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► To cite this version:

Chen Yu, Christophe Roubéix, 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 . inserm-02933686

HAL Id: inserm-02933686

<https://inserm.hal.science/inserm-02933686v1>

Submitted on 8 Sep 2020

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

Chen Yu¹, Christophe Roubeyx², Florian Sennlaub^{2*†}, Daniel R Saban^{1,3*†}

¹ Department of Ophthalmology, Duke University, Durham, NC 27710, USA

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

³ Department of Immunology, Duke University, Durham, NC 27710, USA

*these authors have contributed equally

†Correspondence should be addressed to the lead contacts:

Daniel Saban: daniel.saban@duke.edu

Florian Sennlaub: florian.sennlaub@inserm.fr

Key words: macrophages; inherited retinal dystrophy; retinitis pigmentosa; age-related macular degeneration; AMD

Abstract

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

Microglia and monocytes as distinct lineages in retinal degenerative disease

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

Roles of MNPs in Retinal Health and Photoreceptor Degenerative Disease

The type and pattern of retinal MNPs are largely homologous to those in the brain and spinal cord parenchyma. Microglia are the principal MNPs in the normal retina parenchyma [11]. The perivascular spaces also have resident macrophages that are long-lived [12], albeit radio-sensitive (i.e. succumb to radiation-induced cell death) [11]. Monocytes (**Box 2**) and dendritic 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-1 β (IL-1 β) [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

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

Subretinal Space in Retinal Degenerative States

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

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

a meta-analysis [43]. They also showed that *Cx3cr1*-deficient mice develop age-related MNP infiltration of the SRS, accompanied by photoreceptor degeneration and exaggerated CNV. Given that *Cx3cr1* is a homeostatic microglial checkpoint gene of MNPs [44], this phenotype revealed a link between intrinsic MNP dysregulation and disease pathogenesis. In 2013, the same group showed that AMD patients are characterized by increased intraocular CCL2 concentrations and that MdCs are present in the SRS of intermediate and late AMD patients, as well as in *Cx3cr1*-deficient mice, where they were shown to mediate photoreceptor toxicity [6]. Importantly, the authors demonstrated that in mouse models, two key human AMD genetic risk gene variants, the Apolipoprotein E2 isoform (*APOE*) and Complement Factor H variant Y402H (*CFH*) directly promote pathogenic inflammation in the SRS [26, 27]. In sum, these findings established a link between certain AMD-risk variants and subretinal MdC infiltration, and in turn, the possible pathogenic role of these cells in AMD (**Fig.2a**).

In 2019, another advance in understanding the cellular make-up and contribution of subretinal MNPs in degeneration was reported [3]. Building on their early study [11] and converging subsequent work from another group [36], the authors applied *Cx3cr1*^{CreER/+} microglial lineage tracing mice and single-cell RNA-seq to decipher the ontogeny and function of subretinal MNPs in the *Cx3cr1*-sufficient setting. In a humanized IRD model [45] and in the toxic light-induced photoreceptor degeneration model (LD) [46], microglia were found as the dominant MNP lineage which occupies the SRS, whereas recruited MdCs were largely confined to the parenchyma. Moreover, the authors provided direct evidence that these subretinal microglia restrict but not accelerate disease progression. Identification of transcript-specific inductions of subretinal microglia now opens the door to future elucidation of these disease restricting activities (**Fig.2b**). Collectively, these studies demonstrate the significance of microglia in the SRS of retinal degeneration models and showed that these microglia are not pathogenic, but rather are involved in restricting disease progression.

The following two sections will discuss in detail the evidence leading to the view taken in the current review that MdCs and microglia in the SRS play distinct (and perhaps opposite) roles in photoreceptor degenerative diseases.

Pathogenic Role of MdCs in the Subretinal Space

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

Evidence for pathogenic subretinal MNP accumulation

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

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

receptor CD14 as they locate to non-raft and raft plasma membrane respectively. TLR ligands overcome this separation, as they bind to CD14 and TLR2/4, inducing dimerization and intracellular NF- κ B activation and cytokine expression. Excessive APOE-concentrations extract cholesterol from the lipid rafts of the plasma membrane of MNPs [33, 50], which levitates the separation of TLR and CD14. The receptors dimerize and trigger intracellular NF- κ B activation in the absence of ligands (**Fig.2A'**) [33]. A similar mechanism has been previously described for APOA1 [51]. The resulting increased secretion of IL-6 and CCL2 observed in subretinal *Cx3cr1*-deficient MNPs reduces their RPE-induced elimination and increases monocyte recruitment, respectively (see below) [14, 35]. Additionally, *Cx3cr1*-deficient MNPs overexpress P2rx7, which constitutively activates their inflammasome and facilitates IL-1 β maturation and secretion [48, 52]. The increased IL-1 β secretion resulting from this dysregulation mediates rod and cone degeneration, and significantly increases CNV [34, 48, 53] (**Fig.2A**). In sum, *Cx3cr1* deficiency is sufficient to trigger a pathogenic non-resolving subretinal inflammation, and in *Cx3cr1*-deficient mice, deletion of *ApoE* prevents subretinal accumulation of MNPs and attenuates photoreceptor degeneration and CNV after laser-injury [35].

Of note, the *Cx3cr1*-deficient mice used in these studies did not carry the spontaneous frameshift mutation in *Crb1* (also known as *rd8*) found in C57BL6/N mice that can cause photoreceptor degeneration and spontaneous CNV, and is likely responsible for the AMD-like phenotype described in a number of published studies using inadvertently contaminated mouse strains [54].

In summary, it can be argued that *Cx3cr1*-deficiency represents a model of 'primary' subretinal inflammation. For this concept, *Cx3cr1*-deficient-MNPs 'overreact' to a below-threshold perturbation (e.g. aging, or a non-toxic light challenge [6, 14]), which involves subretinal MdC infiltration that causes retinal degeneration. In 'secondary' inflammation, which is an inflammatory response caused by a supra-threshold insult that induces inflammation in a wildtype animal (such as in the laser CNV or photoreceptor degeneration models) *Cx3cr1*-deficiency exacerbates inflammation and disease progression, observed in laser-induced CNV model [14], paraquat-induced retinopathy [55], and *rd10* mice [56, 57] (autosomal recessive IRD model).

AMD risk factors promote subretinal pathogenic inflammation

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

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

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

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

Evidence implicating subretinal MdCs as a major driver for AMD pathology

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

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

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

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

In IRDs, the participation of MdCs seems to largely depend on the underlying genetic defect and possibly the intensity and speed of the degeneration. For example, *Ccr2* deletion diminished retinal MNP infiltration, yet had a minor protective effect in progressively degenerating *rd10* mice [79]. The faster degeneration *Arrestin-1* (*Arr1*) knockout mice could not

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

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

Protective Role of Microglia in the Subretinal Space

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

Spatial Distribution of MNP lineages in Retinal Degenerative Disease Models

A 2016 paper reported one of the first studies that applied *Cx3cr1^{CreER/+}* mice to decipher *in situ* spatial distributions of retinal microglia versus MdCs during disease [11]. The authors used confocal microscopy to examine retinas from *Cx3cr1^{CreER/+}R26^{tdTomato}* mice subjected to the LD model. This approach allowed microglia to be traced as YFP⁺ tdTomato⁺ and MdCs as YFP⁺ tdTomato⁻. Notably, microglia and MdCs were found to be largely distributed in distinct areas of the retina: microglia predominantly migrated to the SRS, whereas monocytes poured into the neuroparenchyma (presumably via retinal vasculature [7]). This finding was subsequently recapitulated by another group in the model of NaIO₃-induced RPE injury, showing microglial dominance of the SRS through 30 days post injury [36], and by another study using human rhodopsin P23H knock-in mice [3] (this rhodopsin mutant serves as a model of a major autosomal dominant form of IRD [45, 86]). Collectively, these studies revealed a spatial distinction between microglia and MdCs in the *Cx3cr1*-sufficient setting, and pointed to a potentially important role for microglia within the SRS in disease.

More comprehensive studies dissecting the isolated migration patterns of microglia were carried out in a series of *Cx3cr1^{CreER/+}R26^{DTR}* mouse experiments [3]. These subretinal microglia (YFP⁺ DTR⁺) in degeneration were efficiently depleted by DT administration, indicating that they are indeed DTR-expressing microglia. By contrast, MdCs (YFP⁺DTR⁻) still infiltrated the retina, but they were largely located in the parenchyma and incapable of migrating to the SRS, even in the absence of microglia. It remains unclear whether this monocyte infiltration reflects a homeostatic response intended to replace evacuated microglia from the plexiform layers. We argue that this is unlikely, as neither microglia depletion, nor reduced microglia number in *IL34* KO mice, results in monocyte recruitment in steady state [16, 87, 88]. The separate question as to whether microglia depletion in retinal degeneration models leads to a reduced monocyte infiltration was not addressed either, as other glia and retinal neurons express substantial levels of Ccl2 [89]. Collectively, the findings from the depletion experiments suggest that microglia do not outcompete MdCs for the SRS but have restricted access to it in the LD and P23H models. This is also likely the case in the NaIO₃-induced RPE injury model [36]. Whether this pattern can

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

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

Reprogramming of Subretinal Microglia

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

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

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

RPE and Photoreceptor Protection by Microglia

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

transcriptional reprogramming with RPE protection (**Fig.2B**). Moreover, the damage of RPE in this depletion setting included both lateral and/or apical defects to F-actin, resembling key features of RPE dysmorphogenesis in human AMD and certain forms of IRDs [101]. It is noteworthy that DT-mediated depletion has some technical caveats to consider, such as the possible elicitation of pro-inflammatory cytokines shown following brain microglial depletion [87], or perhaps a damaging effect should the RPE be involved in phagocytosing DT-laden apoptotic microglia, although the latter has not been shown. However, indicating such potential caveats are insufficient to induce RPE dysmorphology is that microglia depletion in the steady state does not result in this disease phenotype [3]. Separately, supporting the conclusion that subretinal microglia are protective, RPE dysmorphology is recapitulated in the LD model with reduced microglial numbers in *I134*-deficient mice [3]. Moreover, the aforementioned transcriptomic findings also corroborate this protective phenotype. Collectively, as a better preserved RPE is generally thought to help maintain the function and survival of photoreceptors, these findings suggest that subretinal microglia restrict disease progression.

Still, the question remained as to how microglia confer protection to the RPE. One possible clue in this direction was the accumulation of dead photoreceptors and debris in the SRS in the absence of microglia [3]. Interestingly, as MdCs are spared in this system (as likely are astrocytes and Müller glia), the most parsimonious interpretation, in our view, is that microglia play a role in debris clearance, whereas monocytes less so. Similar microglial protective roles were reported in several other retinal disease models, including retinal detachment [102], RGC excitotoxicity [103], and progressive rod-cone degeneration [104]. A recent study on complement component 3- (C3) and its receptor CR3- dependent phagocytosis supports a similar protective role for microglia in an IRD model of *rd10* mice [105], although there are also reports suggesting that C3 (and C1q) are pathological in LD [106, 107]. Likewise, a microglia-dominant clearance of neuronal debris was described in other models of neurodegeneration, such as optic nerve crush [108] and EAE mice [81]. Hence, we surmise that the failure to clear the photoreceptor debris in the SRS may have cytotoxic effects, potentially explaining the observed RPE defect. Contrasting with this view, a study reported increased photoreceptor survival when using continual tamoxifen administration in *Cx3cr1^{CreER}R26^{DTA}* mice on the *rd10*

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

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

Unified MNP Model in Retinal Degenerative Diseases

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

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

manner. TGFBR1 is phosphorylated by TGFBR2 in order to propagate TGF- β 1 signaling [113], which in turn maintains microglial identity and function [114, 115]. Indeed, conditional deletion of *Tgfb2* in microglia was shown to cause a degenerative disease phenotype in mice [116]. These respective genetic factors might not be sufficient by themselves to drive harmful MNP responses, but can possibly do so when combined with non-genetic factors that confer risk in AMD (e.g., advanced age, high fat and high cholesterol diets, and smoking). Different from those in AMD, evidence in mouse models suggest that MNPs are not driven into a maladaptive state in IRDs that have a monogenic Mendelian etiology involving genes of the visual system [117]. One exception may be some IRDs caused by *MERTK* mutations, a gene that regulates phagocytosis of the RPE and MNPs such as microglia.

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

Concluding Remarks

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

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

Box 2. Monocyte Derived Cells in Health and Disease

Monocytes are bone marrow-derived myeloid cells that circulate in the blood. Monocyte-derived cells do not participate in the resident microglial pool of the retina and CNS. However, they can be recruited during tissue damage or infection of any tissues including the retina and other CNS tissues [62]. They comprise three major subsets (classical, intermediate and non-classical) and are likely sequentially transitioning from one into another [124]. The short-lived CCR2⁺CD14⁺Ly6C⁺ classical monocytes constitute 90% of all circulating monocytes in humans and 60-70% in mice. They circulate the blood for 24h before 99% of them disappear. About 1% of classical monocytes evolve into longer-lived intermediate (CCR2/CD14^{high} CX3CR1/CD16^{high}) monocytes, which finally become CCR2/CD14^{low} CX3CR1/CD16^{high} 'patrolling' monocytes, also referred to as non-classical monocytes [124]. During tissue injury, damage- and pathogen-associated molecular pattern (DAMPs and PAMPs) activate resident macrophages and resident mast cells (absent from the CNS including the retina, but present in the meninges and choroids) that produce chemokines, such as CCL2 (the main ligand of CCR2), which recruit classical monocytes that differentiate into anti-microbial and pro-inflammatory macrophages. These cells release reactive oxygen species and complement components to kill, opsonize and phagocytose pathogens and secrete inflammatory cytokines (such as IL-1 β , TNF- α , IL-6, and CCL2) that alter the tissue to facilitate the immune response [125]. Although these mediators 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	<ul style="list-style-type: none"> • Labels the majority of MNPs 	<ul style="list-style-type: none"> • Unable to distinguish microglia and MdCs • Some markers not specific for MNPs 	[2]
Microglial specific markers, such as P2ry12, Tmem119	<ul style="list-style-type: none"> • Able to specifically label microglia in steady state 	<ul style="list-style-type: none"> • May not distinguish microglia versus MdCs during neuroinflammation 	[3, 97, 100]
Chimaeras			
Bone marrow chimera	<ul style="list-style-type: none"> • Full blood chimerism 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Preservation of CNS in steady state 	<ul style="list-style-type: none"> • Full blood chimerism is not achievable 	[11]
Circulating monocyte-labeling			
Systemic 5-ethynyl-2'-deoxyuridine (EdU)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • A traceable fluorescent probe taken up by circulating monocytes and detectable in cells derived from these monocytes. 	<ul style="list-style-type: none"> • Requires repeated injections; limited to use for short-term study but not chronic conditions. 	[72]
Reporter mice			
<i>Cx3cr1^{GFP}</i> or <i>Cx3cr1^{YFP}</i>	<ul style="list-style-type: none"> • Heterozygotes are good for labeling MNPs • Homozygotes are a full deletion of <i>Cx3cr1</i> gene 	<ul style="list-style-type: none"> • Unable to distinguish microglia and MdCs, as both express <i>Cx3cr1</i> 	[11, 14, 36, 110]
<i>Ccr2^{RFP}</i>	<ul style="list-style-type: none"> • Heterozygotes are good for labeling classical monocytes • Homozygotes are a full deletion of <i>Ccr2</i> gene 	<ul style="list-style-type: none"> • Isn't useful for labeling monocyte-derived macrophages, which lose <i>Ccr2</i> expression • Spares non-classical monocytes 	[6, 7, 36]
<i>Cx3cr1^{CreER}</i> <i>R26^{REPORTER}</i>	<ul style="list-style-type: none"> • Tamoxifen treatment induces the expression of reporter and labels microglia and MdCs • The reporter expression in monocytes is 'washed out', as 	<ul style="list-style-type: none"> • Other long-lived tissue-resident macrophages that maintain <i>Cx3cr1</i> 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	<ul style="list-style-type: none"> • Efficient depletion of Csf1r dependent macrophages • Easy to administer 	<ul style="list-style-type: none"> • Indistinguishably ablate microglia and MdCs • Potential off-target effects on other tyrosine kinase receptors 	[88, 102, 103]
Intravenous clodronate liposome	<ul style="list-style-type: none"> • Efficient depletion of monocytes and their derivatives 	<ul style="list-style-type: none"> • Requires repeated injections, limited to use for short-term study but not chronic conditions. • Potential off-target effects 	[6, 103]
<i>Cx3cr1^{CreER}</i> <i>R26^{DTA}</i>	<ul style="list-style-type: none"> • Efficient depletion of MNPs • Does not require diphtheria toxin 	<ul style="list-style-type: none"> • Indistinguishably depletes all <i>Cx3cr1</i> expressing MNPs • Require multiple tamoxifen administrations 	[39, 118]
<i>Cx3cr1^{CreER}</i> <i>R26^{DTR}</i>	<ul style="list-style-type: none"> • Efficient depletion of microglia following 'wash-out' 	<ul style="list-style-type: none"> • Require tamoxifen and diphtheria toxin administration • Other long-lived tissue-resident macrophages may also be depleted 	[3, 84]
Ccl2- and Ccr2-deletion / inhibition	<ul style="list-style-type: none"> • Efficiently inhibits exit of bone marrow classical monocyte into the blood 	<ul style="list-style-type: none"> • Does not inhibit non-classical monocyte-recruitment 	[6, 7, 73-80]

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Figure Legends

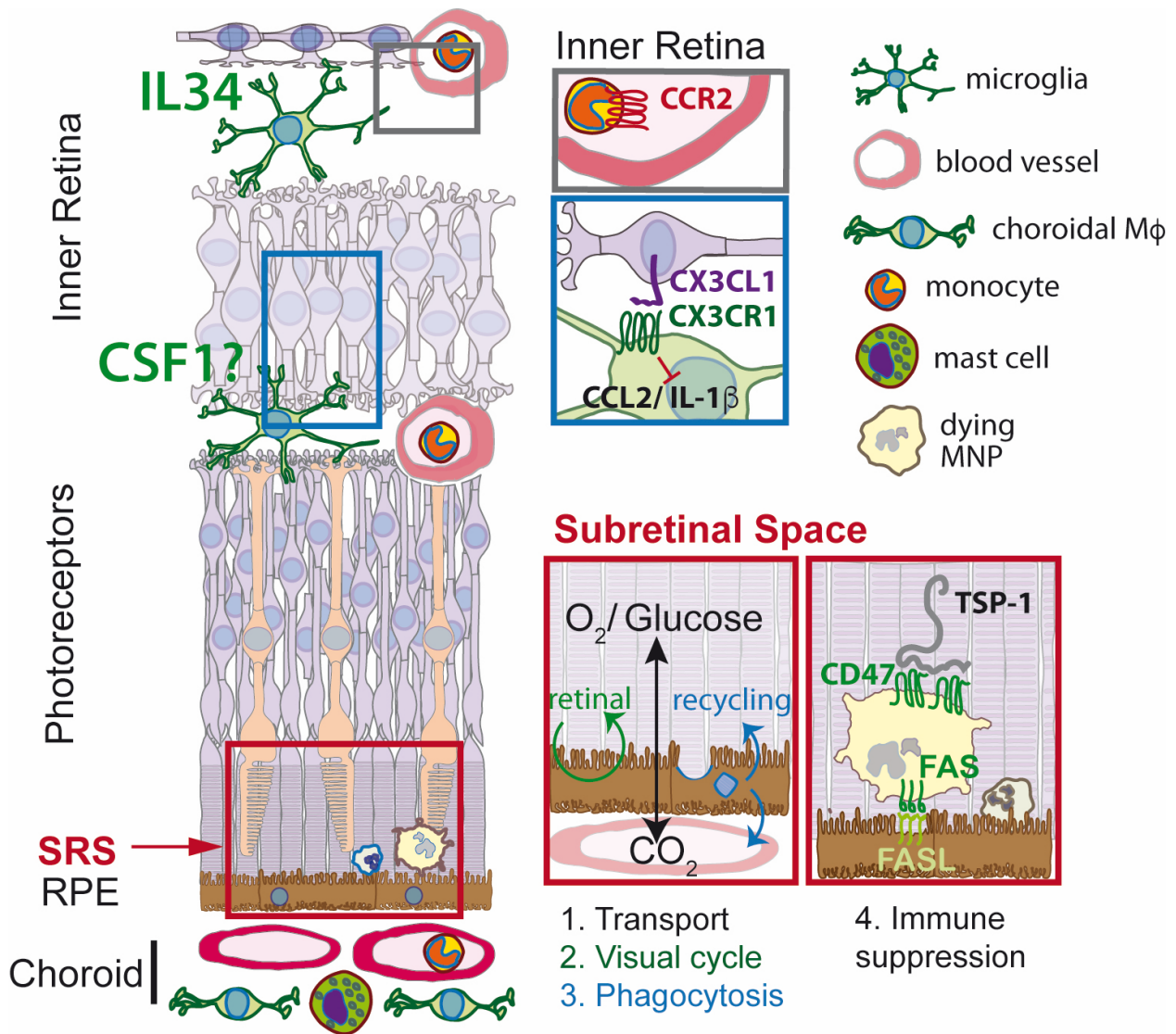


Figure 1: Physiological distribution of retinal MNP and main functions of the subretinal space. Physiologically, the retina (left) is primarily populated by IL34-dependent and CSF-1-dependent microglial cells. CCR2⁺ monocytes are restricted to the circulation separated from the retina by the inner and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

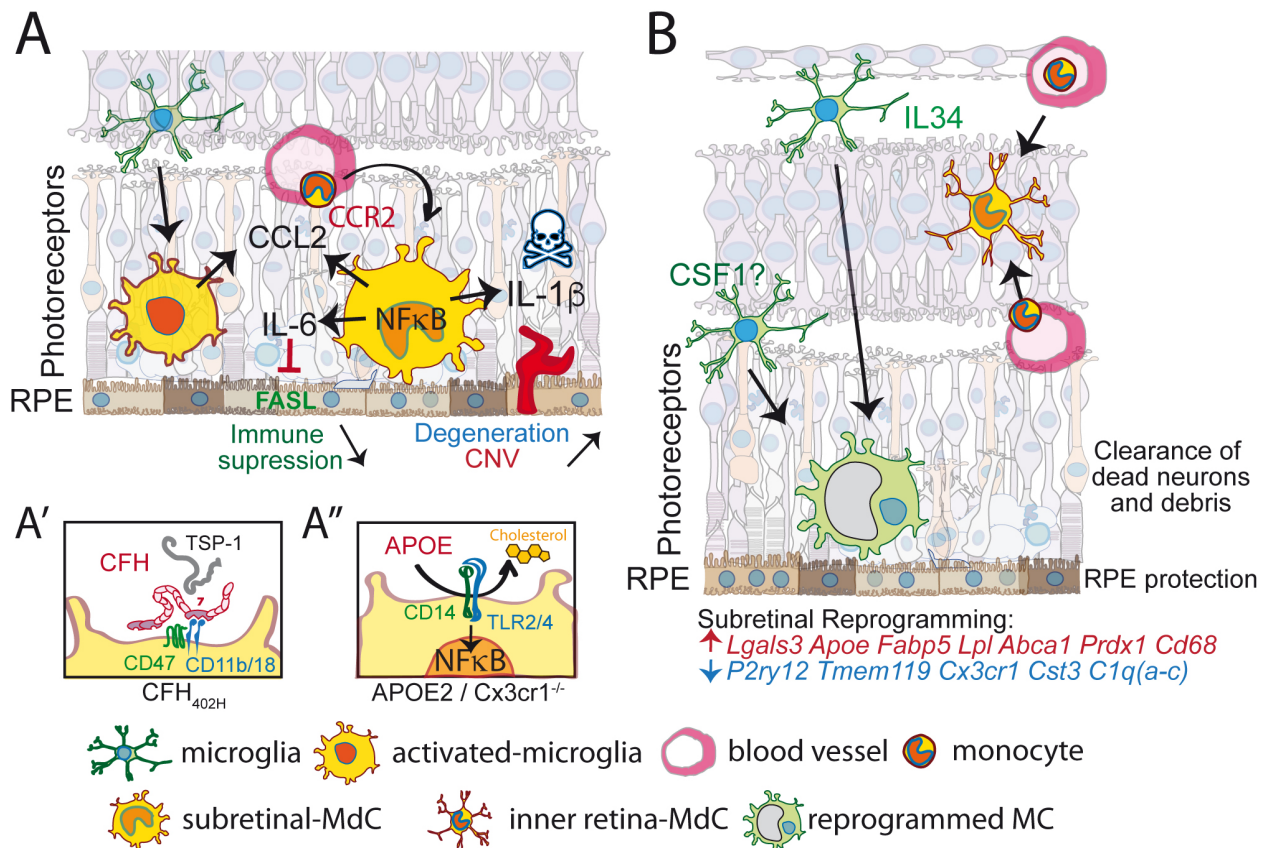


Figure 2: Molecular mechanisms of the role of MNPs in retinal degenerations: (A) participation of mononuclear-derived cells in the infiltration of the SRS: loss of microglial checkpoint genes (such as in *Cx3cr1* deficiency) lowers the threshold of microglial activation and induces NF- κ B signaling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

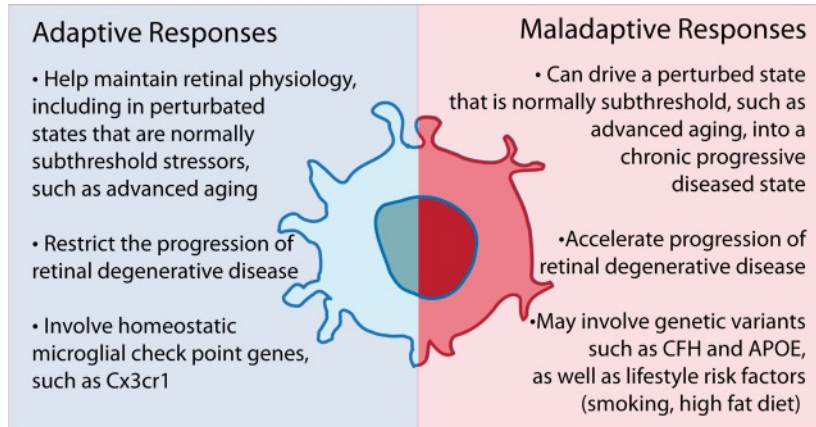


Figure 3: A unified hypothesis for MNP responses in IRDs and AMD: Adaptive responses by resident microglia express low levels of pathogenic cytokines, help eliminate toxic waste and slow down degeneration. When confounded by risk factors, such as genetic variants and environmental factors, microglia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