Vision Research 102 (2014) 71–79

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Rapid light-induced activation of retinal microglia in mice lacking Arrestin-1

Emily S. Levine [a], Azhar Zam [a], Pengfei Zhang [a], Alina Pechko [b], Xinlei Wang [a], Paul FitzGerald [a], Edward N. Pugh Jr. [a,c], Robert J. Zawadzki [c], Marie E. Burns [a,b,c,*]

[a] Dept. of Cell Biology and Human Anatomy, University of California Davis, Davis, CA 95618, United States
[b] Center for Neuroscience, University of California Davis, Davis, CA 95618, United States
[c] Center for Ophthalmology & Vision Science, University of California Davis, Davis, CA 95618, United States

ARTICLE INFO

Article history:
Received 3 July 2014
Received in revised form 22 July 2014
Available online 1 August 2014

Keywords:
Microglia
Photoreceptors
Phototransduction
Retina
Neurodegeneration

ABSTRACT

Microglia dynamically prune synaptic contacts during development, and digest waste that accumulates in degeneration and aging. In many neurodegenerative diseases, microglial activation and phagocytosis gradually increase over months or years, with poorly defined initial triggering events. Here, we describe rapid retinal microglial activation in response to physiological light levels in a mouse model of photoreceptor degeneration that arises from defective rhodopsin deactivation and prolonged signaling. Activation, migration and proliferation of microglia proceeded along a well-defined time course apparent within 12 h of light onset. Retinal imaging *in vivo* with optical coherence tomography revealed dramatic increases in light-scattering from photoreceptors prior to the outer nuclear layer thinning classically used as a measure of retinal neurodegeneration. This model is valuable for mechanistic studies of microglial activation in a well-defined and optically accessible neural circuit, and for the development of novel methods for detecting early signs of pending neurodegeneration *in vivo*.

1. Introduction

In healthy neural tissue, microglia are dynamic, highly ramified cells that monitor their microenvironment and prune neural processes to maintain synaptic homeostasis (reviewed in Aguzzi, Barres, & Bennett, 2013; Kettenmann, Kirchhoff, & Verkhratsky, 2013; Wong, 2013). In response to neuronal infection, injury, or degeneration, microglia are the first and often only responders to phagocytose debris and dying cells. If the disruption of the microenvironment is sufficiently severe, microglia become irreversibly activated, transform into a migratory amoeboid shape, and become capable of large-scale phagocytosis (Langmann, 2007). Overactive microglia, and the macrophages they recruit from the circulation, are thought to participate in the initiation or escalation of neurodegeneration (Hanisch & Kettenmann, 2007). Understanding the earliest stages of microglial activation and the molecular mechanisms that regulate their phagocytic activity is critical for defining the signals that activate them, and for potentially controlling reversible tissue damage.

In the retina, microglial activation accompanies light damage, optic nerve transsection, ischemia, and many inherited degenerations (Karlstetter, Ebert, & Langmann, 2010). In many such instances involving photoreceptor cell death, the transition from healthy to diseased state is difficult to discern due to the slow progression of aging or disease onset. An animal model has been needed whereby photoreceptor development and retinal circuitry can be maintained in a healthy state, but a controllable physiological stimulus can abruptly induce microglial changes. In such a model, the fundamental aspects of microglial activation, migration, proliferation, and phagocytosis, as well as the cause and effect relations of microglia and neural apoptosis, can be mechanistically investigated.

The rapid, light-dependent degeneration of photoreceptors in mice lacking Arrestin1 ($Arr1^{-/-}$) offers such a model. The biochemical mechanisms that transduce light into electrical signals in rods have been extensively studied (Burns & Pugh, 2010) and loss of regulatory factors like Grk1 (rhodopsin kinase; C.K. Chen et al., 1999) and Arr1 (visual arrestin; Xu et al., 1997) that deactivate the photoexcited G-protein coupled receptor, rhodopsin, has been shown to lead to retinal degeneration. Retinas of $Arr1^{-/-}$ mice born and reared in constant darkness initially have normal morphology, but undergo light-dependent photoreceptor degeneration (J. Chen

* Corresponding author at: Center for Neuroscience, University of California Davis, Davis, CA 95618, United States.
E-mail address: meburns@ucdavis.edu (M.E. Burns).

http://dx.doi.org/10.1016/j.visres.2014.07.011
0042-6989/

et al., 1999) within days of exposure to bright light (Burns et al., 2006). The concurrent loss of the rod G protein alpha subunit (Gnat1), which is essential for downstream rhodopsin signaling, rescues rods of Arr1$^{-/-}$ mice from degeneration triggered by dim light (Hao et al., 2002). This finding supports the idea that photoreceptor cell death arises from excessive phototransduction signaling; however, the cellular and physiological mechanisms by which excessive signaling leads to degeneration remain unknown.

Here we show that Arr1$^{-/-}$ retinas exhibit dramatic light-dependent activation of microglia within hours of light exposure. Exposure to even dim light causes microglia to infiltrate the outer nuclear layer and engulf photoreceptor somata. Microglial activation is preceded by a dramatic increase in the light scattering of the photoreceptor layer observed *in vivo* by OCT imaging, revealing this non-invasive imaging modality as a means for detecting cell stress far before classic measures of degeneration are apparent. Thus, the Arr1$^{-/-}$ mouse is an exciting model for studying the acute, physiological induction of microglial activation and the earliest stages of photoreceptor cell death. This system is also valuable for development of *in vivo* metrics of neural stress prior to degeneration and for testing interventions that could reverse the microglial activation.

2. Materials and methods

2.1. Use of animals

Mice were cared for and handled in accordance with National Institutes of Health guidelines for the care and use of experimental animals and approved protocol by the Institutional Animal Care and Use Committee of the University of California, Davis in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Adult wild type C57BL/6J (Jackson Laboratory) were used as control mice. Mice were reared in 24-h darkness, or in the case of a subset of WT mice, reared in cyclic light and dark-adapted overnight prior to experimental light exposure. Mice kept in uniformly illuminated plastic enclosures were either exposed to standard cyclic light with an intensity of ~25 lux for up to 168 h, constant light with an intensity of ~200 lux for up to 96 h, or a brief light with an intensity of ~5000 lux for 2 min followed by up to 72 h in darkness. The intensity of the illumination was measured at the floor of the enclosure with a calibrated meter in photopic lux (lumens m^{-2}). Assuming that the undilated pupils of the mice contracted to a diameter of 0.3 mm, the retinal light flux and rate of rhodopsin isomerization (R* rod^{-1} s^{-1}) can be determined (Lyubarsky, Daniele, & Pugh, 2004). Assuming perfect homogeneity, the illumination producing 25 lux illumination at the pupil plane excited ~250 R* rod^{-1} s^{-1} across the retina, 200 lux generated ~2000 R* rod^{-1} s^{-1}, and 5000 lux generated ~50,000 R* rod^{-1} s^{-1}. The Arr1$^{-/-}$ mice were originally described in Xu et al. (1997), and displayed indistinguishable light responses to those originally described (data not shown). Gnat1$^{-/-}$ mice were originally described in Calvert et al. (2000) and crossed with the Arr1$^{-/-}$ line to homozygosity. The WT, Gnat1$^{-/-}$ and Arr1$^{-/-}$ mice used in these specific experiments were screened for the rd8 mutation (Mattapallil et al., 2012) and found to be lacking the mutation (data not shown).

2.2. Immunohistochemistry

Mice were euthanized by CO_2 narcosis and decapitated, and the eyes were removed and immersed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 5 min at room temperature. The anterior segments were then cut away, the lens removed, and the eyecups fixed for 20 additional minutes. Eyecups were stored in 1× PBS at 4 °C until sectioning. Eyecups were embedded in low-melting agarose (Invitrogen, Cat. #18300-012) preheated to 70 °C and oriented so as to cut planar to the dorsoventral midline as described in Daniele et al. (2011) and Wagner, McCaffery, and Dräger (2000). 150 μm-thick sections were made with a vibratome (Leica Microsystems, model VT 10005) and stored in small base molds of 1× PBS at 4 °C. Sections were incubated with normal sera (goat; Jackson ImmunoResearch) for about 1 h at room temperature. The sections were then incubated in primary antibodies at 4 °C overnight. After three 5 min washes in 1× PBS, secondary antibodies were applied for 1.5–2 h at room temperature. The sections were washed 3 times for 5 min each in 1× PBS, incubated in DAPI (Invitrogen Life Technologies,) then washed three additional times before being mounted onto slides in polyvinyl alcohol mounting medium with DABCO (Sigma Aldrich) or ProLong Gold Antifade Reagent (Invitrogen Life Technologies).

Primary antibodies used for immunohistochemistry include rabbit anti-Iba1 (019-19741; Wako) at a 1/500 dilution and rat anti-CD68 (MCA1957T; AbD Serotec) at a 1/1000 dilution. Alexa 488-conjugated goat anti-rabbit and Alexa 633-conjugated goat anti-rat (Invitrogen Life Technologies) were used as secondary antibodies at 1/300 dilution. Immunosera were diluted in a buffer of PBS with bovine serum albumin (0.5%) and Triton X-100 (0.5%).

Slide-mounted sections were visualized with a Nikon Ti-E A1 multiphoton imaging system using a 40×-water immersion objective and continuous wave lasers. Three-dimensional images (30 μm in Z) were sampled from the center and peripheral regions of each section using the NIS-Elements Microscope Imaging Software (Nikon). Cell numbers, morphological features, and layer thicknesses were counted and measured manually.

2.3. Cryosectioning and TUNEL staining

Retinas were fixed by freeze substitution as described in Yoon and FitzGerald (2009). Briefly, methanol–acetic acid fixative (97% methanol and 3% acetic acid) and propane were chilled to dry ice temperature in advance. Liquid propane was prepared by condensing gaseous propane in the walls of an aluminum PCR block chilled to dry ice temperature. Fresh eyes were rapidly dissected and immersed in liquid propane for 1 min, then transferred into chilled methanol–acetic acid fixative and stored at −80 °C for 48 h. After gradually warming to room temperature, tissue was rehydrated by immersion in fixative with 20%, 40%, 60%, and 80% 1× PBS for 10 min each, then in 100% 1× PBS overnight.

Following rehydration, eyes were embedded in OCT Compound (Tissue-Tek, Sakura, Tokyo, Japan), and cryosectioned at 16 μm thickness in the sagittal plane, allowing dorsal to ventral observation using Microm HM 550 cryostat (Thermo Scientific). Slices were cut adjacent to or through the optic nerve head to minimize variations in retinal thickness. Apoptotic cells were detected using Click-iT TUNEL Alexa Fluor 647 imaging assay (Invitrogen Life Technologies), according to the manufacturer's protocol. Briefly, frozen sections were permeated by 0.25% Triton X-100 in PBS for 20 min. Sections were incubated in a TdT reaction cocktail for 1 h at 37 °C, then protected from light in a Click-iT reaction cocktail for 30 min at room temperature. Sections were mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen Life Technologies) following incubation.

Slide-mounted sections were visualized with a Nikon Ti-E A1 multiphoton imaging system using a 40×-water immersion objective and continuous wave lasers. The mean intensities of DAPI (blue) and TUNEL (red) channels were measured from the outer nuclear layer from central and peripheral regions of each section. The ratio of red to blue intensities was calculated in order to normalize the TUNEL signal by the number of remaining nuclei as