Visual Neuroscience

www.cambridge.org/vns

Retinal microglia polarization in diabetic retinopathy

Xin Li, Zi-Wei Yu, Hui-Yao Li, Yue Yuan, Xin-Yuan Gao** and Hong-Yu Kuang

The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, People's Republic of China

Review Article

Cite this article: Li, X., Yu, Z.-W., Li, H.-Y., Yuan, Y., Gao, X.-Y. & Kuang, H.-Y. (2021). Retinal microglia polarization in diabetic retinopathy. *Visual Neuroscience* 38:E006. https://doi.org/10.1017/S0952523821000031

Received: 26 January 2021
Revised: 17 February 2021
Accepted: 17 February 2021

Keywords:
microglia polarization, retinal microglia, diabetic retinopathy

Address correspondence to:
*Xin-Yuan Gao,
Email: 15369337970@163.com

Abstract

Microglia, the main immune cell of the central nervous system (CNS), categorized into M1-like phenotype and M2-like phenotype, play important roles in phagocytosis, cell migration, antigen presentation, and cytokine production. As a part of CNS, retinal microglial cells (RMC) play an important role in retinal diseases. Diabetic retinopathy (DR) is one of the most common complications of diabetes. Recent studies have demonstrated that DR is not only a microvascular disease but also retinal neurodegeneration. RMC was regarded as a central role in neurodegeneration and neuroinflammation. Therefore, in this review, we will discuss RMC polarization and its possible regulatory factors in early DR, which will provide new targets and insights for early intervention of DR.

1. Introduction

Diabetic retinopathy (DR) is one of the most common complications of diabetes, which is the main cause of the visual defect in working-age people worldwide. Almost all patients with type 1 and more than 60% of patients with type 2 diabetes develop retinopathy (Nentwich & Ulbig, 2015). All this time, microvascular lesions have long been regarded as central to the pathogenesis of DR. In recent years, however, many studies showed that retinal neurovascular units have undergone pathological changes before the vascular injury. Especially, retinal ganglion cells (RGCs) death and retinal microglial cells (RMC) activation are regarded as two early features in DR (Rübsam et al., 2018). Neuroinflammation has been implicated in the pathogenesis of DR. Thus, DR is a microvascular disease as well as chronic inflammation and retinal neurodegeneration (Altmann & Schmidt, 2018). MG activation is often seen in brain diseases, but the role of RMC in DR is rarely addressed. Therefore, in this review, we will summarize the different roles of RMC in early DR to provide some new insights.

2. Origin and function of MG

One century ago, MG was described firstly by *Río Hortega*, which he called these cells "the third element of nerve centers" and later termed "microglia" (Del Rio-Hortega Bereciartu, 2019). And these cells represent 5–12% in the central nervous system (CNS). Generally, as the macrophages of CNS, MG derives from hematopoietic stem cells (Chan et al., 2007; Saijo & Glass, 2011). Unlike other ocular macrophages, RMC arises from the Yolk Sac precursors has been confirmed (O'Koren et al., 2019). Hence, the retina is a well-structured organ within CNS, amoeboid and mesodermal myeloid progenitors enter the retina during development and differentiate into ramified parenchymal MG in the adult retina (Chan et al., 2007; Tay et al., 2019).

Once the tissue is matured, the MG population is maintained in the retina throughout the entire life span where they compose the resident immune cells (Reu et al., 2017). In the adult retina, ramified MG are mainly distributed in the plexiform layers, ganglion cell layer (GCL), and never fiber layer (NFL) (Madeira et al., 2015). In the healthy retina, MG is required for normal retinal growth, neurogenesis, controlling development, vascular formation, aging, and retinal function. They interact with other neurons, glia, and endothelial cells by secreting growth factors, cytokines as well as neuroprotective anti-inflammatory mediators (Altmann & Schmidt, 2018). However, MG is double-edged.

In the normal retina, MG is located in the plexiform layers where they exhibit elaborate ramified processes responsible for immune surveillance of the retina (Karlstetter et al., 2015). Noxious insults such as oxidative stress, hypoxia, inflammation, or inherited mutations trigger MG reactivity manifested by amoeboid morphology, increased proliferation, and migration to the sites of injury (Sierra et al., 2016, Masuda et al., 2017). And sustained microglial inflammatory responses can instigate severe alterations in retinal integrity, and aggravate neuronal demise (Zhao et al., 2015). This is the flip side of MG in the retina, which recent strategies have been shown to suppress microglial inflammatory responses in the retina and prevent neuronal cell death.

© The Author(s), 2021. Published by Cambridge University Press.

CAMBRIDGE UNIVERSITY PRESS

Table 1. Some new targets associated with RMC polarization

New targets	M1/M2 polarization	Mechanism in DR
miR-30a	M1 polarization↓	S100A12 inhibited miR-30a expression and miR-30a downregulated NLRP3
miR-124	M2 polarization↑	Not clear
miR-10	M2 polarization↑	NgR is possible
miR-495	M1 polarization↑	Not clear
TSPO	M2 polarization↓	Not clear
AR	M1 polarization↑	Inhibiting AR may decrease RMC reaction
Ang II/AT1-R	M1 polarization↑	Ang II may directly activate AT1-Rs on RMC to induce an activation response and the production of inflammatory factors
Hcy	M1 polarization↑	HHcy induces RMC activation
GLUT1	M1 polarization↑	RMC expresses high levels of GLUT1 and further upregulate GLUT1 to facilitate glucose uptake and promote anaerobic glycolysis.
MCT1	M1 polarization↑	Not clear

3. The polarization of MG

For a long time, resident ramified MG was described as quiescent cells, waiting for their activation, but now it is known that ramified MG contributes to tissue and neuronal homeostasis (Dereki et al., 2013; Hugh Perry & Teeling, 2013; Kierdorf & Prinz, 2017). Once the stimulus is felt, MG would rapidly change their morphology, gene expression, and functional performance upon any threat to tissue homeostasis, acquiring and activated phenotype (McKimmie et al., 2006), which the procession could be called MG polarization. In DR, RMC is assumed to be the first detector of early signals of hyperglycemia. Located between vasculature and neurons of the retina, RMC activation is often responsible for early harm of the beginning stages of DR (Sorrentino et al., 2016).

Upon activation, ramified MG undergoes a series of stereotypic, morphological, phenotypic, and functional changes (Streit et al., 1989). Firstly, MG exists in two separate morphological states, namely "resting" and "activated" microglia (Kettenmann et al., 2013). During activation, RMC changes from ramified appearance to ameboid-like. Then, there are roughly two phenotypes of MG. In vitro experiments demonstrate that microglial cells develop either a classic pro-inflammatory M1 or an alternative anti-inflammatory and pro-healing M2 polarization phenotypes (Su et al., 2015; Arroba et al., 2016). In vivo, MG appears to acquire intermediate phenotypes, whose ultimate function relies on the combination of different polarization markers ranging from M1 to M2 (Lampron et al., 2013; Arroba et al., 2016).

Generally, M1 is induced by the prototypical Th-1 cytokines interferon-gamma (IFN-c) or lipopolysaccharide (LPS). After activating, M1 phenotypes mainly secrete pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), IL (interleukin)-12, IL-23, IL-1β, IL-6, and chemotactic factors as well as induce cytotoxic mediators such as reactive oxygen species and nitric oxide (NO). By contrast, M2 is usually induced by the Th-2 cytokines, IL-4 or IL-10, and IL-13, which express high levels of anti-inflammatory cytokines, etc. (Martinez et al., 2009; Arroba & Valverde, 2017). The distinction between M1 and M2 microglia is hazy and MG can adapt their phenotypes to meet demands (Graeber et al., 2011). Therefore, the unbalance of MG polarization is a key reason for neuroinflammation.

4. RMC polarization and DR

The involvement of inflammation in DR was developed out of initial studies reporting that diabetic patients under treatment with salicylates for rheumatoid arthritis had a lower-than-expected incidence of this complication of diabetes (Powell & Field, 1964). While recently, neurodegeneration was confirmed to occur in human diabetes even before the development of clinical stages of DR. RMC is the heart of this possess (Simo & Hernandez, 2014) and has a dual phenotype and nature. The study of Arroba has shown that in db/db mice, an early stage of diabetes is characterized by the coexistence of pro-inflammatory and anti-inflammatory cytokines in circulation but, as long as diabetes progresses, this balance is disrupted in favor of a proinflammatory milieu (Arroba & Valverde, 2017). Therefore, the M1/M2 responses are closely related to the activation and polarization of RMC (Arroba & Valverde, 2017). In the health retina, RMC releases neuroprotective and anti-inflammatory factors. However, in response to prolonged pro-inflammatory stimuli, the chronically activated RMC can lead to neuroinflammation by releasing a plethora of cytokines. In a word, in the early stage of DR, hyperglycemia trigger RMC polarization, and M1/M2 is unbalanced.

Several animal models have been used to study the activation of RMC in diabetes and have confirmed the phenotypic changes mentioned herein (Kinuthia et al., 2020). In the streptozotocin (STZ)-induced diabetic rat, MG changes their morphology from a ramified to an amoeboid form and increases in number within the outer plexiform layers (OPL) and outer photoreceptor layer suggesting that activated microglia are highly proliferative and migratory (Zeng et al., 2000). Moreover, in the db/db mouse model of type 2 diabetes, activated RMC was found in the inner nuclear layer and switched from an anti-inflammatory phenotype during early disease toward a pro-inflammatory phenotype in the late stage (Arroba et al., 2016).

DR includes nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Similarly, clinical studies have confirmed microglia activation in the retina of both NPDR and PDR patients whereby, in NPDR microglia migrated into the plexiform layers and proliferated whereas, in PDR, microglia soma were highly hypertrophied and assembled around ischemic regions (Zeng et al., 2008; Kinuthia et al., 2020). These indicated that the activation of RMC is a specific feature of DR.

5. New targets associated with RMC polarization

5.1. miRNAs

MicroRNAs (miRNAs) are universal regulators of differentiation, activation, and polarization of MG in normal and diseased CNS (Ponomarev et al., 2013), such as DR. A growing body of evidence describes the role of miRNAs in microglial cells under defined stimuli, which induce polarized phenotypes (M1/M2) (Graff et al., 2012; Freilich et al., 2013; Cobos Jimenez et al., 2014). Here, we are going to summarize some miRNAs regulating MG polarization.

A recent study shows that miR-30a regulates S100A12-induced RMC activation and inflammation by targeting NLRP3 (Dong & Wang, 2019). In the study, they found S100A12 induces RMC

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press

activation via regulating NLRP3 activation. But *miR-30a* downregulate NLRP3 expression by directly targeting its 3′-UTR in RMC, which could be suppressed consistently by S100A12. These results indicate miR-30a may decrease inflammation induced by S100A12 and play an important role in DR.

Commonly, *miR-124* is the most abundant miRNA expressed in neurons (Mishima et al., 2007). Lots of researches demonstrated that miR-124 determines the phenotype of MG in the CNS that exhibited properties of the M2 macrophages (Ponomarev et al., 2007, Ponomarev et al., 2011). In previous studies of DR, it has been found miR-124 regulates Amador-glycated albumin-induced RMC activation and inflammation by targeting Rac1 (Dong et al., 2016). It demonstrates that miR-124 could modulate RMC polarization and thus become an effective target for DR therapy. However, the specific mechanism needs to be further studied.

In the study that we know of, we find that upregulation of Nogo receptor (NgR) expression induces apoptosis of RGCs in diabetic rats (Li et al., 2014). Moreover, NgR knockdown attenuates DR in STZ-induced diabetic rats (Guo & Li, 2017). These results suggest that NgR may play a role in the progression of DR. According to recent research, in spinal cord injury, *miR-10* could target NgR to modulate MG polarization and the secretion of inflammatory cytokines (Wang et al., 2018). Overexpressed miR-10 could downregulate the expression of NgR, promoted M2 polarization, and inhibited inflammatory cytokine secretion. Although the expression of miR-10 has not been reported in kinds of literatures, it may become a possible view in the study of DR.

Growing evidence has proved that macrophages are vital in obesity and insulin-resistant in type 2 diabetes (T2D) (Kraakman et al., 2014), which has been demonstrated that M1 is associated with insulin resistance and M2 is related to insulin sensitivity (Cucak et al., 2014). Moreover, *miR-495* has been discovered to be involved in the metabolism and immune response in the human body. A recent study found miR-495 could promote the transformation of macrophages into M1 and inhibit M2 (Hu et al., 2019). Also, miR-495 could inhibit the expression of its target gene FTO, aggravate insulin resistance and adipose tissue inflammation in T2D mice. In the previous study of DR, we know the miR-495 expression in RGCs was increased in response to high glucose (HG) (Zhang et al., 2018). However, whether inhibiting miR-495 in DR could delay its progress by inhibiting RMC polarization remains to be further investigated.

5.2. Translocator protein

Translocator protein (TSPO), an 18-kDa protein that expresses on the outer mitochondrial membrane, has been reported as a marker for microglial activation (Thackeray et al., 2018). In CNS, TSPO is present both in neurons and activated glial cells (Kuhlmann & Guilarte, 2000; Maeda et al., 2007). The glial upregulation of TSPO is a major hall marker of neurodegenerative disease (Girard et al., 2012). The study of Karlstetter (Karlstetter et al., 2014) has shown that TSPO is highly expressed in reactive retinal microglia and BV-2 cells stimulated with LPS. They also demonstrated that TSPO is expressed in reactive RMC and modulates microglial inflammation and phagocytosis.

PPAR-γ is a ligand-activated transcription factor that binds to DNA-specific PPAR response elements and regulates the transcription of its target gene. It has demonstrated that TSPO could modulate IL-4-induced M2 polarization via the PPAR-γ pathway. TSPO inhibits the M2 polarization and trophic factors release through the PPAR-γ pathway (Zhou et al., 2020). In DR, whether TSPO can interfere with RMC activation via this pathway remains to be further investigated.

Moreover, macroglia and microglia could also interact with each other. The study has shown that TSPO is acutely and specifically upregulated in RMC in separate mouse models of retinal inflammation and injury. Concomitantly, its endogenous ligand, diazepam-binding inhibitor (DBI), is upregulated in the macroglia of the mouse retina. The study of Wang (Wang et al., 2014) found DBI-TSPO signaling occurs as a form of macroglia–microglia interaction that is activated during retinal inflammation and injury and serves to limit the magnitude of microglial activation responses by negative regulation. These results indicate that TSPO could also regulate RMC activation in this way, but its specific roles in DR remain to be determined.

5.3. Aldose reductase

Aldose reductase (AR), best studied as an enzyme of the polyol pathway, is involved in ocular complications of diabetes, such as DR (Obrosova & Kador, 2011; Hampton et al., 2015). The study of Chang in 2014 (Chang et al., 2014) showed that aldose reductase inhibitors (ARIs) or genetic deficiency substantially reduced LPS-induced cytokine secretion from macrophages and RMC, and also reduced the activation of MMP-9 and attenuated LPS-induced cell migration. Moreover, their further study found that AR mediates RMC activation, which the overexpression of AR elevates RMC activation in the retina (Kun-Che et al., 2016).

Besides, in response to inflammatory stimulation, RMC migrates from the OPL to the subretinal space where they release cytokines, which cause retinal damage (Ma et al., 2009). Therefore, suppressing RMC activation may be a potential strategy to protect the retina. The study of Chang in 2016 and 2019 has found the treatment of mice with ARIs attenuated both RMC activation and its migration (Kun-Che et al., 2016, 2019). In a word, the strategy of ARIs in the early stages of DR may suppress RMC inflammation, and thus delay or prevent vascular disease that is associated with later stages of DR progression and its associated greater risks for permanent vision loss.

5.4. GLUT1 and MCT1

Glucose is the main energy source in CNS, and high-glucose is a major cause of DR. Increasing evidence supports the critical role of multiple metabolic pathways in macrophage reprogramming (Ghesquiere et al., 2014; Kelly & O'Neill, 2015). Rather than depending on mitochondrial ATP production, M1-like macrophages display high glycolysis rates, which can be induced by the expression of glucose transporter 1 (GLUT1) and pro-glycolytic 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3) (Haschemi et al., 2012; Ghesquiere et al., 2014).

Some studies have shown that proinflammatory MG is powered by glycolysis, which relies on high levels of glucose uptake. MG expresses high levels of GLUT1. Under inflammatory conditions, MG further upregulates GLUT1 to facilitate glucose uptake and promote anaerobic glycolysis. The study has demonstrated that GLUT1 critically controls MG activation through facilitating glycolysis (Wang et al., 2019). Moreover, as the sole glucose transporter between blood and retina, GLUT1 would transport more glucose when blood glucose is high. Many studies have confirmed that the suppression of GLUT1 is a new strategy to prevent DR (Lu et al., 2013; You et al., 2017).

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press

Monocarboxylate transporter 1 (MCT1), the main transporters of lactate, is regarded as the special step of anaerobic glycolysis but not the TCA cycle and oxidative phosphorylation. Evidence reveals that MCT1 protein is increased in N11 microglial cells after stimulation with TNF-α (Moreira et al., 2009). Furthermore, hypoxia-inducible factor-1 (HIF-1) is a crucial transcription factor during classical microglial polarization (Bok et al., 2017). The study of Liang found that the protein level of HIF-1α was increased after LPS stimulation and significantly decreased after the knockdown of MCT1 in BV2 cells. And their present findings demonstrate that MCT1 enhances the expression of PFKFB3 via HIF-1α and thereby promotes classical microglial activation and proinflammatory effect. Therefore, inhibiting MCT1 may be potential to prevent neuroinflammation (Kong et al., 2019).

However, the possibility of inhibiting RMC activation by inhibiting GLUT1 or MCT1 to prevent early DR remains to be further investigated.

5.5. Angiotensin II/AT1-R

There is a great deal of evidence indicating that the retina has its independent renin-angiotensin system (RAS), and angiotensin II (Ang II) elicits biological effects via two receptor classes, including angiotensin receptor 1 (AT1-R) and angiotensin receptor 2 (AT2-R). Broadly, AT1-R causes vasoconstriction of retinal vessels (Kawamura et al., 2004) and AT2-R is associated with vasodilation (Fletcher et al., 2010). The role of RAS/AT1-R in DR has been well studied, but little is known about the role of RAS/AT1-R in RMC.

It is well established that all components of the RAS are expressed by retinal cells (Choudhary et al., 2017). Indeed, the enzymatic cleavage of angiotensinogen to Ang II has been shown to occur within glia in the CNS (Wosik et al., 2007). Moreover, the study of Phipps in 2018 (Phipps et al., 2018) has demonstrated the localization of AT1-R to RMC. And they also found Ang II causes selective activation of RMC both immediately and 24 hours following injection of Ang II into the eye. These results suggest that Ang II may directly activate AT1-Rs on RMC to induce an activation response and the production of inflammatory factors. Therefore, Ang II may be involved in the development of early DR by activating RMC. Thus, ACEI/ARB may be a potential therapeutic of DR.

5.6. Homocysteine

Homocysteine (Hcy), an amino acid, its high levels are a pathogenic factor of DR, which could be regarded as a potential biomarker for DR (Tawfik et al., 2019). According to the previous reports, hyperhomocysteinemia (HHcy, elevated Hcy) disrupts blood-retinal barriers (BRBs) (Mohamed et al., 2017) and the retinal pigmented epithelium (RPE) (Ibrahim et al., 2016) and also induces epigenetic modifications (Elmasry et al., 2018). Also, studies have identified a strong association between HHcy and inflammation in both human and experimental models (Liu et al., 2012; Álvarez-Sanchez et al., 2019; Fang et al., 2019). However, the relationship between Hcy and RMC is rarely addressed.

A recent study found that HHcy induces RMC activation and its inflammatory response, which plays a role in the pathogenesis of DR (Elsherbiny et al., 2020). They demonstrated that different concentrations of elevated Hcy induced the activation of NF-κB in cells that play a crucial role in maintaining intact BRB (HRECs and ARPE cells). These data suggest that Hcy induced the activation of RMC and NF-κB to induce neuroinflammation. Thus, the elimination of excess homocysteine could be a potential therapeutic intervention in DR. This could be through adequate nutritional vitamin supplementation of folic acid and vitamin B-12, especially in the diabetic population.

6. New therapies associated with RMC polarization in DR

6.1. Pharmaceutical compositions

Dios/DG is a saponin compound extracted from *Tritulus terrestris* L. The study demonstrated that Dios selectively inhibits the expression of M1 markers in activated MG, but does not alter the expression of M2 markers, and provides neuroprotection by selectively regulating M1 polarization (Wang et al., 2017). These results suggest that Dios could be useful for treating MG-mediated neuroinflammatory diseases. Moreover, a study has found that long-term DG treatment improved glucose tolerance and lipid profiles, reduced production of IL-1β, IL-6, and TNF-α (Wu et al., 2018).

Erianin is the major bibenzyl compound found in the traditional Chinese medicine Dendrobium Chrysostom Lindl, against DR development. The study of Zhang et al. (2019) found that erianin inhibited the activation of RMC both in vivo and in vitro. And they demonstrated that erianin could alleviate DR by reducing retina inflammation by MG via inhibiting the hyperglycemia-mediated ERK1/2-NF-κB signaling pathway.

Saffron (*Crocus sativus* L.) is a traditional Chinese medicine that is frequently used in clinical therapy (Huvnh et al., 2013). The study of Yang et al. (2017) has found that crocin inhibits oxidative stress and the pro-inflammatory response of MG associated with DR via the activation of the PI3K/Akt signaling pathway. This result demonstrated that crocin may be used to control MG activation in DR.

Chuanxiong and its bioactive component,2,3,5,6-tetramethylpyrazine (TMP) may be used in the clinical treatment of neural ocular diseases, such as DR (Zhu et al., 2015). The study of Han et al. (2020) found pretreatment with TMP inhibited RMC activation, migration, and regeneration, especially in GCL. They demonstrated that TMP could attenuate retinal inflammation by inhibiting microglial activation via the TLR4/NF-κB signaling pathway. In addition, they also found TMP also could attenuate the increased expression of cleaved caspase-3 and the decreased ratio of B-cell lymphoma-2 (Bcl-2) to Bcl-2 associated X protein (Bax). These findings suggest that TMP exerted neuroprotective effects in the retina and may provide another way to the treatment of DR.

6.2. Nogo-A antibody

Nogo-A is a potent myelin-associated inhibitor for neuronal plasticity and axonal regeneration in the injured CNS (GrandPre et al., 2000). The study of Baya Mdzomba et al. (2020) found that Nogo-A is upregulated in the retina of patients with DR. And the intravitreal injection of anti-Nogo-A antibody can improve visual function and reduce RMC-mediated inflammation after retinal injury. These data may be useful to develop new therapeutic approaches for DR or neurodegenerative diseases with an important inflammatory component.

6.3. Pregabalin

Pregabalin is the first drug to receive FDA approval for treating diabetic neuropathic pain. Recently, Alia's team found Pregabalin suppressed the diabetes-enhanced retinal expression of IL-1β, TNF-α, CD11b (a surface marker for microglia) while attenuated expression of caspase-3 and DNA fragmentation versus the

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press

diabetic group. This present study provided the first demonstration that pregabalin intervention rescued retinal cells and alleviated retinal apoptosis in diabetic rats. At least partly, it decreased glutamate, suppressed RMC activation, and reduced the oxide-inflammatory burden (Ali et al., 2019). Therefore, pregabalin may have a future utility in treating DR after adequate clinical research.

7. Conclusion

MG is the heart of the neurodegeneration, which is one of the features of the early DR. Therefore, it is important to study the process of RMC polarization in early DR. MG could be divided into pro-inflammatory type M1 and anti-inflammatory type M2, so inhibiting M1 and promoting M2 may be an effective therapy for neuroinflammatory diseases. In this review, we summarize a variety of factors that could influence MG polarization, as well as several active components of drugs that may influence polarization, in the hope of providing new insights for early intervention of DR.

Acknowledgments. This work was supported by grants from the Natural Foundation of Heilongjiang Province (grant numbers H2015057, China), and the Fund of Scientific Research Innovation of the First Affiliated Hospital of Harbin Medical University (grant number 2020 M27, China).

Conflict of Interest. The authors have no conflict of interest to declare.

References

Ali, S.A., Zaitone, S.A., Dessouki, A.A. & Ali, A.A. (2019). Pregabalin affords retinal neuroprotection in diabetic rats: suppression of retinal glutamate, microglia cell expression and apoptotic cell death. *Experimental Eye Research* **184**, 78–90.

Altmann, C. & Schmidt, M.H.H. (2018). The role of microglia in diabetic retinopathy: inflammation, microvasculature defects and neurodegeneration. *International Journal of Molecular Sciences* **19**, 110.

Álvarez-Sanchez, N., Álvarez-Ríos, A.I., Guerrero, J.M., García-García, F.J., Rodríguez-Mañas, L., Cruz-Chamorro, I., Lardone, P.J. & Carrillo-Vico, A. (2019). Homocysteine and C-reactive protein levels are associated with frailty in older Spaniards: the Toledo study for healthy aging. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* **75**, 1488–1494.

Arroba, A.I. & Valverde, Á.M. (2017). Modulation of microglia in the retina: new insights into diabetic retinopathy. *Acta diabetologica* **54**, 527–533.

Arroba, A.I., Alcalde-Estevez, E., García-Ramírez, M., Cazzoni, D., de la Villa, P., Sánchez-Fernández, E.M., Mellet, C.O., García Fernández, J.M., Hernández, C., Simó, R. & Valverde, Á.M. (2016). Modulation of microglia polarization dynamics during diabetic retinopathy in db/db mice. *Biochimica et biophysica acta* **1862**, 1663–1674.

Baya Mdzomba, J., Joly, S., Rodriguez, L., Dirani, A., Lassiaz, P., Behar-Cohen, F. & Pernet, V. (2020). Nogo-A-targeting antibody promotes visual recovery and inhibits neuroinflammation after retinal injury. *Cell Death & Disease* **11**, 101.

Bok, S., Kim, Y.E., Woo, Y., Kim, S., Kang, S.J., Lee, Y., Park, S.K., Weissman, I.L. & Ahn, G.O. (2017). Hypoxia-inducible factor-1α regulates microglial functions affecting neuronal survival in the acute phase of ischemic stroke in mice. *Oncotarget* **8**, 111508–111521.

Chan, W.Y., Kohsaka, S. & Rezaie, P. (2007). The origin and cell lineage of microglia: new concepts. *Brain Research Reviews* **53**, 344–354.

Chang, K.C., Ponder, J., LaBarbera, D.V. & Petrash, J.M. (2014). Aldose reductase inhibition prevents endotoxin-induced inflammatory responses in retinal microglia. *Investigative Ophthalmology & Visual Science* **55**, 2853–2861.

Choudhary, R., Kapoor, M.S., Singh, A. & Bodakhe, S.H. (2017). Therapeutic targets of renin-angiotensin system in ocular disorders. *Journal of Current Ophthalmology* **29**, 7–16.

Cobos Jimenez, V., Bradley, E.J., Willemsen, A.M., van Kampen, Antoine H.C., Baas, F. & Kootstra, N.A. (2014). Next-generation sequencing of microRNAs uncovers expression signatures in polarized macrophages. *Physiological Genomics* **46**, 91–103.

Cucak, H., Grunnet, L.G. & Rosendahl, A. (2014). Accumulation of M1-like macrophages in type 2 diabetic islets is followed by a systemic shift in macrophage polarization. *Journal of Leukocyte Biology* **95**, 149–160.

Del Rio-Hortega Bereciartu, J. (2019). *Pio del Rio-Hortega: The Revolution of Glia*. Anat Rec (Hoboken).

Dereki, N.C., Cronk, J.C. & Kipnis, J. (2013). The role of microglia in brain maintenance: implications for Rett syndrome. *Trends in Immunology* **34**, 144–150.

Dong, N. & Wang, Y. (2019). MiR-30a regulates S100A12-induced retinal microglial activation and inflammation by targeting NLRP3. *Current Eye Research* **44**, 1236–1243.

Dong, N., Xu, B., Shi, H. & Lu, Y. (2016). miR-124 regulates amadori-glycated albumin-induced retinal microglial activation and inflammation by targeting Rac1. *Investigative Ophthalmology & Visual Science* **57**, 2522–2532.

Elmasry, K., Mohamed, R., Sharma, I., Elsherbiny, N.M., Liu, Y., Al-Shabrawey, M. & Tawfik, A. (2018). Epigenetic modifications in hyperhomocysteinemia: potential role in diabetic retinopathy and age-related macular degeneration. *Oncotarget* **9**, 12562–12590.

Elsherbiny, N.M., Sharma, I., Kira, D., Alhusban, S., Samra, Y.A., Jadeja, R., Martin, P., Al-Shabrawey, M. & Tawfik, A. (2020). Homocysteine induces inflammation in retina and brain. *Biomolecules* **10**, 393.

Fang, P., Li, X., Shan, H., Saredy, J.J., Cueto, R., Xia, J., Jiang, X., Yang, X.F. & Wang, H. (2019). Ly6C(+) inflammatory monocyte differentiation partially mediates hyperhomocysteinemia-induced vascular dysfunction in type 2 diabetic db/db mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **39**, 2097–2119.

Fletcher, E.L., Phipps, J.A., Ward, M.M., Vessey, K.A. & Wilkinson-Berka, J.L. (2010). The renin-angiotensin system in retinal health and disease: its influence on neurons, glia and the vasculature. *Progress in Retinal and Eye Research* **29**, 284–311.

Freilich, R.W., Woodbury, M.E. & Ikezu, T. (2013). Integrated expression profiles of mRNA and miRNA in polarized primary murine microglia. *PloS One* **8**, e79416.

Ghesquière, B., Wong, B.W., Kuchnio, A. & Carmeliet, P. (2014). Metabolism of stromal and immune cells in health and disease. *Nature* **511**, 167–176.

Girard, C., Liu, S., Adams, D., Lacroix, C., Sinéus, M., Boucher, C., Papadopoulos, V., Rupprecht, R., Schumacher, M. & Groyer, G. (2012). Axonal regeneration and neuroinflammation: roles for the translocator protein 18 kDa. *Journal of Neuroendocrinology* **24**, 71–81.

Graeber, M.B., Li, W. & Rodriguez, M.L. (2011). Role of microglia in CNS inflammation. *FEBS Letters* **585**, 3798–3805.

Graff, J.W., Dickson, A.M., Clay, G., McCaffrey, A.P. & Wilson, M.E. (2012). Identifying functional microRNAs in macrophages with polarized phenotypes. *The Journal of Biological Chemistry* **287**, 21816–21825.

GrandPre, T., Nakamura, F., Vartanian, T. & Strittmatter, S.M. (2000). Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. *Nature* **403**, 439–444.

Guo, X. & Li, X. (2017). Nogo receptor knockdown and ciliary neurotrophic factor attenuate diabetic retinopathy in streptozotocin-induced diabetic rats. *Molecular Medicine Reports* **16**, 2030–2036.

Hampton, B.M., Schwartz, S.G., Brantley, M.A. & Flynn, H.W. (2015). Update on genetics and diabetic retinopathy. *Clinical Ophthalmology* **9**, 2175–2193.

Han, X., Chen, X., Chen, S., Luo, Q., Liu, X., He, A., He, S., Qiu, J., Chen, P., Wu Y., Zhuang J., Yang M., Wu C., Wu N., Yang Y., Ge J., Zhuang J. & Yu K. (2020). Tetramethylpyrazine attenuates endotoxin-induced retinal inflammation by inhibiting microglial activation via the TLR4/NF-κB signalling pathway. *Biomedicine & Pharmacotherapy* **128**, 110273.

Haschemi, A., Kosma, P., Gille, L., Evans, C.R., Burant, C.F., Starkl, P., Knapp, B., Haas, R., Schmid, J.A., Jandl, C., Amir, S., Lubec, G., Park, J., Esterbauer, H., Bilban, M., Brizuela, L., Pospisilik, J.A., Otterbein, L.E. & Wagner, O. (2012). The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metabolism* **15**, 813–826.

Hu, F., Tong, J., Deng, B., Zheng, J. & Lu, C. (2019). MiR-495 regulates macrophage M1/M2 polarization and insulin resistance in high-fat diet-fed mice via targeting FTO. *Pflugers Archiv: European Journal of Physiology* **471**, 1529–1537.

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press

Hugh Perry, V. & Teeling, J. (2013). Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Seminars in Immunopathology* **35**, 601–612.

Huvnh, T.P., Mann, S.N. & Mandal, N.A. (2013). Botanical compounds: effects on major eye diseases. Evidence-based complementary and alternative medicine. *eCAM* **2013**, 549174.

Ibrahim, A.S., Mander, S., Hussein, K.A., Elsherbiny, N.M., Smith, S.B., Al-Shabrawey, M. & Tawfik, A. (2016). Hyperhomocysteinemia disrupts retinal pigment epithelial structure and function with features of age-related macular degeneration. *Oncotarget* **7**, 8532–8545.

Karlstetter, M., Nothdurfter, C., Aslanidis, A., Moeller, K., Horn, F., Scholz, R., Neumann, H., Weber, B.H., Rupprecht, R. & Langmann, T. (2014). Translocator protein (18 kDa) (TSPO) is expressed in reactive retinal microglia and modulates microglial inflammation and phagocytosis. *Journal of Neuroinflammation* **11**, 3.

Karlstetter, M., Scholz, R., Rutar, M., Wong, W.T., Provis, J.M. & Langmann, T. (2015). Retinal microglia: just bystander or target for therapy? *Progress in Retinal and Eye Research* **45**, 30–57.

Kawamura, H., Kobayashi, M., Li, Q., Yamanishi, S., Katsumura, K., Minami, M., Wu, D.M. & Puro, D.G. (2004). Effects of angiotensin II on the pericyte-containing microvasculature of the rat retina. *The Journal of Physiology* **561**, 671–683.

Kelly, B. & O'Neill, L.A. (2015). Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Research* **25**, 771–784.

Kettenmann, H., Kirchhoff, F. & Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron* **77**, 10–18.

Kierdorf, K. & Prinz, M. (2017). Microglia in steady state. *The Journal of Clinical Investigation* **127**, 3201–3209.

Kinuthia, U., Wolf, A. & Langmann, T. (2020). Microglia and inflammatory responses in diabetic retinopathy. *Frontiers in Immunology* **11**, 564077.

Kong, L., Wang, Z., Liang, X., Wang, Y., Gao, L. & Ma, C. (2019). Monocarboxylate transporter 1 promotes classical microglial activation and pro-inflammatory effect via 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3. *Journal of Neuroinflammation* **16**, 240.

Kraakman, M.J., Murphy, A.J., Jandeleit-Dahm, K. & Kammoun, H.L. (2014). Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? *Frontiers in Immunology* **5**, 470.

Kuhlmann, A.C. & Guilarte, T.R. (2000). Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity. *Journal of Neurochemistry* **74**, 1694–1704.

Kun-Che, C., Shieh, B. & Petrash, J.M. (2016). Aldose reductase mediates retinal microglia activation. *Biochemical and Biophysical Research Communications* **473**, 565–571.

Kun-Che, C., Shieh, B. & Petrash, J.M. (2019). Role of aldose reductase in diabetes-induced retinal microglia activation. *Chemico-Biological Interactions* **302**, 46–52.

Lampron, A., ElAli, A. & Rivest, S. (2013). Innate immunity in the CNS: redefining the relationship between the CNS and its environment. *Neuron* **78**, 214–232.

Liu, X., Zuo, Z., Liu, W., Wang, Z., Hou, Y., Fu, Y. & Han, Y. (2014). Upregulation of Nogo receptor expression induces apoptosis of retinal ganglion cells in diabetic rats. *Neural Regeneration Research* **9**, 815–820.

Liu, Z., Luo, H., Zhang, L., Huang, Y., Liu, B., Ma, K., Feng, J., Xie, J., Zheng, J., Hu, J., Zhan, S., Zhu, Y., Xu, Q., Kong, W. & Wang, X. (2012). Hyperhomocysteinemia exaggerates adventitial inflammation and angiotensin II-induced abdominal aortic aneurysm in mice. *Circulation Research* **111**, 1261–1273.

Lu, L., Seidel, C.P., Iwase, T., Stevens, R.K., Gong, Y.Y., Wang, X., Hackett, S.F. & Campochiaro, P.A. (2013). Suppression of GLUT1; a new strategy to prevent diabetic complications. *Journal of Cellular Physiology* **228**, 251–257.

Ma, W., Zhao, L., Fontainhas, A.M., Fariss, R.N. & Wong, W.T. (2009). Microglia in the mouse retina alter the structure and function of retinal pigmented epithelial cells: a potential cellular interaction relevant to AMD. *PloS One* **4**, e7945.

Madeira, M.H., Boia, R., Santos, P.F., Ambrósio, A.F. & Santiago, A.R. (2015). Contribution of microglia-mediated neuroinflammation to retinal degenerative diseases. *Mediators of Inflammation* **2015**, 673090.

Maeda, J., Higuchi, M., Inaji, M., Ji, B., Haneda, E., Okauchi, T., Zhang, M.R., Suzuki, K. & Suhara, T. (2007). Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine receptor. *Brain Research* **1157**, 100–111.

Martinez, F.O., Helming, L. & Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annual Review of Immunology* **27**, 451–483.

Masuda, T., Shimazawa, M. & Hideaki, H. (2017). Retinal diseases associated with oxidative stress and the effects of a free radical scavenger (Edaravone). *Oxidative Medicine and Cellular Longevity* **2017**, 9208489.

McKimmie, C.S., Roy, D., Forster, T. & Fazakerley, J.K. (2006). Innate immune response gene expression profiles of N9 microglia are pathogen-type specific. *Journal of Neuroimmunology* **175**, 128–141.

Mishima, T., Mizuguchi, Y., Kawahigashi, Y., Takizawa, T. & Takizawa, T. (2007). RT-PCR-based analysis of microRNA (miR-1 and -124) expression in mouse CNS. *Brain Research* **1131**, 37–43.

Mohamed, R., Sharma, I., Ibrahim, A.S., Saleh, H., Elsherbiny, N.M., Fulzele, S., Elmasry, K., Smith, S.B., Al-Shabrawey, M. & Tawfik, A. (2017). Hyperhomocysteinemia alters retinal endothelial cells barrier function and angiogenic potential via activation of oxidative stress. *Scientific Reports* **7**, 11952.

Moreira, T.J.T.P., Pierre, K., Maekawa, F., Repond, C., Cebere, A., Liljequist, S. & Pellerin, L. (2009). Enhanced cerebral expression of MCT1 and MCT2 in a rat ischemia model occurs in activated microglial cells. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* **29**, 1273–1283.

Nentwich, M.M. & Ulbig, M.W. (2015). Diabetic retinopathy—ocular complications of diabetes mellitus. *World Journal of Diabetes* **6**, 489–499.

Obrosova, I.G. & Kador, P.F. (2011). Aldose reductase/polyol inhibitors for diabetic retinopathy. *Current Pharmaceutical Biotechnology* **12**, 373–385.

O'Koren, E.G., Yu, C., Klingeborn, M., Wong, A.Y.W., Prigge, C.L., Mathew, R., Kalnitsky, J., Msallam, R.A., Silvin, A., Kay, J.N., Bowes Rickman, C., Arshavsky, V.Y., Ginhoux, F., Merad, M. & Saban, D.R. (2019). Microglial function is distinct in different anatomical locations during retinal homeostasis and degeneration. *Immunity* **50**, 723–737 e7.

Phipps, J.A., Vessey, K.A., Brandli, A., Nag, N., Tran, M.X., Jobling, A.I. & Fletcher, E.L. (2018). The role of angiotensin II/AT1 receptor signaling in regulating retinal microglial activation. *Investigative Ophthalmology & Visual Science* **59**, 487–498.

Ponomarev, E.D., Maresz, K., Tan, Y. & Dittel, B.N. (2007). CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **27**, 10714–10721.

Ponomarev, E.D., Veremeyko, T., Barteneva, N., Krichevsky, A.M. & Weiner, H. L. (2011). MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. *Nature Medicine* **17**, 64–70.

Ponomarev, E.D., Veremeyko, T. & Weiner, H.L. (2013). MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. *Glia* **61**, 91–103.

Powell, E.D. & Field, R.A. (1964). Diabetic retinopathy and rheumatoid arthritis. *Lancet* **2**, 17–18.

Reu, P., Khosravi, A., Bernard, S., Mold, J.E., Salehpour, M., Alkass, K., Perl, S., Tisdale, J., Possnert, G., Druid, H. & Frisén, J. (2017). The lifespan and turnover of microglia in the human brain. *Cell Reports* **20**, 779–784.

Rübsam, A., Parikh, S. & Fort, P. (2018). Role of inflammation in diabetic retinopathy. *International Journal of Molecular Sciences* **19**

Saijo, K. & Glass, C.K. (2011). Microglial cell origin and phenotypes in health and disease. *Nature Reviews Immunology* **11**, 775–787.

Sierra, A., de Castro, F., Del Río-Hortega, J., Rafael Iglesias-Rozas, J., Garrosa, M. & Kettenmann, H. (2016). The "Big-Bang" for modern glial biology: Translation and comments on Pío del Río-Hortega 1919 series of papers on microglia. *Glia* **64**, 1801–1840.

Simo, R. & Hernandez, C. (2014). Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends in Endocrinology and Metabolism: TEM* **25**, 23–33.

Sorrentino, F.S., Allkabes, M., Salsini, G., Bonifazzi, C. & Perri, P. (2016). The importance of glial cells in the homeostasis of the retinal microenvironment and their pivotal role in the course of diabetic retinopathy. *Life Science* **162**, 54–59.

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press

Streit, W.J., Graeber, M.B. & Kreutzberg, G.W. (1989). Peripheral nerve lesion produces increased levels of major histocompatibility complex antigens in the central nervous system. *Journal of Neuroimmunology* **21**, 117–123.

Su, F., Yi, H., Xu, L. & Zhang, Z. (2015). Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia in vitro. *Neuroscience* **294**, 60–68.

Tawfik, A., Mohamed, R., Elsherbiny, N.M., DeAngelis, M.M., Bartoli, M. & Al-Shabrawey, M. (2019). Homocysteine: a potential biomarker for diabetic retinopathy. *Journal of Clinical Medicine* **8**, 121.

Tay, T.L., Carrier, M. & Tremblay, M.È. (2019). Physiology of microglia. *Advances in Experimental Medicine and Biology* **1175**, 129–148.

Thackeray, J.T., Hupe, H.C., Wang, Y., Bankstahl, J.P., Berding, G., Ross, T.L., Bauersachs, J., Wollert, K.C. & Bengel, F.M. (2018). Myocardial inflammation predicts remodeling and neuroinflammation after myocardial infarction. *Journal of the American College of Cardiology* **71**, 263–275.

Wang, M., Wang, X., Zhao, L., Ma, W., Rodriguez, I.R., Fariss, R.N. & Wong, W.T. (2014). Macroglia-microglia interactions via TSPO signaling regulates microglial activation in the mouse retina. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **34**, 3793–3806.

Wang, S., Wang, F., Yang, H., Li, R., Guo, H. & Hu, L. (2017). Diosgenin glucoside provides neuroprotection by regulating microglial M1 polarization. *International Immunopharmacology* **50**, 22–29.

Wang, L., Pavlou, S., Du, X., Bhuckory, M., Xu, H. & Chen, M. (2019). Glucose transporter 1 critically controls microglial activation through facilitating glycolysis. *Molecular Neurodegeneration* **14**, 2.

Wang, W., Zhan, R., Zhou, J., Wang, J. & Chen, S. (2018). MiR-10 targets NgR to modulate the proliferation of microglial cells and the secretion of inflammatory cytokines. *Experimental and Molecular Pathology* **105**, 357–363.

Wosik, K., Cayrol, R., Dodelet-Devillers, A., Berthelet, F., Bernard, M., Moumdjian, R., Bouthillier, A., Reudelhuber, T.L. & Prat, A. (2007). Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **27**, 9032–9042.

Wu, Y., Ye, F., Lu, Y., Yong, H., Yin, R., Chen, B. & Yong, Y. (2018). Diosgenin glucoside protects against myocardial injury in diabetic mice by inhibiting RIP140 signaling. *American Journal of Translational Research* **10**, 3742–3749.

Yang, X., Huo, F., Liu, B., Liu, J., Chen, T., Li, J., Zhu, Z. & Lv, B. (2017). Crocin inhibits oxidative stress and pro-inflammatory response of microglial cells associated with diabetic retinopathy through the activation of PI3K/Akt signaling pathway. *Journal of Molecular Neuroscience: MN* **61**, 581–589.

You, Z.P., Zhang, Y.L., Shi, K., Shi, L., Zhang, Y.Z., Zhou, Y. & Wang, C.Y. (2017). Suppression of diabetic retinopathy with GLUT1 siRNA. *Scientific Reports* **7**, 7437.

Zeng, X., Ng, Y. & Ling, E. (2000). Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. *Visual Neuroscience* **17**, 463–471.

Zeng, H., Green, W. & Tso, M. (2008). Microglial activation in human diabetic retinopathy. *Archives of Ophthalmology (Chicago, Ill: 1960)* **126**, 227–232.

Zhang, X., Yuhong, Y. & Feng, Z. (2018). Suppression of microRNA-495 alleviates high-glucose-induced retinal ganglion cell apoptosis by regulating Notch/PTEN/Akt signaling. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* **106**, 923–929.

Zhang, T., Hao, O., Mei, X., Lu, B., Yu, Z., Chen, K., Wang, Z. & Ji, L. (2019). Erianin alleviates diabetic retinopathy by reducing retinal inflammation initiated by microglial cells via inhibiting hyperglycemia-mediated ERK1/2-NF-κB signaling pathway. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* **33**, 11776–11790.

Zhao, L., Zabel, M.K., Wang, X., Ma, W., Shah, P., Fariss, R.N., Qian, H., Parkhurst, C.N., Gan, W.B. & Wong, W.T. (2015). Microglial phagocytosis of living photoreceptors contributes to inherited retinal degeneration. *EMBO Molecular Medicine* **7**, 1179–1197.

Zhou, D., Ji, L. & Chen, Y. (2020). TSPO modulates IL-4-induced microglia/macrophage M2 polarization via PPAR-γ pathway. *Journal of Molecular Neuroscience* **70**, 542–549.

Zhu, X., Wang, K., Zhang, K., Tan, X., Wu, Z., Sun, S., Zhou, F. & Zhu, L. (2015). Tetramethylpyrazine protects retinal capillary endothelial cells (TR-iBRB2) against IL-1β-induced nitrative/oxidative stress. *International Journal of Molecular Sciences* **16**, 21775–21790.

https://doi.org/10.1017/S0952523821000031 Published online by Cambridge University Press