Review Article

Inflammatory pathways in pathological neovascularization in retina and choroid: a narrative review on the inflammatory drug target molecules in retinal and choroidal neovascularization

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Objective: We review inflammatory drug targets in retinal and choroidal neovascularization (NV) in narrative manner.

Background: Vascular remodeling and angiogenesis are processes typically associated with wound-healing mechanisms intended to minimize ischemia and maintain tissue homeostasis. In the eye, however, these actions primarily deteriorate tissue homeostatic recovery, and could even contribute to the progress of severe conditions, e.g., blindness. Angiogenesis in diabetic retinopathy (DR) and age-related macular degeneration (AMD) is the primary cause of vision loss in working-age and elderly populations. Current treatment of anti-vascular endothelial growth factor (VEGF) agents has limited action efficacy, working in less than 50% patients. Understanding cellular and molecular networks associated in retinal vascular remodeling may provide an insight to develop novel therapeutic strategies.

Methods: Here, we highlight ocular cells—endothelial, mural, retinal pigment epithelium (RPE), glial and macrophages, as well as inflammatory molecules—such as the complement system, stromal derived factor-1, chemokine CXC receptor-4, inflammasome, interleukin-18, programed cell death ligand-1, insulin-like growth factor (IGF) and sphigosin-1-phosphate receptor, associated with retinal and choroidal NV, and discuss their recent and future therapeutic approaches.

Conclusions: A deeper understanding on pathogenesis, pathobiology including ocular immunobiology of retinal and choroidal NV will pave the way to expand and overleap the current therapeutic approach.

Keywords: SDF-1/CXCR4 axis; inflammasome; programmed cell death ligand-1 (PD-L1); insulin-like growth factor (IGF); sphigosin-1-phosphate (S1P)

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Introduction

The retina works with a high metabolic demand, and vascular maintenance is vital to sustaining normal metabolic function, visual processing and retina homeostasis (1). Improper vascular maintenance and/or blood supply affect normal visual function and cause disease conditions. Dysregulated angiogenesis is one pathological aspect of vision-impairing retinal diseases, such as diabetic retinopathy (DR) and age-related macular degeneration (AMD). In these diseases, neovascularization (NV) accelerates structural and functional damage in the retina (asterisks in *Figure 1*). At the early and intermediate stages of DR and AMD, prior to NV, there are activated endothelial cells (ECs) and immune cells, local and systemic inflammatory responses, and retina degeneration (2).

DR and AMD are the leading causes of the blindness among working adults, and elderly population, respectively. The prevalence of diabetes mellitus (DM) was 442 million in 2014 and continues to increase throughout the world (3). More than one-third of DM patients develop DR, one of the most common complications (4,5). DR patients may appear asymptomatic at early stages, but the ongoing progression of leukocyte adherence, capillary dropout, and mural cell activation and loss can advance to a later stage known as proliferative DR (PDR). PDR is characterized by retinal NV at the vitreoretinal interface and/or the inner retina (*Figure 1A*). In contrast, AMD, a multifactorial degenerative disorder of the central retina, advances to geographic atrophy and/or choroidal NV (*Figure 1B*). DR and AMD are characterized by the loss of inner and outer blood retina barrier (BRB) integrity, respectively. Inner BRB is maintained by tight junctions of endothelia, mural and glial cells (*Figure 1A*), whereas outer BRB is maintained by tight junctions of retinal pigment epithelium (RPE) and Bruch's membrane (BrM) (*Figure 1B*).

In this review article, we aim to describe and discuss the pathological aspects of cells associated in inner and outer BRB and retinal and choroidal NV, as well as the role of inflammatory molecules. Finally, we will discuss current advances in the therapeutic targets for retinal and choroidal angiogenic conditions. We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/aes-21-4)

Cells in retinal and choroidal NV

The final effector cells in angiogenesis are ECs, but the main pathological cells that initiate the process are mural and RPE cells in retinal and choroidal NV, respectively. Mural and RPE cells are essential in maintaining the inner and outer BRB. Further, there are many cell types associated with the pathological processes of retinal and choroidal NV. We categorize them for the convenience of description: EC and mural cell, RPE, glial cells, and microglia and macrophages.

Cells in retinal and choroidal NV: vascular EC and mural cell

Retinal vasculature consisting of ECs, basement membrane and mural cells, is the essential component for inner BRB and protects the inner retina (*Figure 1A*). Retinal blood vessels contain an abundance of mural cells (1:1 ratio with EC), which is quite uncommon in other body regions. For an example, the varying ratio of mural cell to EC is 1:100 in skeletal muscle (6,7). Mural cells support EC survival and vascular stability, inhibiting angiogenesis in normal retina (8). During DR progression, the loss of mural cells is an early feature in retinal capillaries (9), causing capillary dilation, microaneurysms, leakage and edema, as well as EC death, leading to vascular closure and eventual retinal NV (8,10,11). There are some notable differences in fenestration between retinal and choroidal vasculatures. The choriocapillaris (innermost layer of choroid) is highly permeable and consists of only ECs, with very little basement membrane and mural cells (12,13) (*Figure 1B*), whereas RPE contains tight junctions which protect the outer neural retina.

Cells in retinal and choroidal NV: RPE

RPE is a polarized monolayer, with apical microvilli processes adjacent to the photoreceptors and a folded basal aspect on the BrM (*Figure 1B*). RPE tight junctions, located apical-laterally to the adjacent cells, provide an essential property of the outer BRB. RPE controls the passage of ions, water, and metabolites between the retina and choriocapillaris, and takes up nutrients, such as glucose and fatty acids, from the choroid to nourish photoreceptors, and removes metabolic wastes from the photoreceptors to the choriocapillaris. RPE, also participates in the visual cycles, providing 11-cis retinal to photoreceptors and taking all-trans retinal from photoreceptors (14,15). RPE dysfunction, therefore, disturbs the healthy outer retinal environment. Aged RPE increases lipofuscin and

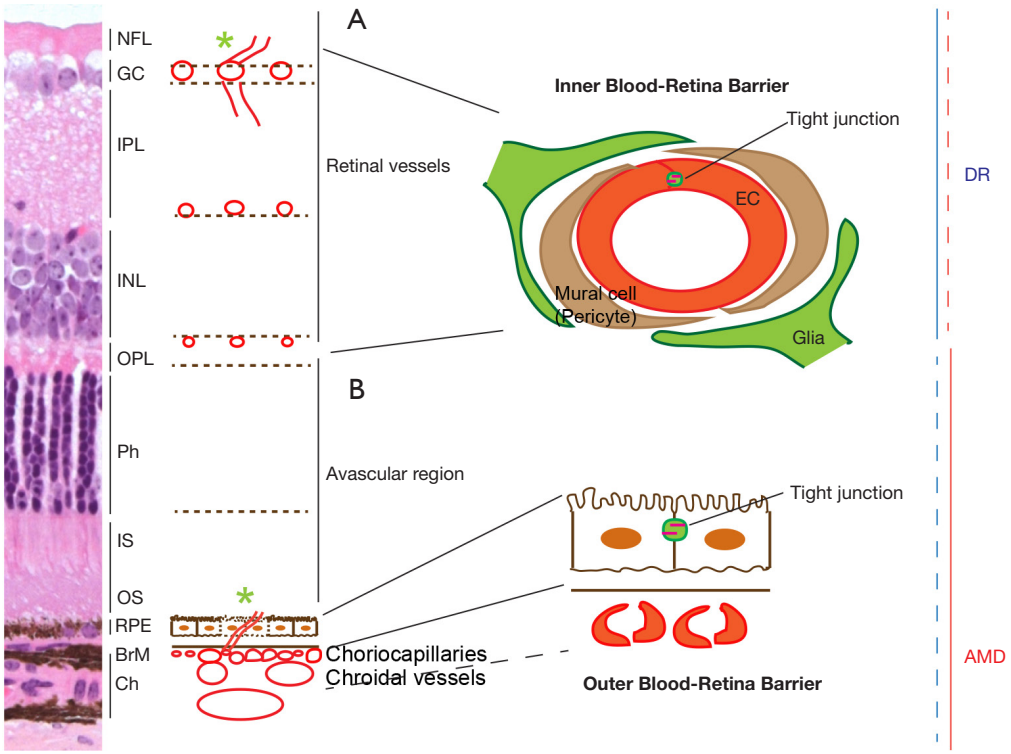

Figure 1 Retina and choroidal neovascularization in DR and AMD. The diagram depicts retinal and choroidal blood vessels, as well as structures and cells associated with inner and outer blood-retina barriers, and their association with DR or AMD. Inner blood-retina barrier is more associated with DR (A), whereas outer blood-retina barrier is more associated with AMD (B). Blue line indicates close association with DR pathogenesis, and red line indicates close association with AMD pathogenesis. Dotted lines indicate less association. DR, diabetic retinopathy; AMD, age-related macular degeneration; BrM, Bruch's membrane; Ch, choroid; EC, endothelial cell; GC, ganglion cell; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segment of photoreceptor; NFL, nerve fiber layer; OPL, outer plexiform layer; OS, outer segment of photoreceptors; Ph, photoreceptors; RPE, retinal pigment epithelium.

basal deposits, forming drusen. In AMD, RPE is a major degenerated cell type and secretes vascular endothelial growth factor (VEGF), an angiogenic molecule, and the increased VEGF induces choroid NV (14) (*Figure 2*). RPE disturbance, along with reduced phagocytic and electrophysiological function, is observed in DR as well (16-18) (*Figure 2*).

RPE secretes a variety of growth factors to support photoreceptors and ECs and regulate angiogenesis: fibroblast growth factor (FGF), transforming growth factor β (TGFβ), ciliary neurotrophic factor (CNTF), pigment-epithelium-derived factor (PEDF) and VEGF, etc. FGF2 (19) and TGFβ (20) are upregulated in RPE/choroid tissue of choroidal NV, and the inhibition of FGF2 (21,22) and TGFβ (20,23) prevents the NV formation in animals. However, there are controversial observations that the absence of TGFβ signaling in microglia (24) or EC (25) exacerbates choroidal NV. Therefore, TGFβ function is either pro- or anti-angiogenic, depending on the intracellular signaling pathways and the cell types (26). CNTF (27) and PEDF (28), secreted from the apical surface of RPE, support photoreceptor survival and suppress NV in retina, while VEGF is mainly secreted from the basolateral surface of RPE to support choroid (28,29). VEGF expression decreases with age but continues to persist (30-32). Furthermore, RPE secretes cytokines and chemokines, such as monocyte chemoattractant protein 1 (MCP1), in a polarized manner (33,34), recruiting leukocytes and amplifying inflammation (*Figure 3A*). The balance and polarized secretion of anti- and pro-angiogenic and inflammatory molecules maintains a healthy outer retinal environment (*Figure 3B-3D*).

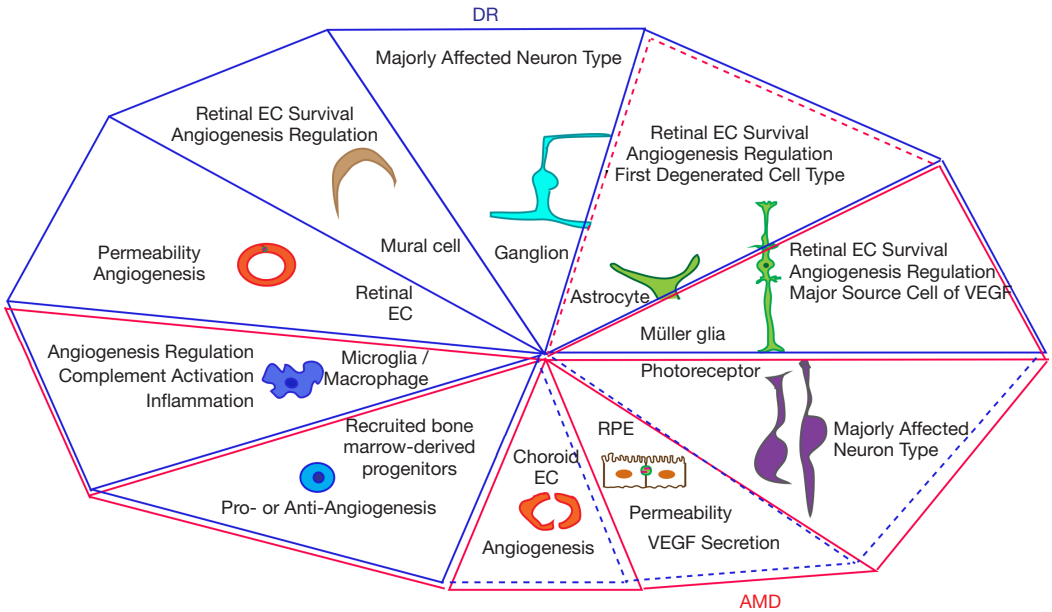

Figure 2 Cells associated in pathogenesis of DR and AMD. Major pathological function of cells associated with DR and AMD is described. Blue line indicates association with DR, and red line indicates association with AMD. Solid lines indicate close whereas dotted lines less association. DR, diabetic retinopathy; AMD, age-related macular degeneration; EC, endothelial cell; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor.

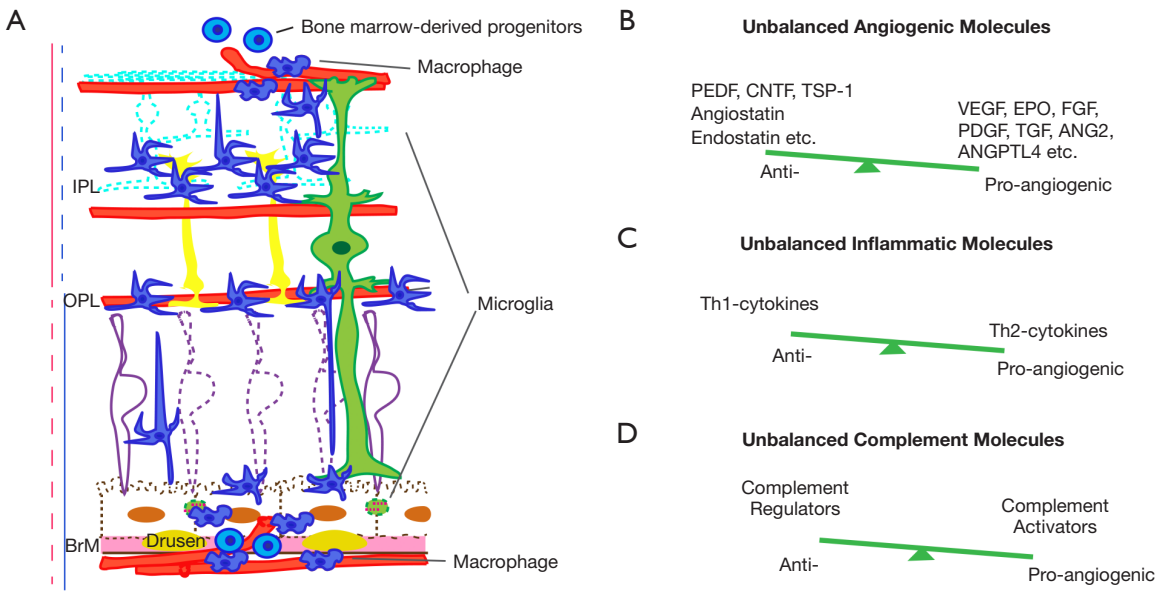

Figure 3 Neovascularization and inflammation in DR and AMD. (A) Glia and macrophage infiltration, (B) unbalanced molecules of angiogenesis, (C) unbalanced molecules of inflammation, and (D) uncontrolled complements. DR, diabetic retinopathy; AMD, age-related macular degeneration; IPL, inner plexiform layer; OPL, outer plexiform layer; BrM, Bruch's membrane; PEDF, pigment epithelium derived factor; CNTF, ciliary neurotrophic factor; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor; EPO, erythropoietin; FGF, fibroblast growth factor; PDGF, platelet derived growth factor; TGF, transforming growth factor; ANG2, angiopoietin-2; ANGPTL4, angiopoietin-like 4.

Cells in retinal and choroidal NV: astrocytes and Müller glia

Astrocytes and Müller glia in neurovascular units of the retinal vascular plexus (*Figure 1A*) are altered and associated with pathogenesis of retinal and choroidal NV (*Figure 2*). Astrocytes located in nerve fibers and ganglion cell layers are initially damaged before Müller cell activation during DR progression (35,36) and astrocyte alteration is also reported in AMD patients (37,38) (*Figure 2*). Müller glia, located across the entire retina, support and modulate all neuron types in the retina and is the major cell type to secrete the pro-angiogenic molecule VEGF, in DR (15,39,40). Müller glia activation and alteration are also observed in AMD: Glial fibrillary acidic protein-positive Müller glia processes are extended to the outer limiting membrane (41,42), and are sprouted on the vitreal surface of the inner limiting membrane (38) (*Figure 2*).

Cells in retinal and choroidal NV: microglia and macrophages

Microglia, the retina resident immune cell type (*Figures 2,3A*), are normally found in two plexiform layers, but they are relocated to damaged areas (43) (*Figure 3A*). The activated microglia produce inflammatory cytokines and other molecules, such as metalloproteases and nitrous oxide, which potentiate chronic pathological inflammation (44). There is further direct evidence that microglia are involved in NV: the lack of microglia exhibits a reduced vascular density (45,46) and co-culture of microglia and aortic ring enhances the vascular branches from the ring (47). The NVs recruit cells from peripheral blood stream and bone marrow as well (48-50) (*Figures 2,3A*). The resident microglia and recruited monocytes and macrophages activate into two subgroups: M1 and M2 microglia and macrophages, and neutrophils activate into, typically, N1 and N2 (51-53). M1 microglia and macrophages, and N1 neutrophils secrete Th1-derived cytokines, such as interferon (IFN)-γ, whereas M2 macrophages and N2 neutrophils secrete Th2-derived cytokines, such as IL-4. IL-4 is either pro-angiogenic (54) or anti-angiogenic (55,56) (*Figure 3C*). M2 macrophages and N2 neutrophils additionally secrete pro-angiogenic molecules, such as VEGF and matrix metalloprotinase 9 (51,57-59) (*Figure 3C*).

Besides local unbalanced inflammation, peripheral inflammation is also associated with DM/DR and AMD. The deletion of peroxisome proliferator activated receptor-γ (PPAR-γ), a nuclear receptor transcription factor that mediates M2 macrophage phenotype (60), contributes to obesity and insulin resistance (61,62). On the other hand, choroidal NV patients have higher counts of macrophages and neutrophils, and elevated levels of inflammation markers, such as C-reactive protein, and platelets in blood (63,64). VEGF+ M2 activated macrophages are peripherally observed at the initial choroidal NV stage (65) as well, and CCR2 monocyte depletion suppresses choroidal NV formation (66). Transgenic *ccl2*, *ccr2*, *cx3cr1* and *ccl2/cx3cr1* deficient mice are associated with aberrant monocyte trafficking and exhibit choroidal NV features with other AMD phenotypes (67-70). Finally, peripheral monocyte depletion induced by clodronate (48) and neutrophil depletion (CC chemokine receptor 2 knockout mice) (66) result in a reduced size of laser-induced choroidal NV, indicating the direct contribution of peripherally circulating immune cells in the NV process.

Inflammatory pathways in retinal and choroidal NV

It is well known that inflammatory pathways are highly activated in retinal and choroidal NV, owing to dysregulation of complement activities in DR and AMD (71,72), as well as increased environmental risk factors such as obesity, hypertension, smoking and high-fat diet (73-76). Genetic studies have indicated that a dysregulated complement system is linked with DR (77,78) and AMD (72,79). The inflammatory features of local resident and recruited cells from peripheral blood stream and bone marrow contribute to the NV environment of DR and AMD. We review the current advances in inflammatory molecular pathways of the complement system, stromal derived factor-1 (SDF-1) /chemokine CXC receptor-4 (CXCR4), inflammasome nucleotide-binding oligomerization domain (Nod)-like receptor containing domain 3 (NLRP3), interleukin 18 (IL-18), programed cell death ligand-1 (PD-L1), insulin-like growth factor (IGF) and sphigosin-1-phosphate receptor (S1PR), to gain insights into potential candidates of therapeutic targets to treat retinal and choroidal NV.

Inflammatory pathways in retinal and choroidal NV: complement system

The complement system, part of the innate immune system, participates in pathogen elimination, but also becomes involved in diverse biological processes including

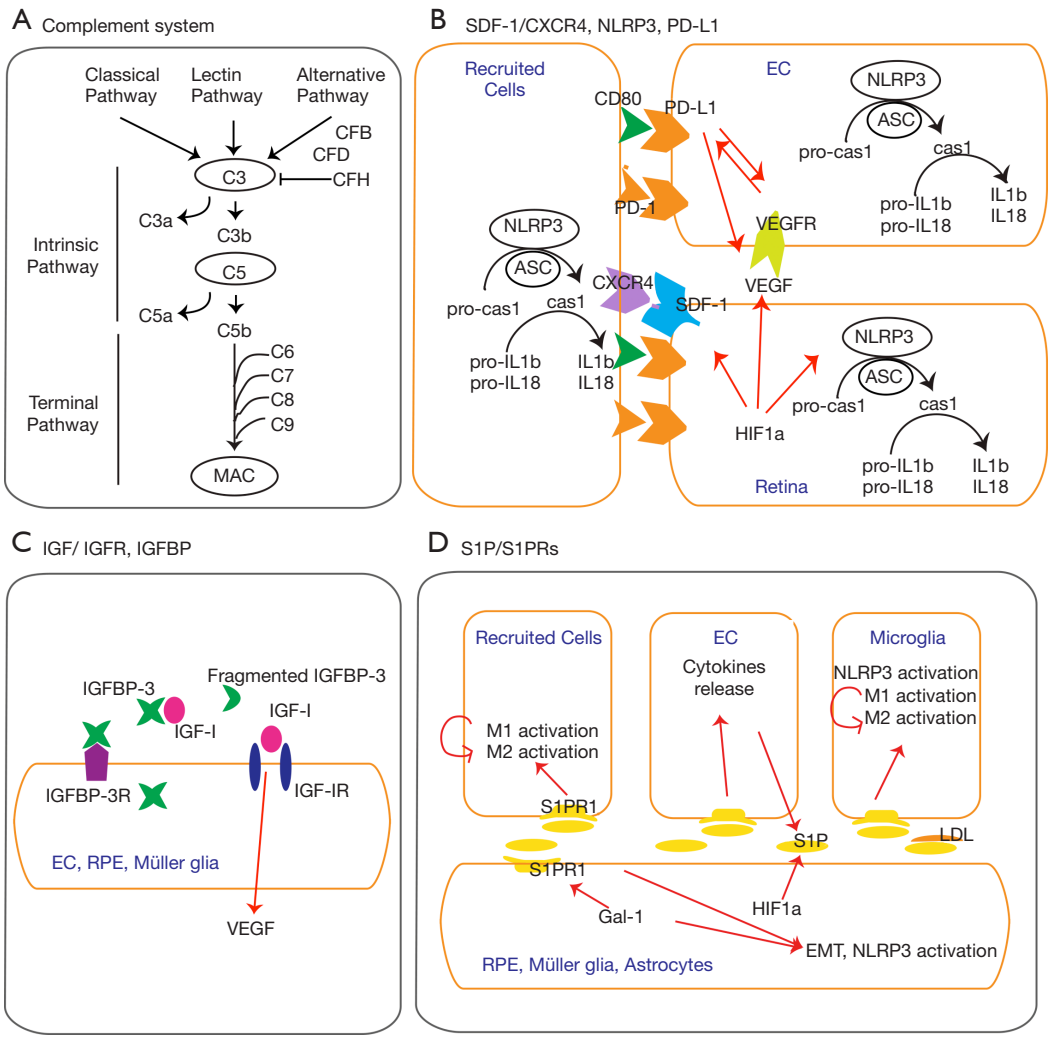

Figure 4 Molecular pathways associated with retina inflammation and neovascularization. (A) Complement system, (B) SDF-1/CXCR4, NLRP3, PD-L1. (C) IGF/IGFR, IGFBP, and (D) S1P/S1PRs. CFB, complement factor B; CFD, complement factor D; CFH, complement factor H; MAC, membrane attack complex; SDF-1, stromal derived factor-1; CXCR4, chemokine CXC receptor 4; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor containing pyrin domain 3; PD-L1, programed cell death ligand-1; IL, interleukin; VEGFR, vascular endothelial growth factor receptor; CD80, cluster of differentiation 80; PD-L1, programmed cell death ligand-1; PD-1, programmed cell death protein-1; HIF-1a, hypoxia inducible factor-1a; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; IGFBP, IGF binding protein; S1P, sphigosin-1-phosphate; S1PR, sphigosin-1-phosphate receptor; Gal-1, Galectin-1; LDL, low-density lipoprotein; EMT, epithelial mesenchymal transition.

cell differentiation, synaptogenesis, tissue clearance, degeneration, regeneration, lipid metabolism, tumorigenesis and angiogenesis (80). Activation of the complement system is accomplished through three pathways: classical, lectin, and alternative (*Figure 4A*). Classical pathway is associated with humoral immune system, activated by antigen-antibody binding, and lectin pathway is associated with innate immune system, activated by glycoproteins and glycolipids, whereas the alternative pathway is particularly implicated in sterile/pathogen-free inflammation of chronic neurodegenerative diseases (80,81).

While the initiation of each pathway is distinct, all three eventually converge to produce C3 and C5 convertases (80,81). Complement factor B (CFB), complement factor D (CFD) and serine proteases are required for the generation of C3 convertase, while complement factor

H (CFH) is the main inhibitor of C3 activation. The anaphylatoxins C3a and C5a are the chemo-attractants that guide monocytes, macrophages, neutrophils and the other immune cells. Complement activation is implicated in DR and AMD pathogenesis and pathobiology (71,81,82). In retinal and choroidal NV, there are high levels of complement activators, such as CFD, C3 and C5, and low level of regulators, such as CFH (*Figures 3B* and *4A*). Resistance to streptozotocin (STZ)-induced diabetes was observed in C3 knockout mice (83), and the genetic association of C5 genes in type 2 diabetes and PDR was observed (78,84). Complement components are found in patient choroidal NV (71,72), and the deficiency of C3a and C5a receptors, as well as C3 and C5 knockouts, have shown reduced VEGF expression and resistance to laser-induced choroidal NV formation (85,86). In addition, the overexpression of C3 and C5 induces choroidal NV (86). However, mice lacking receptors for C3a and C5a show early onset of retinal degeneration, and were more susceptible to light-induced retinal dysfunction (87). These findings indicate that the complement system is a double-edged sword regarding tissue protection and degeneration, and should be tightly balanced: The plenty contributes to NV, but sparsity contributes to retina degeneration. Clinical trials have often attempted to target complement components, not only for geographic atrophy Dry AMD, but also for choroid NV Wet AMD (82,88). It is of note that while CFD inhibitor Lampalizumab (NCT02247479; NCT02247531, Phase 3, Genentech), C3 inhibitor Compstatin (NCT01157065, Phase 2, Apellis) and C5 inhibitor Eculizumab (NCT00935883, Phase 2, Alexion pharmaceuticals) failed in clinical trials to treat Dry AMD (89), c3 inhibitor APL-2 Pegcetacoplan (NCT03525600, NCT03525613, Phase 3, Apellis Pharmaceuticals) and c5 inhibitor (Zimura; NCT02686658; Phase 2/3, IVERIC bio) are currently in clinical trials and awaiting the results (90,91). IB1302 Bispecific antibody fusion protein targeting VEGF and complement cascade (NCT04820452, Innovent Biologics, Inc.) finished Phase 1 and just started Phase 2 for Wet AMD in April, 2021.

Inflammatory pathways in retinal and choroidal NV: stromal cell derived factor-1 (SDF-1)/chemokine CXC receptor-4 (CXCR4)

Chemokines and their cognate receptors are involved in the migration of peripheral cells to injury sites. SDF-1 (also known as chemokine ligand 12, CXCL12) and the receptor CXCR4 are considered an essential chemokine signaling pathway in NV (92) (*Figure 4B*). CXCR4 was initially cloned from leukocytes (93,94) and is cofactor for HIV-1 entry into T cells (95). Both SDF-1 and CXCR4 contain hypoxia response elements within the promoters and are induced by a major transcription factor, hypoxia-inducible factor-1 (HIF-1). SDF-1/CXCR4 axis is involved in recruitment and differentiation of hematopoietic progenitors to hypoxic sites. In either ischemic retinopathy or laser-induced choroidal NV, both SDF-1 and CXCR4 are up-regulated and co-localized in astrocytes, Müller glia, RPE, choroid (96,97), hematopoietic and bone marrow-derived CD45+ cells and microglia/macrophage, but not in ECs (97). CXCR4 blockade (97,98) reduces movement of bone marrow-derived CD45+ cells and F4/80+ macrophages into ischemic retina, and suppresses the formation of retinal and choroidal NV (97), but has no apparent regression effect for established choroidal NV (98).

Inflammatory pathways in retinal and choroidal NV: Nucleotide-binding oligomerization domain (Nod)-like receptor containing pyrin domain3 (NLRP3)

Chronic and sterile inflammation is a hallmark of chronic diseases, including DR and AMD. The inflammasomes, as one of the pattern recognition receptors in the innate immune system, regulate secretion of pro-inflammatory cytokines via caspase-1 activation, in response to endogenous damage and infectious signals. Inflammasome activation leads to conversion of procaspase-1 into active caspase-1, and the maturation of pro-interleukin (IL)-1beta and pro-IL-18 (*Figure 4B*). The NLRP3 (known as cryopyrin) is mostly characterized among other NLR inflammasomes and has been a growing area of interest, given its association with chronic degenerative and metabolic diseases (99,100). Upregulated activation of NLRP3 inflammasome was observed in macrophages, liver and kidney of diabetic patients (101-104), and in vitreous fluid and retina of DR patients (105,106), as well as animals (107). The attenuation of NLRP3 inflammasome activation and oxidative stress, as a result of treatment with IL-22, curcumin, cepharanthine or piperine or fenofibrate, has mitigated diabetic nephropathy (108-111), and recently, intravitreal injection of MCC950, a specific inhibitor of NLRP3 ameliorated retinal NV in an oxygen-induced ischemic retinopathy model (112). Similarly, AMD animal models of Dry and Wet forms have shown increased levels of NLRP3 inflammasome activation (113-116),

and the clinical aspects of AMD, such as drusen and complement activation, suggest inflammasome's association in pathophysiology conditions of AMD. However, it is still unclear how NLRP3 is directly associated with choroidal NV. Laser-induced choroidal NV in NLRP3 genetic deficiency exhibited exacerbation (114), but VEGF-A^{hyper} choroidal NV model in NLRP3 genetic deficiency exhibited decline (113). When it is considered that laser-induced acute model does not fully represent clinical Wet AMD pathophysiology and VEGF-A^{hyper} is relatively chronic, studies in other animal models such as knockout of very-low-density lipoprotein receptor (VLDLR) (117) may confirm NLRP3 association further. Since inflammation itself is neutral like weapon, NLRP3 inflammasome might take either protective or deleterious roles, depending on the stages and conditions of diseases, and the studies on the associated-downstream pathways and cell specific mechanisms will be important.

Inflammatory pathways in retinal and choroidal NV: interleukin (IL)-18

Proinflammatory IL-1beta and IL-18 are downstream cytokines of NLRP3 activation. Like NLRP3 upregulation in retinal and choroidal NV, as mentioned above, IL-1beta and IL-18 are also upregulated in aqueous and vitreous fluids of non-PDR and PDR (105,106,118), and are considered increased in choroidal NV patients as well based on cell and animal studies (114,115,119-121). The upregulation of IL-1beta seems to be associated with oxidative stress, BRB permeability and tissue deterioration in DR and AMD (113,114,121-125). However, there are controversial results of IL-18 function in retinal and choroidal NV, indicating the physiological complexity of IL-18 functions. Deficiency of IL-18 resulted in severe choroidal NV development and treatment of IL-18 attenuated choroidal NV formation via reducing VEGF in laser-induced choroidal NV model (114,119), and IL-18 deficiency in VEGF-A^{hyper} model increased choroidal NV compared to that in NLRP3 and IL-1beta deficiency in VEGF-A^{hyper} (113). Interestingly, NLRP3 and IL-1beta targeting resulted in inhibition of Dry AMD pathology but IL-18 inhibition deteriorated AMD pathology in CEP-adducted serum albumin-immunized model. However, any deficiency of either IL-1beta, NLRP3 or IL-18 did not inhibit choroidal NV formation in VEGF-A^{hyper} model. It was later argued that IL-18 does not exhibit either pro- or anti-angiogenic effects on laser-induced choroidal NV formation (120,126). Nevertheless, anti-VEGF treatment increases ocular IL-18 levels, and the increased IL-18 level is correlated with good visual outcome in patients with macular edema. Similarly, upregulation of IL-18 by anti-VEGF treatment occurs in ischemic retinopathy animal model, suggesting anti-angiogenic activity of IL-18 (127). It is noteworthy that the expression level of pro-IL-18 and IL-18 is known to be permanent in RPE cells, and not increased by NLRP3 inflammasome activity (125). It is also well known that endothelium and epithelium cells are hardly activated by inflammation, thus the cytokine roles of resident and recruited immune cells will be important. Finally, the level of IL-18 concentration may have a protective role in retinal and choroidal NV, whereas higher amounts of IL-18 combined with other cytokines, may be detrimental.

Inflammatory pathways in retinal and choroidal NV: programed cell death ligand-1 (PD-L1)

Programmed cell death receptor (PD-1) and its ligand PD-L1, immune checkpoint proteins, are essential in ocular immune privilege. PD-L1 is found in ocular tissues, including ECs and RPE, and PD-L1 expression is increased under inflammatory conditions (128,129). PD-1 is expressed on lymphocytes and antigen presenting cells. PD-L1 blocking/downregulation using anti-PD-L1 antibody and siRNA treatment enhances pro-inflammatory cytokine production (128) and increases EC proliferation with upregulation of VEGFR2 expression. Further, the cornea in PD-L1 knockout mice induces higher level of angiogenic responses than wild type, via CD80 not PD-1 (130), suggesting that the ratio of CD80 and PD-1 expression on the recruited immune cells could determine either angiogenesis or immune deviation (*Figure 4B*). CD80, known as B7.1, is expressed on antigen presenting cells such as monocytes. In addition, VEGF treatment induces PD-L1 in tumors (131). Taken together, PD-L1 is essential in ocular immunobiology and its molecular signaling pathway could be part of the NV process. Further understanding of PD-1/PD-L1 in retinal and choroidal NV should be pursued and compared with cancer studies.

Inflammatory pathways in retinal and choroidal NV: IGF system

The IGF system consists of IGF-I and IGF-II ligands and

their cognate receptors, IGF-binding proteins (IGFBPs), and IGFBP-specific proteases, all regulating cell growth and differentiation. IGF-I and IGF-I receptor (IGF-IR) are detected in retinal ECs, RPE and Müller cells (132-134) (*Figure 4C*). IGF-I is considered one of pro-angiogenic factors in PDR (135) and Wet AMD (136). In PDR, vitreous IGF-I levels are elevated (137-139), and genetic (140) and injected overexpression (139,141,142) of intravitreal IGF-l results in BRB breakdown, VEGF upregulation, loss of vascular integrity and retinal NV. On the other hand, IGF-lR inhibitors decrease VEGF secretion and retinal and choroidal NV (143,144).

IGFBP-3 is a major IGFBP species in circulation and its binding to IGF-I decreases IGF-I bioactivity by preventing IGF-1 binding to IGF-IR (145). IGFBP-3 further exerts IGF/IGF-R-independent cellular functions, including anti-inflammation, either pro- or anti-apoptosis and DNA damage repair (145-147). Interestingly, obese populations display a decrease in functional IGFBP-3 levels and an increase in proteolytic IGFBP-3 fragments in circulation (148,149). Cell and animal studies have shown the protective role of IGFBP-3 in retinal NV. The restoration of intravitreal IGFBP-3 results in protection of ischemic retinal injury and DR in murine models (150,151). IGFBP-3 reduces retina vascular permeability, inhibits pro-inflammation and protects from retinal EC apoptosis (152-155). IGFBP-3 further recruits bone marrow-derived cells, vascular progenitors and hematopoietic stem cells to sites of retinal hypoxia and ischemic injury, and stabilizes mural cells, maintains endothelial integrity, and reduces inflammation (150,154). Taken together, increased IGF-I and nonfunctional IGFBP-3 fragments seem to be early players in retinal and choroidal NV formation.

Inflammatory pathways in retinal and choroidal NV: sphigosine-1-phosphate (S1P)/S1P receptors (S1PRs)

S1P is a sphingosine-containing bioactive lipid generated from ceramide, mainly secreted by RBCs, ECs and activated platelets. It binds five G protein-coupled surface receptors (S1PRs1-5). S1PRs are found on retinal ganglion cells, RPE, ECs, astrocytes, microglia, monocytes, dendric cells and lymphocytes (156-159). The function of S1P/S1PRs is not fully understood, but they are associated with vascular barrier function and inflammatory responses (159). Increased level of S1P is observed at inflammation sites and recruits immune cells. SIP/SIPRs signaling also induces NLRP3 activation (158), and triggers M1 and Th1-polarized proinflammatory responses (160,161). However, the S1P/S1PRs axis also activates negative feedback, reducing vascular leakage (162), as well. In retina (*Figure 4D*), RPE is considered a major source of S1P during choroidal NV progression (163), and astrocytes and Müller glia might be other sources of S1P during retinal NV. Photoreceptors also express S1P in response to light damage (159,164,165), and S1P is generated by sphingosine kinases that are upregulated by HIF1α (166). Hypoxia upregulates S1P/S1PRs axis, subsequently generates nitric oxide and increases vasodilation of ECs (167). Since S1PR modulator, FTY720 (fingolimod, S1PR agonist) was approved for multiple sclerosis in 2010 (168,169), fingolimod and other S1P/S1PR modulators are under clinical trial for brain diseases such as acute stroke, amyotrophic lateral sclerosis, schizophrenia, Rett syndrome, glioblastoma and other autoimmune and inflammatory diseases, like psoriasis and Crohn's (170). The modulation of S1P/S1PR using anti-S1P antibody (Sonepcizumab, S1P antagonist) suppresses retinal and choroidal NV by suppressing inflammation and reducing the recruited microglia and macrophages in animals (166). However, Sonepcizumab did not show a statistically significant improvement in the visual acuity of Wet AMD patients, as a monotherapy and adjunctive to anti-VEGF agents in the Phase 2 clinical trial (166,171). Since S1P and complex signaling of S1P/S1PRs1-5 have a dual role in inflammatory responses (170), the cellular and molecular knowledge of SIP and S1PRs in retina diseases might provide a possible intervention strategy for retina inflammatory diseases. Of note, S1PR1 expression is upregulated by galectin-1 (Gal-1) and overexpressed Gal-1 and SIP/S1PRs are known to be associated with epithelial to mesenchymal transition (EMT) in cancer (172). EMT is a process where epithelial cells lose polarity and adhesion but obtain migratory and differentiated properties. These similar mechanisms might be associated with RPE inward migration, causing broken outer BRB.

Conclusions

We reviewed cells and inflammatory pathways associated with retinal and choroidal NV in DR and AMD here to provide the current available knowledge and insight in their therapeutic targets. Therapy of anti-VEGF agents for ocular NV diseases has offered substantial improvement in outcomes (173-175), but therapeutic innovation is still a requisite, as approximately up to 40–50% of both Wet AMD (163,176) and PDR (177,178) patients do

not fully respond to anti-VEGF therapy. Given that the pathogenesis and pathobiology of DR and AMD has yet to be clearly elucidated, a deeper understanding of unbalanced inflammation will warrant a continued effort in expanding our knowledge. Furthermore, additional studies may complement current anti-VEGF therapeutic approaches and pave the way for next generation therapeutics to treat chronic retinal and choroidal NV.

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