Thus, we seek to describe and quantify signs of microglia activation and differences depending on the retinal layer, 24 h after unilateral laser-induced OHT. Two groups of adult Swiss mice were used: age-matched control (naïve) and lasered. In the lasered animals, OHT eyes as well as contralateral eyes were analyzed. Retinal whole-mounts were immunostained with antibodies against Iba-1 and MHC-II. We quantified the number of microglial cells in the photoreceptor layer (OS), outer plexiform layer (OPL), and inner plexiform layer (IPL); the number of microglial vertical processes connecting the OPL and OS; the area of the retina occupied by Iba-1+ cells (Iba1-RA) in the nerve fiber layer-ganglion cell layer (NFL-GCL), the total arbor area of microglial cells in the OPL and IPL and; Iba-1+ cell body area in the OPL, IPL and NFL-GCL. In contralateral and OHT eyes the morphological features of Iba-1+ cell activation were: migration, enlargement of the cell body, higher degree of branching and reorientation of the processes, radial disposition of the soma and processes toward adjacent microglial plexuses, and presence of amoeboid cells acting as macrophages. These signs were more pronounced in OHT eyes. Most of Iba-1+ cells did not express MHC-II; rather, only dendritic and rounded cells expressed it. In comparison with naïve eyes, in OHT eyes and contralateral eyes no significant differences were found in the microglial cell number; but there was a significant increase in Iba1-RA. The total arbor area of microglial cells was significantly decreased in: i) OHT eyes with respect contralateral eyes and naïve-eyes in IPL; ii) OHT eyes with respect to naïve eyes in OPL. The number of microglial vertical processes connecting the OPL and OS were significantly increased in contralateral eyes compared with naïve-eyes and OHT eyes. In OPL, IPL and NFL-GCL, the cell body area of Iba-1+ cells was significantly greater in OHT eyes than in naïve and contralateral eyes, and greater in contralateral eyes than in naïve eyes. A non-proliferative microglial reactivation was detected both in contralateral eyes and in OHT eyes in an early time after unilateral laser-induced OHT (24 h). This fast microglial activation, which involves the contralateral eye, could be mediated by the immune system.

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Abbreviations

GCL	ganglion cell layer
IOP	intraocular pressure
IPL	inner plexiform layer
Iba1-RA	area of the retina occupied by Iba-1 + cells
MHC-II	major histocompatibility complex class II
NFL	nerve fiber layer
OHT	ocular hypertension
OS	photoreceptor layer
OPL	outer plexiform layer
RGC	retinal ganglion cell

1. Introduction

Glaucoma is a neurodegenerative disease characterized by retinal ganglion cell (RGC) loss. Classically, intraocular pressure (IOP) has been considered the main glaucoma risk factor treatable at present (Quigley and Broman, 2006). However, IOP control does not always delay the progression of the disease.

It has been demonstrated that in glaucomatous neurodegeneration the immune system plays an important role (Tezel and the Fourth ARVO/Pfizer Ophthalmics Research Institute Conference, Working Group, 2009). In the central nervous system, the microglia is the principal immunocompetent cell (Bosco et al., 2011; Streit et al., 2005). This cell responds to neuronal damage by adopting an activated phenotype (Davis et al., 2017; de Hoz et al., 2013; Gallego et al., 2012; Rojas et al., 2014), which is characterized by morphological changes (retraction, reorientation and hyper-ramification of the processes and presence of different morphological types such as hyper-ramified, rod-like, and amoeboid microglia), migration, proliferation, and accumulation around the injured areas. In addition, activated microglia can alter the expression of receptors (CX3CR1, P2Y12 and CD200R1) and enzymes (metalloproteinases, collagenases and furin) and secrete cytotoxic substances such as proinflammatory cytokines, proteases, and oxygen-free radicals. In addition, these activated cells can act as antigen-presenting cells, and can transform into phagocytes (Karlstetter et al., 2015; Karlstetter et al., 2015; Kettenmann et al., 2011; Kettenmann et al., 2011; Luo et al., 2010; Luo et al., 2010; Ramírez et al., 2015; Ransohoff and Perry, 2009; Kezic et al., 2013; Wang et al., 2017). Microglial reactivation has been associated with RGC loss in human glaucoma (Yuan and Neufeld, 2001) and in experimental models of glaucoma (de Hoz et al., 2013; Gallego et al., 2012; Rojas et al., 2014), which could contribute to the glaucoma progression (Madeira et al., 2015). The inhibition of microglial activation by minocycline (Bosco et al., 2008) or with a high dose of irradiation (Bosco et al., 2012) can decrease RGC death.

In our previous studies in a mouse model of unilateral laser-induced ocular hypertension (OHT), we demonstrated that, 15 days after laser treatment, microglia showed several quantitative and qualitative signs of activation. These signs mainly included: i) shortening and widening of microglial processes (consistent with the significant reduction of the microglia arbor area); the presence of a high degree of branching (hyper-ramified microglia); microglial migration across the retinal parenchyma; increased microglial number; presence of CD68 amoeboid microglia acting as macrophages; presence of rod-like microglia (only in OHT eyes) which could be related with the synaptic stripping (a process in which microglia selectively remove synapses from injured neurons) (Blinzinger and Kreutzberg, 1968; Trapp et al., 2007); and MHC-II up-regulation in cells of all retinal layers. These microglial activation signs were detected in the OHT eye but also in the untreated contralateral normotensive eye. The microglial activation in the contralateral eye 15 days after laser treatment could reflect the initial events of OHT-induced neurodegeneration, probably mediated by inflammatory mechanisms (de Hoz et al., 2013; Gallego et al., 2012; Rojas et al., 2014). However, it is unknown whether microglial activation is an early process occurring after laser induced IOP elevation through photocoagulation of the limbal and episcleral veins, not only in OHT eye but also in the normotensive contralateral eye. If this activation occurs early after the induction of the OHT (24 h), it could be informative to analyze the specific characteristics of retinal microglial activation in both OHT and contralateral normotensive eyes.

Few studies examine early retinal microglial activation (2 h–4 days) after OHT induction in experimental models (Wang et al., 2000, 2014; Naskar et al., 2002; Zhang et al., 2005; Fu and Sretavan, 2010; Ha et al., 2015; Trost et al., 2015), and even more, those who use the mouse for that purpose (Fu and Sretavan, 2010; Naskar et al., 2002; Wang et al., 2000). Only three studies (Bodeutsch et al., 1999; Wang et al., 2000; Zhang et al., 2005) analyze microglial activation at 24 h after retinal injury, and these have been performed in rats. Two of these studies, use models to induce OHT different from ours, such as the acute elevation of intraocular pressure (raised to 120 mmHg for 60 min by saline injection in the anterior chamber) (Zhang et al., 2005) and the episcleral vein cauterization (Wang et al., 2000). The third uses another mechanism to inflict retinal damage, the optic-nerve crush (Bodeutsch et al., 1999; Liu et al., 2012). Thus, no available studies using the OHT mouse model of the present study analyze the early (24 h) changes in retinal microglia. Nevertheless, none describes in detail the microglial activation in the damaged eye and in its contralateral undamaged eye.

Thus, the aim of the present study was to analyze in retinal whole-mounts, at an early time after laser-induced OHT (24 h), the distinctive signs of microglial activation in the different retinal layers, both in OHT eyes and in contralateral eyes, including: microglia cell number, cell arbor area in the plexiform layers, area occupied by Iba-1 + cells (Iba 1-RA) in the NFL-GCL, number of microglial vertical processes connecting the OPL and OS, MHC-II- upregulation, and cell body area of Iba-1 + cells in the OPL, IPL and NFL- GCL, -.

2. Materials and methods

2.1. Ethics statement

Mice were treated in accordance with Spanish law and the Guidelines for Humane Endpoints for Animals Used in Biomedical Research. This study was approved by the Ethics Committee for Animal Research of Murcia University (Murcia, Spain) and the Animal Health Service of the Murcia Regional Ministry of Agriculture and Water (approval ID number: A1310110807). In addition, animal procedures followed institutional guidelines, European Union regulations for the use of animals in research, and the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

2.2. Animals and anesthetics

The experiments were performed on adult male albino Swiss mice (between 40 and 45 g) obtained from the breeding colony of the University of Murcia. The animals were housed in temperature- and light-controlled rooms with a 12 h light/dark cycle and *ad libitum* access to food and water. Light intensity within the cages ranged from 9 to 24 lux. Surgical procedures, including IOP measurement, were performed under general anesthesia induced with an intraperitoneal (ip) injection of a mixture of ketamine (75 mg/kg, Ketolar®, Parke-Davies, Barcelona, Spain) and xylazine (10 mg/kg, Rompún®, Bayer, Barcelona, Spain). During recovery from anesthesia, the mice were placed in their cages and an ointment containing tobramycin (Tobrex®; Alcon, Barcelona, Spain) was applied to the cornea to prevent corneal desiccation and

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