Microglia versus Monocytes: Distinct Roles in Degenerative Diseases of the Retina.

Chen Yu, Christophe Roubeix, Florian Sennlaub, Daniel R Saban

To cite this version:

Chen Yu, Christophe Roubeix, Florian Sennlaub, Daniel R Saban. Microglia versus Monocytes: Distinct Roles in Degenerative Diseases of the Retina.. Trends in Neurosciences, 2020, 43 (6), pp.433-449. 10.1016/j.tins.2020.03.012 . hal-02879944

HAL Id: hal-02879944

https://hal.sorbonne-universite.fr/hal-02879944v1

Submitted on 24 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Microglia versus Monocytes: Distinct Roles in Degenerative Diseases of the Retina**

2 Chen Yu[1], Christophe Roubeix[2], Florian Sennlaub[2*†], Daniel R Saban[1,3*†]
3
4
5 [1] Department of Ophthalmology, Duke University, Durham, NC 27710, USA

6 [2] Sorbonne Université, INSERM, CNRS, Institut de la Vision, 17 rue Moreau, F-75012 Paris,
7 France

8 [3] Department of Immunology, Duke University, Durham, NC 27710, USA

9
10
11 *these authors have contributed equally
12
13 [†]Correspondence should be addressed to the lead contacts:

14 Daniel Saban: daniel.saban@duke.edu

15 Florian Sennlaub: florian.sennlaub@inserm.fr

16

17
18
19 **Key words:** macrophages; inherited retinal dystrophy; retinitis pigmentosa; age-related macular
20 degeneration; AMD

21 ## Abstract

22 Unlike in the healthy mammalian retina, macrophages in retinal degenerative states are not
23 solely comprised of microglia but may include monocyte-derived recruits. Recent studies have
24 applied transgenics, lineage-tracing and transcriptomics to help decipher the distinct roles of
25 these two cell-types in the disease settings of inherited retinal degenerations and age-related
26 macular degeneration. Literature discussed here focuses on the ectopic presence of both
27 macrophage types in the extracellular site surrounding the outer aspect of photoreceptor cells
28 (i.e., the subretinal space), which is crucially involved in the pathobiology. From these studies
29 we propose a working model in which perturbed photoreceptor states cause microglial
30 dominant migration to the subretinal space as a protective response, whereas the abundant
31 presence of monocyte-derived cells there instead drives and accelerates pathology. The latter,
32 we propose, is underpinned by specific genetic and non-genetic determinants that lead to a
33 maladaptive macrophage state.

34 ## Microglia and monocytes as distinct lineages in retinal degenerative disease

35 As part of the central nervous system (CNS), the retina is endowed with mononuclear
36 phagocytes (MNPs) that continually surveil its neuronal parenchyma and border tissues. These
37 consist of microglia, a specialized type of yolk-sac derived macrophage that reside in the
38 parenchyma [1-3] and border-associated macrophages [4] (i.e. long-lived retinal perivascular
39 macrophages and short-lived choroidal macrophages adjacent to the retina [3, 5]). In certain
40 disease states, blood derived monocytes can transiently invade the retina and differentiate into
41 macrophages [2, 6, 7]. These monocyte-derived cells (MdCs) and retinal microglia, have
42 received considerable research attention in photoreceptor degenerative diseases, including
43 inherited retinal degenerations (IRDs) and age-related macular degeneration (AMD) (**Box 1**). In
44 these conditions, MNPs infiltrate the affected photoreceptor layers and other outer retinal
45 structures, and in mouse models, manipulation of MNPs can alter the disease course. However,
46 given the more definitive demonstration in recent years that that microglia and monocytes
47 represent distinct MNP lineages [1, 8-10], there is growing appreciation for their non-redundant
48 functions in disease and the importance of studying these cells as separate entities. As such,
49 this review focuses on the distinctive roles for MdCs and microglia in retinal degeneration. We
50 discuss these concepts in the context of the subretinal space (SRS, **see Glossary**), the
51 extracellular site where photoreceptor outer segment tips contact or closely oppose the retinal
52 pigment epithelium (RPE), which is crucially involved in pathobiology. Finally, from recent
53 findings in animal models, we propose a unified model that classifies MNP responses as
54 adaptive versus maladaptive, and further extrapolate this idea to retinal degenerative diseases
55 in humans.

56 ## Roles of MNPs in Retinal Health and Photoreceptor Degenerative Disease

57 The type and pattern of retinal MNPs are largely homologous to those in the brain and spinal
58 cord parenchyma. Microglia are the principal MNPs in the normal retina parenchyma [11]. The
59 perivascular spaces also have resident macrophages that are long-lived [12], albeit radio-
60 sensitive (i.e. succumb to radiation-induced cell death) [11]. Monocytes **(Box 2)** and dendritic
61 cells are generally not thought to gain access to the retinal parenchyma in non-diseased states.

Like elsewhere in the CNS, retinal microglia are highly ramified, tiled, and express C-X3-C Motif Chemokine Receptor-1 (Cx3cr1), a homeostatic microglial immune checkpoint factor that inhibits expression of inflammatory cytokines such as Interleukin-1β (IL-1β) [13, 14] and C-C Motif Chemokine-2 (Ccl2) [6]. However, also like in the brain and spinal cord, retinal degeneration can result in large-scale monocyte recruitment, along with microglial reactivity. Yet, due to overlapping phenotypic markers of these two MNP lineages, tools to study their isolated contributions have only recently been available, which we summarize in **Table 1**.

Normal Physiological Conditions

The retina is organized into distinct laminae. The inner layers are endowed with blood vessels. These vessels are bordered by vascular endothelial cells with tight junctions, which are a major component of the inner blood retina barrier (BRB). Within the innermost layers, some microglia reside around somata of retinal ganglion cell (RGC) or their axon projections within the nerve fiber layer. These axons connect the eye, via the optic nerve, to the brain, with the primary target brain regions being the lateral geniculate nucleus and the superior colliculus. However, most microglia reside within the two distinct synaptic layers, inner and outer plexiform layers (OPL and IPL, respectively). The OPL and IPL are where photoreceptors synapse with bipolar cells, and bipolar cells synapse with RGC, respectively. Despite some commonalities between OPL and IPL microglia, for instance the fact that colony stimulating factor-1 receptor (Csf1r) is essential for their survival, recent work has revealed key differences between IPL and OPL microglial pools in the steady state [3]. The maintenance of IPL microglia is mostly dependent on RGC-produced IL-34 (an alternate ligand to Csf1r) [15-17], whereas OPL microglia are IL-34 independent (possibly maintained by glial-derived Csf1). Importantly, these respective pools have distinct electrophysiological contributions to visual processing **(Box 3)**.

In many mammalian species, including pigmented rodents and humans, the outermost retinal layers in adulthood are physiologically devoid of MNPs and blood vessels [6, 18, 19]. This includes photoreceptor somata (outer nuclear layer) and their ciliated segments (inner and outer segment layers), all the way through to the SRS and the RPE, a specialized monolayer of neural crest-derived border cells [20] **(Fig.1).** Absence of microglia in the SRS is of critical importance, as this site is essential for photoreceptor homeostasis and function. It contains the

91 interphotoreceptor matrix and surrounds apical microvilli of the RPE. There, the RPE delivers
92 neurotrophic factors, nutrients, ions, and visual cycle chromophores to photoreceptors. The
93 RPE also phagocytoses spent photoreceptor outer segment discs in the SRS, a process carried
94 out via conserved phagocytic machinery, such as with certain integrins [21, 22] and Mer
95 tyrosine kinase (Mertk) [23] in a circadian-dependent manner. Lastly, RPE cells are laterally
96 connected via tight junctions, forming thereby the outer BRB and structurally separating
97 choroidal vasculatures from the inner retina. Additionally, the RPE is highly immunosuppressive
98 [24-26], and MNPs reaching the SRS that ligate thrombospondin-1 (Tsp-1) via their CD47
99 receptor are eliminated by the RPE expressing Fas ligand (FasL) [26, 27] **(Fig.1)**.

100 *Subretinal Space in Retinal Degenerative States*

101 Invasion of the photoreceptor layers and SRS by MNPs is a disease phenotype of IRDs and AMD
102 [6, 14, 18, 19, 28]. It is accompanied by the loss of ramified microglia (to ameboid shaped cells)
103 [29] and a general increase in total MNPs. The increase in MNPs numbers is due to microglia
104 proliferation, and monocyte infiltration in certain conditions [5, 30]. A subpopulation of Cd11c^{+}
105 MNPs was also described [31, 32]. Histological studies of human postmortem retinas suggest
106 that subretinal MNPs are invariably present in intermediate and advanced (or 'late') forms of
107 AMD [6, 19, 33-35]. A pronounced presence of subretinal MNPs has also been documented in
108 mouse mutants with accelerated age-associated photoreceptor degeneration [14], RPE injury
109 model [36] and laser-induced choroidal neovascularization (CNV), an experimental model for
110 wet AMD [37]. Subretinal MNPs have also been described in human postmortem IRD subjects
111 [18], and in adult rodents with IRD mutations [3, 7, 38, 39], as well as other injury models [7,
112 11]. In fact, the presence of subretinal MNPs in IRDs was appreciated as early as the 1970's
113 using Royal College of Surgeons rats that have dystrophic retinas [40]. The cause of this
114 degenerative condition was later determined as a *Mertk* mutation that renders RPE incapable
115 of phagocytosing spent photoreceptor discs [41], and *Mertk* mutation was also found in human
116 IRDs [42]. However, it took several more decades before the distinction between endogenous
117 microglia and recruited MdCs was definitively made [1, 9].

118 An important advance in the field was reported in 2007 [14]. In this study, the authors
119 demonstrated the association of a variant of *CX3CR1* with AMD (in humans), later supported by

120 a meta-analysis [43]. They also showed that *Cx3cr1*-deficient mice develop age-related MNP
121 infiltration of the SRS, accompanied by photoreceptor degeneration and exaggerated CNV.
122 Given that *Cx3cr1* is a homeostatic microglial checkpoint gene of MNPs [44], this phenotype
123 revealed a link between intrinsic MNP dysregulation and disease pathogenesis. In 2013, the
124 same group showed that AMD patients are characterized by increased intraocular CCL2
125 concentrations and that MdCs are present in the SRS of intermediate and late AMD patients, as
126 well as in *Cx3cr1*-deficient mice, where they were shown to mediate photoreceptor toxicity [6].
127 Importantly, the authors demonstrated that in mouse models, two key human AMD genetic risk
128 gene variants, the Apolipoprotein E2 isoform (*APOE*) and Complement Factor H variant Y402H
129 (*CFH*) directly promote pathogenic inflammation in the SRS [26, 27]. In sum, these findings
130 established a link between certain AMD-risk variants and subretinal MdC infiltration, and in
131 turn, the possible pathogenic role of these cells in AMD (**Fig.2a**).
132 In 2019, another advance in understanding the cellular make-up and contribution of
133 subretinal MNPs in degeneration was reported [3]. Building on their early study [11] and
134 converging subsequent work from another group [36], the authors applied *Cx3cr1*$^{CreER/+}$
135 microglial lineage tracing mice and single-cell RNA-seq to decipher the ontogeny and function
136 of subretinal MNPs in the *Cx3cr1*-sufficient setting. In a humanized IRD model [45] and in the
137 toxic light-induced photoreceptor degeneration model (LD) [46], microglia were found as the
138 dominant MNP lineage which occupies the SRS, whereas recruited MdCs were largely confined
139 to the parenchyma. Moreover, the authors provided direct evidence that these subretinal
140 microglia restrict but not accelerate disease progression. Identification of transcript-specific
141 inductions of subretinal microglia now opens the door to future elucidation of these disease
142 restricting activities **(Fig.2b)**. Collectively, these studies demonstrate the significance of
143 microglia in the SRS of retinal degeneration models and showed that these microglia are not
144 pathogenic, but rather are involved in restricting disease progression.
145 The following two sections will discuss in detail the evidence leading to the view taken
146 in the current review that MdCs and microglia in the SRS play distinct (and perhaps opposite)
147 roles in photoreceptor degenerative diseases.

148 ## Pathogenic Role of MdCs in the Subretinal Space

149 Infiltration of MNPs in wet AMD (and laser-induced experimental CNV) characterized by a
150 breach of the BRB (edema and subretinal hemorrhages), has long been recognized as an
151 important factor of the pathogenesis (reviewed in [33]). In contrast, in slowly evolving
152 geographic atrophy (GA) and IRDs, MNPs, in particular MdCs, were not commonly assumed to
153 infiltrate the photoreceptor cell layer or inner retina, as no clinical signs of BRB rupture are
154 visible. Over recent years, it has become increasingly clear that MNPs, including MdCs, infiltrate
155 the outer retina in intermediate AMD and GA [33]. However, it is still passionately debated
156 whether and how the MNP infiltration influences CNV and degeneration.

157 ### *Evidence for pathogenic subretinal MNP accumulation*

158 One of the first pieces of evidence that MNPs could be more than bystanders in retinal
159 degeneration came from the study of *Cx3cr1*-deficient mice. The Cx3cr1 receptor is strongly
160 expressed by MNPs, where it mediates a tonic inhibitory signal, but not on neurons or the RPE
161 in the adult retina. *Cx3cr1*-deficient mice develop accelerated and exaggerated subretinal MNP
162 accumulation in conditions that do not trigger inflammation in wildtype mice (such as 12-18m
163 old mice under normal light conditions, and a non-toxic light-challenge that does not induce
164 degeneration in pigmented wildtype mice) [6, 14, 26, 27]. In aged *Cx3cr1*-deficient mice, the
165 prolonged presence of MNPs in the SRS and their continued ingestion of lipid-, retinol-rich
166 photoreceptor outer segments [14] leads to sizeable, 'foam cell' macrophages that are visible *in*
167 *vivo* by clinical (fundus) examination [47] and share a similar appearance with pseudodrusen in
168 AMD (**Box 1**). Interestingly, pseudodrusen are also associated with subretinal MNPs in patients
169 [28]. Importantly, this age-dependent accumulation of subretinal MNPs in *Cx3cr1*-deficient
170 mice is associated with a significant degeneration of rods and cones [6, 14, 26, 27, 34, 48], but
171 not with RPE atrophy (**Fig.2A**). They therefore model aspects of a subtype of GA called
172 incomplete outer retinal atrophy [49].

173 Mechanistically, *Cx3cr1*-deficient MNPs are characterized by an over-expression of
174 APOE. The APOE excess activates toll-like receptor (TLR) signaling on MNPs in the absence of
175 TLR ligands, damage- and pathogen-associated molecular patterns (DAMPs and PAMPs) [35].
176 Normally, in the absence of DAMPS and PAMPS, TLR2 and TLR4 are separated from their co-

177 receptor CD14 as they locate to non-raft and raft plasma membrane respectively. TLR ligands
178 overcome this separation, as they bind to CD14 and TLR2/4, inducing dimerization and
179 intracellular NF-κB activation and cytokine expression. Excessive APOE-concentrations extract
180 cholesterol from the lipid rafts of the plasma membrane of MNPs [33, 50], which levitates the
181 separation of TLR and CD14. The receptors dimerize and trigger intracellular NF-κB activation in
182 the absence of ligands (**Fig.2A'**) [33]. A similar mechanism has been previously described for
183 APOA1 [51]. The resulting increased secretion of IL-6 and CCL2 observed in subretinal *Cx3cr1*-
184 deficient MNPs reduces their RPE-induced elimination and increases monocyte recruitment,
185 respectively (see below) [14, 35]. Additionally, *Cx3cr1*-deficient MNPs overexpress P2rx7, which
186 constitutively activates their inflammasome and facilitates IL-1β maturation and secretion [48,
187 52]. The increased IL-1β secretion resulting from this dysregulation mediates rod and cone
188 degeneration, and significantly increases CNV [34, 48, 53] (**Fig.2A**). In sum, *Cx3cr1* deficiency is
189 sufficient to trigger a pathogenic non-resolving subretinal inflammation, and in *Cx3cr1*-deficient
190 mice, deletion of *Apoe* prevents subretinal accumulation of MNPs and attenuates
191 photoreceptor degeneration and CNV after laser-injury [35].
192 Of note, the *Cx3cr1*-deficient mice used in these studies did not carry the spontaneous
193 frameshift mutation in *Crb1* (also known as *rd8*) found in C57BL6/N mice that can cause
194 photoreceptor degeneration and spontaneous CNV, and is likely responsible for the AMD-like
195 phenotype described in a number of published studies using inadvertently contaminated
196 mouse strains [54].
197 In summary, it can be argued that *Cx3cr1*-deficiency represents a model of 'primary'
198 subretinal inflammation. For this concept, *Cx3cr1*-deficient -MNPs 'overreact' to a below-
199 threshold perturbation (e.g. aging, or a non-toxic light challenge [6, 14]), which involves
200 subretinal MdC infiltration that causes retinal degeneration. In 'secondary' inflammation, which
201 is an inflammatory response caused by a supra-threshold insult that induces inflammation in a
202 wildtype animal (such as in the laser CNV or photoreceptor degeneration models) *Cx3cr1*-
203 deficiency exacerbates inflammation and disease progression, observed in laser-induced CNV
204 model [14], paraquat-induced retinopathy [55], and *rd10* mice [56, 57] (autosomal recessive
205 IRD model).

206 *AMD risk factors promote subretinal pathogenic inflammation*

207 AMD is a highly heritable disease and several haplotypes encoding for protein variants have
208 been established as risk-determinants in AMD. An isoform of *APOE*, along with a common
209 variant of *CFH* accounts for a substantial part of the genetic risk [58, 59].

210 With respect to *APOE*, carriers of the *APOE2*-isoform, which is associated with higher
211 APOE concentrations, are at increased risk for AMD, whereas carriers of the APOE4-isoform,
212 associated with lower APOE concentrations and impaired cholesterol transport, are protected
213 against AMD compared to the common *APOE3*-allele [60, 61]. The MNPs of transgenic mice
214 expressing human *APOE2* (*TRE2*-mice) were shown to express high levels of APOE that activate
215 TLR signaling and induce inflammatory cytokines, similar to the phenotype seen in the
216 aforementioned *Cx3cr1*-deficient MNPs. Analogously to *Cx3cr1*-knockout mice, TRE2-mice
217 develop age- and stress-related pathogenic subretinal MNP accumulation [26, 35]. In *Cx3cr1*-
218 deficient mice, in the context of APOE-dependent subretinal inflammation, the *APOE4*-allele led
219 to diminished APOE and CCL2 levels and protected against harmful subretinal MNP
220 accumulation observed in *Cx3cr1*-deficient *APOE3*-allele carrying mice [26] (**Fig.2A'**). .

221 As mentioned, a variant of CFH has also been linked to AMD. CFH binds to the integrin
222 CD11b/CD18 complex that is strongly expressed on MNPs, and this complex was shown to curb
223 TSP-1 activation of its integrin-associated CD47 receptor that is necessary for MNP elimination.
224 The AMD-associated CFH(H402) variant markedly increases this effect, promoting chronic
225 subretinal inflammation in mouse models and in vitro settings [27] (**Fig.2A''**).

226 Taken together, these results show that APOE2 and the CFH(H402) variant promote
227 subretinal pathogenic inflammation. In other words, these two causative genetic AMD risk
228 variants, essential triggers for disease development, promote subretinal MNP accumulation and
229 associated degeneration in mouse models. This provides strong evidence, we would argue, to
230 implicate a pathogenic role for subretinal MNP accumulation in AMD.

231 *Evidence implicating subretinal MdCs as a major driver for AMD pathology*

232 The approaches used in most studies so far did not enable differentiating between MdCs and
233 microglia, as non-specific markers were used for their identification (**Table 1**). The receptor
234 CCR2, strongly expressed on inflammatory monocytes, constitutes one of the few specific

235 markers that distinguish monocytes and early MdCs, as it is not expressed by microglia [6, 62,
236 63]. Studies of postmortem human sections showed that CCR2$^+$ MdCs invariably participated in
237 the SRS in intermediate AMD and GA [6]. Additionally, increased intra-ocular levels of CCL2, a
238 cytokine that recruits CCR2$^+$ monocytes to the tissue [62], were found in neovascular [64-69]
239 and atrophic-AMD [6, 68].

240 Experimentally, *Cx3cr1*-deficient MNPs overexpress CCL2 in aged and light-challenged
241 mice [6]. After a light-challenge of *Cx3cr1*$^{GFP/GFP}$*Ccr2*$^{RFP/+}$ mice that express RFP under the *Ccr2*
242 promoter, around 10% of subretinal MNPs were RFP$^+$ and therefore monocyte-derived [6].
243 However, *Ccr2* is quickly down-regulated when monocytes differentiate to macrophages [3, 6,
244 30, 70], which leads to an underestimation of MdC numbers. To overcome this, monocytes and
245 their derivative MdCs were permanently labeled by repeated systemic injections of the
246 traceable nucleotide 5-ethynyl-2′-deoxyuridine (EdU). These experiments revealed that ~50% of
247 the subretinal MNPs are MdCs [6]. Monocyte depletion with systemic clodronate liposome
248 completely prevented EdU$^+$ MdC recruitment. Concordantly, deletion of *Ccl2* or *Ccr2,* inhibition
249 of Ccr2, diminished the subretinal MNPs by ~50-60% in acute and chronic models. Interestingly,
250 the inhibition of CCR2$^+$-monocyte recruitment in *Cx3cr1*-deficient mice nearly completely
251 prevented their inflammation-associated photoreceptor degeneration, suggesting that MdCs
252 are responsible for the inflammation-induced degeneration. Similar results were observed in
253 the carboxyethyl pyrrole immunization-induced AMD model [71].

254 In laser-induced CNV, MdCs participate significantly in subretinal MNP accumulation as
255 determined by *in vivo* imaging of mice injected with an intra-peritoneal or subcutaneous depot
256 tracer that labels monocytes and MdCs [72], and in bone marrow transplantation experiments
257 [37, 73]. Functionally, the depletion of circulating monocytes [73-75] and the inhibition of
258 monocyte-recruitment by genetic deletion of Ccr2 and Ccl2 [76-78], significantly inhibited
259 subretinal MNP accumulation and CNV formation [75].

260 In IRDs, the participation of MdCs seems to largely depend on the underlying genetic
261 defect and possibly the intensity and speed of the degeneration. For example, *Ccr*2 deletion
262 diminished retinal MNP infiltration, yet had a minor protective effect in progressively
263 degenerating *rd10* mice [79]. The faster degeneration *Arrestin-1* (*Arr1*) knockout mice could not

264 be rescued when monocyte recruitment was prevented by CCL2 inhibition [7]. However, in
265 *Mertk*$^{-/-}$ mice, which lack this important phagocytosis receptor on the RPE but also on MNPs,
266 there is evidence that MdCs may be recruited to the SRS in these mice [38], and *Ccl2* deletion
267 significantly protected *Mertk*$^{-/-}$ mice from degeneration [80].

268 Taken together, MdCs appear to participate with varying degrees in IRD models,
269 whereas they play a strong role in 'primary' inflammation, where the genetic variant primarily
270 activates MNPs. Indeed, in models of primary inflammation such as *Cx3cr1*$^{-/-}$ mice and
271 transgenic TRE2-mice, subretinal MdCs have been shown to be the main culprits of
272 photoreceptor degeneration. The fact that well-established causative AMD-risk variants results
273 in subretinal MdC accumulation in these models emphasizes the role of the AMD-risk variants
274 in inflammation and the role of MNPs, in particular MdCs, in AMD. In IRDs, this might not
275 necessarily be the case and additionally depend on the patients' genetic variants affecting MNP
276 function.

277 ## Protective Role of Microglia in the Subretinal Space

278 With the new appreciation in the late 2000's that microglia and MdCs represent distinct
279 lineages [1] and early studies pointing to their possible differential functions in experimental
280 autoimmune encephalomyelitis (EAE) and other models of CNS conditions [81-83], a pressing
281 need had emerged for new tools to distinguish these two cell types. A breakthrough came in
282 2013 with the development of *Cx3cr1*^{CreER} knock-in mice [9, 84, 85], now enabling conditional
283 genetic manipulation of microglia by taking advantage of their long half-lives and self-
284 renewability [84]. Importantly, these mice could be used to decipher the ontogeny of microglia
285 versus MdCs in disease, to conditionally knockout genes, or to selectively deplete microglia
286 when paired with inducible diphtheria toxin receptor (DTR) gene (**Table 1**). In the following
287 sections, we review recent evidence from studies leveraging this lineage tracing technique in
288 the Cx3cr1-sufficient mice indicating differential distributions of microglia and MdCs in retinal
289 degeneration models, and we further discuss new evidence implicating a cytoprotective role for
290 microglia in the SRS.

291 *Spatial Distribution of MNP lineages in Retinal Degenerative Disease Models*

292 A 2016 paper reported one of the first studies that applied *Cx3cr1*$^{CreER/+}$ mice to decipher *in situ*
293 spatial distributions of retinal microglia versus MdCs during disease [11]. The authors used
294 confocal microscopy to examine retinas from *Cx3cr1*$^{CreER/+}$*R26*^{tdTomato} mice subjected to the LD
295 model. This approach allowed microglia to be traced as YFP$^+$ tdTomato$^+$ and MdCs as YFP$^+$
296 tdTomato$^-$. Notably, microglia and MdCs were found to be largely distributed in distinct areas of
297 the retina: microglia predominantly migrated to the SRS, whereas monocytes poured into the
298 neuroparenchyma (presumably via retinal vasculature [7]). This finding was subsequently
299 recapitulated by another group in the model of $NaIO_3$-induced RPE injury, showing microglial
300 dominance of the SRS through 30 days post injury [36], and by another study using human
301 rhodopsin P23H knock-in mice [3] (this rhodopsin mutant serves as a model of a major
302 autosomal dominant form of IRD [45, 86]). Collectively, these studies revealed a spatial
303 distinction between microglia and MdCs in the Cx3cr1-sufficient setting, and pointed to a
304 potentially important role for microglia within the SRS in disease.

305 More comprehensive studies dissecting the isolated migration patterns of microglia
306 were carried out in a series of *Cx3cr1*$^{CreER/+}$*R26*^{DTR} mouse experiments [3]. These subretinal
307 microglia (YFP$^+$ DTR$^+$) in degeneration were efficiently depleted by DT administration, indicating
308 that they are indeed DTR-expressing microglia. By contrast, MdCs (YFP$^+$DTR$^-$) still infiltrated the
309 retina, but they were largely located in the parenchyma and incapable of migrating to the SRS,
310 even in the absence of microglia. It remains unclear whether this monocyte infiltration reflects
311 a homeostatic response intended to replace evacuated microglia from the plexiform layers. We
312 argue that this is unlikely, as neither microglia depletion, nor reduced microglia number in *Il34*
313 KO mice, results in monocyte recruitment in steady state [16, 87, 88]. The separate question as
314 to whether microglia depletion in retinal degeneration models leads to a reduced monocyte
315 infiltration was not addressed either, as other glia and retinal neurons express substantial levels
316 of Ccl2 [89]. Collectively, the findings from the depletion experiments suggest that microglia do
317 not outcompete MdCs for the SRS but have restricted access to it in the LD and P23H models.
318 This is also likely the case in the $NaIO_3$-induced RPE injury model [36]. Whether this pattern can

319 change with disease progression or when there is a breach of Bruch's membrane such as in
320 neovascular ('wet') AMD requires further investigation.

321 Further support of the concept that microglia may have restricted access to certain sites
322 can be gleaned from elsewhere in the CNS. For instance, there is a spatial distinction of
323 microglia versus MdCs in Alzheimer's disease (AD) with respect to amyloid-β plaques in the
324 cortex and hippocampus. Two independent studies demonstrated that unlike endogenous
325 microglia, peripheral-derived myeloid cells do not cluster around these plaques and fail to
326 modify amyloid load in the APP23 and APP/PS1 transgenic mouse models of AD [90, 91]. This
327 close spatial association between microglia and amyloid plaques was recently corroborated
328 using *Cx3cr1*$^{CreER/+}$ lineage tracing in 5xFAD transgenic mice [92]. Another example of microglia-
329 restricted access might also be gleaned in the normal mouse brain, where a distinctive
330 microglial population, but not the choroid plexus macrophage population, was found to reside
331 on the apical epithelium of the choroid plexus [93]. Together, these reports reveal restricted
332 microglial access to specific parenchymal or non-parenchymal niches throughout the CNS.

333 *Reprogramming of Subretinal Microglia*

334 With this characterization of subretinal microglia in degenerative conditions, the question of
335 their functional significance was now raised. This was addressed through single cell RNA-seq
336 analysis of *Cx3cr1*$^{YFP+}$ cells sorted from retinas, which led to the observation that subretinal
337 microglia have a unique transcriptomic signature [3]. Compared to microglia in homeostatic
338 conditions, subretinal microglia in LD had upregulated expression of genes encoding for
339 proteins characterized by antioxidant and lipid activity (e.g. *Srxn1*, *Apoe*, *Lpl, Abca1*), and
340 downregulated expression of genes related to immune responses (e.g. *Ifngr1*, *C1qa*, *Aif1, Jun*).
341 This profile suggests that subretinal microglia can respond to increased levels of oxidative stress
342 and extracellular lipids, which are typical characteristics of a neurodegenerative environment,
343 and to do so in a manner that involves reduced pro-inflammatory responses (**Fig.2B**). In
344 addition, downregulation of *Abca1* through senescence impairs cholesterol efflux by
345 macrophages and promotes disease in the CNV model [94]. Separately, subretinal microglia
346 upregulate *Ifnar1* expression, the loss of which increases lesion size and vascular leakage in the
347 CNV model [95]. Interestingly, there is additional evidence that some elements of this microglial

348 reprogramming are relevant in other retinal disease settings. A recent study showed the most
349 highly expressed subretinal microglial genes, namely *Cd68* and *Lgals3*, were also prominent in a
350 model of autoimmune uveoretinitis (an ocular autoimmune disease)[96]. However, in contrast
351 to the LD model, both subretinal microglia and parenchymal microglia highly expressed these
352 gene markers, indicating that microenvironmental cues specific to the SRS can be indispensable
353 in inducing some of these gene changes.

354 In the broader context of neuroimmunology, questions regarding protective *versus*
355 pathogenic roles of microglia continue to be intensely debated. Further arguing for a protective
356 role of subretinal microglia, in our view, is that the transcriptional profile of these cells exhibits
357 similarities to disease-associated microglia (DAM). DAM cells were first described as a subtype
358 of microglia that surrounds brain amyloid-β plaques in the 5xFAD model of AD and restricts
359 disease progression in a *Trem2*-dependent fashion [97]. Overlapping expression signatures
360 between subretinal microglia and DAM cells include increased expression of *Lgals3, Lpl, Spp1,*
361 *Apoe, Trem2, and Cstb*, and downregulation of *Tmem119, P2ry12, Cx3cr1* and *Siglech.*
362 Interestingly, a related microglia gene signature was reported during normal postnatal brain
363 development [98], which was also thought to be crucial for normal RGC development [99]. It is,
364 therefore, tempting to hypothesize that the DAM phenotype represents a stereotypic response
365 which supports physiological processes under environments with substantial cell death, as
366 shared in postnatal development and intermediate to advanced stages of certain
367 neurodegenerative diseases. However, further work is needed to fully address the functional
368 significance of the DAM signature in various conditions. As one example, a similar microglial
369 gene profile was reported in the EAE model, and in this context, the DAM signature as a
370 pathological response could be argued [100].

371 *RPE and Photoreceptor Protection by Microglia*

372 The transcriptional reprogramming of subretinal microglia raises the question as to the isolated
373 role of microglia in retinal pathobiology. $Cx3cr1^{eYFP\text{-}CreER/+}R26^{DTR}$ mice were used for conditional
374 depletion of microglia prior to exposure to LD, or in P23H mice prior to when major
375 degeneration takes place to address this question [3]. The absence of microglia resulted in
376 massive structural damage of the RPE, thereby directly linking their subretinal localization and

377 transcriptional reprogramming with RPE protection (**Fig.2B**). Moreover, the damage of RPE in
378 this depletion setting included both lateral and/or apical defects to F-actin, resembling key
379 features of RPE dysmorphogenesis in human AMD and certain forms of IRDs [101]. It is
380 noteworthy that DT-mediated depletion has some technical caveats to consider, such as the
381 possible elicitation of pro-inflammatory cytokines shown following brain microglial depletion
382 [87], or perhaps a damaging effect should the RPE be involved in phagocytosing DT-laden
383 apoptotic microglia, although the latter has not been shown. However, indicating such
384 potential caveats are insufficient to induce RPE dysmorphology is that microglia depletion in the
385 steady state does not result in this disease phenotype [3]. Separately, supporting the conclusion
386 that subretinal microglia are protective, RPE dysmorphology is recapitulated in the LD model
387 with reduced microglial numbers in *Il34*-deficient mice [3]. Moreover, the aforementioned
388 transcriptomic findings also corroborate this protective phenotype. Collectively, as a better
389 preserved RPE is generally thought to help maintain the function and survival of
390 photoreceptors, these findings suggest that subretinal microglia restrict disease progression.
391 Still, the question remained as to how microglia confer protection to the RPE. One
392 possible clue in this direction was the accumulation of dead photoreceptors and debris in the
393 SRS in the absence of microglia [3]. Interestingly, as MdCs are spared in this system (as likely are
394 astrocytes and Müller glia), the most parsimonious interpretation, in our view, is that microglia
395 play a role in debris clearance, whereas monocytes less so. Similar microglial protective roles
396 were reported in several other retinal disease models, including retinal detachment [102], RGC
397 excitotoxicity [103], and progressive rod-cone degeneration [104]. A recent study on
398 complement component 3- (C3) and its receptor CR3- dependent phagocytosis supports a
399 similar protective role for microglia in an IRD model of *rd10* mice [105], although there are also
400 reports suggesting that C3 (and C1q) are pathological in LD [106, 107]. Likewise, a microglia-
401 dominant clearance of neuronal debris was described in other models of neurodegeneration,
402 such as optic nerve crush [108] and EAE mice [81]. Hence, we surmise that the failure to clear
403 the photoreceptor debris in the SRS may have cytotoxic effects, potentially explaining the
404 observed RPE defect. Contrasting with this view, a study reported increased photoreceptor
405 survival when using continual tamoxifen administration in *Cx3cr1*^{CreER}*R26*^{DTA} mice on the *rd10*

406 background to deplete microglia [39]. However, the specter of whether tamoxifen
407 administration *per se* was responsible for this phenotype was raised following the report that
408 tamoxifen alone rescues degeneration in *rd10* mice [109].

409 In summary, these studies demonstrate that in *Cx3cr1*-sufficient mice, the SRS is a
410 microglia-dominant macrophage-niche in several models of retinal degeneration, where few
411 subretinal MdCs were observed. The net-effect of *Cx3cr1*-sufficient microglia during LD and
412 P23H degeneration, we would argue, is the restriction of disease progression. Evidence
413 suggests that this protective response is mediated in the SRS via swift clearance of otherwise
414 toxic debris, but whether there are other processes involved in protection or pathogenesis
415 remains to be further explored.

416 Unified MNP Model in Retinal Degenerative Diseases

417 From these studies we propose a new conceptual framework to help understand the roles of
418 MNPs in retinal degeneration. Though the model we put forth here is based on conclusions
419 made in different experimental settings, our thinking stems from the current knowledge of
420 *Cx3cr1* and other homeostatic checkpoint genes (reviewed in [44]). In *Cx3cr1*-sufficient mice,
421 microglia have a beneficial net-effect in retinal degeneration through performing functions such
422 as controlled clearance of otherwise toxic neuronal debris [3, 11, 110]. By contrast, in *Cx3cr1*-
423 deficient mice, microglia are primed to drive responses that have a harmful net-effect, such as
424 uncontrolled proinflammatory cytokine production and further recruitment of inflammatory
425 MdCs [6, 13]. From this distinction we can categorize MNP responses as beneficial/'adaptive'
426 versus harmful/'maladaptive' and further that certain signaling factors, such as Cx3cr1, can 'tip
427 the scale' from the former type of response to the latter [6, 14] (**Fig.3**).

428 Extrapolating this model to human disease, it can be argued that certain genetic variants
429 that confer risk in developing AMD could serve as such factors that help drive maladaptive
430 responses. Indeed, *CX3CR1* polymorphisms T280M or V249I in humans have been considered to
431 be a risk factor in AMD, which may be dependent on other factors or independent from them
432 [43, 111]. Other major risk factors, including certain *APOE2* isoforms and the *CFH402H* variant,
433 may also be involved in driving harmful MNP responses [26, 27]. Likewise, *TGFBR1*
434 polymorphisms identified in GWAS studies that confer AMD risk [112] may act in a similar

435 manner. TGFBR1 is phosphorylated by TGFBR2 in order to propagate TGF-β1 signaling [113],
436 which in turn maintains microglial identity and function [114, 115]. Indeed, conditional deletion
437 of *Tgfbr2* in microglia was shown to cause a degenerative disease phenotype in mice [116].
438 These respective genetic factors might not be sufficient by themselves to drive harmful MNP
439 responses, but can possibly do so when combined with non-genetic factors that confer risk in
440 AMD (e.g., advanced age, high fat and high cholesterol diets, and smoking). Different from
441 those in AMD, evidence in mouse models suggest that MNPs are not driven into a maladaptive
442 state in IRDs that have a monogenic Mendelian etiology involving genes of the visual system
443 [117]. One exception may be some IRDs caused by *MERTK* mutations, a gene that regulates
444 phagocytosis of the RPE and MNPs such as microglia.

445 There are limitations and unresolved questions concerning this proposed model.
446 Regarding the former, it is clear that not all activities in either *Cx3cr1*-sufficient or -deficient
447 settings are binary, i.e. solely beneficial or harmful. For instance, microglial migration out of
448 plexiform layers in order to take up the SRS is likely to cause synaptic deficits at the inner retina
449 [3, 118]. Likewise, certain situations exist in which a robust inflammatory response could be
450 categorized as adaptive rather than maladaptive, such as in ocular infections or some
451 malignancies from the perspective of protecting host survival. In terms of open questions, it is
452 not fully understood how immune checkpoint genes regulate the transition between adaptive
453 and maladaptive responses. For instance, *Cx3cr1* deletion primes the maladaptive response,
454 while downregulation of this gene in subretinal microglia is associated with protection.

455 Concluding Remarks

456 Whether MNPs which ectopically accumulate in the SRS in photoreceptor degeneration
457 contribute to, or restrict disease pathology, is a controversial topic. We argue that the answer
458 can be either/or, depending on the responding MNP cell lineage and the impact of other
459 factors. In mice, evidence indicates that endogenous microglia that migrate to the SRS function
460 in restricting disease progression, whereas MdCs there are pathologic. Still, there are
461 **Outstanding Questions** that will need to be addressed to better understand this proposed
462 dichotomy. Also, the potential factors that lead to one fate or the other in the context of
463 human AMD have yet to be elucidated. Addressing this knowledge gap is a future challenge

that involves the development of animal models, which incorporate genetic and non-genetic risk factors of AMD, paired with lineage tracing applications to distinguish the two MNP cell types. For human postmortem retinas, the lack of phenotypic markers to discern microglia and MdCs means that future efforts will have to rely on single cell RNA seq applications. Such continued research is critical given the current immense societal burden of retinal degenerative diseases.

Acknowledgement

This work was supported by grants from NIH R01EY021798, NIH P30EY005722, Bright Focus MDR, Research to Prevent Blindness (Unrestricted) for Saban. For Sennlaub, INSERM, ANR MACLEAR (ANR-15-CE14-0015-01), LABEX LIFESENSES [ANR-10-LABX-65] supported by the ANR (Programme d'Investissements d'Avenir [ANR-11-IDEX-0004-02]), BrightFocus Foundation grant (M2018096), AVIESAN-UNADEV Maladies de la Vision 2018-2019 (N°UU159-00-C18/1616, N°UU113-00-C17/2086), Carnot, and a generous donation by Doris and Michael Bunte.

Declaration of Interests

The authors declare no competing interests.

Glossary

- **Blood retinal barrier (BRB)**: the physiological barrier that mediates selective diffusion of molecules and regulates infiltration of cells from the blood to the retina. The BRB is comprised of both an inner and an outer barrier. The inner BRB is formed by tight junctions between vascular endothelial cells, together with pericytes, perivascular macrophages as well as processes of astrocytes and Muller cells, whereas the outer BRB is primarily formed by the retinal pigment epithelium.
- **Drusen**: the extracellular deposits that build up between the retinal pigment epithelium and Bruch's membrane. Drusen is primarily composed of lipids and lipoproteins and is a risk factor of developing AMD. Reticular drusen, also known **Pseudodrusen**, is the subretinal drusenoid deposits.
- **Geographic atrophy (GA)**: the late-stage of atrophic age-related macular degeneration (also known as dry AMD), in which the patches of RPE loss become confluent.
- **Retinal pigment epithelium (RPE)**: a polarized monolayer of epithelial cells located between retinal parenchyma and choroids. Apart from its role as the barrier, the RPE is essential for function and renewal of photoreceptors. Dysfunction of the RPE can cause vision loss, and its death along with photoreceptor loss are pathological hallmarks of atrophic age-related macular degeneration.
- **Subretinal space (SRS)**: the anatomical site where photoreceptor outer segment tips and the RPE are closely opposed or in contact. This site is important for a variety of molecular and cellular activities of the photoreceptors and the RPE. Physiologically, it is devoid of immune cells. In photoreceptor degeneration, MNPs accumulate there with the progression of disease.

Box 1. IRDs and AMD

Inherited retinal degenerations (IRDs) and age-related macular disease (AMD) are both characterized by the degeneration of rods, cones, the RPE, and the choroid, while the overlying inner retina remains morphologically intact to a certain extent. However, from an etiological and symptomatic standpoint, IRDs and AMD are very different. IRDs are rare (1/4000) monogenetic diseases [117], most often due to mutations of rod genes (rod-cone dystrophy). The lack of the essential gene (recessive e.g. phosphodiesterase 6B, *rd10* mutation) or the

513 accumulation of a dysfunctional variant (dominant e.g. rhodopsin P23H mutation) leads to rod
514 cell death and night blindness (age of onset 10-20 years), followed by a centripetal
515 degeneration of the RPE and cones that lead to tunnel vision and eventually blindness. AMD is a
516 late onset (above 55 years), prevalent, complex and multifactorial disease caused by the
517 interplay of age, environmental, and genetic risk factors. The common AMD risk variants are
518 not specific for the RPE or photoreceptors but instead concern factors of the complement
519 cascade and lipoproteins, among others [112, 119]. Early AMD is characterized by
520 lipoproteinaceous debris in the subretinal space (termed pseudodrusen, reviewed in [120]) or
521 below the RPE (soft drusen). Advanced or 'late' debilitating AMD is characterized by central
522 choroidal neovascularization (wet or 'exudative' AMD, late form) and ultimately a disciform scar
523 (wet AMD end stage), or by a central, centrifugally extending lesion of the photoreceptors, RPE,
524 and choroid that often starts parafoveally (geographic atrophy, GA, late form [33]). Loss of rods
525 and to a lesser extent of cones, occurs prior to RPE loss above pseudodrusen [121] and in a
526 transitional zone adjacent to GA lesions [34] and disciform scars [122, 123]. Finally, the
527 degeneration of the RPE and choroid leads to the typical atrophic lesions in the fundus
528 appearance, which extends centrifugally. AMD only affects the central retina that contains the
529 macula and the cone-rich fovea, necessary for high acuity vision that does not exist in mice.
530

531 **Box 2. Monocyte Derived Cells in Health and Disease**

532 Monocytes are bone marrow-derived myeloid cells that circulate in the blood. Monocyte-
533 derived cells do not participate in the resident microglial pool of the retina and CNS. However,
534 they can be recruited during tissue damage or infection of any tissues including the retina and
535 other CNS tissues [62]. They comprise three major subsets (classical, intermediate and non-
536 classical) and are likely sequentially transitioning from one into another [124]. The short-lived
537 $CCR2^{+}CD14^{+}Ly6C^{+}$ classical monocytes constitute 90% of all circulating monocytes in humans
538 and 60-70% in mice. They circulate the blood for 24h before 99% of them disappear. About 1%
539 of classical monocytes evolve into longer-lived intermediate ($CCR2/CD14^{high}$ $CX3CR1/CD16^{high}$)
540 monocytes, which finally become $CCR2/CD14^{low}$ $CX3CR1/CD16^{high}$ 'patrolling' monocytes, also
541 referred to as non-classical monocytes [124]. During tissue injury, damage- and pathogen-
542 associated molecular pattern (DAMPs and PAMPs) activate resident macrophages and resident
543 mast cells (absent from the CNS including the retina, but present in the meninges and choroids)
544 that produce chemokines, such as CCL2 (the main ligand of CCR2), which recruit classical
545 monocytes that differentiate into anti-microbial and pro-inflammatory macrophages. These
546 cells release reactive oxygen species and complement components to kill, opsonize and
547 phagocytose pathogens and secrete inflammatory cytokines (such as IL-1β, TNF-α, IL-6, and
548 CCL2) that alter the tissue to facilitate the immune response [125]. Although these mediators
549 are crucial to combat infection and ensure the survival of the organism, they can also cause

considerable collateral damage, in particular in the CNS (including the retina) with poor regenerative potential [33]. Once the lesion is disinfected, the infiltrating macrophages produce cytokines such as VEGF that facilitate angiogenesis and tissue repair, scar formation, and inflammation resolution [126, 127]. Finally, pro-inflammatory macrophages disappear from the site and the tissue is left with the tissue-specific resident macrophages, as before the injury [128]. If the inflammatory response is not quickly controlled, it can become pathogenic and contribute to degeneration, pathological neovascularization and fibrosis, as seen in many diseases such as metabolic diseases (obesity, atherosclerosis), cancers and neurodegenerative diseases including in photoreceptor degeneration [125, 129-131].

Box 3. Retinal Microglial Roles in Visual Function

There is a growing appreciation for the role of microglia in adult retinal neurophysiology. Retinal microglia's contributions to visual processing in mice has been shown to vary according to their anatomical location [3]. Mice that lack of *Il34*, which is normally expressed by RGC, have a specific microglial loss in the IPL but not OPL. This IPL-specific defect is associated with a diminished electrophysiologic responses to light stimuli by cone bipolar cells, but normal photoreceptor responses [3]. *Il34* knockout mice have no obvious quantitative loss of synapses, thus pointing to a possible qualitative role for IL-34-dependent microglia in synaptic function within the IPL. Hence, this study established the existence of an IL-34 dependent pool of microglia restricted to the IPL that plays a key role in cone bipolar cell responses during visual processing. It was demonstrated in another study that transient microglial depletion leads to reduced electrophysiological responses to light stimuli in both photoreceptors and bipolar cells [118]. Transient microglial depletion does not result in major retinal morphological changes or neuronal loss [118]. Another study further showed that microglial repopulation after depletion restored the impaired visual functions [88]. Collectively, in addition to the RPE [132] and Muller glia [133], these studies identified microglia as another non-neuronal cells that are involved in neuro-retinal electrophysiology.

Table 1. Technical Challenges for Discerning MNP Lineages in Retinal Degeneration.

Tools	Pros	Cons	Refs
Marker staining			
Conventional markers, such as Iba-1, F4/80, Cd11b	• Labels the majority of MNPs	• Unable to distinguish microglia and MdCs • Some markers not specific for MNPs	[2]
Microglial specific markers, such as P2ry12, Tmem119	• Able to specifically label microglia in steady state	• May not distinguish microglia versus MdCs during neuroinflammation	[3, 97, 100]
Chimaeras			
Bone marrow chimera	• Full blood chimerism	• Side effects of irradiation and transplantation, such as temporary disruption of BRB and myelomonocytic engraftment in the CNS	[2]
Bone marrow chimera with lead head shielding	• Preservation of CNS in steady state	• Full blood chimerism is not achievable	[11]
Circulating monocyte-labeling			
Systemic 5-ethynyl-2'-deoxyuridine (EdU)	• A traceable nucleotide that is integrated into the DNA of dividing cells • Efficient labeling of short-lived circulating monocytes and MdCs but not long-lived microglia in steady state. • Nuclear stain, avoids confounding with (i) MNP auto-fluorescence and (ii) positivity due to transfer (phagocytosis) of reporter proteins	• Requires repeated injections; limited to use for short-term study but not chronic conditions. • Microglia may replicate during degeneration; local injections necessary for distinction	[6]
Intravenously injected fluorescent tracers	• A traceable fluorescent probe taken up by circulating monocytes and detectable in cells derived from these monocytes.	• Requires repeated injections; limited to use for short-term study but not chronic conditions.	[72]
Reporter mice			
Cx3cr1^{GFP} or *Cx3cr1*^{YFP}	• Heterozygotes are good for labeling MNPs • Homozygotes are a full deletion of *Cx3cr1* gene	• Unable to distinguish microglia and MdCs, as both express *Cx3cr1*	[11, 14, 36, 110]
Ccr2^{RFP}	• Heterozygotes are good for labeling classical monocytes • Homozygotes are a full deletion of *Ccr2* gene	• Isn't useful for labeling monocyte-derived macrophages, which lose *Ccr2* expression • Spares non-classical monocytes	[6, 7, 36]
Cx3cr1^{CreER} *R26*^{REPORTER}	• Tamoxifen treatment induces the expression of reporter and labels microglia and MdCs • The reporter expression in monocytes is 'washed out', as	• Other long-lived tissue-resident macrophages that maintain *Cx3cr1* expression may also be labeled	[9, 11, 36, 84, 85]

	they are quickly replaced by non-labeled cells once tamoxifen decayed		
Depletion / recruitment inhibition studies			
Csf1r antagonists	• Efficient depletion of Csf1r dependent macrophages • Easy to administer	• Indistinguishably ablate microglia and MdCs • Potential off-target effects on other tyrosine kinase receptors	[88, 102, 103]
Intravenous clodronate liposome	• Efficient depletion of monocytes and their derivatives	• Requires repeated injections, limited to use for short-term study but not chronic conditions. • Potential off-target effects	[6, 103]
$Cx3cr1^{CreER}$ $R26^{DTA}$	• Efficient depletion of MNPs • Does not require diphtheria toxin	• Indistinguishably depletes all *Cx3cr1* expressing MNPs • Require multiple tamoxifen administrations	[39, 118]
$Cx3cr1^{CreER}$ $R26^{DTR}$	• Efficient depletion of microglia following 'wash-out'	• Require tamoxifen and diphtheria toxin administration • Other long-lived tissue-resident macrophages may also be depleted	[3, 84]
Ccl2- and Ccr2-deletion / inhibition	• Efficiently inhibits exit of bone marrow classical monocyte into the blood	• Does not inhibit non-classical monocyte-recruitment	[6, 7, 73-80]

578

579 ## Reference

580 1. Ginhoux, F. et al. (2010) Fate mapping analysis reveals that adult microglia derive from
581 primitive macrophages. Science 330 (6005), 841-5.

582 2. Reyes, N.J. et al. (2017) New insights into mononuclear phagocyte biology from the visual
583 system. Nat Rev Immunol 17 (5), 322-332.

584 3. O'Koren, E.G. et al. (2019) Microglial Function Is Distinct in Different Anatomical Locations
585 during Retinal Homeostasis and Degeneration. Immunity 50 (3), 723-737 e7.

586 4. Goldmann, T. et al. (2016) Origin, fate and dynamics of macrophages at central nervous
587 system interfaces. Nat Immunol 17 (7), 797-805.

588 5. McMenamin, P.G. et al. (2019) Immune cells in the retina and choroid: Two different tissue
589 environments that require different defenses and surveillance. Prog Retin Eye Res 70, 85-98.

590 6. Sennlaub, F. et al. (2013) CCR2(+) monocytes infiltrate atrophic lesions in age-related macular
591 disease and mediate photoreceptor degeneration in experimental subretinal inflammation in
592 Cx3cr1 deficient mice. EMBO Mol Med 5 (11), 1775-93.

593 7. Karlen, S.J. et al. (2018) Monocyte infiltration rather than microglia proliferation dominates
594 the early immune response to rapid photoreceptor degeneration. J Neuroinflammation 15 (1),
595 344.

596 8. Liu, Z. et al. (2019) Fate Mapping via Ms4a3-Expression History Traces Monocyte-Derived
597 Cells. Cell 178 (6), 1509-1525 e19.

598 9. Yona, S. et al. (2013) Fate mapping reveals origins and dynamics of monocytes and tissue
599 macrophages under homeostasis. Immunity 38 (1), 79-91.

600 10. Gomez Perdiguero, E. et al. (2015) Tissue-resident macrophages originate from yolk-sac-
601 derived erythro-myeloid progenitors. Nature 518 (7540), 547-51.

602 11. O'Koren, E.G. et al. (2016) Fate mapping reveals that microglia and recruited monocyte-
603 derived macrophages are definitively distinguishable by phenotype in the retina. Sci Rep 6,
604 20636.

605 12. Lapenna, A. et al. (2018) Perivascular macrophages in health and disease. Nat Rev Immunol
606 18 (11), 689-702.

607 13. Cardona, A.E. et al. (2006) Control of microglial neurotoxicity by the fractalkine receptor.
608 Nat Neurosci 9 (7), 917-24.

609 14. Combadiere, C. et al. (2007) CX3CR1-dependent subretinal microglia cell accumulation is
610 associated with cardinal features of age-related macular degeneration. J Clin Invest 117 (10),
611 2920-8.

612 15. Lin, H. et al. (2008) Discovery of a cytokine and its receptor by functional screening of the
613 extracellular proteome. Science 320 (5877), 807-11.

614 16. Wang, Y. et al. (2012) IL-34 is a tissue-restricted ligand of CSF1R required for the
615 development of Langerhans cells and microglia. Nat Immunol 13 (8), 753-60.

616 17. Greter, M. et al. (2012) Stroma-derived interleukin-34 controls the development and
617 maintenance of langerhans cells and the maintenance of microglia. Immunity 37 (6), 1050-
618 1060.

619 18. Gupta, N. et al. (2003) Activated microglia in human retinitis pigmentosa, late-onset retinal
620 degeneration, and age-related macular degeneration. Exp Eye Res 76 (4), 463-71.

621 19. Lad, E.M. et al. (2015) Abundance of infiltrating CD163+ cells in the retina of postmortem
622 eyes with dry and neovascular age-related macular degeneration. Graefes Arch Clin Exp
623 Ophthalmol 253 (11), 1941-5.

624 20. Strauss, O. (2005) The retinal pigment epithelium in visual function. Physiol Rev 85 (3), 845-
625 81.

626 21. Finnemann, S.C. et al. (1997) Phagocytosis of rod outer segments by retinal pigment
627 epithelial cells requires alpha(v)beta5 integrin for binding but not for internalization. Proc Natl
628 Acad Sci U S A 94 (24), 12932-7.

629 22. Yu, C. et al. (2019) Annexin A5 regulates surface alphavbeta5 integrin for retinal clearance
630 phagocytosis. J Cell Sci.

631 23. Dransfield, I. et al. (2015) Mer receptor tyrosine kinase mediates both tethering and
632 phagocytosis of apoptotic cells. Cell Death Dis 6, e1646.

633 24. Griffith, T.S. et al. (1995) Fas ligand-induced apoptosis as a mechanism of immune privilege.
634 Science 270 (5239), 1189-92.

635 25. Wenkel, H. and Streilein, J.W. (2000) Evidence that retinal pigment epithelium functions as
636 an immune-privileged tissue. Invest Ophthalmol Vis Sci 41 (11), 3467-73.

637 26. Levy, O. et al. (2015) APOE Isoforms Control Pathogenic Subretinal Inflammation in Age-
638 Related Macular Degeneration. J Neurosci 35 (40), 13568-76.

639 27. Calippe, B. et al. (2017) Complement Factor H Inhibits CD47-Mediated Resolution of
640 Inflammation. Immunity 46 (2), 261-272.

641 28. Greferath, U. et al. (2016) Correlation of Histologic Features with In Vivo Imaging of
642 Reticular Pseudodrusen. Ophthalmology 123 (6), 1320-31.

643 29. Silverman, S.M. and Wong, W.T. (2018) Microglia in the Retina: Roles in Development,
644 Maturity, and Disease. Annu Rev Vis Sci 4, 45-77.

645 30. Ronning, K.E. et al. (2019) Molecular profiling of resident and infiltrating mononuclear
646 phagocytes during rapid adult retinal degeneration using single-cell RNA sequencing. Sci Rep 9
647 (1), 4858.

648 31. McPherson, S.W. et al. (2019) The retinal environment induces microglia-like properties in
649 recruited myeloid cells. J Neuroinflammation 16 (1), 151.

650 32. Dando, S.J. et al. (2019) Regional and functional heterogeneity of antigen presenting cells in
651 the mouse brain and meninges. Glia 67 (5), 935-949.

652 33. Guillonneau, X. et al. (2017) On phagocytes and macular degeneration. Prog Retin Eye Res
653 61, 98-128.

654 34. Eandi, C.M. et al. (2016) Subretinal mononuclear phagocytes induce cone segment loss via
655 IL-1beta. Elife 5.

656 35. Levy, O. et al. (2015) Apolipoprotein E promotes subretinal mononuclear phagocyte survival
657 and chronic inflammation in age-related macular degeneration. EMBO Mol Med 7 (2), 211-26.

658 36. Ma, W. et al. (2017) Monocyte infiltration and proliferation reestablish myeloid cell
659 homeostasis in the mouse retina following retinal pigment epithelial cell injury. Sci Rep 7 (1),
660 8433.

661 37. Caicedo, A. et al. (2005) Blood-derived macrophages infiltrate the retina and activate Muller
662 glial cells under experimental choroidal neovascularization. Exp Eye Res 81 (1), 38-47.

663 38. Kohno, H. et al. (2015) Expression pattern of Ccr2 and Cx3cr1 in inherited retinal
664 degeneration. J Neuroinflammation 12, 188.

665 39. Zhao, L. et al. (2015) Microglial phagocytosis of living photoreceptors contributes to
666 inherited retinal degeneration. EMBO Mol Med 7 (9), 1179-97.

667 40. Essner, E. and Gorrin, G. (1979) An electron microscopic study of macrophages in rats with
668 inherited retinal dystrophy. Invest Ophthalmol Vis Sci 18 (1), 11-25.

669 41. D'Cruz, P.M. et al. (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal
670 dystrophic RCS rat. Hum Mol Genet 9 (4), 645-51.

671 42. Gal, A. et al. (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal
672 dystrophy gene, cause retinitis pigmentosa. Nat Genet 26 (3), 270-1.

673 43. Zhang, R. et al. (2015) Associations Between the T280M and V249I SNPs in CX3CR1 and the
674 Risk of Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci 56 (9), 5590-8.

675 44. Deczkowska, A. et al. (2018) Microglial immune checkpoint mechanisms. Nat Neurosci 21
676 (6), 779-786.

677 45. Sakami, S. et al. (2011) Probing mechanisms of photoreceptor degeneration in a new mouse
678 model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin
679 mutations. J Biol Chem 286 (12), 10551-67.

680 46. Noell, W.K. et al. (1966) Retinal damage by light in rats. Invest Ophthalmol 5 (5), 450-73.

681 47. Raoul, W. et al. (2008) Lipid-bloated subretinal microglial cells are at the origin of drusen
682 appearance in CX3CR1-deficient mice. Ophthalmic Res 40 (3-4), 115-9.

683 48. Hu, S.J. et al. (2015) Upregulation of P2RX7 in Cx3cr1-Deficient Mononuclear Phagocytes
684 Leads to Increased Interleukin-1beta Secretion and Photoreceptor Neurodegeneration. J
685 Neurosci 35 (18), 6987-96.

686 49. Sadda, S.R. et al. (2018) Consensus Definition for Atrophy Associated with Age-Related
687 Macular Degeneration on OCT: Classification of Atrophy Report 3. Ophthalmology 125 (4), 537-
688 548.

689 50. Smoak, K.A. et al. (2010) Myeloid differentiation primary response protein 88 couples
690 reverse cholesterol transport to inflammation. Cell Metab 11 (6), 493-502.

691 51. Sullivan, P.M. et al. (2011) Reduced levels of human apoE4 protein in an animal model of
692 cognitive impairment. Neurobiol Aging 32 (5), 791-801.

693 52. Netea, M.G. et al. (2009) Differential requirement for the activation of the inflammasome
694 for processing and release of IL-1beta in monocytes and macrophages. Blood 113 (10), 2324-35.

695 53. Lavalette, S. et al. (2011) Interleukin-1beta inhibition prevents choroidal neovascularization
696 and does not exacerbate photoreceptor degeneration. Am J Pathol 178 (5), 2416-23.

697 54. Mattapallil, M.J. et al. (2012) The rd8 mutation of the Crb1 gene is present in vendor lines of
698 C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes.
699 Invest Ophthalmol Vis Sci 53, 2921-7.

700 55. Chen, M. et al. (2013) Paraquat-Induced Retinal Degeneration Is Exaggerated in CX3CR1-
701 Deficient Mice and Is Associated with Increased Retinal Inflammation. Invest Ophthalmol Vis Sci
702 54 (1), 682-90.

703 56. Zabel, M.K. and Kirsch, W.M. (2013) From development to dysfunction: microglia and the
704 complement cascade in CNS homeostasis. Ageing Res Rev 12 (3), 749-56.

705 57. Peng, B. et al. (2014) Suppression of microglial activation is neuroprotective in a mouse
706 model of human retinitis pigmentosa. J Neurosci 34 (24), 8139-50.

707 58. Swaroop, A. et al. (2007) Genetic susceptibility to age-related macular degeneration: a
708 paradigm for dissecting complex disease traits. Hum Mol Genet 16 Spec No. 2, R174-82.

709 59. McKay, G.J. et al. (2011) Evidence of association of APOE with age-related macular
710 degeneration: a pooled analysis of 15 studies. Hum Mutat 32 (12), 1407-16.

711 60. Heeren, J. et al. (2004) Impaired recycling of apolipoprotein E4 is associated with
712 intracellular cholesterol accumulation. J Biol Chem 279 (53), 55483-92.

713 61. Mahley, R.W. et al. (2009) Apolipoprotein E: structure determines function, from
714 atherosclerosis to Alzheimer's disease to AIDS. J Lipid Res 50 Suppl, S183-8.

715 62. Geissmann, F. et al. (2010) Development of monocytes, macrophages, and dendritic cells.
716 Science 327 (5966), 656-61.

717 63. Mizutani, M. et al. (2011) The fractalkine receptor but not CCR2 is present on microglia from
718 embryonic development throughout adulthood. J Immunol 188 (1), 29-36.

719 64. Fauser, S. et al. (2015) Intraocular and systemic inflammation-related cytokines during one
720 year of ranibizumab treatment for neovascular age-related macular degeneration. Acta
721 Ophthalmol 93 (8), 734-8.

722 65. Guymer, R.H. et al. (2011) Identification of Urinary Biomarkers for Age-related Macular
723 Degeneration. Invest Ophthalmol Vis Sci.

724 66. Jonas, J.B. et al. (2010) Monocyte chemoattractant protein 1, intercellular adhesion
725 molecule 1, and vascular cell adhesion molecule 1 in exudative age-related macular
726 degeneration. Arch Ophthalmol 128 (10), 1281-6.

727 67. Kramer, M. et al. (2012) Monocyte chemoattractant protein-1 in the aqueous humour of
728 patients with age-related macular degeneration. Clin Exp Ophthalmol 40 (6), 617-25.

729 68. Newman, A.M. et al. (2012) Systems-level analysis of age-related macular degeneration
730 reveals global biomarkers and phenotype-specific functional networks. Genome Med 4 (2), 16.

731 69. Rezar-Dreindl, S. et al. (2016) The Intraocular Cytokine Profile and Therapeutic Response in
732 Persistent Neovascular Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci 57 (10),
733 4144-50.

734 70. Saederup, N. et al. (2010) Selective chemokine receptor usage by central nervous system
735 myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5 (10), e13693.

736 71. Cruz-Guilloty, F. et al. (2013) Infiltration of proinflammatory m1 macrophages into the outer
737 retina precedes damage in a mouse model of age-related macular degeneration. Int J Inflam
738 2013, 503725.

739 72. Sim, D.A. et al. (2015) A simple method for in vivo labelling of infiltrating leukocytes in the
740 mouse retina using indocyanine green dye. Dis Model Mech 8 (11), 1479-87.

741 73. Shi, Y.Y. et al. (2011) Monocyte/macrophages promote vasculogenesis in choroidal
742 neovascularization in mice by stimulating SDF-1 expression in RPE cells. Graefes Arch Clin Exp
743 Ophthalmol 249 (11), 1667-79.

744 74. Liu, J. et al. (2013) Myeloid cells expressing VEGF and arginase-1 following uptake of
745 damaged retinal pigment epithelium suggests potential mechanism that drives the onset of
746 choroidal angiogenesis in mice. PLoS One 8 (8), e72935.

747 75. Sakurai, E. et al. (2003) Macrophage depletion inhibits experimental choroidal
748 neovascularization. Invest Ophthalmol Vis Sci 44 (8), 3578-85.

749 76. Luhmann, U.F. et al. (2009) The drusen-like phenotype in aging Ccl2 knockout mice is caused
750 by an accelerated accumulation of swollen autofluorescent subretinal macrophages. Invest
751 Ophthalmol Vis Sci 50 (12), 5934-43.

752 77. Robbie, S.J. et al. (2016) Enhanced Ccl2-Ccr2 signaling drives more severe choroidal
753 neovascularization with aging. Neurobiol Aging 40, 110-9.

754 78. Tsutsumi, C. et al. (2003) The critical role of ocular-infiltrating macrophages in the
755 development of choroidal neovascularization. J Leukoc Biol 74 (1), 25-32.

756 79. Guo, C. et al. (2012) Knockout of ccr2 alleviates photoreceptor cell death in a model of
757 retinitis pigmentosa. Exp Eye Res 104, 39-47.

758 80. Kohno, H. et al. (2014) CCL3 production by microglial cells modulates disease severity in
759 murine models of retinal degeneration. J Immunol 192 (8), 3816-27.

760 81. Yamasaki, R. et al. (2014) Differential roles of microglia and monocytes in the inflamed
761 central nervous system. J Exp Med 211 (8), 1533-49.

762 82. Ajami, B. et al. (2007) Local self-renewal can sustain CNS microglia maintenance and
763 function throughout adult life. Nat Neurosci 10 (12), 1538-43.

764 83. Ajami, B. et al. (2011) Infiltrating monocytes trigger EAE progression, but do not contribute
765 to the resident microglia pool. Nat Neurosci 14 (9), 1142-9.

766 84. Parkhurst, C.N. et al. (2013) Microglia promote learning-dependent synapse formation
767 through brain-derived neurotrophic factor. Cell 155 (7), 1596-609.

768 85. Goldmann, T. et al. (2013) A new type of microglia gene targeting shows TAK1 to be pivotal
769 in CNS autoimmune inflammation. Nat Neurosci 16 (11), 1618-26.

770 86. Dryja, T.P. et al. (1990) A point mutation of the rhodopsin gene in one form of retinitis
771 pigmentosa. Nature 343 (6256), 364-6.

772 87. Bruttger, J. et al. (2015) Genetic Cell Ablation Reveals Clusters of Local Self-Renewing
773 Microglia in the Mammalian Central Nervous System. Immunity 43 (1), 92-106.

774 88. Zhang, Y. et al. (2018) Repopulating retinal microglia restore endogenous organization and
775 function under CX3CL1-CX3CR1 regulation. Sci Adv 4 (3), eaap8492.

776 89. Feng, C. et al. (2017) Expression of CCL2 and its receptor in activation and migration of
777 microglia and monocytes induced by photoreceptor apoptosis. Mol Vis 23, 765-777.

778 90. Prokop, S. et al. (2015) Impact of peripheral myeloid cells on amyloid-beta pathology in
779 Alzheimer's disease-like mice. J Exp Med 212 (11), 1811-8.

780 91. Varvel, N.H. et al. (2015) Replacement of brain-resident myeloid cells does not alter
781 cerebral amyloid-beta deposition in mouse models of Alzheimer's disease. J Exp Med 212 (11),
782 1803-9.

783 92. Reed-Geaghan, E.G. et al. (2020) Plaque-associated myeloid cells derive from resident
784 microglia in an Alzheimer's disease model. J Exp Med 217 (4).

785 93. Van Hove, H. et al. (2019) A single-cell atlas of mouse brain macrophages reveals unique
786 transcriptional identities shaped by ontogeny and tissue environment. Nat Neurosci 22 (6),
787 1021-1035.

788 94. Sene, A. et al. (2013) Impaired cholesterol efflux in senescent macrophages promotes age-
789 related macular degeneration. Cell Metab 17 (4), 549-61.

790 95. Luckoff, A. et al. (2016) Interferon-beta signaling in retinal mononuclear phagocytes
791 attenuates pathological neovascularization. EMBO Mol Med 8 (6), 670-8.

792 96. Heng, J.S. et al. (2019) Comprehensive analysis of a mouse model of spontaneous
793 uveoretinitis using single-cell RNA sequencing. Proc Natl Acad Sci U S A.

794 97. Keren-Shaul, H. et al. (2017) A Unique Microglia Type Associated with Restricting
795 Development of Alzheimer's Disease. Cell 169 (7), 1276-1290 e17.

796 98. Hammond, T.R. et al. (2019) Single-Cell RNA Sequencing of Microglia throughout the Mouse
797 Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. Immunity 50 (1), 253-271
798 e6.

799 99. Anderson, S.R. et al. (2019) Developmental Apoptosis Promotes a Disease-Related Gene
800 Signature and Independence from CSF1R Signaling in Retinal Microglia. Cell Rep 27 (7), 2002-
801 2013 e5.

802 100. Krasemann, S. et al. (2017) The TREM2-APOE Pathway Drives the Transcriptional
803 Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 47 (3), 566-581
804 e9.

805 101. Handa, J.T. et al. (2019) A systems biology approach towards understanding and treating
806 non-neovascular age-related macular degeneration. Nat Commun 10 (1), 3347.

807 102. Okunuki, Y. et al. (2018) Microglia inhibit photoreceptor cell death and regulate immune
808 cell infiltration in response to retinal detachment. Proc Natl Acad Sci U S A 115 (27), E6264-
809 E6273.

810 103. Todd, L. et al. (2019) Reactive microglia and IL1beta/IL-1R1-signaling mediate
811 neuroprotection in excitotoxin-damaged mouse retina. J Neuroinflammation 16 (1), 118.

812 104. Spencer, W.J. et al. (2019) PRCD is essential for high-fidelity photoreceptor disc formation.
813 Proc Natl Acad Sci U S A 116 (26), 13087-13096.

814 105. Silverman, S.M. et al. (2019) C3- and CR3-dependent microglial clearance protects
815 photoreceptors in retinitis pigmentosa. J Exp Med 216 (8), 1925-1943.

816 106. Natoli, R. et al. (2017) Retinal Macrophages Synthesize C3 and Activate Complement in
817 AMD and in Models of Focal Retinal Degeneration. Invest Ophthalmol Vis Sci 58 (7), 2977-2990.

818 107. Jiao, H. et al. (2018) Subretinal macrophages produce classical complement activator C1q
819 leading to the progression of focal retinal degeneration. Mol Neurodegener 13 (1), 45.

820 108. Norris, G.T. et al. (2018) Neuronal integrity and complement control synaptic material
821 clearance by microglia after CNS injury. J Exp Med 215 (7), 1789-1801.

822 109. Wang, X. et al. (2017) Tamoxifen Provides Structural and Functional Rescue in Murine
823 Models of Photoreceptor Degeneration. J Neurosci 37 (12), 3294-3310.

824 110. Zabel, M.K. et al. (2016) Microglial phagocytosis and activation underlying photoreceptor
825 degeneration is regulated by CX3CL1-CX3CR1 signaling in a mouse model of retinitis
826 pigmentosa. Glia 64 (9), 1479-91.

827 111. Schaumberg, D.A. et al. (2014) Prospective study of common variants in CX3CR1 and risk of
828 macular degeneration: pooled analysis from 5 long-term studies. JAMA Ophthalmol 132 (1), 84-
829 95.

830 112. Fritsche, L.G. et al. (2013) Seven new loci associated with age-related macular
831 degeneration. Nat Genet 45 (4), 433-9, 439e1-2.

832 113. Wrana, J.L. et al. (1994) Mechanism of activation of the TGF-beta receptor. Nature 370
833 (6488), 341-7.

834 114. Butovsky, O. et al. (2014) Identification of a unique TGF-beta-dependent molecular and
835 functional signature in microglia. Nat Neurosci 17 (1), 131-43.

836 115. Zoller, T. et al. (2018) Silencing of TGFbeta signalling in microglia results in impaired
837 homeostasis. Nat Commun 9 (1), 4011.

838 116. Ma, W. et al. (2019) Absence of TGFbeta signaling in retinal microglia induces retinal
839 degeneration and exacerbates choroidal neovascularization. Elife 8.

840 117. Duncan, J.L. et al. (2018) Inherited Retinal Degenerations: Current Landscape and
841 Knowledge Gaps. Transl Vis Sci Technol 7 (4), 6.

842 118. Wang, X. et al. (2016) Requirement for Microglia for the Maintenance of Synaptic Function
843 and Integrity in the Mature Retina. J Neurosci 36 (9), 2827-42.

844 119. Ratnapriya, R. et al. (2019) Retinal transcriptome and eQTL analyses identify genes
845 associated with age-related macular degeneration. Nat Genet 51 (4), 606-610.

846 120. Spaide, R.F. et al. (2018) Subretinal drusenoid deposits AKA pseudodrusen. Surv
847 Ophthalmol 63 (6), 782-815.

848 121. Spaide, R.F. (2013) Outer retinal atrophy after regression of subretinal drusenoid deposits
849 as a newly recognized form of late age-related macular degeneration. Retina 33 (9), 1800-8.

850 122. Bird, A.C. et al. (2014) Geographic atrophy: a histopathological assessment. JAMA
851 Ophthalmol 132 (3), 338-45.

852 123. Curcio, C.A. (2001) Photoreceptor topography in ageing and age-related maculopathy. Eye
853 (Lond) 15 (Pt 3), 376-83.

854 124. Patel, A.A. et al. (2017) The fate and lifespan of human monocyte subsets in steady state
855 and systemic inflammation. J Exp Med 214 (7), 1913-1923.

856 125. Wynn, T.A. et al. (2013) Macrophage biology in development, homeostasis and disease.
857 Nature 496 (7446), 445-55.

858 126. Fadok, V.A. et al. (2001) Phagocyte receptors for apoptotic cells: recognition, uptake, and
859 consequences. J Clin Invest 108 (7), 957-62.

860 127. Huynh, M.L. et al. (2002) Phosphatidylserine-dependent ingestion of apoptotic cells
861 promotes TGF-beta1 secretion and the resolution of inflammation. J Clin Invest 109 (1), 41-50.

862 128. Gautier, E.L. et al. (2013) Local apoptosis mediates clearance of macrophages from
863 resolving inflammation in mice. Blood 122 (15), 2714-2722.

864 129. Glass, C.K. et al. (2010) Mechanisms underlying inflammation in neurodegeneration. Cell
865 140 (6), 918-34.

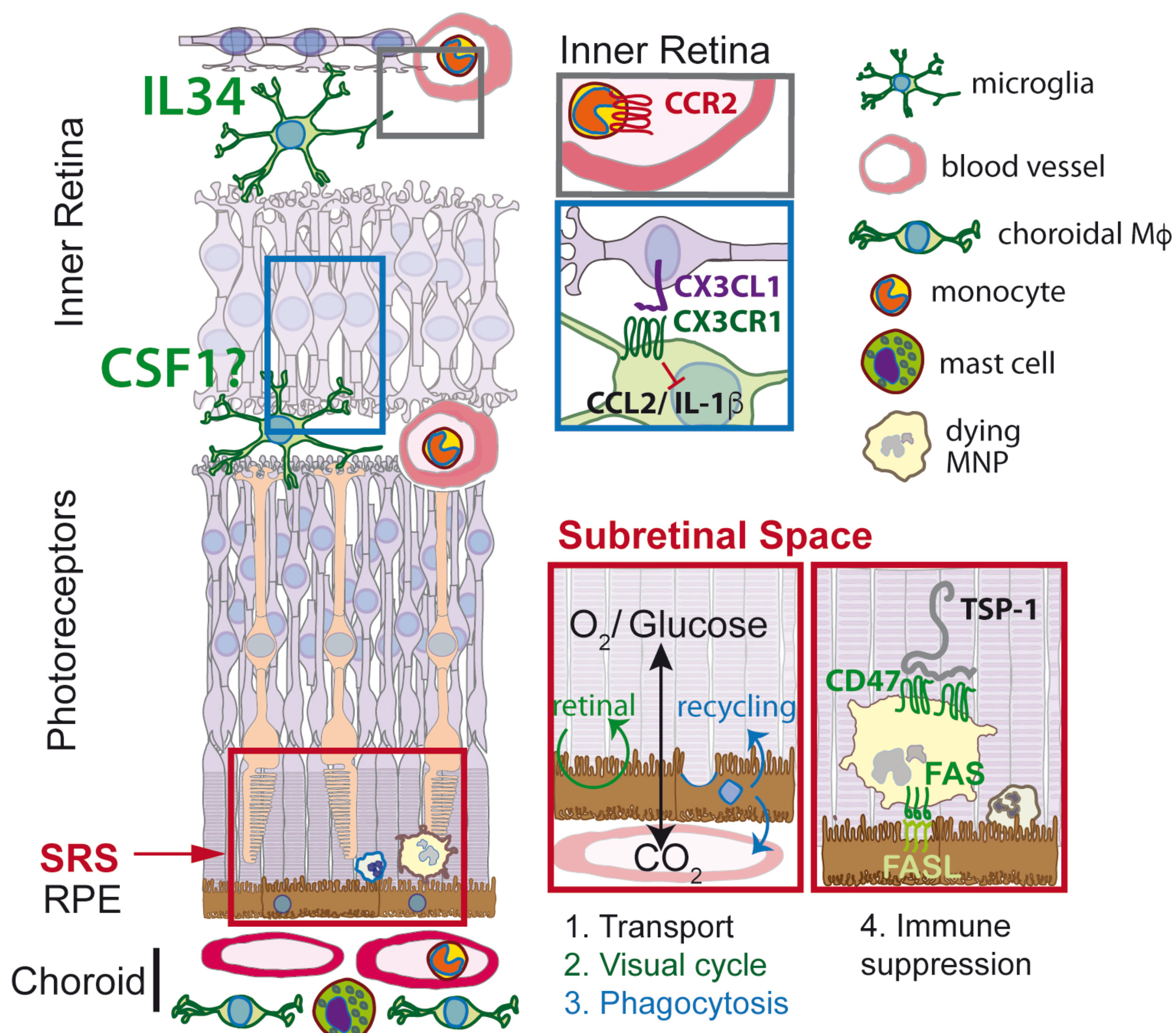
866 130. Grivennikov, S.I. et al. (2010) Immunity, inflammation, and cancer. Cell 140 (6), 883-99.

867 131. Hotamisligil, G.S. (2010) Endoplasmic reticulum stress and the inflammatory basis of
868 metabolic disease. Cell 140 (6), 900-17.

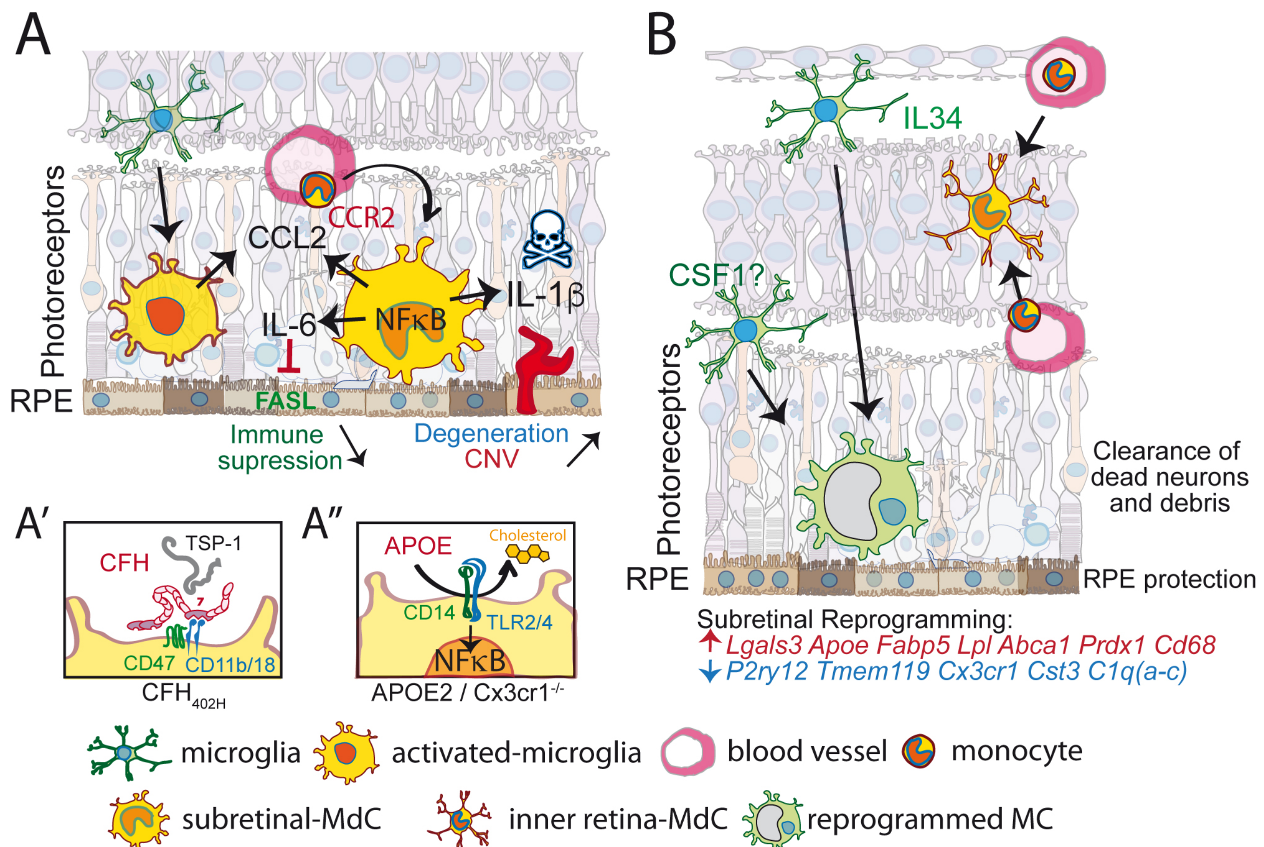
869 132. Jin, M. et al. (2005) Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium.
870 Cell 122 (3), 449-59.

871 133. Morshedian, A. et al. (2019) Light-Driven Regeneration of Cone Visual Pigments through a
872 Mechanism Involving RGR Opsin in Muller Glial Cells. Neuron 102 (6), 1172-1183 e5.
873

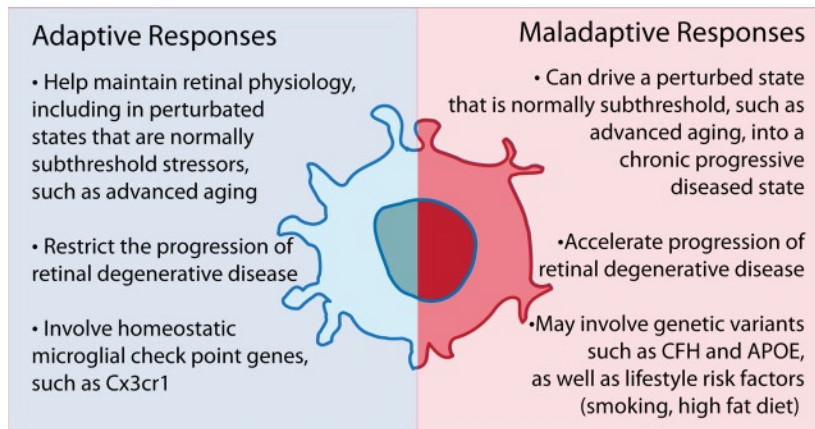
874 **Figure Legends**



875
876 **Figure 1: Physiological distribution of retinal MNPs and main functions of the subretinal**
877 **space.** Physiologically, the retina (left) is primarily populated by IL34-dependent and CSF-1-
878 dependent microglial cells. $CCR2^{+}$ monocytes are restricted to the circulation separated from
879 the retina by the inner and outer blood retinal barrier. CX3CL1 is constitutively expressed as a
880 transmembrane protein in inner retinal neurons (top right) and provides a tonic inhibitory
881 signal to the microglial checkpoint factor CX3CR1, expressed by retinal microglia. The subretinal
882 space (bottom right), demarcated by the tight junctions of the outer limiting membrane and of
883 the RPE is the site of 1.) Oxygen and glucose uptake and CO2 excretion; 2.) the visual cycle; 3.)
884 phagocytosis of used photoreceptor outer segments and excretion or recycling the material; 4.)
885 immune suppression, in which FasL induces elimination of infiltrating leukocytes after
886 activation of the integrin associated protein CD47 by thrombospondin 1 (TSP-1). SRS: subretinal
887 space; RPE: retinal pigment epithelium; MNP: mononuclear phagocyte; Mϕ: macrophage.
888



889
890 **Figure 2: Molecular mechanisms of the role of MNPs in retinal degenerations:** (A) participation
891 of mononuclear-derived cells in the infiltration of the SRS: loss of microglial checkpoint genes
892 (such as in *Cx3cr1* deficiency) lowers the threshold of microglial activation and induces NF-κB
893 signaling and inflammatory cytokine secretion in conditions of normally sub-threshold stress.
894 Increased levels of CCL2 lead to monocyte recruitment; increased levels of IL-6 lead to down-
895 regulation of FasL and immune-suppression; and lastly increased levels of IL-1β lead to
896 photoreceptor degeneration and neovascularization. (A') In AMD, NF-κB activation can be due
897 to the AMD-associated APOE2 allele that, similar to Cx3cr1-deficiency in mice, leads to
898 increased APOE concentrations, lipid-raft destabilization of the plasma membrane and the
899 activation of the innate immunity receptor cluster (CD14 and TLR2/4). (A'') Furthermore, the
900 AMD-associated Complement Factor H (CFH) 402H variant inhibits MNP elimination as it blocks
901 TSP-1 mediated CD47 activation particularly efficiently. Both AMD-risk factors thereby promote
902 pathogenic subretinal inflammation. (B) Dominance of microglia in the SRS in *Cx3cr1* sufficient
903 settings: both IL34-dependent and independent microglia migrate to the SRS, which is poorly
904 accessible to MdCs. These subretinal microglia are transcriptionally reprogrammed by
905 downregulating homeostatic marker genes and upregulating genes involved in lipid
906 metabolism, anti-oxidant activity and others as indicated. Functionally, these cells clear dead
907 neurons and cellular debris, and protect the RPE and photoreceptors. CNV choroidal
908 neovascularization; RPE: retinal pigment epithelium.
909



910
911 **Figure 3: A unified hypothesis for MNP responses in IRDs and AMD:** Adaptive responses by
912 resident microglia express low levels of pathogenic cytokines, help eliminate toxic waste and slow
913 down degeneration. When confounded by risk factors, such as genetic variants and
914 environmental factors, microglia more readily express pathogenic cytokines and recruit
915 monocytes. Such maladaptive responses by MNPs exacerbate retinal degenerative diseases.