

Role of microglia/macrophage polarisation in intraocular diseases (Review)

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Abstract. Macrophages form a crucial component of the innate immune system, and their activation is indispensable for various aspects of immune and inflammatory processes, tissue repair, and maintenance of the balance of the body's state. Macrophages are found in all ocular tissues, spanning from the front surface, including the cornea, to the posterior pole, represented by the choroid/sclera. The neural retina is also populated by specialised resident macrophages called microglia. The plasticity of microglia/macrophages allows them to adopt different activation states in response to changes in the tissue microenvironment. When exposed to

various factors, microglia/macrophages polarise into distinct phenotypes, each exhibiting unique characteristics and roles. Furthermore, extensive research has indicated a close association between microglia/macrophage polarisation and the development and reversal of various intraocular diseases. The present article provides a review of the recent findings on the association between microglia/macrophage polarisation and ocular pathological processes (including autoimmune uveitis, optic neuritis, sympathetic ophthalmia, retinitis pigmentosa, glaucoma, proliferative vitreoretinopathy, subretinal fibrosis, uveal melanoma, ischaemic optic neuropathy, retinopathy of prematurity and choroidal neovascularization). The paradoxical role of microglia/macrophage polarisation in retinopathy of prematurity is also discussed. Several studies have shown that microglia/macrophages are involved in the pathology of ocular diseases. However, it is required to further explore the relevant mechanisms and regulatory processes. The relationship between the functional diversity displayed by microglia/macrophage polarisation and intraocular diseases may provide a new direction for the treatment of intraocular diseases.

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Abbreviations: RPE, retinal pigment epithelium; HIF-1 α , hypoxia-inducible factor- α ; TNF- α , tumor necrosis factor- α ; LPS, lipopolysaccharide; TLR, Toll-like receptor; PPAR γ , peroxisome proliferator-activated receptor γ ; NO, nitric oxide; iNOS, inducible NO synthase; VEGF, vascular endothelial growth factor; EAU, experimental autoimmune uveitis; MDSCs, myeloid-derived suppressor cells; ROS, reactive oxygen species; AhR, aryl hydrocarbon receptor; BMDMs, bone marrow-derived macrophages; MS, multiple sclerosis; RGCs, retinal ganglion cells; EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; FA, fatty acids; SO, sympathetic ophthalmia; RP, retinitis pigmentosa; PONT, partial optic nerve transection; LBP, *Lycium barbarum*; PACAP, pituitary adenylate cyclase-activating polypeptide; PVR, proliferative vitreoretinopathy; CNV, choroidal neovascularization; nAMD, neovascular age-related macular degeneration; ECM, extracellular matrix; Arg-1, Arginase-1; NAION, nonarteritic anterior ischemic optic neuropathy; rAION, rat AION; ROP, retinopathy of prematurity; HUVEC, human umbilical vein endothelial cell; CSF1/M-CSF, macrophage colony-stimulating factor 1; CSF2/(GM)-CSF, granulocyte-macrophage colony-stimulating factor 2

Key words: microglia/macrophage polarization, ocular disease, retinopathy of prematurity, choroidal neovascularization, autoimmune uveitis, proliferative vitreoretinopathy, subretinal fibrosis, diabetic retinopathy

Contents

1. Introduction
2. Intraocular inflammation-related diseases
3. Intraocular fibrosis-related diseases
4. Intraocular malignancy
5. Intraocular neovascularization-related diseases
6. Conclusion

1. Introduction

Macrophages are vital components of the innate immune system and are ubiquitous throughout the body. First identified and elucidated by Metchnikoff in the 19th century, macrophages have been recognised for their pivotal roles in the phagocytosis and elimination of microorganisms (1). Their multifaceted functions encompass the maintenance of tissue equilibrium, orchestration and resolution of immune responses during pathogenic assaults and the facilitation of tissue repair and restructuring in both developmental and injury-induced contexts (2,3). Furthermore, macrophages exhibit diverse

cellular responses and adapt to distinct stimuli or sources within tissues or the environment. For instance, exposure to microbial stimulation triggers an M1 or inflammatory state in macrophages, which is characterised by increased production of pro-inflammatory cytokines and microbe eradication. Conversely, during helminthic or parasitic infections, macrophages transition to an M2 or alternative state, specialising in tissue regeneration and remodelling (4).

The eye is a remarkably specialised sensory organ that encompasses several intricately interconnected tissue types, each of which is pivotal for the formation of clear visual images by the neural retina. Among the intraocular tissues, the pigmented iris, ciliary body and choroid collectively form the uvea. Similar to the brain, the retina represents neural tissue sheltered by the blood-eye barrier, comprising a complex network of interconnected neurons. Positioned adjacent to the neural retina, the choroid, a highly vascularised connective tissue, serves a crucial role in providing metabolic and nutritional support to the outer retina. In the unique microenvironment of intraocular tissue, there are different populations of resident tissue macrophages that are adept at maintaining tissue homeostasis and coordinating inflammatory responses when encountering abnormal stimuli.

The neural retina contains specialised resident macrophages known as microglia (5). Originating early in embryogenesis from precursor cells in the embryonic yolk sac, microglia migrate to specific regions within the central nervous system (CNS) at approximately embryonic day 8.5 (6,7). Their developmental pathways overlap with those of tissue macrophages. In cases of radiation-induced complete microglial apoptosis, bone marrow-derived macrophages (BMDMs) can supplement and express a phenotype similar to that of microglia. However, these cells constitute a distinct population capable of self-renewal and are not typically substituted for BMDMs (8). In the retina, microglia are primarily found in three specific locations: The nerve fibre, inner and outer plexiform layers (5).

Macrophages are crucial components of the innate immune system and exhibit various functions. They serve as a primary defence against microorganisms and orchestrate adaptive immune responses. Apart from generating essential pro-inflammatory cytokines and chemokines, macrophages also have pivotal roles in phagocytosis, clearing apoptotic cells and tissue debris (9). In addition, macrophages engage in immune regulation by expressing anti-inflammatory cytokines like interleukin (IL)-10, transforming growth factor (TGF- β) and lipid mediators, such as lipoxins. Macrophages are implicated in tissue remodelling and the development of various organs, such as the breast tissue, bones, kidneys and brain (10). Macrophage dysregulation may trigger autoimmune conditions and persistent inflammatory diseases (11).

In the CNS, microglia constitute 5-12% of total brain cells and share functional similarities with peripheral macrophages. Microglia actively contribute to synaptic plasticity and debris clearance in the healthy brain (12,13). Studies revealed that even in a relatively quiescent state, microglia have pivotal roles in tissue repair and infection control (14). Following injury or infection, microglia in the CNS promptly respond to stimuli by releasing cytokines that induce phagocytosis and direct cytotoxicity (15). Peripheral macrophages can replenish the microglia. Although microglia and macrophages share several

functions, such as antigen presentation and production of cytokines, including oxidative free radicals, chemokines and nitric oxide (NO), they possess distinct characteristics. In the initial stages of CNS inflammatory responses, microglia demonstrate lower cytokine production levels (CD45, C-C chemokine receptor type (CCR)1 and CCR5) alongside higher TGF- β expression. Conversely, infiltrating macrophages exhibit elevated expression of CD45, CCR1, CCR2 and CCR5, accompanied by reduced TGF- β expression (6,16). These differences in biomarker profiles aid in distinguishing the CNS-resident microglia from the infiltrating macrophages (17). Nonetheless, both resident microglia and infiltrating macrophages have analogous roles in the CNS during inflammatory responses.

Macrophages demonstrate responsiveness to endogenous signals after infection or injury, assuming both pathogenic and protective roles (2,18,19). Upon appropriate stimulation, M1 macrophages serve as the frontline defence of the innate immune system during the early stages of a disease. Microglia share phenotypic traits with peripheral macrophages and detect detrimental stimuli through various immune receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerisation domains (NODs) and NOD-like receptors (20,21). Microglia exhibit different activation states within injured tissues (22,23). Upon injury, microglia or macrophages infiltrating from the circulation polarise toward a pro-inflammatory (M1) phenotype upon exposure to pro-inflammatory cytokines such as interferon- γ (IFN- γ) and tumour necrosis factor (TNF)- α .

Typically, M1 classically activated macrophages express TNF- α , IL-1 α , IL-1 β , IL-6, IL-12, IL-23, C-X-C motif chemokine ligand (CXCL)9, CXCL10 and other cytokines and chemokines. They are distinguished by their high secretion ratio of IL-12 and IL-23 but produce relatively less IL-10. Furthermore, M1 macrophages engage in the type I T-helper cell (Th1) immune response as both inducer and effector cells, apart from their roles in defence against parasites and tumours (24-26). Similar to infiltrating macrophages, microglia respond by producing M1-associated factors, including pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-6, IL-12, IL-23 and TNF- α), chemokines, redox molecules [e.g. NADPH oxidase and inducible NO synthase (iNOS)], macrophage receptors with collagen structure, costimulatory proteins (CD40) and major histocompatibility complex class II (18,21,27-30).

M2 macrophages have multifaceted roles in allergic responses, parasite clearance, inflammation suppression, tissue remodelling, angiogenesis, immune regulation and tumour promotion (31). Within this subset, M2 macrophages consist of four distinct subpopulations: i) M2a, predominantly induced by IL-4 and IL-13; ii) M2b, primarily triggered by immune complexes, IL-1 β and TLR ligands; iii) M2c macrophages, produced in response to IL-10, glucocorticoids and TGF- β (25,32-36); and iv) M2d, primarily induced by TLR antagonists (33). The polarisation of microglia toward the M2 phenotype mirrors that of peripheral macrophages (37-40), leading to distinctive mRNA profiles following stimulation with IL-4 and IL-10, including the expression of arginase 1 (Arg-1), chitinase-like protein 3, Fizz1 and peroxisome proliferator-activated receptor (PPAR) (41). Although these connections have been demonstrated *in vitro*, the induction

Table I. Phenotypes, inducible factors, surface markers and functions of macrophage polarization.

Cell type	Inducible factor	Surface marker	Phenotype	Function	(Refs.)
M1	IFN- γ , TNF- α , (GM)-CSF, LPS	TLR-2, TLR-4, CD80, CD86, iNOS, MHC-II	TNF- α , IL-1 α , IL-1 β , IL-6, IL-12, IL-23, CXCL1-3, CXCL8-10, CCL2-5, CCL11	Th1 immune reaction, proinflammation, anti-tumor	(24-26)
M2					
M2a	IL-4, IL-13	CD206, MHC-II, IL-1R, Arg-1, Ym1/2, FIZZ1	TGF- β , Arg-1, IL-10, CCL17, CCL22, CCL18	Cell growth, anti-inflammation, tissue repair, Th2 immune response, anaphylaxis, fibrosis	(25,35,36)
M2b	Immune complex, TLR, IL-1 β	CD206, MHC-II, CD86, IL-10R, IL-12R, IL-6R	TNF- α , IL-1 β , IL-10, IL-6, IL-12	Regulation of immune responses, inflammatory reactions	(32,35,36)
M2c	IL-10, glucocorticoids, TGF- β	CD206, CD163, TLR-1, TLR-8, Arg-1	IL-10, TGF- β , Arg-1, CXCL13, CCL16, CCL18	Phagocytosis, immunosuppression, tissue remodeling	(34-36)
M2d	TLR antagonists	CD206, IL-10R, IL-12R	VEGF, TNF- α , IL-10, IL-12	Promotion of angiogenesis and tumor progression	(33,35,36)

LPS, lipopolysaccharide, TLR, Toll-like receptor; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; MHC, major histocompatibility complex; iNOS, inducible nitric oxide synthase; CCL-2, C-C motif chemokine ligand 2; CXCL13, C-X-C motif chemokine ligand 13; Th1, type 1 T-helper cell; Arg-1, Arginase-1.

of M2 occurs *in vivo* in sterile wounds, even in the absence of IL-4 or IL-13 (42). In this model, M2 macrophages were observed to originate from M1 macrophages transitioning into repair-oriented macrophages within the tissue after recruitment from circulation (43). As a result, the intrinsic phenotype of these cells may diverge based on their origin and local microenvironment.

By contrast, although M2 macrophages are divided into different subpopulations, they share a common phenotype characterised by low production of IL-12 and IL-23 but a high release of IL-10. M2a macrophages express IL-10, TGF- β , C-C motif chemokine ligand (CCL1)7, CCL22 and other cytokines. In general, M2 macrophages are unique in that they release a low proportion of pro-inflammatory cytokines, such as IL-1, TNF- α and IL-6. However, M2b subpopulations are distinctive for high expression of IL-10 and CD86 but low production of IL-12 and Arg-1. Like M1 macrophages, they are proficient producers of IL-1, TNF- α and IL-6 (35,36). Furthermore, M2b macrophages express high levels of reactive nitrogen intermediates and iNOS (35,36).

M2c macrophages, also known as inactivated macrophages, secrete IL-10, TGF- β , CCL16 and CCL18, having a key role in the phagocytosis of apoptotic cells (34). In addition, M2d macrophages induce IL-10 and vascular endothelial growth factor (VEGF) production, thereby promoting angiogenesis and pathological tumour processes (33). The phenotypes, inducer factors, surface markers and functions of macrophage polarisation are shown in Table I, and a schematic diagram of the M1 and M2 macrophage subsets is shown in Fig. 1.

2. Intraocular inflammation-related diseases

Macrophages have pivotal roles in maintaining tissue homeostasis and regulating inflammation (44,45). M1 macrophages undergo polarisation triggered by lipopolysaccharide (LPS) alone or in conjunction with Th1 cytokines such as IFN- γ and granulocyte-macrophage colony-stimulating factor (GM-CSF). Consequently, M1 macrophages secrete pro-inflammatory cytokines, including IL-1 β , IL-6, IL-12, IL-23 and TNF- α , through the activation of various transcription factors, such as signal transducer and activator of transcription (STAT)1, nuclear factor κ B (NF- κ B) and IFN regulatory factor 5. Thus, M1 macrophages are characterised by a pro-inflammatory phenotype (46). Conversely, M2 macrophages receive polarisation signals primarily from Th2 cytokines, such as IL-4 and IL-13, and exhibit anti-inflammatory and immunomodulatory phenotypes (47). M2 macrophages produce anti-inflammatory cytokines, including IL-10 and TGF- β , by activating multiple transcription factors such as STAT3, STAT6, IFN regulatory factor 4 and PPAR- γ (48). The association between macrophage polarisation and inflammatory cytokine levels is illustrated in Fig. 2

Autoimmune uveitis. The experimental autoimmune uveitis (EAU) model is a noninfectious uveitis animal model that closely resembles human uveitis in both clinical and histological features (49-51). EAU in mice is induced through the subcutaneous injection of an emulsified antigen, which disrupts immune tolerance within the body. Following immunisation, naïve T cells receive antigens delivered by presenting cells.

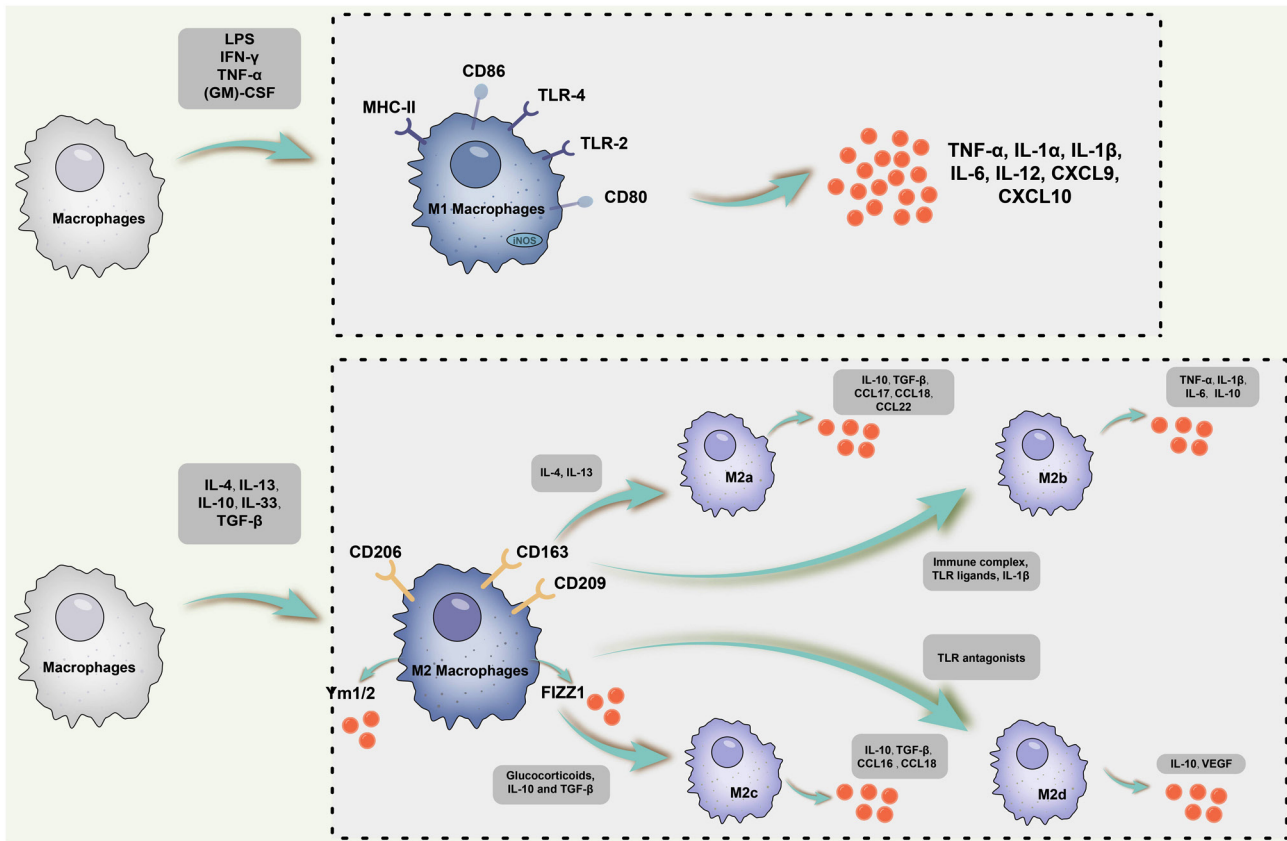


Figure 1. Schematic diagram of M1 and M2 macrophage subsets. Typically, M1-classically activated macrophages are polarized in response to IFN- γ , TNF- α and other cytokines, and express TNF- α , IL-1 α , IL-1 β , IL-6, IL-12, IL-23, CXCL9, CXCL10 and other cytokines and chemokines. M2 macrophages consist of four distinct subpopulations: i) M2a, induced primarily by IL-4 and IL-13; ii) M2b is mainly triggered by immune complexes, IL-1 β and TLR ligands; iii) M2c macrophages, produced in response to IL-10, glucocorticoids and TGF- β ; iv) M2d, induced primarily by TLR antagonists. Although M2 macrophages are divided into distinct subpopulations, they share a common phenotype characterized by low production of IL-12 and IL-23 but high release of IL-10. M2a macrophages express IL-10, TGF- β , CCL17 as well as CCL22 and other cytokines. However, the M2b subpopulation is unique in its high expression of IL-10 and CD86 but lower production of IL-12 and Arg-1. Like M1 macrophages, they are capable of producing IL-1, TNF- α and IL-6. In addition, M2b macrophages express high levels of RNI and iNOS. As for M2c macrophages, also known as inactivated macrophages, these cells secrete IL-10, TGF- β , CCL16 and CCL18 and have a key role in the phagocytosis of apoptotic cells. Furthermore, M2d macrophages induce IL-10 and VEGF production, thereby promoting angiogenesis and tumor pathological processes. CXCL9, C-X-C motif chemokine ligand 9; TLR, Toll-like receptor; iNOS, inducible nitric oxide synthase; RNI, reactive nitrogen intermediates; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; MHC, major histocompatibility complex; LPS, lipopolysaccharide; Arg-1, Arginase-1.

Subsequently, these cells are converted into Th0 cells, and T-cell subpopulations such as Th1 and Th17 differentiated from these cells have important functions in numerous autoimmune diseases, including EAU (52,53). These T cells multiply in the peripheral system and translocate to the retina, where they release inflammatory factors and promote macrophage migration, causing tissue damage (50).

Diverse immune-cell infiltration is a hallmark of the EAU retina, involving macrophages, neutrophils, dendritic cells and other immune cells (54). Furthermore, the proportions of various immune cells were observed to vary across different phases of EAU. During the acute EAU phase, macrophages accounted for 40% of all retinal immune cells. However, in the late chronic stage, the percentage decreased to 19%. By contrast, the percentage of immune cells, such as CD8 T cells and myeloid-derived suppressor cells (MDSCs), increased during the transition from the acute to the chronic phase (54).

In addition, the phenotypes of immune constituents infiltrating different EAU stages undergo dynamic changes. For instance, in the acute phase, most macrophages exhibit the M1 phenotype, whereas in the chronic (angiogenic) phase,

M2 macrophages are predominant (55). These results suggest that the retinal microenvironment under inflammatory conditions determines the subsets of infiltrating cells, in addition to controlling the phenotypes of different types of immune cell.

Studies have underscored the involvement of innate immune cells, particularly macrophages and microglia, in antigen presentation in EAU (56). Macrophages are recognised as crucial effector cells in EAU and contribute to the inflammatory process by releasing inflammatory cytokines (57). Retinal microglia exhibit phagocytic and pathogenic characteristics similar to those of macrophages. Upon activation, both macrophages and retinal microglia release pathogenic factors such as TNF- α and iNOS, resulting in the nitration of cytochrome c, which is known to cause EAU-cell apoptosis (58-60).

The aryl hydrocarbon receptor (AhR), a high-molecular-weight transcription factor, has been demonstrated to exert a negative regulatory effect on LPS-mediated inflammatory responses in macrophages (61). This suggests that AhR may be involved in the negative regulation of M1 polarisation. After EAU induction, AhR-/- mice had more severe

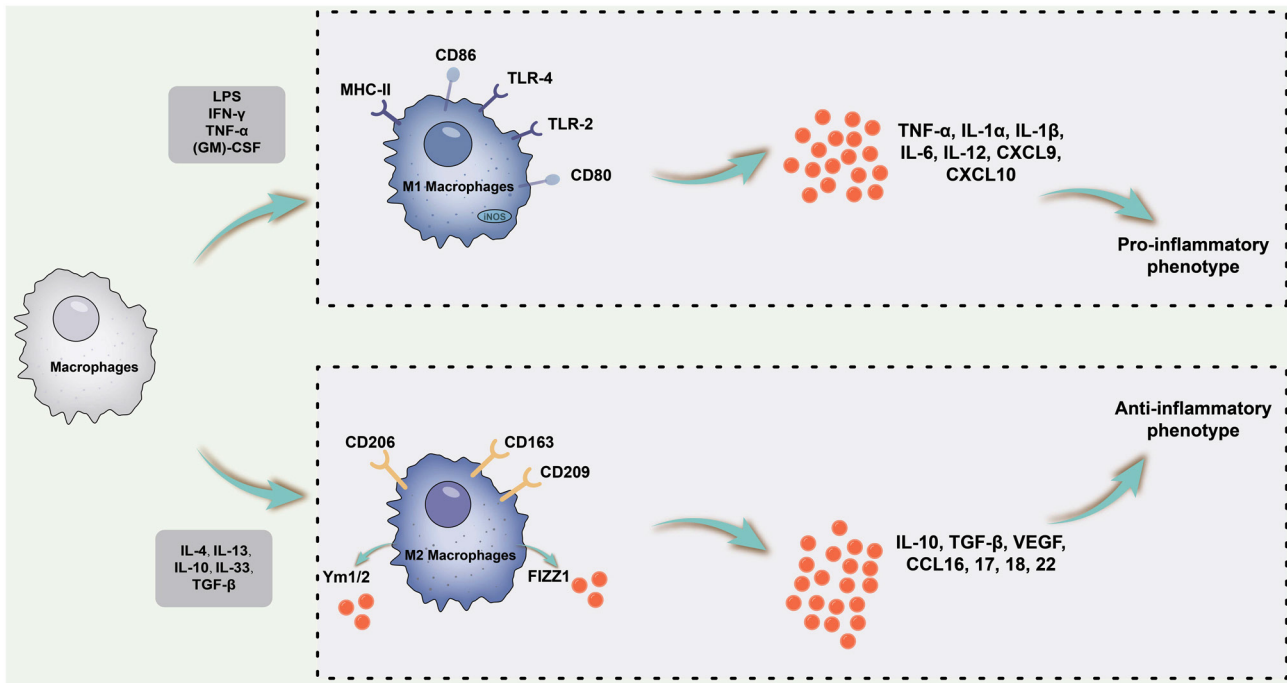


Figure 2. Schematic diagram of the association between macrophage polarization and inflammatory cytokines. M1 macrophage polarization is primarily triggered by LPS alone or in combination with Th1 cytokines, such as IFN- γ and GM-CSF. M1 macrophages are characterized by a pro-inflammatory phenotype and secrete pro-inflammatory cytokines, including IL-1 β , IL-6, IL-12, IL-23 and TNF- α . By contrast, M2 macrophages primarily receive polarizing signals from Th2 cytokines, such as IL-4 and IL-13, exhibiting an anti-inflammatory and immunomodulatory phenotype. M2 macrophages produce anti-inflammatory cytokines including IL-10 and TGF- β . MHC, major histocompatibility complex; LPS, lipopolysaccharide; Th1, type 1 T-helper cell; CXCL9, C-X-C motif chemokine ligand 9; TLR, Toll-like receptor; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; iNOS, inducible nitric oxide synthase.

clinical and histopathological manifestations of uveitis than AhR mice. Compared with AhR EAU mice, AhR^{+/+/-} EAU mice showed evidence of a significant increase in macrophages/microglia and a greater polarisation of phenotypes from M2 to M1 (62).

Furthermore, research has shown that the use of 2,3,7,8-tetrachlorodibenzo-p-dioxin (an AhR activator) can activate AhR through the NF- κ B, STAT1 and STAT3 signalling pathways. This induces macrophage M2 polarisation, reducing the production of apoptotic cells and the release of pro-inflammatory factors. Consequently, the clinical manifestations of EAU are alleviated (62).

IL-33 is a member of the IL-1 cytokine family and signals through a heterodimeric receptor composed of suppression of tumorigenicity 2 (ST2) and IL-1R accessory protein (63). Studies have highlighted the crucial role of the IL-33/ST2 pathway in enhancing the polarisation of alternatively activated macrophages (M2) (64).

Following 21 days of EAU induction, ST2-deficient mice showed worse clinical symptoms than non-knockout mice, whereas treatment of wild-type (WT) mice with IL-33 significantly improved uveitis lesions. This improvement was accompanied by a significant increase in the proportion of CD206 and CD273 cells, suggesting that the upregulation of the IL-33/ST2 signalling pathway drives macrophage (M2) polarisation, thus attenuating the clinical manifestations of EAU (65).

Furthermore, glucocorticoids have been reported to mediate the P38-MAPK/myocyte enhancer factor-2c axis, thereby promoting the polarisation transition of macrophages

from M2 to M1 and the release of anti-inflammatory factors. Consequently, this process inhibits EAU and fosters the healing of damaged eye tissue (66).

Suppressor of cytokine signalling (SOCS) proteins, particularly SOCS1 and SOCS3, regulate macrophage polarisation and cytokine expression. For instance, in BMDMs, SOCS3 acts as a negative regulator of GM-CSF-induced expression of CCL2, Arg-1 and matrix metalloproteinase 12 (67,68). LysM^{Cre/+}SOCS3^{fl/fl} mice (LysM^{Cre/+}SOCS3^{fl/fl} mice were obtained by crossing SOCS3^{fl/fl} mice with LysM-Cre mice, a type of mouse with SOCS3 deficiency in myeloid cells) showed an increased proportion of GM-CSF in the intraretinal milieu, which may have triggered the release of CCL2 and Arg-1 from macrophages.

Research has demonstrated that mice deficient in SOCS3 (LysM^{Cre/+}SOCS3^{fl/fl}) experience enhanced retinal degeneration and accelerated retinal angiogenesis owing to inflammation (69). In the acute phase of EAU, LysM^{Cre/+}SOCS3^{fl/fl} mice exhibited increased numbers of infiltrating neutrophils and decreased numbers of macrophages compared to WT mice. Real-time reverse transcription PCR analysis revealed a significant upregulation in the release of TNF- α , IL-1 β , IFN- γ , GM-CSF and Arg-1 in the retina of LysM^{Cre/+}SOCS3^{fl/fl} mice compared to that in WT mice. Furthermore, the percentage of Arg-1+ infiltrating cells was notably higher in LysM^{Cre/+}SOCS3^{fl/fl} EAU retinas than in WT EAU retinas. In the absence of SOCS3, both macrophages and neutrophils expressed higher levels of Arg-1, CCL2, IL-6 and VEGF, promoting angiogenesis, suggesting that deletion of SOCS3 partially induced M2 polarisation. Both isoforms of

arginase have been implicated in vascular cell dysfunction and vessel wall remodelling in various diseases (70). Arg-1 is a characteristic marker of M2-type macrophages. To treat EAU, researchers utilised an Arg inhibitor, amino-2-borono-6-hexanoic acid, which effectively inhibits retinal angiogenesis without improving inflammation (69). This suggests that the development of retinal fibrovascular membranes in EAU is associated with the polarisation of macrophages to the M2 phenotype.

Optic neuritis. Optic neuritis, an acute inflammatory demyelinating disease of the optic nerve, is an initial symptom of multiple sclerosis (MS). It is characterised by optic nerve degeneration and loss of retinal ganglion cells (RGCs), resulting in permanent visual impairment; however, reliable treatments for this condition are currently lacking. The well-established experimental autoimmune encephalomyelitis (EAE) mouse model used for studying MS has also proven useful for investigating optic neuritis. The mouse model is characterised by the upregulation of molecules involved in inflammation, gliosis and macrophage infiltration (71). Accumulation of inflammatory factors leads to macrophage infiltration, which subsequently produces a large number of potentially harmful cytokines, further fuelling the inflammatory process (72).

EAE, triggered primarily by autoimmune Th1 and Th17 cells, is an inflammatory disease of the CNS (73). These cells produce various cytokines, including IFN- γ , TNF and GM-CSF, which participate in the M1 polarisation process of macrophages. Approximately 70% of the immune cells in the inner environment of the CNS in an inflammatory state are macrophages that are responsible for most neuronal tissue damage by releasing TNF, NO and other inflammatory factors (74-77). During EAE pathology, macrophages exhibit a dual-activated phenotype that expresses both M1 and M2 markers, such as CD86 and chitinase-like protein 3 (78). During EAE, both M1 (IFN- γ) and M2 (IL-4) cytokines are present in the inflamed CNS. Therefore, promoting the conversion of M1 subpopulation macrophages to the M2 subpopulation can promote the repair of MS-related damage and ameliorate functional impairment (6). Studies have demonstrated that fatty acids (FAs) have a positive impact on neuronal rescue by modulating macrophage phenotypes, reducing pro-inflammatory capacity and enhancing tissue recovery capacity (79).

Among M1-related factors, IL-12 and IL-23 are largely involved in the progression of EAE (80) by inducing macrophage recruitment through the upregulation of CXCL-10 and CXCL-11 release (81). Conversely, M2-related markers, such as CCL-2, promote the repair of neuronal axons in the EAE model (82), and CCL-22 upregulates the migration of anti-inflammatory immune cells during EAE progression (83). Furthermore, studies have demonstrated that treatment with ω -3 FAs reduces RGC damage by regulating the conversion of the M1 to the M2 subpopulation (84).

In the context of optic neuritis in an EAE model, suppressing M1 subpopulations and activating M2 subpopulations can effectively prevent retinal inflammatory processes (85). This intervention holds promise for hindering optic nerve damage and protecting RGCs from death.

Sympathetic ophthalmia (SO). SO is a type of uveitis characterised by granulomatous lesions that occur after ocular surgery or penetrating trauma (86). It appears to occur as a delayed-type hypersensitivity reaction to antigens in tissues exposed to traumatic events (87,88). The histopathology of SO is often characterised by choroidal capillary involvement, the presence of eosinophils, inflammation within the scleral canal, and substantial infiltration by B lymphocytes and macrophages (89,90).

To further investigate the role of macrophages in the inflammatory process of SO, researchers have performed immunohistochemical staining of choroid tissue obtained from patients clinically diagnosed with SO. Their analysis revealed a significant presence of infiltrating CD68 cells, along with the infiltration of TNF- α , INF- γ and other cytokines (91). These findings strongly suggest that macrophages are involved in the pathological processes underlying SO.

The presence of Dalen-Fuchs nodules suggests granulomatous inflammation in the middle of the retinal pigment epithelial (RPE) and Bruch's zones (88,89,92). Granulomas primarily consist of activated macrophages (93,94) and may arise within the retina. Studies have shown that M1 macrophage-specific cytokines, such as IL-23 and CCL19, account for a large proportion of granulation tissue in SO (93), suggesting that most inflammatory cells in SO Dalen-Fuchs nodules and granulation tissue are M1 macrophages.

Retinitis pigmentosa (RP). RP is a retinal degenerative disease accompanied by the apoptosis of photoreceptor cells, often leading to severe visual impairment. Degeneration of photoreceptors is initiated by microglial activation, infiltration of macrophages, and accumulation of immunoglobulins and complement factors, resulting in persistent inflammation, proliferation of microglial cells and progressive apoptosis of retinal neurons (95,96). Consequently, it is crucial to explore avenues for RP intervention therapy that involve the regulation of microglial activation and suppression of the inflammatory response.

As RP advances, the blood-retinal barrier becomes disrupted, leading to the recruitment of macrophages into the retina. This recruitment has an important role in activating immune cells and triggering the release of pro-inflammatory factors, which further exacerbates disease progression and ultimately leads to the loss of the retinal photoreceptor layer (97). Blood-borne immune cells have a significant role in the microenvironment associated with RP and are considered key mediators of the development of neurodegenerative diseases (98,99).

Resident microglia and invading macrophages coordinate responses to CNS injury by restoring tissue loss and causing neuroinflammation (14,100). These immune cells have distinct phenotypes, such as M1 macrophages that foster inflammation and M2 macrophages that facilitate tissue repair and regeneration (101). Given the contrasting roles of these macrophage subsets, recent therapeutic approaches to nervous system inflammation are being tuned from immune cell suppression to achieving a balance through molecules that regulate the M1/M2 phenotypic polarisation switch (102).

Studies have demonstrated that olfactory ensheathing cell transplantation holds promise for regulating the polarisation of retinal macrophages from M1 to M2 in Royal College of

Surgeons rats via the JAK2/STAT3 pathway. This approach reduces the infiltration of activated M1 macrophages and fosters a less inflammatory microenvironment (103). Significant improvements in both the functional and structural aspects of vision can be achieved through this intervention. Similarly, essential FA supplementation improves retinal dysfunction and degeneration by reducing inflammation and microglial activation, weakening M1 markers, and inducing the transformation of rd10 mice (a model of autosomal recessive RP) retinas and LPS-stimulated BV10 cells to the M2 phenotype (104).

Glaucoma. Glaucoma is a neurodegenerative disease characterised by optic nerve atrophy and irreversible loss of RGCs (105). Secondary degeneration of RGCs has a critical role in the progression of glaucomatous damage (106), as RGC apoptosis may continue even after intraocular pressure is reduced. Hence, delaying secondary RGC degeneration holds promise as a potential therapeutic approach for glaucoma treatment.

Damage to RGCs can be categorised as primary (resulting from direct injury to the axon or cell body, such as axonal extrusion or transection) or secondary (resulting from the release of toxic effectors from adjacent dying cells) (107-110). To investigate the secondary degeneration of RGCs, researchers have developed the partial optic nerve transection (PONT) model. In comparison with the complete optic nerve transection and optic nerve crush models, which damage all axons simultaneously, the PONT model offers an advantage, as it only damages a portion of the inner axons of the optic nerve, leaving others intact. This enables the separation of the primary from the secondary injury (111).

In glaucoma, the mechanisms leading to the death of RGCs are multifaceted and encompass the activation of microglia/macrophages, autophagy, disturbances in calcium regulation, apoptosis, oxidative stress, expression of pro-apoptotic proteins and neurotrophic deprivation (112). Microglia and macrophages have essential roles in inflammation, tissue restoration and homeostasis regulation following inflammation or CNS injury (113). Various subsets of macrophages contribute to the pro-inflammatory, anti-inflammatory, cell growth and tissue repair processes. Therefore, manipulating the activation state of microglia/macrophage subsets to foster favourable cytoprotection in response to injury may be a promising approach for glaucoma treatment.

The study revealed a significant increase in the release of CD68, iNOS and Arg-1 one week after PONT modelling, indicating an increase in M1 microglia/macrophages, which may contribute to RGC death. However, researchers found that polysaccharides extracted from *Lycium barbarum* could delay RGC degeneration by four weeks after PONT. This delay was accompanied by an increase in the number of activated microglia/macrophages and a higher count of M2-type microglia/macrophages. This suggests that *L. barbarum* regulates microglia/macrophage phagocytic activity and induces M2 polarisation, ultimately leading to delayed RGC damage (111).

Furthermore, in a glaucoma ganglion cell injury model (N-methyl-D-aspartic acid-induced retinal injury model), studies demonstrated that pituitary adenylate cyclase-activating

polypeptide (PACAP) increased the proportion of M2 subpopulations. In addition, PACAP promoted the release of factors such as TGF- β 1 and IL-10 mRNA (112), both of which are markers of the M2 subtype of microglia/macrophages. The M2 subtype is associated with an acquired inactive state and is linked to reduced tissue damage, enhanced phagocytosis, increased synthesis of trophic factors, and decreased secretion of pro-inflammatory cytokines (25). These findings suggest that PACAP regulates the activation of microglia/macrophages toward the M2 subtype, thereby offering retinal protection against damage.

Ischaemic optic neuropathy. Clinically, non-arteritic anterior ischaemic optic neuropathy (NAION) often presents as optic disc oedema accompanied by acute painless visual loss (114). NAION is thought to arise from ischaemic damage to the optic nerve, which triggers an inflammatory response and oedema (115,116).

The optic nerve head is highly sensitive to changes in blood flow and is easily affected by factors such as autoregulation, vasospasm and systemic vascular diseases (117). In the context of NAION, inflammation is thought to be partially responsible for optic nerve damage (115,118). In a rat model of anterior ischaemic optic neuropathy (rAION), extracellular macrophages in the hypoxic region were recruited early and activated the resident microglia (119). Macrophages improve neuronal survival by secreting relevant factors and effectively phagocytosing myelin components to promote axonal regeneration (120). Furthermore, activated M2 microglia/macrophages have been linked to neuroprotection (121). However, it is important to acknowledge that in CNS diseases, activated microglia/macrophages may also release harmful factors, including pro-inflammatory cytokines and free radicals, which can cause damage to the nervous system (72). The proportion and subpopulation type of M2 microglia/macrophages in different pathological states may have distinct effects on the survival of RGCs and/or axon repair.

Research findings indicate that early treatment of the rAION model with G-CSF stabilises optic nerve vascular permeability, reduces macrophage recruitment near the optic nerve and induces M2 microglia/macrophage polarisation within the optic nerve (122). This treatment approach subsequently leads to a decreased expression of pro-inflammatory factors, prevents apoptosis induced by such factors and exerts neuroprotective effects in the rAION model.

Another study demonstrated that the binding complex of icariin and CCAAT enhancer-binding protein β significantly induces endogenous G-CSF expression by promoting alternative phosphorylation of I κ B kinase- β , inhibitor of NF- κ B (123). The elevated G-CSF expression then triggers noncanonical NF- κ B activation, which further activates the PI3K/serine/threonine protein kinase B-a (AKT1) signalling pathway and promotes M2 microglia/macrophage polarisation, thereby preventing neuroinflammation and RGC apoptosis after optic nerve infarction in a rAION. In addition, ω -3 polyunsaturated FAs have also been found to possess neuroprotective effects in rAION by promoting the transformation of M1 macrophages into M2 macrophages. This transformation subsequently reduces the release of pro-inflammatory factors, such as TNF- α , iNOS and IL-1 β , exerting anti-inflammatory

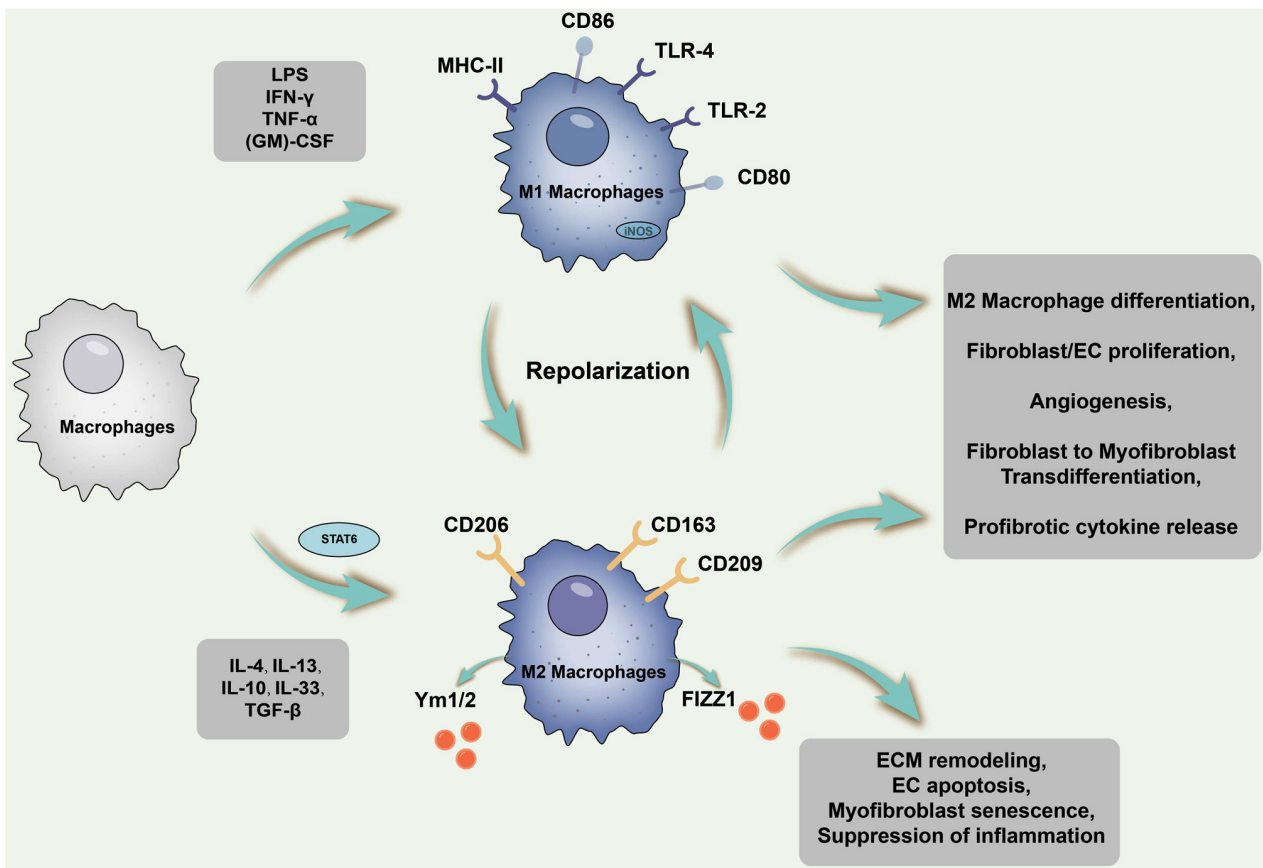


Figure 3. Schematic diagram of the processes related to macrophage polarization and fibrosis. During tissue remodeling and profibrotic phases, M2 macrophages emerge through differentiation of recruited infiltrating monocytes or polarization of infiltrating M1 macrophages. STAT6 is activated during this period to promote IL-4/IL-13-mediated M2 macrophage differentiation by upregulating the expression of Arg-1 and various other pro-fibrotic phenotype genes. M2 macrophages exhibit an anti-inflammatory phenotype and stimulate fibroblasts to enhance ECM production, among other effects. ECM, extracellular matrix; MHC, major histocompatibility complex; LPS, lipopolysaccharide; TLR, Toll-like receptor; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; iNOS, inducible nitric oxide synthase; STAT6, signal transducer and activator of transcription 6; Arg-1, Arginase-1; EC, endothelial cell.

effects that mitigate cytokine-induced optic nerve damage and help maintain RGC survival after infarction (84).

Furthermore, puerarin treatment has been found to stimulate the PI3K-AKT signalling pathway and sustain AKT1 activation, resulting in microglia/macrophages releasing CCAAT enhancer-binding protein β and hallmark M2 markers, such as Arg-1 and IL-10 (124,125). Polarisation of M1 microglia/macrophages into M2 microglia/macrophages reduces the proportion of TNF- α and IL-1 β after optic nerve infarction, effectively preventing subsequent cytokine-induced optic nerve injury.

3. Intraocular fibrosis-related diseases

Intraocular fibrosis-related diseases exhibit molecular mechanisms similar to fibrosis in organs, including the lungs, liver, kidneys, heart and skin (126). Following tissue injury, epithelial cells have a pivotal role in the recruitment and activation of inflammatory cells, endothelial cells and fibroblasts. Furthermore, epithelial cells undergo epithelial-mesenchymal transition (EMT), facilitating their transdifferentiation into myofibroblasts (127,128). These myofibroblasts are responsible for extracellular matrix (ECM) production, proliferation and migration across the basal layer, facilitating the coverage and regeneration of damaged tissue.

During this stage, M2 macrophages emerge either through the differentiation of recruited infiltrating monocytes or through the polarisation of infiltrating M1 macrophages. STAT6 activation occurs during this period, fostering IL-4/IL-13-mediated M2 macrophage differentiation by upregulating the expression of Arg-1 and various other profibrotic phenotype genes. M2 macrophages exhibit an anti-inflammatory phenotype and stimulate fibroblasts to enhance ECM production (129). The processes related to macrophage polarisation and fibrosis are illustrated in Fig. 3.

Proliferative vitreoretinopathy (PVR). PVR is characterised by extensive proliferation and shrinkage of cell tissues at the posterior interface of the vitreous and inner surface of the retina (130). Shrinkage of these cell membranes can lead to traction retinal detachment or the reopening of previously treated retinal tears, resulting in severe visual impairment. Although the exact pathogenesis of PVR remains to be fully elucidated, the prevailing view is that it is a long-term injury repair process involving the activation of inflammatory cells, cytokine production, ocular cell proliferation and scarring (131).

A pivotal characteristic of PVR is the formation of myofibroblast membranes from transdifferentiated RPE cells and other cell types, including macrophages (132). Macrophages

are considered one of the most crucial inflammatory cell types (133). By comparing vitreous samples from patients with PVR and uncomplicated retinal detachment, researchers have noted a significant increase in the number of monocytes/macrophages in vitreous samples from patients with PVR. Monocytes/macrophages were found to peak in the first 30 days after symptom onset in PVR and gradually decline over the subsequent three months (134). The abundance of macrophages in the intraocular microenvironment in the early stages of PVR and their sustained presence during progression underscore their vital role in the pathological process of PVR.

Macrophages have an irreplaceable role in the development of PVR through their ability to phagocytose damaged cells and tissues and release various growth factors and cytokines that mediate fibroblast chemotaxis and proliferation (135). Among the macrophage subsets, M2 macrophages, identified by the expression of Arg-1 and CD206, are particularly important in tissue repair and fibrogenesis (136).

In studies conducted in a rabbit model of PVR (137), researchers observed a swift onset of intense inflammation within the initial two weeks following PVR induction, with inflammation continuing to escalate until the 4-week mark post-induction. After the inflammatory phase, RPE cells undergo EMT, transitioning into fibroblast-like cells, which then give rise to contractile membranes. Throughout this process, the levels of growth factors, such as IFN- γ , VEGF, platelet-derived growth factor BB, placental growth factor and angiopoietin-2, surge, potentially fostering the survival, proliferation and EMT of RPE cells. The formation of contractile membranes and the secretion of growth factors are closely linked to M2 macrophages (133,138). Studies based on vitreous samples from human patients have indicated that M2 macrophage-derived microparticles can stimulate the proliferation and migration of RPE cells by activating the PI3K/AKT/mTOR signalling pathway (139), thereby contributing to the pathogenesis of vitreoretinal diseases.

Furthermore, studies based on vitreous samples from human patients suggested that M2 macrophages may contribute to the development of fibrovascular membranes in diabetic proliferative retinopathy (140). In a mouse model of PVR, CD206-positive M2 macrophages were found near the surface of the fibrous proliferation membrane, and the γ -secretase inhibitor DAPT was shown to inhibit RPE cell-induced PVR formation (decreased α -smooth muscle actin expression) and inhibit the infiltration of M2 macrophages by specifically targeting the Notch signalling pathway, thereby ameliorating PVR (141). These findings underscore the critical involvement of M2 macrophages in the pathogenesis of PVR and present a potential therapeutic target for intervention.

Subretinal fibrosis. Choroidal neovascularisation (CNV) is the primary cause of vision loss in neovascular age-related macular degeneration (nAMD), with CNV possibly progressing to end-stage fibrous plaques and disc scarring (142). In nAMD, the accumulation of drusen may lead to reduced oxygen diffusion in the choriocapillary plexus, eventually culminating in CNV. The subsequent growth of new abnormal blood vessels in the subretinal space often results in haemorrhage, triggering a wound-healing response that eventually leads to subretinal fibrosis (126).

Fibrosis is a healing process that occurs in response to tissue injury (128). During the healing phase, angiogenesis is triggered to facilitate tissue repair, enhance oxygen supply and facilitate the migration of inflammatory cells to the lesion area (143). In nAMD, CNV develops in the subretinal and/or subpigmented epithelial spaces, causing haemorrhages and leaks that ultimately lead to subretinal fibrosis. This process involves the recruitment and/or migration of various cell types. These cells interact with inflammatory factors, causing significant remodelling of the ECM (144).

Histopathological examination of human eyes revealed significant recruitment of macrophages during CNV, where they have a role in the development of pathological neovascularisation, drusen formation and fibroblast scaffolds (145). Direct anatomical and functional evidence suggests that circulating macrophages rather than resident macrophages are responsible for laser-induced CNV. To construct subretinal fibrosis models, researchers commonly use a laser-induced acute wound-healing model of CNV (146,147). In this experimental setup, macrophages were found to promote the formation of fibroblast scaffolds during the early wound-healing response of laser-affected CNV lesions, with most macrophages at the laser injury sites being activated M2 macrophages (147). CNV membranes infiltrated by M2 macrophages were more susceptible to fibrosis than those with M1 macrophage infiltration (148).

Studies based on the laser-induced subretinal fibrosis model have demonstrated that inhibiting macrophage transition to the M2 subpopulation via the PI3K/Akt axis contributes to the improvement of fibrotic lesions in subretinal fibrosis (146). In addition, the application of triptolide has shown promise in reducing subretinal fibrosis by inhibiting the polarisation of M2 subpopulations and suppressing the activation of the TGF- β 1/Smad axis, thereby downregulating TGF- β 1-induced EMT/endothelial-MT (149).

4. Intraocular malignancy

Macrophage phagocytic activity has a crucial role in clearing dead and dying cells. However, tumours can regulate macrophage function, thwart macrophage-triggered inflammation and kill tumour cells. This metabolic reprogramming drives the transformation of macrophages into either the M1 or M2 subpopulations, which are influenced by various cytokine stimuli. Although tumour-associated macrophages do not strictly conform to the M1 and M2 subpopulations, they often have similarities to M2 and actively promote tumour growth by upregulating immunosuppression (150).

Research indicates that, in the tumour microenvironment, M1-polarised macrophages primarily depend on glucose flux and the conversion of glucose to lactate, along with the production of reactive oxygen species and NO to combat tumours. Conversely, M2-polarised macrophages predominantly rely on FA β -oxidation and the tricarboxylic acid cycle while stimulating the production of polyamines and L-proline to facilitate tumour growth (150). Macrophage polarisation and tumour-related processes are shown in Fig. 4.

Uveal melanoma. Uveal melanoma is the most prevalent primary intraocular malignancy in adults, with an incidence of ~6-7 new cases per million individuals (151). The current

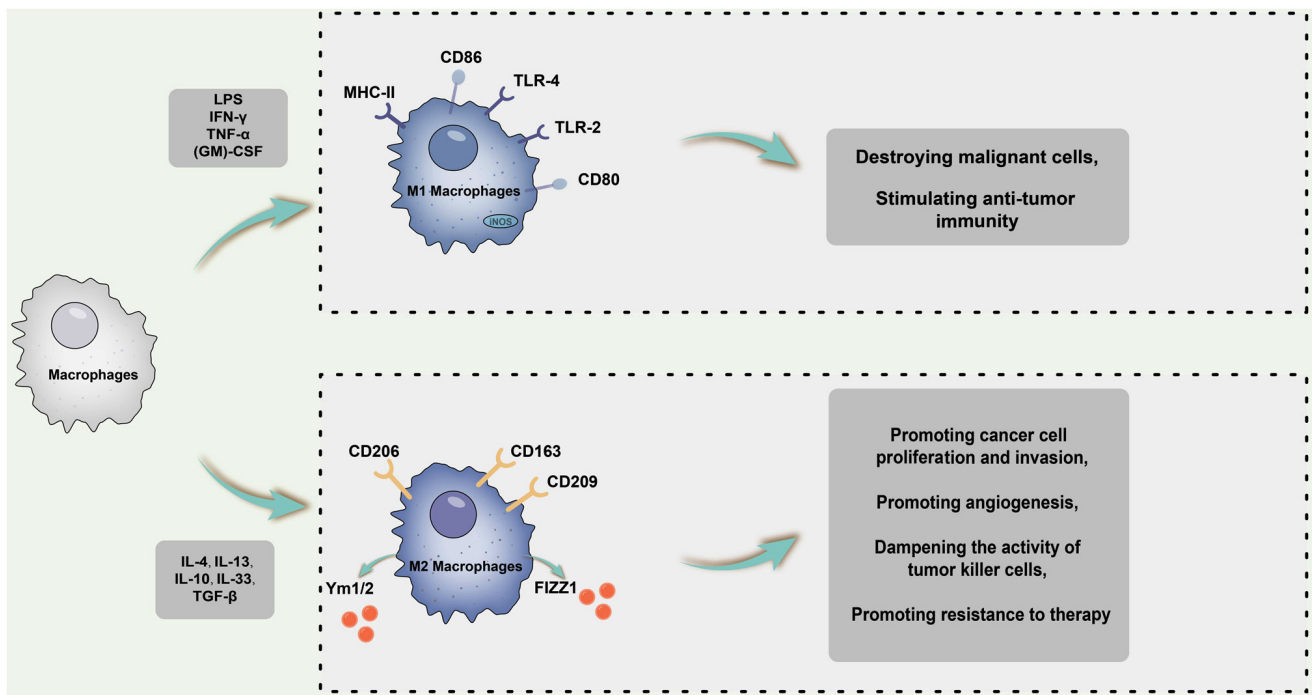


Figure 4. Schematic diagram of macrophage polarization and tumor-related processes. In the tumor microenvironment, M1-polarized macrophages fight tumors primarily by killing tumor cells and stimulating anti-tumor inflammation. By contrast, M2-polarized macrophages promote tumor growth mainly by promoting tumor cell proliferation and invasion and promoting angiogenesis. Although tumor-associated macrophages do not strictly fit into the M1 and M2 subpopulations, they often resemble M2 and actively promote tumor growth by upregulating immunosuppression. LPS, lipopolysaccharide; TLR, Toll-like receptor; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; iNOS, inducible nitric oxide synthase.

treatment modalities include enucleation and radiation therapy. Although these treatments can curtail primary tumour growth, they remain ineffective in preventing tumour metastasis, leading to death within ~1-3 years (152).

Studies have provided compelling evidence for the pivotal role of macrophages in melanoma growth and survival. Melanoma-derived exosomes have also been identified as mediators of immunosuppression (153). These exosomes exert their effects by directly interacting with and suppressing various lymphocytes or by inducing MDSCs. In turn, MDSCs promote M2 subset transformation and recruit tumour-promoting regulatory T cells (154).

Researchers have postulated that lysing IFN- γ released by both tumour cells and immune cells in the microenvironment may be a contributing factor in transforming macrophages from a tumour-promoting M2 phenotype to an anti-tumour M1 phenotype, primarily through the IFN- γ /JAK-STAT1 pathway (155,156). In a mouse xenograft model, treatment with the oncolytic herpes simplex virus 1-enhanced green fluorescence protein through vitrectomy injection led to increased IFN- γ levels, an elevation in M1 macrophages and a reduction in M2 macrophages in peripheral blood, intraocular sites and distant tumours. *In vitro* experiments have further demonstrated a significant increase in IFN- γ at both the RNA and protein levels following oncolytic virus infection (157). Consequently, this treatment approach effectively reduced intraocular and subcutaneous tumours throughout the body.

However, it has been observed that melanoma exosomes can induce both M1 and M2 representative factors, namely TNF- α and IL-10, respectively (154). Furthermore, macrophage

function assays have revealed an increasing trend from iNOS (M1) to Arg-1 (M2) activity, indicating that melanoma exosomes can induce a 'mixed' M1 and M2 tumour-promoting macrophage activation phenotype. Thus, in the pathological progression of uveal melanoma, M1 and M2 macrophage subpopulations seem to have flexible adaptability to tumour survival.

5. Intraocular neovascularisation-related diseases

Macrophages have a role in angiogenesis, albeit to a limited extent, by promoting the production of pro-angiogenic and growth factors, such as VEGF-A and fibroblast growth factor 2 (FGF2). Research indicates that M1 macrophages may facilitate vascular sprouting through the secretion of VEGF, IL-1 β and TNF- α (158). Conversely, investigations have demonstrated that M2 macrophages, rather than M1 macrophages, enhance angiogenesis *in vivo* with increased expression of VEGF, FGF2, insulin-like growth factor 1, CCL2 and placental growth factor (33,159). Furthermore, a study determined that M2-polarised macrophages exhibit greater angiogenic potential than other subpopulations (159). However, the precise mechanisms underlying macrophage-mediated angiogenesis and the cellular interactions between endothelial cells and macrophage subsets remain to be fully elucidated. Although the categorisation of macrophages into distinct subpopulations offers a simplified overview of their intricate functional activities in the body, the specific mechanisms involved have not been determined. Macrophage polarisation and angiogenesis-related cytokines are shown in Fig. 5.

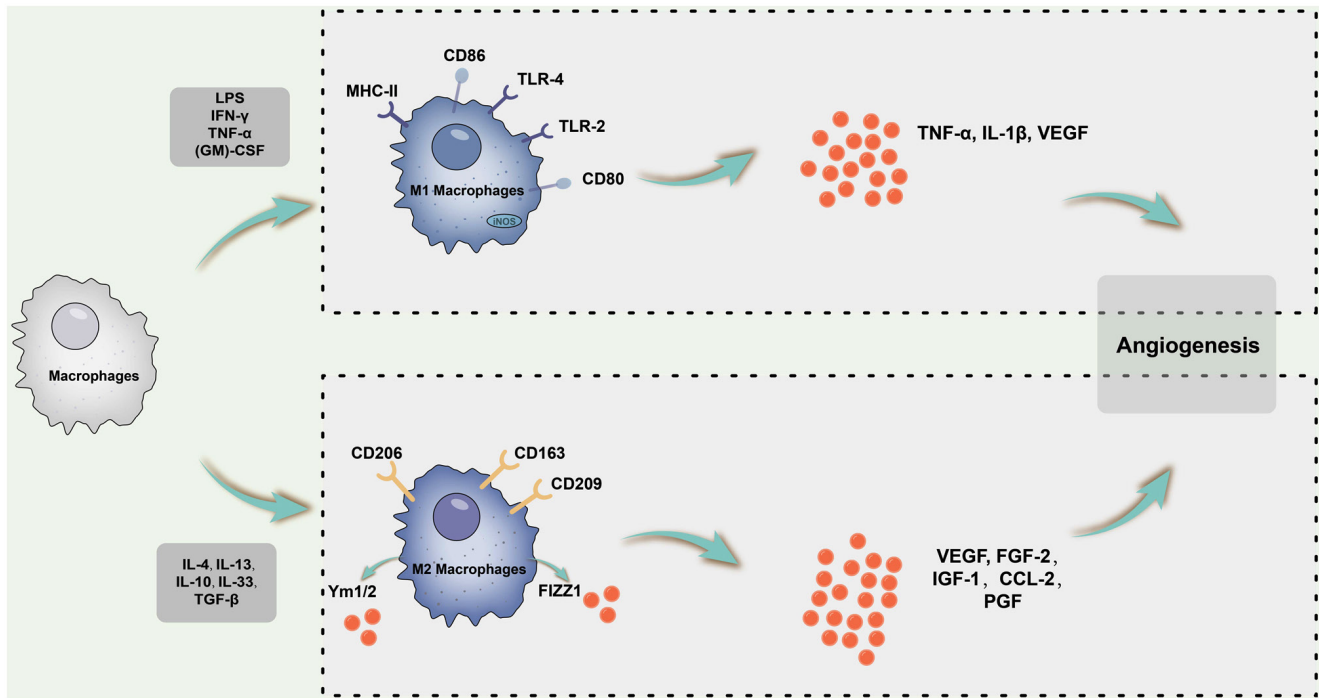


Figure 5. Schematic diagram of the macrophage polarization process and angiogenesis-related cytokines. M1 macrophages may promote vascular sprouting by secreting VEGF, IL-1 β and TNF- α . By contrast, M2 macrophages enhance angiogenesis *in vivo* and enhance the expression of VEGF, FGF-2, IGF-1, CCL-2 and PGF. LPS, lipopolysaccharide; TLR, Toll-like receptor; (GM)-CSF, granulocyte-macrophage colony-stimulating factor; iNOS, inducible nitric oxide synthase; IGF, insulin-like growth factor; CCL2, C-C motif chemokine ligand 2; FGF, fibroblast growth factor; PGF, placental growth factor.

Diabetic retinopathy (DR). Diabetes is a metabolic disorder primarily characterised by hyperglycaemia stemming from abnormal insulin secretion and insulin resistance. Among patients with DR, microvascular complications are the most common manifestation. DR is categorised into non-proliferative DR (non-PDR) and PDR (PDR), with a distinction based on the presence of retinal neovascularisation (160). Non-PDR is typically characterised by asymptomatic microvascular changes, whereas PDR is involved in angiogenesis (161). DR is characterised by the abnormal growth and leakage of small blood vessels, leading to local oedema and associated tissue dysfunction. Dysregulation of vascular regeneration and inflammation are thought to be involved in the pathogenesis of DR (162,163).

Researchers have observed that upon high-glucose stimulation, microglia initially polarise toward the M2a phenotype, a response that initially mitigates tissue damage (164). However, as time progresses, there is an escalation in the production of M1 pro-inflammatory factors, accompanied by a decrease in the production of M2 anti-inflammatory factors, gradually shifting the macrophages toward the M2b phenotype. In advanced stages, microglia tend to exhibit an M1 phenotype with pronounced pro-inflammatory effects. In a rat model of streptozotocin-induced DR, there was an increase in M1 polarisation and a decrease in M2 polarisation, and microglia tended toward M1 polarisation with increasing glucose concentrations (165). The levels of iNOS (an M1 marker) and Arg-1 (an M2 marker) were higher in the retinas of db/db mice at five weeks of age. However, at eight weeks of age, iNOS levels continue to increase, whereas Arg-1 levels return to baseline (166).

It has been indicated that melatonin inhibits the excessive activation of microglia in the retina of diabetic rats by inhibiting the PI3K/AKT/Stat3/NF- κ B signalling pathway, i.e.,

reducing the number of microglia cells and promoting their anti-inflammatory properties (167). It was speculated that this is related to melatonin promoting the transformation of microglia from pro-inflammatory (M1) to anti-inflammatory (M2) cells. Similarly, it was demonstrated that inhibiting M1 polarisation and promoting M2 polarisation of retinal microglia in DR rats through the TLR4/MyD88/NF- κ B p65 pathway can effectively improve early DR (168).

Retinopathy of prematurity (ROP). ROP is characterised by impaired retinal blood vessel growth and development in preterm infants, which frequently leads to visual impairment and blindness (169). The pathological process of ROP is divided into two stages: An initial phase marked by delayed vascular growth after birth accompanied by vascular regression, followed by a second phase of hypoxia-induced pathological angiogenesis (170). An abnormal vascular state disrupts the inner retinal environment, thereby exacerbating the ischaemic state and leading to retinal leakage, scarring and eventually blindness. Although treatments such as laser and cryotherapy have improved ROP-related blindness, visual outcomes remain suboptimal for treated patients. Laser treatment has been successful in resolving most threshold ROP cases (171) and 100% of pre-threshold ROP cases (172). However, eyes treated with cryofixation or laser photocoagulation often manifest structural sequelae (171), underscoring the pressing need for preventive and less invasive therapeutic approaches.

Vascular abnormalities and inflammatory cell recruitment are primary contributors to the progression of abnormal retinal vascular diseases, including PDR and ROP (173). Studies have shown that macrophages have a role in promoting abnormal angiogenesis during pathological retinal vessel growth and

that M1 and M2 subpopulations of macrophages are present in the intraretinal environment of ROP (174).

Studies have demonstrated that microglia/macrophages are activated after P12 once oxygen-induced retinopathy (OIR) models are established. M1 microglia/macrophages were observed in neovascular tufts located in the retina, starting at P12 and reaching their peak at P17 upon returning to normoxic conditions. At this time-point, the NF- κ B/STAT3 axis is triggered, which results in an increased proportion of M1 microglia/macrophages and an enhanced proportion of TNF- α and IL-6. Consequently, the neovascular clusters exhibit a progressive increase in volume from P12 to P17. However, a shift to M2-type microglia/macrophage activity occurs from P17 onwards during the advanced stages of OIR. The IL-4/STAT6/PPAR- γ axis is triggered from P17 and reaches its maximum at P20, promoting M2 microglia/macrophage transformation. This, in turn, results in a decrease in inflammatory factors and regression of neovascular clusters (175).

Furthermore, investigations have revealed that cytokines TNF- α and VEGF, released by the M1 subpopulation, promote abnormal angiogenesis through interactions with endothelial cells. By contrast, M2 macrophages promote vascular anastomosis. The involvement of Notch1 signalling has been reported, although the exact secretory factors remain to be elucidated (176). These findings underscore the coordinated engagement of M1 and M2 macrophage subsets in guiding retinal neovascularisation.

Promoting macrophage transition from the M1 to the M2 phenotype during the pathological process of OIR is thought to have anti-angiogenic benefits. To investigate this, Marchetti *et al.* (177) used human umbilical cord blood to obtain enriched progeny CD14(+) cell populations, which were then injected into the eyes of OIR mice. The results demonstrated that only CD14(+) cells polarised into M2-type macrophages could promote the normalisation of retinal vasculature and control pathological neovascularisation. Consequently, areas of vascular occlusion and associated tissue hypoxia were reduced. A separate study found that in the OIR retina, activated microglia/macrophages were predominantly of the M1 type rather than the M2 type. Treatment with ferulic acid was shown to decrease the proportion of iNOS+ microglia/macrophages while increasing the release of Arg-1, suggesting its potential to transform microglia/macrophages from the M1 type (expressing iNOS, CD86, IL-6 and TNF- α) to the M2 type (expressing Arg-1, IL-10 and CD206), thus exerting a strong anti-angiogenic effect (178).

It has been demonstrated that blocking the activation of NF- κ B signalling can effectively promote the transformation of M1 macrophages into the M2 phenotype in OIR mice and subsequently reduce the number of neovascular clusters (179). In addition, IL-17A neutralisation attenuates ocular neovascularisation by increasing the proportion of M2 macrophages and downregulating the release of VEGF from M1 macrophages (180).

To explore macrophage polarisation in an OIR mouse model, researchers assessed the retina and found a significant increase in both M1- and M2-like macrophages compared to normal controls. Both M1 and M2 macrophages exhibit a pro-angiogenic effect, promoting human umbilical vein endothelial cell (HUVEC) proliferation and contributing to retinal

pathological neovascularisation (181). Similarly, Ma *et al.* (174) investigated patients with advanced ROP and revealed a pro-angiogenic and pro-inflammatory microenvironment, with M1 macrophages predominant over M2.

In studies focusing on the role of pigment epithelium-derived factor, it was shown to inhibit macrophage polarisation in the retinas of an OIR mouse model through the regulation of adipose triglyceride lipase in the MAPK and Notch1 pathways. Specifically, pigment epithelium-derived factor suppressed the Notch1 and MAPK signalling pathways by inhibiting adipose triglyceride lipase, leading to a significant reduction in the release of iNOS and Arg-1, which are characteristic factors of M1 macrophages and M2 subpopulations, respectively. This ultimately resulted in a reduction in retinal neovascularisation (181).

Consequently, the role of macrophages in neovascularisation in OIR models remains a subject of debate, as both M1 and M2 macrophages may be involved. On the one hand, in the OIR model, retinal neovascularisation can manifest in two forms: Pathological neovascularisation, characterised by the emergence of abnormal blood vessels sprouting from the retinal surface into the vitreous, and physiological revascularisation, which involves the restoration of avascular regions with functional intraretinal vessels (182). Therefore, their proportions at different pathological stages may have contradictory effects. Ritter *et al.* (183) found that a large number of migrating cells were localised in the retinal ischaemic area with a large loss of microglia, which may replace the function of microglia and promote vascular remodelling in the damaged area by releasing an appropriate amount of VEGF. Therefore, their location within the retina may also be one of the reasons for their contradictory roles in OIR models.

CNV. Wet AMD is a disease that causes vision loss due to the growth of CNV in the macula (184). Aberrant neovascularisation initially proliferates under the RPE band and then breaches the RPE band, causing intraocular haemorrhage and exudative serous retinal detachment, and later, discoid scarring (126). This localised loss of the retinal photoreceptor layer and RPE zone results in irreversible macular function loss and vision impairment.

CNV is considered involved in the submacular healing process (126,144). Angiogenesis has a crucial role in this process, and current clinical strategies predominantly focus on reducing the levels of VEGF, which is the primary factor that promotes angiogenesis (185,186). However, despite these efforts, only ~30% of patients with exudative AMD experience a three-line improvement in visual acuity, and ~15% of patients experience progressive deterioration, leading to legal blindness, even after receiving VEGF-inhibiting drugs (187-189). These results were expected, considering angiogenesis is an integral part of the complex healing phase. Hence, the search for alternative therapeutic approaches for CNV beyond anti-angiogenic treatments continues.

Multiple studies have explored AMD pathology and identified inflammation as a key driver of neovascular AMD progression (99,126,144,190,191). AMD is characterised by a chronic inflammatory response. Within this inflammatory milieu, macrophage recruitment and cytokine regulation are key mediators of CNV development (191).

Studies have indicated that macrophages are involved in abnormal angiogenesis in the pathology of CNV. M1 macrophages, characterised by specific markers such as iNOS, IL-6 and TNF- α , have been shown to inhibit angiogenesis (159). Conversely, M2 macrophages, identified using specific markers such as Arg-1, CD206 and CD163, promote pathological angiogenesis in CNV (192). Nakamura *et al* (193) revealed that increased IL-10 release in the eyes of aged mice activates associated signalling pathways, resulting in an increased proportion of M2 macrophages and the activation of vascular proliferative processes. Macrophage polarisation has emerged as a potential therapeutic target for CNV treatment.

In laser-impacted CNV, dynamic patterns of M1 macrophages and M2 subpopulations were observed, showing an early and immediate shift to M1, followed by a sustained shift to M2. M1 macrophages appear to be involved in the initial stages of CNV, whereas M2 macrophages have a critical role in the middle and late stages of CNV development and remodelling (194). For instance, during experimental CNV, upregulation of M1 signature factors (TNF- α and iNOS) was observed at day 3, suggesting inflammation at the onset of CNV lesions. By contrast, CD206 reached its maximum expression on day 7 of CNV formation, whereas CD86 and CD163 reached their maximum expression on day 14 of lesion formation. These marker genes represent different M2 macrophage subpopulations, suggesting that different macrophage subtypes have distinct roles at different time-points during the pathological process of CNV. Specifically, M2a macrophages may be associated with neovascularisation, whereas M2b and M2c macrophages may be involved in fibrous scarring (195).

CSF1, also known as macrophage CSF, has a crucial role in macrophage recruitment (196) and the transition to the M2 subpopulation (197). When the CSF1 receptor receives CSF1, the PI3K/AKT/forkhead box (FOX)O1 axis is activated, promoting M2 polarisation. Furthermore, under hypoxic conditions, HUVECs release more CSF1, thereby promoting macrophage migration and transition to the M2 subpopulation by upregulating the PI3K/AKT/FOXO1 axis. In a CSF1/CSF1 receptor (CSF1R)-associated manner, the M2 subpopulation upregulates the proliferation, recruitment and lumen formation of HUVECs. Inhibition of the CSF/CSFR axis has been shown to suppress M2 polarisation of macrophages and attenuate laser-induced CNV formation in mice (198).

In addition, studies have revealed that long non-coding RNA nuclear paraspeckle assembly transcript 1 (NEAT1) promotes the expression of M2 macrophage markers by targeting phosphatase and tensin homolog via microRNA (miR)-148a-3p. Downregulation of NEAT1 can effectively inhibit CNV by suppressing the transformation of M2 macrophage subsets (199).

Researchers have proposed that the Rho-associated protein kinase (ROCK) signalling pathway is a key pathway in regulating macrophage polarisation, and that the expression of ROCK pathway-related factors and pathway signal transduction processes affect the pathological process of CNV. They conducted experiments by differentiating mouse BMDMs into M1 or M2 phenotypes and injecting them into the eyeballs of laser-modelled WT mice. They observed that CNV lesions were not altered by native-morphological macrophages but that M2 subpopulation macrophages promoted lesion progression,

which was reversed in ROCK2 inhibitor-treated animals. By contrast, the M1 subpopulation ameliorated the damage caused by the CNV. Further intravitreal injection of the M1 subpopulation in laser-modelled mice treated with a ROCK2 inhibitor did not ameliorate CNV-induced damage, confirming that ROCK2 inhibits CNV lesions *in vivo* by promoting the polarisation transition of macrophages to M1 (200). Ras homolog family member A (RhoA) expression and myosin phosphatase target subunit 1 and myosin light chain phosphorylation are also upregulated in CNV and decreased by melatonin administration (201). The RhoA/ROCK axis promotes the transition of macrophages to the M2 subpopulation and prevents conversion of the M1 subpopulation, thereby triggering CNV lesions. Melatonin converts M2 microglia/macrophages to the M1 subset by inhibiting the RhoA/ROCK axis, resulting in the downregulation of CNV lesions, reduced associated vascular leakage and inhibition of abnormal vascular status in laser-affected CNV lesions.

Other studies have shown that miR-505 is abnormally upregulated in laser-induced CNV lesions. Transmembrane protein 229 B (TMEM229B) was identified as a direct target of miR-505-5p, and administration of an miR-505 inhibitor significantly upregulated the expression of endogenous TMEM229B in CNV mice. This specific inhibition of M2 polarisation in mice with CNV led to reduced VEGF expression and suppressed CNV formation. *In vitro* experiments further demonstrated that exogenous TMEM229B significantly inhibited the expression of the M2-specific markers Ym-1 and Arg-1 (202).

In addition, the IL-4 mutant protein IL-4/Q116E was found to regulate the inflammatory response of laser-induced CNV through the Notch/delta-like canonical Notch ligand 4/monocyte to macrophage differentiation-associated signalling pathway, increasing the expression of CD68 and CD80 and reducing the expression of Arg-1 in RPE choroidal tissue. Induction of macrophage polarisation from M2 to M1 attenuated CNV development (203).

Furthermore, in the context of a laser-induced CNV model, injured RPE upregulated 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3)-driven glycolysis in macrophages, resulting in the induction of hypoxia-inducible factor (HIF)-1 α /HIF-2 α and NF- κ B. This subsequently induced the expression of macrophage subset-associated signature factors and pro-angiogenic factors, ultimately promoting the transformation of the M1 subpopulation of macrophages into the M2 subpopulation and promoting CNV development. However, the PFKFB3 inhibitor AZ67 effectively downregulated the expression of HIF-1 α /HIF-2 α and NF- κ B signalling and largely prevented laser-affected CNV lesions (204).

Furthermore, researchers have used small interfering RNAs (siRs) to suppress TIMP metalloproteinase inhibitor 3 (TIMP-3) expression in BMDMs and RPE/choroidal tissues in a laser-induced mouse model of CNV. They found that the release of M2 biomarkers CD206, CD163, Arg-1 and Ym-1 was correspondingly upregulated *in vitro* and *in vivo* in the siR-TIMP-3 group, indicating that a lack of TIMP-3 may promote M2 macrophage differentiation. Furthermore, intraocular injection of siR-TIMP-3 was shown to upregulate the progression of CNV lesions, as detected using optical coherence tomography angiography, suggesting that TIMP-3

inhibition is associated with the M2 macrophage subset and has a key role in CNV formation (205).

Finally, studies have shown a greater proportion of M2 macrophages compared with the M1 subpopulation during three and seven days of buffer treatment in a laser-induced mouse model (206). Triptolide significantly downregulated the accumulation of the M2 subpopulation at the lesion site over the 3 and 7-day periods. Triptolide also reduced the proportion of M2 macrophages during the same periods. In addition, triptolide decreased the release of VEGF, intercellular adhesion molecule 1 and TNF- α in local CNV injury, consistent with a reduction in the total number of aggregated macrophages and a lower ratio of the M2 subpopulation. Consequently, intraperitoneal injection of triptolide inhibited the transformation of the M2 subpopulation in CNV focal lesion areas, resulting in the downregulation of inflammatory and angiogenic factors, thereby inhibiting CNV progression and macrophage infiltration in CNV focal areas (207).

6. Conclusion

The present review provides an overview of key findings on the role of microglia/macrophage polarisation in intraocular diseases. It also provides ideas for further research on macrophage polarisation and the role of different subpopulations in intraocular diseases and a summary of the association between macrophage polarisation and different diseases. In intraocular tissues, there are not only BMDMs but also specialised resident macrophages called microglia, which provide the initial defence against microorganisms and participate in immune regulation. They have key roles in phagocytosis by clearing apoptotic cells and tissue debris. Dysfunction of macrophages/microglia may lead to autoimmune and persistent inflammatory diseases. An increasing number of studies have shown that macrophage or microglial polarisation has a key role in the pathological process of intraocular diseases and that regulating the polarisation process can effectively delay the progression of related diseases.

The present study provides the first review of the association between macrophage polarisation and intraocular disease. Although studies have been published on the relationship between macrophage polarisation and intraocular neovascular diseases, the publication time was relatively early and the literature included was not comprehensive. Simultaneously, there is a lack of an overview of the relationship between macrophage polarisation and intraocular fibrosis- and inflammation-related diseases. In addition, the present review covers, as much as possible, the typical literature on the association between intraocular disease and macrophage polarisation to provide a more comprehensive overview.

The present study also has certain limitations. On the one hand, certain studies have divided macrophages into different subgroups during the study to simplify their complex functional activities in the body, but not enough to elucidate the specific mechanisms involved. In addition, most studies simply divided macrophages into M1/M2 subgroups and did not further subdivide M2a, M2b or other subgroups in terms of research methods. Therefore, only an overview of the relevant mechanisms can be provided, rather than discussing them in depth. Although all attempts were made to unify the

differences in research models in the process of literature inclusion, there may still be differences in methods among certain studies. Different models can only simulate the pathological process of diseases locally, which may lead to differences in the relevant research conclusions. In the present study, it was attempted to determine the association between macrophage polarisation and intraocular diseases. Therefore, the discussion section provided a simplified overview of the mechanism and did not discuss its relevance to other cell types (neutrophils, T cells, fibroblasts, etc.) in depth. Furthermore, because there are few clinical studies on the association between macrophage polarisation and intraocular diseases, it is difficult to collect clinical literature on the pathogenesis of diseases.

As mentioned earlier, the current study lacks further delineation of the macrophage subsets; therefore, future studies need to identify the macrophage subsets involved in the disease process to further elucidate the specific mechanisms involved. Although numerous basic studies are related to intraocular diseases, relevant clinical studies are still lacking. Therefore, in the future, more clinical studies on the association between macrophage polarisation and intraocular diseases are required to further explore the disease mechanism.

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Availability of data and materials

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Authors' contributions

HL analyzed and summarized the literature on the association of macrophage polarization with intraocular disease and was a major contributor in writing the manuscript. BL is responsible for the search and collection of literature related to macrophage polarization and intraocular diseases. YLZ optimized the writing structure and developed the idea of the article. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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