1–11 • The Journal of Neuroscience, November 13, 2024 • 44(46):e0938242024

Development/Plasticity/Repair

Monocyte Invasion into the Retina Restricts the Regeneration of Neurons from Müller Glia

Nicolai Blasdel,[1] Sucheta Bhattacharya,[2] Phoebe C. Donaldson,[1] Thomas A. Reh,[1] and Levi Todd[1,2*]
[1]Department of Biological Structure, University of Washington, Seattle, Washington 98195 and [2]Department of Ophthalmology and Visual Sciences, SUNY Upstate Medical University, Syracuse, New York 13210

Endogenous reprogramming of glia into neurogenic progenitors holds great promise for neuron restoration therapies. Using lessons from regenerative species, we have developed strategies to stimulate mammalian Müller glia to regenerate neurons in vivo in the adult retina. We have demonstrated that the transcription factor Ascl1 can stimulate Müller glia neurogenesis. However, Ascl1 is only able to reprogram a subset of Müller glia into neurons. We have reported that neuroinflammation from microglia inhibits neurogenesis from Müller glia. Here we found that the peripheral immune response is a barrier to CNS regeneration. We show that monocytes from the peripheral immune system infiltrate the injured retina and negatively influence neurogenesis from Müller glia. Using CCR2 knock-out mice of both sexes, we found that preventing monocyte infiltration improves the neurogenic and proliferative capacity of Müller glia stimulated by Ascl1. Using scRNA-seq analysis, we identified a signaling axis wherein Osteopontin, a cytokine highly expressed by infiltrating immune cells is sufficient to suppress mammalian neurogenesis. This work implicates the response of the peripheral immune system as a barrier to regenerative strategies of the retina.

Key words: **neurogenesis; neuroimmunology; regeneration; retina**

Significance Statement

Regeneration of neurons in the central nervous system is extremely limited in mammals. Transgenic overexpression of the proneural transcription factor Ascl1 enables mammalian retinal glia to regenerate some neurons lost to injury. We found that during this regenerative response to injury, monocytes from the periphery invade the neural retina, and these inflammatory cells negatively regulate the ability of Müller glia to reprogram into neurogenic progenitors. When monocytes are inhibited from infiltrating the retina, regeneration of neurons from Müller glia is significantly enhanced. This work implicates peripheral immunomodulation as a tool to enhance endogenous neuronal replacement strategies.

Introduction

Neurodegeneration of the retina causes permanent blindness because the mammalian central nervous system (CNS) lacks the ability to regenerate. Some non-mammalian species are able to regenerate retina and restore lost vision (Todd and Reh, 2022). In fish, this remarkable feat of regeneration is accomplished by Müller glia (MG), the primary glial cell of the vertebrate retina (Fausett and Goldman, 2006). MG in fish respond to neuronal loss by reprogramming into proliferating neurogenic progenitors that can regenerate all retinal cell types (Lenkowski and Raymond, 2014). MG in mice and human retina do not spontaneously reprogram into neurogenic progenitors after neuronal loss and instead activate an injury–response program typically referred to as "gliosis."

The injury–response program in mammalian MG involves structural remodeling and an upregulation of inflammatory factors (Bringmann et al., 2009). Importantly, mammalian MG do not induce proneural transcription factors in response to injury like their fish counterparts (Karl et al., 2008; Hoang et al., 2020). We have had success in stimulating a neurogenic response in mouse MG by forcing overexpression of the proneural factor Ascl1 in the damaged retina (Ueki et al., 2015; Jorstad et al., 2017). However, only a subset of Ascl1-overexpressing MG undergo neurogenesis, while the majority do not reprogram and instead express inflammatory gene programs (Jorstad et al., 2017, 2020; Todd et al., 2020; Palazzo et al., 2022).

Input from immune cells can drive glia to an inflammatory phenotype (Liddelow et al., 2017; Dulken et al., 2019; Guttenplan

Received May 16, 2024; revised Sept. 20, 2024; accepted Sept. 24, 2024.
Author contributions: N.B., T.A.R., and L.T. designed research; N.B., S.B., P.C.D., and L.T. performed research; N.B., S.B., P.C.D., T.A.R., and L.T. analyzed data; T.A.R. and L.T. wrote the paper.
We thank the Todd, Reh, and the Bermingham-McDonogh laboratory members for technical advice and critical feedback throughout the project. This work was supported by National Institutes of Health grant NEI R01EY021482 to T.A.R. and National Institutes of Health grants NEI K99EY033402 and NEI R00EY033402 to L.T. and Mary Gates Undergraduate Scholarship to P.C.D.
*L.T. is the lead contact of this study.
The authors declare no competing financial interests.
Correspondence should be addressed to Thomas A. Reh at tomreh@uw.edu or Levi Todd at toddl@upstate.edu.
https://doi.org/10.1523/JNEUROSCI.0938-24.2024
Copyright © 2024 the authors

et al., 2020). In our previous work, we found that retinal microglia induce an inflammatory nonregenerative state in MG and ablation of these cells significantly improves neurogenesis (Todd et al., 2020). This work, and additional studies has implicated the neuroimmune response as a significant component of endogenous regeneration strategies (Todd et al., 2016; White et al., 2017; Hoang et al., 2020; Jorstad et al., 2020; Palazzo et al., 2022). However, microglia are not the only immune cell type that responds to neural injury. The broader invasion of the peripheral immune system is being increasingly recognized to have significant consequences on neurodegeneration (Gadani et al., 2015; Guillonneau et al., 2017; Yu et al., 2020). How the peripheral immune response impacts endogenous cell replacement strategies in the nervous system has not been investigated.

In our previous scRNA-seq analysis of sorted myeloid cells after retinal injury, we noticed monocytes invade the retina after NMDA damage (Todd et al., 2020). Monocytes are critical mediators of innate immunity and can serve as precursors to macrophages and dendritic cells, while exerting their own functions (Auffray et al., 2007; Geissmann et al., 2008). Monocytes are derived from adult hematopoiesis and are absent in healthy retinas but are rapidly recruited into damaged tissue (Yu et al., 2020). Monocyte infiltration has been observed in retinal degeneration models in rodents and in diseased human retina (Sennlaub et al., 2013; Karlen et al., 2018). Monocyte infiltration often exacerbates the inflammatory response and has been suggested to be a primary driver of pathology in retinal degenerative diseases (Guo et al., 2012; Guillonneau et al., 2017; Yu et al., 2020). It is important to distinguish the contributions of these innate immune cell types for cellular targeting in the case of disease or endogenous cell replacement therapies where the impact of monocyte infiltration on retinal regeneration might be significant.

In this study, we found that monocytes invade the neural retina after injury; we tested the consequence of monocyte infiltration on MG-mediated retinal regeneration by genetically removing the CCR2 receptor in Ascl1-overexpressing mice. We found that prevention of monocyte invasion into the retina significantly improves MG neurogenesis in two different retinal injury models. We then performed scRNA-seq analysis of infiltrating immune cells and damaged MG, and by analyzing intercellular communication networks, we identified Osteopontin (Spp1) as a potential regulatory cytokine expressed by monocytes; we confirmed this prediction, finding that Osteopontin is sufficient to suppress retinal regeneration. Taken together, this study implicates the response of the peripheral immune system as counterproductive to in vivo neuronal regeneration in the adult retina.

Materials and Methods

Animals. All animals were treated in accordance with University of Washington Institutional Animal Care and Use Committee (IACUC) and SUNY Upstate-approved protocols. The *Glast-CreER:LNL-tTA:tetO-mAscl1-ires-GFP* and the *Glast-CreER:LNL-tTA:tetO-mAscl1-CCR2^{RFP}* mice were from a mixed background of C57BL/6 and B6SJF1. The CCR2^{RFP} mice are from a mixed C57BL/6J and C57BL/6N background. The tetO-mAscl1-ires-GFP mice were a gift from M. Nakafuku (University of Cincinnati), and the Glast-CreER, LNL-tTa mice, and the CCR2^{RFP} lines were from Jackson Laboratory. Males and females were both used in experiments. All in vivo experiments were performed on adult mice that were over 40 d old.

Immunohistochemistry. After euthanasia and enucleation, anterior portions of eyes were removed, and globes were fixed for 30 min in 4% PFA in PBS. Fixed eyes were incubated overnight in 30% sucrose at 4°C. Retinas were then frozen in O.C.T. and cryosectioned at 18 μm. For immunostaining, sections were washed two times for 10 min in PBS and then incubated in primary antibody in 0.5% Triton X-100 and PBS overnight. Slides were then washed in PBS and incubated in secondary antibodies for 1 h. Finally, slides were washed again in PBS and coverslipped with Fluoromount-G (SouthernBiotech). See Table 1 for antibodies and their concentrations used in this study.

EdU labeling. For EdU administration, animals were provided EdU (Abcam) in the drinking water at a concentration of 0.5 mg/ml for 7 d following NMDA injections. For EdU labeling, instructions were followed according to the Click-iT EdU Assay from Invitrogen.

Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling. To identify dying cells that contained fragmented DNA, the TUNEL method was used on 18 μm cryosections. We used an In Situ Cell Death Kit (TMR red; Roche Applied Science) following the manufacturer's instructions.

Retinal dissociation and fluorescence-activated cell sorting. To obtain single cells for RNA-sequencing, animals were killed and retinas were dissociated into single cells following the Worthington Kit instructions. Retinas are incubated in a mixture of papain and deoxyribonuclease (DNase) for 10 min at 37°C, followed by trituration to break up the remaining retinal clumps. Following this, an equal volume of ovomucoid is added to stop the dissociation. Cells are then spun at 4°C at 300 × *g* for 10 min and resuspended in Neurobasal and passed through a 35 μm filter for sorting. FACS was performed on GFP+ cells and CD45+ immune cells using a BD FACSAria III cell sorter.

Injections. Intravitreal injections were performed with a 32 ga Hamilton syringe on isoflurane anesthetized mice. NMDA was injected at a concentration of 100 mM at a volume of 1 μl in PBS. TSA (Sigma-Aldrich) was injected at a concentration of 1 μg/μl. Tamoxifen was injected intraperitoneally at 1.5 mg per 100 μl of corn oil on adult mice for 4 consecutive days. Recombinant OPN (R&D Systems) was injected at 1 μg/μl alongside NMDA or TSA injections in a 1:1 ratio for 2 μl total per injection.

Microscopy/image quantification. Images were taken on either a ZeissLSM770, LSM880 confocal microscope, or upright NiE Nikon. For cell counts, four images were taken per retina with a 20× objective at the same magnification, and four images were quantified and averaged

Table 1. List of antibodies and concentration used in this study

Antibody	Concentration	Vendor	Catalog #
Rabbit anti-Cabp5	1:500	Gift from F. Haesleer	n/a
Rabbit anti-Calretinin	1:500	Swant	CR7697
Rat anti-Cd44	1:500	BD Biosciences	550538
Rat anti-Cd45, (A95-1), PE-Cy7	1:1,000	BD Biosciences	552849
Chicken anti-GFP	1:1,000	Abcam	ab13970
Rabbit anti-Iba1	1:1,000	Wako	11031
Mouse anti-HuC/D	1:500	Invitrogen	A-21271
Goat anti-Osteopontin	1:500	R&D Systems	AF808
Goat anti-Otx2	1:500	R&D Systems	BAF1979
Rabbit anti-Pax6	1:500	BioLegend	901302
Rat anti-RFP	1:500	Proteintech	5f8
Goat anti-Sox2	1:1,000	Santa Cruz Biotechnology	SC-17320
Secondaries			
Donkey anti-chicken 488	1:1,000	Jackson ImmunoResearch	03-545-155
Donkey anti-rabbit 488	1:1,000	Thermo Fisher Scientific	A212065
Donkey anti-rabbit 568	1:1,000	Thermo Fisher Scientific	A100042
Donkey anti-rat 594	1:1,000	Thermo Fisher Scientific	A21209
Donkey anti-goat 568	1:1,000	Thermo Fisher Scientific	A11057
Donkey anti-goat 647	1:1,000	Jackson ImmunoResearch	705-605-147
Donkey anti-mouse 647	1:1,000	Jackson ImmunoResearch	715-605-150

as representative of a single eye. Counts from two individual eyes are then summed to represent one biological replicate and significance of difference was determined using a Mann–Whitney *U* test.

Single-cell RNA library construction. Following FACS purification of Cd45+/CD11b+ immune cells and GFP+ Muller glia, cells were spun down at 300 × *g* at 4°C with a target concentration of 1,000 cells/µl. Library Construction was done using 10x Genomics 3′ single-cell RNA Chip and further processed following the protocol of Chromium Single Cell 3′ Reagents Kit Users guide.

Libraries were sequenced using an Illumina NextSeq 500/550 versus kit and aligned to the mm10 genome using Cell Ranger version 3.0. Filtered output files were further analyzed in R using Seurat version ≥4.6 and CellChat version 1.6.1. Low-quality cells were identified as having low read depth and were removed from datasets. Initial processing of data was done using built-in Seurat functions, whereby cells were normalized, variable features were identified, data was scaled, the principal components were determined, and a UMAP was created using the first 30 dimensions. Clusters were identified and labeled using the Louvain algorithm with a resolution of 0.4 and using markers found with the Wilcoxon rank sum test as well as identifying percent and average expression using the tools available in the Seurat R package version 4.6.

Cell–cell communication analysis. Cells not identified as Cd45+/Aif1− were removed from the immune cell dataset, and cells identified as Rlbp1−/Aqp4− (not Muller glia) were removed from the 5 d postinjured retina dataset prior to CellChat Analysis. Additional Hexb+/Aif1+ microglia were subsetted from four previously published regeneration datasets to bolster downstream analysis (Hoang et al., 2020; Todd et al., 2020, 2022). Comparisons between datasets was then done using the Harmony integration of the immune and glial datasets using the function RunHarmony(). The new integrated dataset was then processed using the same pipeline as the initial datasets.

Cell–cell communication analysis was then performed using the CellChatDB mouse database and following the provided tutorial from the Nie Lab (https://github.com/jinworks/CellChat/blob/master/tutorial/Comparison_analysis_of_multiple_datasets.html). Following preprocessing of the CellChat object, the average gene expression of each cell group was calculated using the Trimean Method in the function computeCommunProb(). The communication probability of each pathway was then computed by summarizing the communication probabilities of all ligands–receptors interactions associated with each signaling pathway using computeCommunProbPathway() and the network was aggregated using aggregateNet(). To compute the network centrality and flow betweenness of the communication network, we used the function netAnalysis_computeCentrality(). Finally, signaling contribution from each cell group was visualized using netAnalysis_signalingRole_heatmap().

Results

Monocytes invade into the retina in response to excitotoxic injury

In our previous study examining the effects of microglia on retinal regeneration, we performed scRNA-seq on FAC-sorted CD45+/Cd11b+ immune cells after NMDA injury (Todd et al., 2020). We noted that a cluster of cells in our analysis belonged to CCR2+/Ly6C+ monocytes, revealing that monocytes from the peripheral immune system invaded into damaged retinas. To gain a better understanding of the infiltration dynamics of monocytes, we used the CCR2:RFP transgenic mouse which allows for fluorescent labeling of CCR2+ monocytes (Saederup et al., 2010). Microglia do not express CCR2, allowing for short-term delineation of these two distinct immune components (Saederup et al., 2010). However, after monocytes differentiate into macrophages, CCR2:RFP is downregulated leaving this tool unable to discern the ontogeny at later time points (Chen et al., 2020; Silvin et al., 2022).

We performed intravitreal injections of NMDA to kill inner retinal neurons via excitotoxicity and performed immunohistochemistry for Iba1 to image microglia/monocytes and RFP to image invading monocytes (Fig. 1*a–c*). As expected, in control PBS injected retinas, microglia displayed a homeostatic ramified morphology and monocytes were not present in the retinal parenchyma (Fig. 1*c*). The few RFP+ monocytes that could be found were located inside of blood vessels (Fig. 1*c*). In contrast, we found that in NMDA-damaged retinas microglia shift to an ameboid "reactive" morphology and significant monocyte invasion occurred into the retina parenchyma (Fig. 1*c*). We quantified the dynamics of monocyte invasion; while RFP+ monocytes were not immediately present after injury (4 h), the cells invaded in abundant numbers from 24 to 96 h and started declining by 144 h postinjury (Fig. 1*d*). NMDA predominantly kills retinal ganglion cells and amacrine cells of the inner retina and this was the location that monocytes accumulated (Fig. 1*e*).

When the CCR2:RFP allele is bred to homozygosity, it leads to a functional knock-out of CCR2 as the locus is replaced with the RFP transgene (Saederup et al., 2010). This CCR2 knock-out strategy has been validated to prevent monocyte invasion into the CNS in various models, including stroke, stab wound, and retinal light damage (Fang et al., 2018; Frik et al., 2018; Karlen et al., 2018). In alignment with these findings, we found a lack of monocyte invasion in NMDA-injured retinas where CCR2: RFP allele was homozygous (Fig. 1*f*). We also found that CCR2 deletion did not impact the extent of TUNEL+ cells after NMDA injury (Fig. 1*g*,*h*).

Prevention of monocyte invasion improves retinal regeneration

The monocytic invasion into the retina after NMDA injury coincides with the period when, in previous studies, we found that transgenic overexpression of the proneural transcription factor Ascl1 can reprogram the MG to a neurogenic state (Jorstad et al., 2017). This suggests that their presence might affect this process. Our regeneration studies use a mouse line that allows for inducible expression of Ascl1 specifically in MG while also fate mapping the MG lineage (Jorstad et al., 2017). For brevity, this *Glast-ErCre/LnL-tTA/teto-Ascl1-GFP* line will be referred to as Ascl1-MG. We established a retinal regeneration paradigm wherein MG-specific Ascl1 induction via tamoxifen is followed by NMDA injury and histone deacetylase inhibitor (TSA). This Ascl1-NMDA-TSA (ANT) treatment stimulates MG to produce functional retinal neurons predominantly of the bipolar lineage (Jorstad et al., 2017, 2020).

To test whether monocyte depletion impacts MG-mediated retinal regeneration, we crossed our Ascl1-MG regeneration mouse to the CCR2:RFP reporter/knock-out line (Fig. 2*a*). We next performed our retinal regeneration paradigm in the absence of monocytes (Fig. 2*b*). We found that by preventing monocyte invasion, MG neurogenesis was significantly enhanced (Fig. 2*c–f*). The percentage of MG-derived bipolar neurons (GFP/Otx2+) in monocyte-depleted retinas was increased threefold (Fig. 2*e*). We also found that MG-derived cells in the CCR2ko retinas contained more regenerated neurons expressing the mature bipolar maker Cabp5 and fewer cells retaining the MG-marker Sox2 (Fig. 2*f*,*g*). Surprisingly, we found that contrary to our previous findings with Ascl1 alone, TSA was not required, nor did it promote neurogenesis in the context of CCR2 deletion (Fig. 2*e*).

The increase in neurogenesis in the CCR2ko mice could be the result of increased MG proliferation and/or increased transdifferentiation of Ascl1-expressing MG directly to neurons. We have

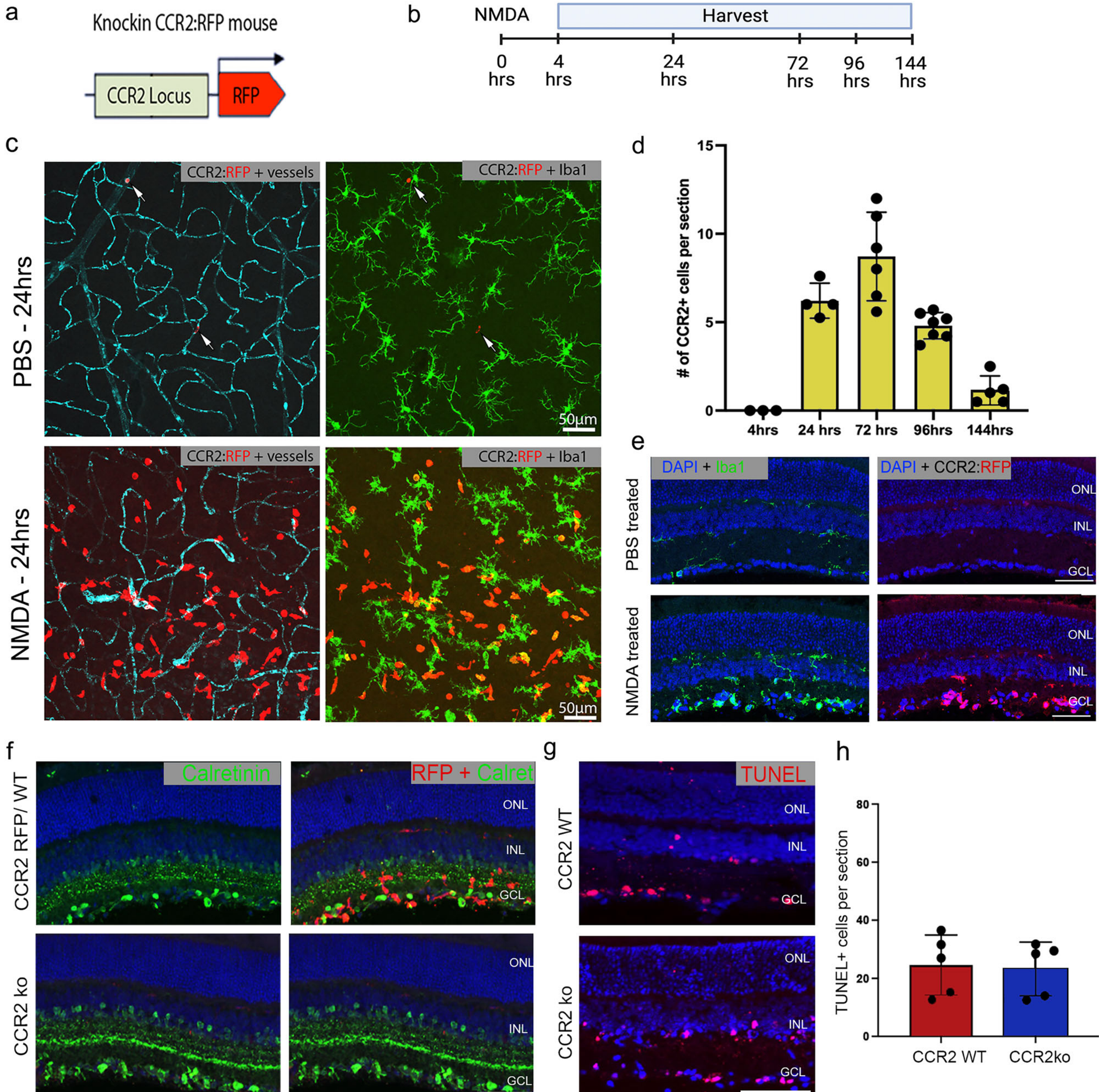

Figure 1. Monocytes invade into NMDA-damaged retinas. ***a***, Schematic depicting the transgenic mouse used to track CCR2+/RFP+ monocytes. ***b***, Experimental paradigm to induce neuronal death in the retina via NMDA and showing time points of tissue harvest. ***c***, Representative images of retinal whole mounts in control PBS-injected retinas versus NMDA-injected retinas labeled for Iba1+ microglia/macrophage (green) and RFP+ monocytes (red). The arrow depicts monocytes in healthy retinas are only found inside vessels. ***d***, Quantification of the average number of CCR2+/RFP+ cells found per retinal section imaged at 20×. ***e***, Representative images of retinal cross sections between PBS control injected eyes versus NMDA-injected retinas labeled for microglia and monocytes (CCR2) showing monocyte accumulation in the GCL at the site of cell death. ***f***, Representative images showing lack of RFP+ (red) monocytes in the CCR2 RFP/RFP knock-out retina stained with calretinin (green). ***g***, Representative images showing TUNEL+ apoptotic cells after NMDA damage in CCR2 WT versus CCR2 knock-out retinas. ***h***, Quantification representing TUNEL+ cells per section in CCR2 WT versus CCR2 knock-out retinas. Abbreviations are as follows: GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. All scale bars are 50 µm.

previously found that a subset of MG undergo proliferation prior to regenerating neurons (Jorstad et al., 2020; Todd et al., 2021, 2022). Monocyte infiltration into the brain has been reported to reduce the proliferative ability of astrocytes following traumatic injury (Frik et al., 2018). Therefore, we sought to test whether CCR2ko impacted the ability of MG to proliferate in response to NMDA injury. We included EdU in the drinking water at the onset of retinal injury and left mice exposed to EdU for 10 d during the regeneration paradigm. We found that MG proliferate more in the absence of monocytes (Fig. 2*h*,*i*) and ∼82% of these proliferating MG differentiate into neurons (Fig. 2*j*). These data suggest that monocytes suppress both the neurogenic and proliferative capacity of Ascl1-MG.

Monocytes also inhibit neurogenesis in response to light damage

Prior studies have shown that different types of neural injury result in different neuroimmune responses (Gadani et al., 2015). To test whether monocyte invasion inhibits regeneration after a different injury stimulus, we subjected our Ascl1-MG mouse line

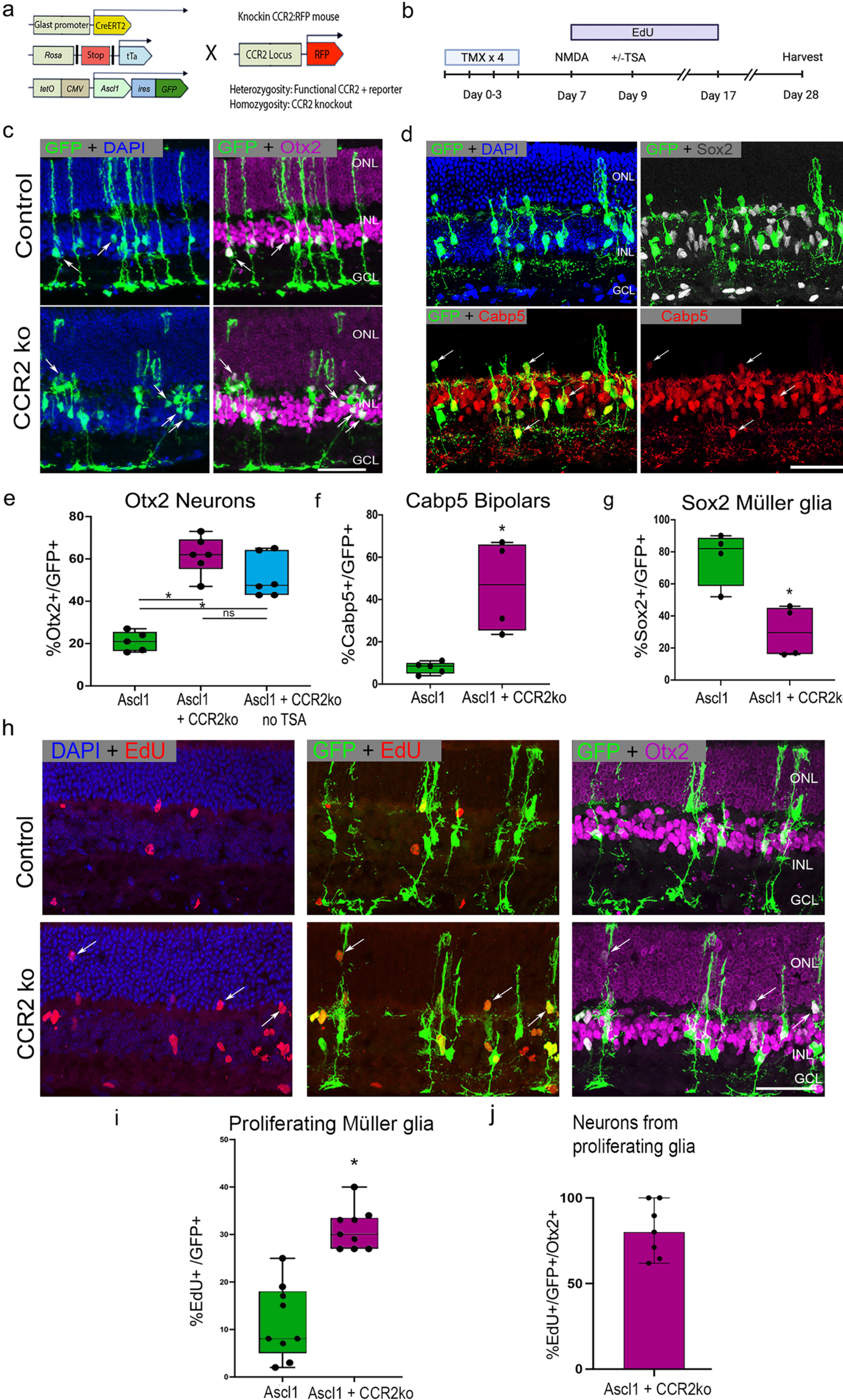

Figure 2. Prevention of monocyte invasion increases neurogenesis from Müller glia. ***a***, Schematic of transgenic mouse to stimulate retinal regeneration by MG-specific induction of Ascl1 crossed to CCR2:RFP line to allow for monocyte inhibition simultaneously. ***b***, Experimental paradigm to stimulate retinal regeneration by tamoxifen induction of Ascl1 and GFP in MG followed by intravitreal injections of NMDA with or without TSA and EdU added to drinking water to trace newborn cells. ***c***, Representative images of GFP+ MG-derived Otx2 (purple) with Ascl1-only versus Ascl1 plus CCR2 knock-out versus without TSA treatment. The arrows depict examples of Otx2/GFP+ MG-derived neurons. ***d***, Representative image of GFP+ MG-derived cells expressing the cone-bipolar marker Cabp5 (red) and not expressing the MG marker Sox2 (silver) in a monocyte-deficient mouse. The arrows show example of glia-derived GFP/Cabp5+ bipolar. ***e–g***, Quantification of the percent of MG-derived cells that differentiated into Otx2+ neurons (Ascl1 vs Ascl1 CCR2ko $*p < 0.0001$, Ascl1 vs no TSA $p < 0.0001$; ***e***), Cabp5 bipolars ($*p = 0.02$; ***f***), or remained Sox2+ ($*p = 0.03$; ***g***) in Ascl1-only versus Ascl1 plus monocyte knockdown. ***h***, Representative images of EdU (red) pulse labeling of GFP+ glia giving rise to newborn neurons (Otx2+). The arrow shows examples of Edu+/GFP+/Otx2+ cells. ***i***, Quantification of the percent of GFP+ glia that underwent cell cycle re-entry in control versus CCR2 knock-out retinas ($*p = 0.0001$). ***j***, Percent of Edu+ cells that differentiated into Otx2+ cells. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar, is 50 µm. Significance of difference ($*p < 0.05$) for ***e*** was determined using a one-way ANOVA followed by Tukey's multiple-comparisons test and ***f–i*** used a Mann–Whitney *U* test; individual dots represent biological replicates using both retinas combined.

to intense acute light damage, with and without monocytes (Fig. 3*a*). In brief, mice underwent exposure to ∼10,000 lux of white light for 8 h, a paradigm known to kill 30% of photoreceptors as early as 24 h and 90% of photoreceptors by 1 week (Kang et al., 2020). Light damage has been validated to lead to invasion of monocyte-derived cells (Joly et al., 2009; Sennlaub et al., 2013; O'Koren et al., 2016). We tested whether prevention of monocyte invasion increased neural regeneration after light injury (Fig. 3*a*). It has been recently reported that MG can respond to photoreceptor damage and generate neurons similar to what occurs after retinal ganglion cell death (Pavlou et al., 2024). We found that inhibition of monocyte recruitment significantly increased the amount of neurogenesis from MG following photoreceptor ablation (Fig. 3*b*,*c*), much like what we found after the NMDA injury. As reported previously, we found that death of photoreceptors leads to neurogenesis of Otx2+ bipolar neurons (Fig. 3*b*,*c*) from MG and does not stimulate a shift to the photoreceptor fate. The majority of new neurons were found in the INL with Otx2+ morphology similar to bipolars and not photoreceptors (inverted chromatin), the few regenerated neurons that were found in the ONL lacked expression of photoreceptor markers (data not shown). These data show that in two diverse injury stimuli, peripheral monocytes inhibit neural regeneration.

scRNA-seq of monocytes and Müller glia during retinal regeneration

To identify putative mechanisms underlying the repression of neurogenesis from MG that is caused by monocytes, we analyzed their transcriptome after NMDA; we sorted all immune cells in Ascl1-expressing mice (CD45+) 5 d after NMDA injury and processed them for scRNA-sequencing. Consistent with our data from the monocyte reporter mice, scRNA-seq data from NMDA-treated mice confirmed that monocytes are present in regenerating retinas alongside microglia, T cells, and neutrophils (Fig. 4*a*,*b*; Table 2). To focus on the potential cross talk between microglia, monocytes, and MG, we subsetted monocytes and microglia and combined this dataset with a previously published dataset of MG (Todd et al., 2020) and to increase the representation of microglia in our analysis, we bioinformatically isolated microglia from four previously published datasets from retinas undergoing NMDA-induced regeneration (Todd et al., 2020, 2021, 2022). These datasets were integrated with Harmony and plotted as a UMAP (Korsunsky et al., 2019; Fig. 4*c*). The UMAP plots show two distinct large clusters: MG and monocytes/microglia. Both larger clusters have subclusters that can be identified by known marker genes. The monocyte/microglial cluster contains Hexb+ microglia and CCR2+ monocytes (Fig. 4*d*). Similar to our previous report, we found that the MG take on diverse states after retinal injury, with a Rlbp1+ homeostatic cluster, a H2-Q4+ MHC-high cluster, and a Ifit3+ cluster high in interferon genes (Fig. 4*d*; Todd et al., 2020).

To better understand how infiltrating monocytes may communicate with reactive MG, we performed CellChat, a tool to infer cell signaling inputs and outputs from scRNA-seq datasets (Jin et al., 2021). By cross-referencing the expression patterns of the cells in our dataset to a curated database of ligand–receptor interactions pulled from the KEGG Pathway database and predicting significant communications through differentially expressed ligands and receptors, CellChat can infer intercellular communications. CellChat models these ligand–receptor interactions using the law of mass action, whereby concentrations of a given ligand and its receptor are thought to indicate the probability of meaningful interaction. These intercellular interactions are then grouped into 1 of the 229 functionally related signaling pathways identified in the literature (Jin et al., 2021). This dataset revealed signaling patterns between immune cells and MG as significantly enriched in the scRNA-seq data (Fig. 4*e*). Providing confidence in this approach, these analyses revealed signaling pathways known to influence MG inflammatory response to injury, including MDK, Notch, CCL, TNF, and TGFb (Lenkowski et al., 2013; Nelson et al., 2013; Conner et al., 2014; Todd et al., 2017, 2020; Karlen et al., 2018; Nagashima et al., 2020; Campbell et al., 2021; Palazzo et al., 2022; Le et al., 2023).

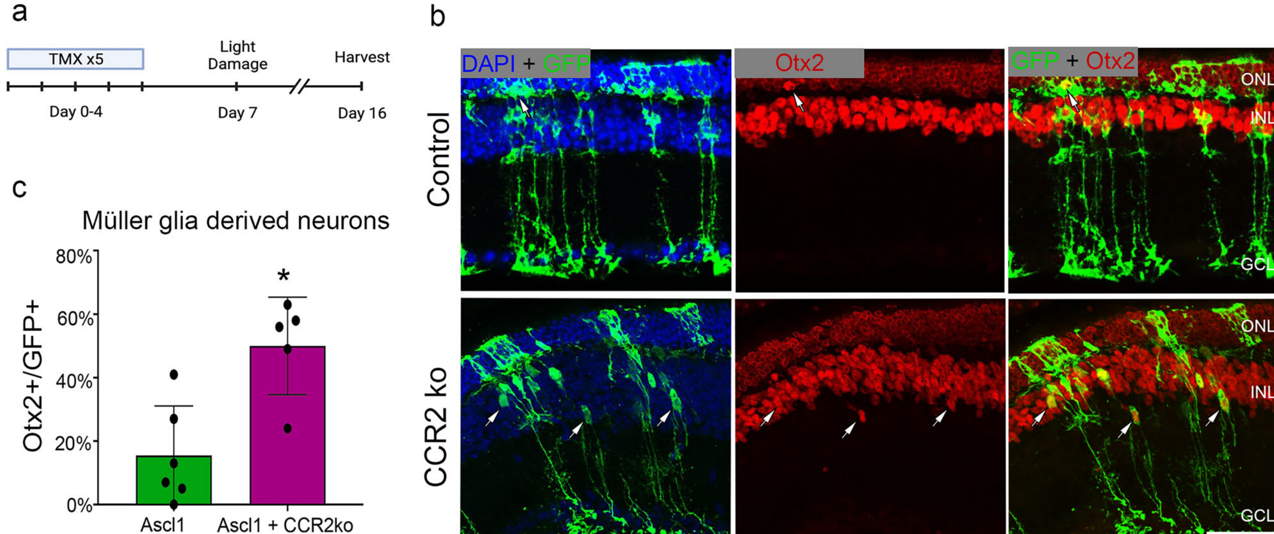

Figure 3. Monocytes suppress Müller glia neurogenesis in a phototoxic injury paradigm. ***a***, Experimental paradigm to induce Müller glia neurogenesis in a light injury paradigm. ***b***, Representative images of Ascl1 expressing GFP+ MG (green) and Otx2+ bipolar neurons (red) in Ascl1-only (control) and Ascl1 + CCR2 ko mice after undergoing light damage. The arrows show example of double positive Otx2/GFP+ cells. ***c***, Quantification of the percent of neurogenesis (GFP+ MG/Otx2+ neurons) in Ascl1-only versus Ascl1 + CCR2 knock-out mice after phototoxic injury. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar, is 50 µm. Significance of difference (*$p = 0.0173$) was determined using Mann–Whitney *U* test; individual dots represent biological replicates using both retinas combined.

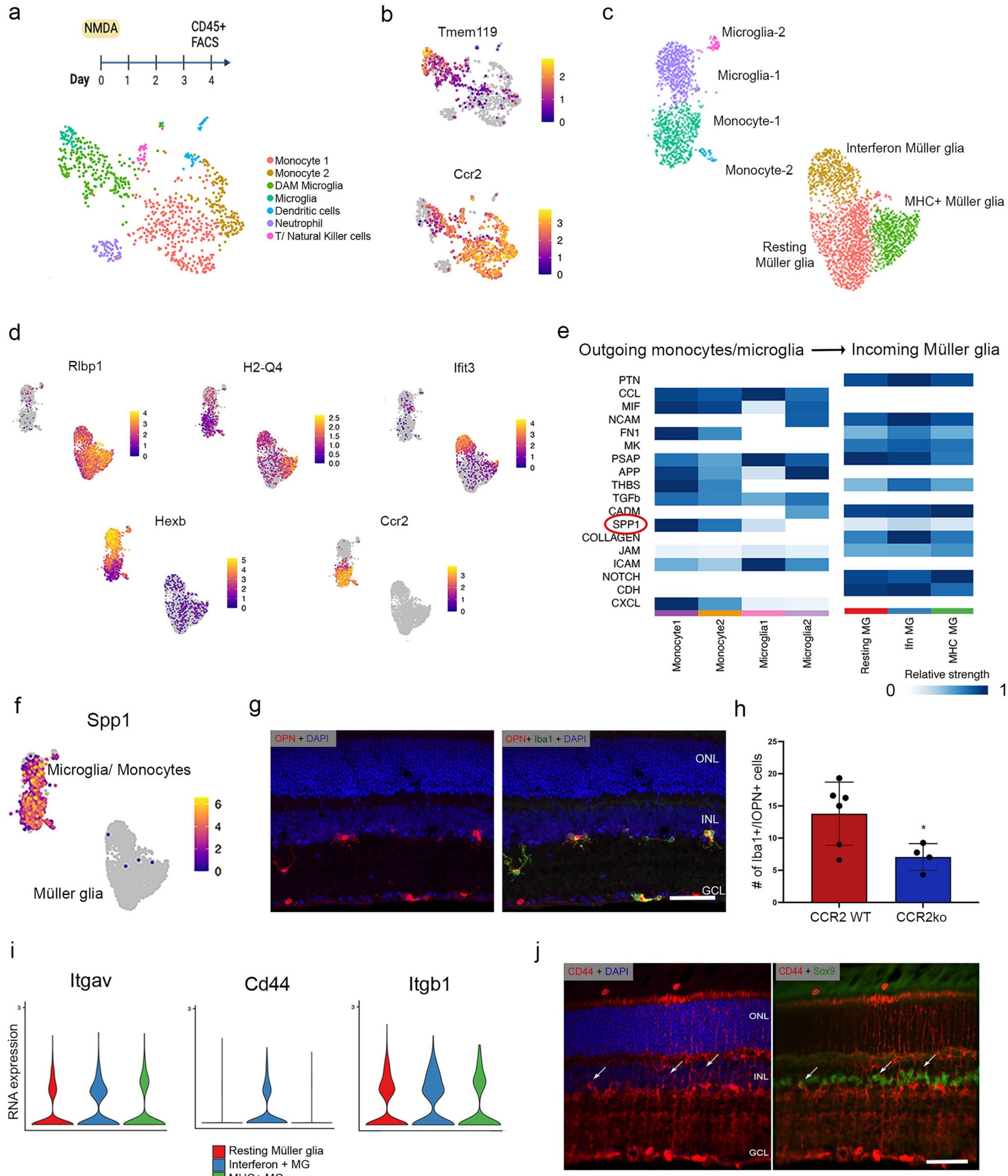

Figure 4. scRNA-seq analysis of immune cells and glia using CellChatDB identifies putative signaling mechanisms between monocytes and Müller glia. ***a***, Paradigm and UMAP plot for scRNA-seq analysis of CD45+ immune cells that invade NMDA-damaged retinas. ***b***, Feature plots highlighting the clusters of microglia (Tmem119) and monocytes (Ccr2) that are subsetted for integration with MG in ***c***. ***c***, UMAP of integrated dataset of sorted MG during the regeneration paradigm with microglia/monocytes. ***d***, Feature plots highlighting MG (Rlbp1+) and the MHC+ (H2-QR) and Interferon+ (Ifit3) subtypes that form after injury. Feature plots also the Ccr2+ monocyte and Hexb+ microglia clusters. ***e***, Heat map of the CellChat generated network analysis showing the relative signaling strength of incoming and outgoing signaling pathways between cell groups. ***f***, Feature plot showing OPN expression (Spp1) is specifically expressed by microglia/monocytes. ***g***, Representative image of OPN expression (red) in Iba1+ (green) immune cells 24 h after NMDA injury. ***h***, Quantification of the number of OPN+/Iba1+ cells 24 h after NMDA injury in WT versus CCR2ko retinas. ***i***, Violin plots from scRNA-seq data showing the OPN receptors Itgav, Cd44, and Itgb1 are expressed by MG. ***j***, Representative image of Cd44 (red) expression in Sox9+ (green) MG. Abbreviations are as follows: GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. All scale bars represent 50 µm.

Osteopontin represses Müller glia neurogenesis

We were particularly interested in Osteopontin (Spp1) signaling, as the ligand was highly enriched in monocytes/microglia and the receptors Cd44, Itgav, and Itgb1 are expressed in damaged MG (Fig. 4*e–j*). We confirmed OPN is expressed in Iba1+ retinal immune cells after NMDA injury and that CCR2 deletion reduced the number of OPN+ cells in damaged retinas (Fig. 4*g,h*). We also confirmed protein expression of one of the OPN receptors, CD44, in Sox9+ MG, consistent with other reports (Fig. 4*j*; Rich et al., 1995; Shinoe et al., 2010) Recently, it was reported that exogenous Osteopontin (OPN) protein can induce NfkB in mouse MG, a signaling pathway known to counteract MG reprogramming (Palazzo et al., 2020, 2022, 2023). Therefore, we reasoned if monocyte-derived OPN induces a nonregenerative state in MG, adding back OPN in monocyte knockdown retinas should reverse the neurogenic increase seen with monocyte inhibition.

To test this, we performed in vivo reprogramming experiments in monocyte knockdown retinas while intravitreally treating mice with recombinant OPN (Fig. 5*a,b*). Similar to previous experiments, we noted high neurogenic rates in Ascl1-MG mice combined with CCR2ko, and we found that exogenous OPN counteracted this effect (Fig. 5*c,d*). OPN-treated retinas had a significantly lower amount of MG neurogenesis, a rate similar to Ascl1 only (Fig. 5*d*). It had previously been reported that OPN is neuroprotective in mouse models of glaucoma, and this could potentially underlie the reduction we see in neurogenesis (Li and Jakobs, 2022; M. Zhao et al., 2023). Therefore, we tested whether OPN was neuroprotective after NMDA damage and found that OPN did not influence the survival of RGCs in this context (Fig. 5*e,f*). Taken together, these data suggest that OPN is one of the molecules released by immune cells that inhibit the neurogenic reprogramming of MG independent of neuroprotection.

Table 2. Top genes used to identify clusters in Figure 4*a*

Cluster ID	Top genes
Monocyte 1	*Thbs1, Pirb, Tgfbi, Arg1, Fn1, Ccr2, Cybb, F13a1, F10, Saa3*
Monocyte 2	*Ms4a6c, Lyz2, Ms4a4c, Timp1, Cd74, S100a4, Ifi27l2a*
DAM microglia	*Sparc, Cd81, C1qa, C1qc, C1qb, Hexb, Cx3cr1, Cst3, Ccl12, Ctsd*
Microglia	*P2ry12, Gpr34, Ccl2*
Dendritic	*Ccl22, Ccr7, Il4i1, Tbc1d4, Serpinb6b, Bcl2a1a, Crip1, Lsp1, Ccl5, Fscn1*
Neutrophil	*Mmp9, S100a9, S100a8, Asprv1, Lcn2, G0s2, Stfa21, Gm5483, Wfdc21, Retnlg*
T cells/natural killer cells	*Cxcr6, Trbc2, Cd3g, Trdc, Trbc1, Il17a, Nkg7, Cd3d, AW112010, Ramp1*

Discussion

Strategies to stimulate endogenous regeneration of neurons from glia in the mammalian retina have made rapid progress over the past few years (Jorstad et al., 2017, 2020; Hamon et al., 2019;

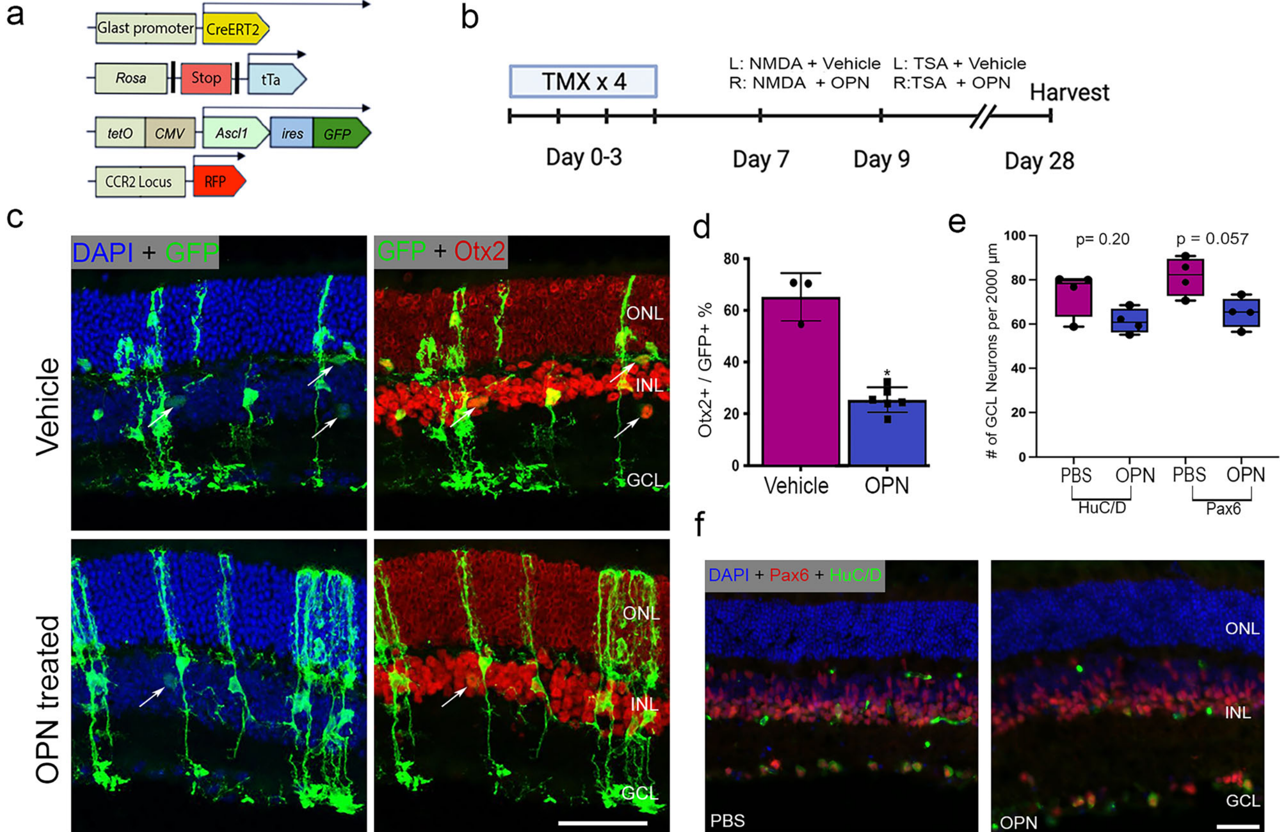

Figure 5. A monocyte enriched ligand OPN is sufficient to inhibit neurogenesis from Müller glia. ***a***, Transgenic mouse to stimulate regeneration in the absence of monocytes. ***b***, Paradigm to test whether recombinant OPN inhibits the increase of neurogenesis seen in Ascl1-mediated regeneration in the absence of monocytes. ***c***, Representative images of GFP+ MG and their GFP+/Otx2+ neuronal progeny with and without OPN treatment. The arrows show examples of GFP/Otx2+ cells. ***d***, Quantification of the percent of MG-derived neurons in Ascl1-CCR2 knock-out retinas with and without recombinant OPN. Significance of difference ($*p = 0.0121$) was determined using Mann–Whitney *U* test; individual dots represent biological replicates using both retinas combined. ***e***, Quantification of the number of Pax6+ and HuC/D+ surviving neurons after NMDA damage in the GCL with and without OPN treatment. ***f***, Representative pictures of Pax6+ (red) and HuC/D+ (green) neurons 48 h after NMDA treatment with and without recombinant OPN. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars are 50 µm.

Rueda et al., 2019; Hoang et al., 2020; Todd et al., 2020, 2021, 2022; Palazzo et al., 2022; Le et al., 2023). In addition to Ascl1 overexpression, alternative routes to stimulate mammalian glia to make neurons have been identified including manipulation of the NFI factors, Hippo/Yap signaling, and Notch (Rueda et al., 2019; Hoang et al., 2020; Le et al., 2023). The majority of work to date has focused on the intrinsic gene regulatory networks controlling neurogenesis from glia. While this work is critical, it is important to consider the signals from the microenvironment affecting the MG-derived progenitors. For therapeutic purposes, this means the retina will typically be in a diseased or aged state where inflammation will be prominent.

In this study, we found that monocytes, bone marrow-derived peripheral immune cells, invade the neural retina after damage and limit the ability of MG to undergo proliferation and neurogenesis. Using a transgenic approach to block CCR2-mediated recruitment of monocytes, we demonstrate that regeneration can be significantly enhanced with prevention of monocyte invasion. These data are consistent with findings from a mouse model of stroke where depletion of monocytes enhanced neurogenesis from the subventricular zone (Laterza et al., 2017) and suggests that neurogenesis in the mammalian CNS more broadly may be limited by inflammation (Monje et al., 2003; Dulken et al., 2019; Navarro Negredo et al., 2020). It should be noted that CCR2 is not exclusively expressed by monocytes but also shows expression on T cells of which a small amount were detected in our scRNA-seq dataset. To definitively establish monocytes as the sole cell type responsible for the regenerative enactment seen by CCR2 knock-out, adoptive transfer of wild-type monocytes would be required.

We previously reported that microglia negatively influenced MG neurogenesis. This was shown in vivo by ablating microglia pharmacologically with the CSF1R inhibitor PLX5622 (Todd et al., 2020). Several cell types in addition to microglia including monocytes express CSF1R and could potentially be affected by PLX5622, and the literature does not present a consistent picture: some groups have reported that PLX5622 reduces monocytes directly (Lei et al., 2020), another report found that ablation of microglia indirectly reduces monocyte numbers in the retina because microglia recruit monocytes (Okunuki et al., 2019) and others have found PLX5622 does not reduce monocyte numbers (Spangenberg et al., 2019). Our previous data also found PLX5622 improved MG neurogenesis in vitro in primary cultures where only microglia and MG were present. Taken together, our data is consistent with the possibility that microglia act as a negative regulator of retinal neurogenesis; however, whether the effect of PLX5622 is ultimately through monocyte recruitment, altered monocyte behavior, or whether there is parallel monocyte–microglia mechanisms will take future experiments and additional tool development to untangle.

How monocyte invasion impacts the phenotype of the resident microglia will be interesting to untangle in future studies. Prevention of monocyte invasion alters the RNA-seq profiles of microglia in models of stroke by reducing inflammatory genes (Somebang et al., 2021). Additionally, in Alzheimer's models CCR2+ monocytes are necessary to polarize microglia into an inflammatory substate that clears plaque burden (El Khoury et al., 2007; Naert and Rivest, 2011). It is possible that prevention of monocyte accumulation in the retina polarizes microglia to responses that are more proregenerative.

The fact that both microglia and monocyte-mediated inflammation negatively influence MG neurogenesis is in contrast to what occurs in zebrafish and chick, where inflammation promotes MG reprogramming (Fischer et al., 2014; White et al., 2017; Conedera et al., 2019; Todd et al., 2020; Zhang et al., 2020; El-Hodiri et al., 2023). Taken together, these studies suggest that the neuroimmune response to injury has a critical role in limiting mammalian retinal regeneration, and the difference in the MG response to inflammation between regenerating and nonregenerating species may be a key factor underlying the difference among species in regenerative potential.

One of the ways monocytes may limit neurogenesis is by inhibiting MG proliferation. We found that MG have an increased propensity to proliferate after NMDA injury in the absence of monocytes. Similarly, in a mouse model of traumatic brain injury, monocyte knockdown led to less astrocyte scarring and more astrocyte proliferation, a phenotype associated with better repair outcomes (Frik et al., 2018). Interestingly, we also observed that MGs that re-enter the cell cycle are heavily biased to differentiate into neurons. Though proliferation is not necessary for reprogramming (Heinrich et al., 2010; Fishman et al., 2015), high proliferation rates have been associated with higher reprogramming and differentiation rates (Babos et al., 2019; Wang et al., 2023). In zebrafish, retinal regeneration, cell division of MG via downregulation of Pten and activation of the Yap pathway, is necessary for neurogenesis (Lourenco et al., 2021; Gupta et al., 2023). Therefore, increasing the proliferation of MG may be a fruitful avenue to increase neurogenic reprogramming in the mammalian retina.

Previously, we found that the HDAC inhibitor TSA was required in NMDA-damaged retinas to stimulate neurogenesis from MG in adult, but not young mice (Ueki et al., 2015; Jorstad et al., 2017). This effect of TSA promoting reprogramming was attributed to reopening of development chromatin sites that are naturally shut down in adult MG (Jorstad et al., 2017; VandenBosch et al., 2020). Here, we surprisingly found that HDAC inhibition is not required for MG reprogramming when monocyte invasion is prevented. This suggests that in previous studies, TSA may have served as an immunomodulator "resetting" the phenotype of inflammatory cells. Alternatively, HDAC inhibition may shift the chromatin state in MG away from and inflammatory state induced by immune cells, and this is no longer necessary in the absence of monocytes.

To identify the molecular mechanisms that underlie the suppression of neurogenesis mediated by monocytes, we used a computational approach. Using scRNA-seq and CellChat analysis, we identified potential interacting signaling pathways that underlie monocyte–MG interactions (Jin et al., 2021). One of these interactions involved Osteopontin/OPN (Spp1) ligand and the Itgav, Igtb, and Cd44 receptors on MG. We show that Osteopontin (Spp1) is highly expressed by infiltrating monocytes and specific subtypes of inflammatory microglia similar to what has been observed in prior studies (Benhar et al., 2023; Lan et al., 2024). Depending on the context of ligand processing, tissue location and disease phase Osteopontin have been implicated in both repair and toxicity of the nervous system (Cappellano et al., 2021). However, OPN signaling is thought to exacerbate inflammation, and high levels of OPN have been associated with worse outcomes in patients with Alzheimer's, Parkinson's, and multiple sclerosis (Cappellano et al., 2013). One of the inflammatory pathways OPN is known to interact with and induce is NfKb (Das et al., 2003; W. Zhao et al., 2011). Induction of NFkb inhibits Ascl1-mediated reprogramming of MG (Palazzo et al., 2022). Interestingly, the exogenous application of OPN intravitreally induces the NFkb reporter in MG (Palazzo et al., 2023). These findings suggest one of the ways monocyte infiltration may

inhibit neurogenesis is via OPN inducing Nkfb signaling in MG. This is consistent with our finding that OPN injections restore the inhibition of neurogenesis in the absence of monocytes.

In sum, this study demonstrates a key role for monocytes in suppressing the Ascl1-induced neural regenerative response in MG. At least one of the factors that underlie this suppression is Osteopontin. Together, with our prior work demonstrating a similar repressive activity from microglia, it appears that the response of the adult mammalian CNS to injury is not supportive of regeneration. The response to CNS injury is quite different in species that do not regenerate and those that do, where the innate immune system is required for regeneration. This suggests that modulating the immune response in the mammalian CNS after injury might create a more favorable environment for neural repair.

References

Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, Sarnacki S, Cumano A, Lauvau G, Geissmann F (2007) Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science 317:666–670.

Babos KN, Galloway KE, Kisler K, Zitting M, Li Y, Shi Y, Quintino B, Chow RH, Zlokovic BV, Ichida JK (2019) Mitigating antagonism between transcription and proliferation allows near-deterministic cellular reprogramming. Cell Stem Cell 25:486–500.e9.

Benhar I, et al. (2023) Temporal single-cell atlas of non-neuronal retinal cells reveals dynamic, coordinated multicellular responses to central nervous system injury. Nat Immunol 24:700–713.

Bringmann A, Iandiev I, Pannicke T, Wurm A, Hollborn M, Wiedemann P, Osborne NN, Reichenbach A (2009) Cellular signaling and factors involved in Muller cell gliosis: neuroprotective and detrimental effects. Prog Retin Eye Res 28:423–451.

Campbell WA, Fritsch-Kelleher A, Palazzo I, Hoang T, Blackshaw S, Fischer AJ (2021) Midkine is neuroprotective and influences glial reactivity and the formation of Muller glia-derived progenitor cells in chick and mouse retinas. Glia 69:1515–1539.

Cappellano G, Carecchio M, Fleetwood T, Magistrelli L, Cantello R, Dianzani U, Comi C (2013) Immunity and inflammation in neurodegenerative diseases. Am J Neurodegener Dis 2:89–107.

Cappellano G, Vecchio D, Magistrelli L, Clemente N, Raineri D, Barbero Mazzucca C, Virgilio E, Dianzani U, Chiocchetti A, Comi C (2021) The Yin-Yang of osteopontin in nervous system diseases: damage versus repair. Neural Regen Res 16:1131–1137.

Chen HR, Sun YY, Chen CW, Kuo YM, Kuan IS, Tiger Li ZR, Short-Miller JC, Smucker MR, Kuan CY (2020) Fate mapping via CCR2-CreER mice reveals monocyte-to-microglia transition in development and neonatal stroke. Sci Adv 6:eabb2119.

Conedera FM, Pousa AMQ, Mercader N, Tschopp M, Enzmann V (2019) Retinal microglia signaling affects Muller cell behavior in the zebrafish following laser injury induction. Glia 67:1150–1166.

Conner C, Ackerman KM, Lahne M, Hobgood JS, Hyde DR (2014) Repressing notch signaling and expressing TNFalpha are sufficient to mimic retinal regeneration by inducing Muller glial proliferation to generate committed progenitor cells. J Neurosci 34:14403–14419.

Das R, Mahabeleshwar GH, Kundu GC (2003) Osteopontin stimulates cell motility and nuclear factor kappaB-mediated secretion of urokinase type plasminogen activator through phosphatidylinositol 3-kinase/Akt signaling pathways in breast cancer cells. J Biol Chem 278:28593–28606.

Dulken BW, et al. (2019) Single-cell analysis reveals T cell infiltration in old neurogenic niches. Nature 571:205–210.

El-Hodiri HM, Bentley JR, Reske AG, Taylor OB, Palazzo I, Campbell WA, Halloy NR, Fischer AJ (2023) Heparin-binding epidermal growth factor and fibroblast growth factor 2 rescue Muller glia-derived progenitor cell formation in microglia- and macrophage-ablated chick retinas. Development 150:dev202070.

El Khoury J, Toft M, Hickman SE, Means TK, Terada K, Geula C, Luster AD (2007) Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat Med 13:432–438.

Fang W, et al. (2018) CCR2-dependent monocytes/macrophages exacerbate acute brain injury but promote functional recovery after ischemic stroke in mice. Theranostics 8:3530–3543.

Fausett BV, Goldman D (2006) A role for alpha1 tubulin-expressing Muller glia in regeneration of the injured zebrafish retina. J Neurosci 26:6303–6313.

Fischer AJ, Zelinka C, Gallina D, Scott MA, Todd L (2014) Reactive microglia and macrophage facilitate the formation of Muller glia-derived retinal progenitors. Glia 62:1608–1628.

Fishman VS, Shnayder TA, Orishchenko KE, Bader M, Alenina N, Serov OL (2015) Cell divisions are not essential for the direct conversion of fibroblasts into neuronal cells. Cell Cycle 14:1188–1196.

Frik J, Merl-Pham J, Plesnila N, Mattugini N, Kjell J, Kraska J, Gomez RM, Hauck SM, Sirko S, Gotz M (2018) Cross-talk between monocyte invasion and astrocyte proliferation regulates scarring in brain injury. EMBO Rep 19: e45294.

Gadani SP, Walsh JT, Lukens JR, Kipnis J (2015) Dealing with danger in the CNS: the response of the immune system to injury. Neuron 87:47–62.

Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, Narni-Mancinelli E, Lauvau G (2008) Blood monocytes: distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. Immunol Cell Biol 86:398–408.

Guillonneau X, Eandi CM, Paques M, Sahel JA, Sapieha P, Sennlaub F (2017) On phagocytes and macular degeneration. Prog Retin Eye Res 61:98–128.

Guo C, Otani A, Oishi A, Kojima H, Makiyama Y, Nakagawa S, Yoshimura N (2012) Knockout of ccr2 alleviates photoreceptor cell death in a model of retinitis pigmentosa. Exp Eye Res 104:39–47.

Gupta S, Sharma P, Chaudhary M, Premraj S, Kaur S, Vijayan V, Arun MG, Prasad NG, Ramachandran R (2023) Pten associates with important gene regulatory network to fine-tune Muller glia-mediated zebrafish retina regeneration. Glia 71:259–283.

Guttenplan KA, Stafford BK, El-Danaf RN, Adler DI, Munch AE, Weigel MK, Huberman AD, Liddelow SA (2020) Neurotoxic reactive astrocytes drive neuronal death after retinal injury. Cell Rep 31:107776.

Hamon A, Garcia-Garcia D, Ail D, Bitard J, Chesneau A, Dalkara D, Locker M, Roger JE, Perron M (2019) Linking YAP to Muller glia quiescence exit in the degenerative retina. Cell Rep 27:1712–1725.e6.

Heinrich C, Blum R, Gascon S, Masserdotti G, Tripathi P, Sanchez R, Tiedt S, Schroeder T, Gotz M, Berninger B (2010) Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol 8:e1000373.

Hoang T, et al. (2020) Gene regulatory networks controlling vertebrate retinal regeneration. Science 370:eabb8598.

Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan CH, Myung P, Plikus MV, Nie Q (2021) Inference and analysis of cell-cell communication using CellChat. Nat Commun 12:1088.

Joly S, et al. (2009) Cooperative phagocytes: resident microglia and bone marrow immigrants remove dead photoreceptors in retinal lesions. Am J Pathol 174:2310–2323.

Jorstad NL, et al. (2020) STAT signaling modifies Ascl1 chromatin binding and limits neural regeneration from Muller glia in adult mouse retina. Cell Rep 30:2195–2208.e5.

Jorstad NL, Wilken MS, Grimes WN, Wohl SG, VandenBosch LS, Yoshimatsu T, Wong RO, Rieke F, Reh TA (2017) Stimulation of functional neuronal regeneration from Muller glia in adult mice. Nature 548:103–107.

Kang S, Larbi D, Andrade M, Reardon S, Reh TA, Wohl SG (2020) A comparative analysis of reactive Muller glia gene expression after light damage and microRNA-depleted Muller glia-focus on microRNAs. Front Cell Dev Biol 8:620459.

Karl MO, Hayes S, Nelson BR, Tan K, Buckingham B, Reh TA (2008) Stimulation of neural regeneration in the mouse retina. Proc Natl Acad Sci U S A 105:19508–19513.

Karlen SJ, Miller EB, Wang X, Levine ES, Zawadzki RJ, Burns ME (2018) Monocyte infiltration rather than microglia proliferation dominates the early immune response to rapid photoreceptor degeneration. J Neuroinflammation 15:344.

Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, Baglaenko Y, Brenner M, Loh PR, Raychaudhuri S (2019) Fast, sensitive and accurate integration of single-cell data with Harmony. Nat Methods 16:1289–1296.

Lan Y, et al. (2024) Fate mapping of Spp1 expression reveals age-dependent plasticity of disease-associated microglia-like cells after brain injury. Immunity 57:349–363.e9.

Laterza C, Wattananit S, Uoshima N, Ge R, Pekny R, Tornero D, Monni E, Lindvall O, Kokaia Z (2017) Monocyte depletion early after stroke promotes neurogenesis from endogenous neural stem cells in adult brain. Exp Neurol 297:129–137.

Le N, Vu TD, Palazzo I, Pulya R, Kim Y, Blackshaw S, Hoang T (2023) Robust reprogramming of glia into neurons by inhibition of Notch signaling and NFI factors in adult mammalian retina. bioRxiv.

Lei F, Cui N, Zhou C, Chodosh J, Vavvas DG, Paschalis EI (2020) CSF1R inhibition by a small-molecule inhibitor is not microglia specific; affecting hematopoiesis and the function of macrophages. Proc Natl Acad Sci U S A 117:23336–23338.

Lenkowski JR, Qin Z, Sifuentes CJ, Thummel R, Soto CM, Moens CB, Raymond PA (2013) Retinal regeneration in adult zebrafish requires regulation of TGFbeta signaling. Glia 61:1687–1697.

Lenkowski JR, Raymond PA (2014) Muller glia: stem cells for generation and regeneration of retinal neurons in teleost fish. Prog Retin Eye Res 40:94–123.

Li S, Jakobs TC (2022) Secreted phosphoprotein 1 slows neurodegeneration and rescues visual function in mouse models of aging and glaucoma. Cell Rep 41:111880.

Liddelow SA, et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481–487.

Lourenco R, Brandao AS, Borbinha J, Gorgulho R, Jacinto A (2021) Yap regulates Muller glia reprogramming in damaged zebrafish retinas. Front Cell Dev Biol 9:667796.

Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760–1765.

Naert G, Rivest S (2011) CC chemokine receptor 2 deficiency aggravates cognitive impairments and amyloid pathology in a transgenic mouse model of Alzheimer's disease. J Neurosci 31:6208–6220.

Nagashima M, D'Cruz TS, Danku AE, Hesse D, Sifuentes C, Raymond PA, Hitchcock PF (2020) Midkine-a is required for cell cycle progression of Muller glia during neuronal regeneration in the vertebrate retina. J Neurosci 40:1232–1247.

Navarro Negredo P, Yeo RW, Brunet A (2020) Aging and rejuvenation of neural stem cells and their niches. Cell Stem Cell 27:202–223.

Nelson CM, Ackerman KM, O'Hayer P, Bailey TJ, Gorsuch RA, Hyde DR (2013) Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. J Neurosci 33:6524–6539.

O'Koren EG, Mathew R, Saban DR (2016) Fate mapping reveals that microglia and recruited monocyte-derived macrophages are definitively distinguishable by phenotype in the retina. Sci Rep 6:20636.

Okunuki Y, Mukai R, Nakao T, Tabor SJ, Butovsky O, Dana R, Ksander BR, Connor KM (2019) Retinal microglia initiate neuroinflammation in ocular autoimmunity. Proc Natl Acad Sci U S A 116:9989–9998.

Palazzo I, Deistler K, Hoang TV, Blackshaw S, Fischer AJ (2020) NF-kappaB signaling regulates the formation of proliferating Muller glia-derived progenitor cells in the avian retina. Development 147:dev183418.

Palazzo I, Kelly L, Koenig L, Fischer AJ (2023) Patterns of NFkB activation resulting from damage, reactive microglia, cytokines, and growth factors in the mouse retina. Exp Neurol 359:114233.

Palazzo I, Todd LJ, Hoang TV, Reh TA, Blackshaw S, Fischer AJ (2022) NFkB-signaling promotes glial reactivity and suppresses Muller glia-mediated neuron regeneration in the mammalian retina. Glia 70:1380–1401.

Pavlou M, Probst M, Blasdel N, Prieve AR, Reh TA (2024) The impact of timing and injury mode on induced neurogenesis in the adult mammalian retina. Stem Cell Reports 19:239–253.

Rich KA, Figueroa SL, Zhan Y, Blanks JC (1995) Effects of Muller cell disruption on mouse photoreceptor cell development. Exp Eye Res 61:235–248.

Rueda EM, Hall BM, Hill MC, Swinton PG, Tong X, Martin JF, Poche RA (2019) The hippo pathway blocks mammalian retinal Muller glial cell reprogramming. Cell Rep 27:1637–1649.e6.

Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou CL, Ransohoff RM, Charo IF (2010) Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5:e13693.

Sennlaub F, et al. (2013) CCR2(+) monocytes infiltrate atrophic lesions in age-related macular disease and mediate photoreceptor degeneration in experimental subretinal inflammation in Cx3cr1 deficient mice. EMBO Mol Med 5:1775–1793.

Shinoe T, Kuribayashi H, Saya H, Seiki M, Aburatani H, Watanabe S (2010) Identification of CD44 as a cell surface marker for Muller glia precursor cells. J Neurochem 115:1633–1642.

Silvin A, et al. (2022) Dual ontogeny of disease-associated microglia and disease inflammatory macrophages in aging and neurodegeneration. Immunity 55:1448–1465.e6.

Somebang K, et al. (2021) CCR2 deficiency alters activation of microglia subsets in traumatic brain injury. Cell Rep 36:109727.

Spangenberg E, et al. (2019) Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. Nat Commun 10:3758.

Todd L, et al. (2021) Efficient stimulation of retinal regeneration from Muller glia in adult mice using combinations of proneural bHLH transcription factors. Cell Rep 37:109857.

Todd L, et al. (2022) Reprogramming Muller glia to regenerate ganglion-like cells in adult mouse retina with developmental transcription factors. Sci Adv 8:eabq7219.

Todd L, Finkbeiner C, Wong CK, Hooper MJ, Reh TA (2020) Microglia suppress Ascl1-induced retinal regeneration in mice. Cell Rep 33:108507.

Todd L, Palazzo I, Squires N, Mendonca N, Fischer AJ (2017) BMP- and TGFbeta-signaling regulate the formation of Muller glia-derived progenitor cells in the avian retina. Glia 65:1640–1655.

Todd L, Reh TA (2022) Comparative biology of vertebrate retinal regeneration: restoration of vision through cellular reprogramming. Cold Spring Harb Perspect Biol 14:a040816.

Todd L, Squires N, Suarez L, Fischer AJ (2016) Jak/Stat signaling regulates the proliferation and neurogenic potential of Muller glia-derived progenitor cells in the avian retina. Sci Rep 6:35703.

Ueki Y, Wilken MS, Cox KE, Chipman L, Jorstad N, Sternhagen K, Simic M, Ullom K, Nakafuku M, Reh TA (2015) Transgenic expression of the proneural transcription factor Ascl1 in Muller glia stimulates retinal regeneration in young mice. Proc Natl Acad Sci U S A 112:13717–13722.

VandenBosch LS, Wohl SG, Wilken MS, Hooper M, Finkbeiner C, Cox K, Chipman L, Reh TA (2020) Developmental changes in the accessible chromatin, transcriptome and Ascl1-binding correlate with the loss in Muller glial regenerative potential. Sci Rep 10:13615.

Wang NB, Lende-Dorn BA, Adewumi HO, Beitz AM, Han P, O'Shea TM, Galloway KE (2023) Proliferation history and transcription factor levels drive direct conversion. bioRxiv.

White DT, Sengupta S, Saxena MT, Xu Q, Hanes J, Ding D, Ji H, Mumm JS (2017) Immunomodulation-accelerated neuronal regeneration following selective rod photoreceptor cell ablation in the zebrafish retina. Proc Natl Acad Sci U S A 114:E3719–E3728.

Yu C, Roubeix C, Sennlaub F, Saban DR (2020) Microglia versus monocytes: distinct roles in degenerative diseases of the retina. Trends Neurosci 43: 433–449.

Zhang Z, et al. (2020) Inflammation-induced mammalian target of rapamycin signaling is essential for retina regeneration. Glia 68:111–127.

Zhao M, et al. (2023) Osteopontin drives retinal ganglion cell resiliency in glaucomatous optic neuropathy. Cell Rep 42:113038.

Zhao W, Wang L, Zhang M, Wang P, Zhang L, Yuan C, Qi J, Qiao Y, Kuo PC, Gao C (2011) NF-kappaB- and AP-1-mediated DNA looping regulates osteopontin transcription in endotoxin-stimulated murine macrophages. J Immunol 186:3173–3179.