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Seeing through VEGF: Innate and adaptive immunity in pathologic angiogenesis in the eye

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Abstract

The central role of VEGF signaling in regulating normal vascular development and pathological angiogenesis has been documented in multiple studies. Ocular anti-VEGF therapy is highly effective for treating a subset of patients with blinding eye disorders such as diabetic retinopathy and neovascular age-related macular degeneration (AMD). However, chronic VEGF suppression can lead to adverse effects associated with poor visual outcomes due to the loss of pro-survival and neurotrophic capacities of VEGF. In this review, we discuss emerging evidence for immune-related mechanisms that regulate ocular angiogenesis in a VEGF-independent manner. These novel molecular and cellular pathways may provide potential therapeutic avenues for a multitarget strategy, preserving the neuroprotective functions of VEGF in those patients whose disease is unresponsive to VEGF neutralization.

Keywords

Angiogenesis; VEGF; immune regulation; neovascularization; AMD; diabetic retinopathy

Modulation of VEGF signaling: the therapeutic hub

Angiogenesis (see Glossary) is a physiological process involving the growth of new blood vessels from pre-existing vessels. In the eye, physiologic angiogenesis is critical during early development and indispensable for normal vision. In contrast, many disease states involving the eye such as diabetic retinopathy, retinal vein occlusions, age-related macular degeneration (AMD), and retinopathy of prematurity (ROP), have been linked to the

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disrupted regulation of this process leading to pathologic angiogenesis and blindness (Figure 1) [1].

Diabetes often results in significant tissue damage as manifested by nephropathy, neuropathy and in the eye, diabetic retinopathy. The systemic changes associated with elevated blood glucose and metabolic syndrome associated with diabetes lead to significant end organ damage in the eye, and especially in the retinal microcirculation [2]. Diabetic retinopathy is the leading cause of blindness in working age adults in the U.S., and it is associated with functional and structural alterations of retinal vasculature that later result in capillary degeneration and retinal ischemia. This is a public health crisis. In the U.S. alone, there are almost 750,000 people with proliferative diabetic retinopathy and over 60,000 new cases are reported each year. The earliest manifestation of diabetic retinopathy is in the form of pericyte loss that leads to instability of the retinal capillaries, formation of capillary microaneurysms, development of ischemic zones within the retina, and capillary leakage with subsequent retinal exudation and edema. Progressive damage to retinal microvascular network leads to tissue ischemia, upregulation of hypoxia inducible factor-1 (HIF-1), stimulation of vascular endothelial growth factor (VEGF) secretion and retinal neovascularization characterized by the development of new retinal and pre-retinal vascular fronds [3].

AMD is multifactorial disease and aging is the main risk factor. There are two forms of AMD; neovascular or wet AMD, and non-neovascular or dry AMD, which precedes the wet form [4]. Advanced AMD is characterized by an alteration of regulatory processes that either affect cell survival or induce cell proliferation, specifically neovascularization. AMD is the leading cause of blindness in people over 50 years of age in the industrialized world. Dry AMD is characterized by the development of lipid rich deposits called drusen underneath the retina and within the macula. The composition of these deposits has been reviewed recently [5]. As the disease progresses, drusen components induce inflammation and alterations in the retinal pigmented epithelium (RPE). In some patients, disease progresses to the advanced stages that can be associated with severe central vision loss. In advanced dry AMD, vision loss is secondary to RPE damage and cell death that subsequently leads to photoreceptor cell death and atrophic changes in the neurosensory retina. In the neovascular form, blindness is caused by pathogenic and proliferative neovascularization underneath the retina (choroidal neovascularization, CNV). The majority of acute vision loss in AMD is from the wet form of disease. These features can be overlapping as patients with advanced dry AMD can develop CNV and vice versa. In addition, as therapeutic agents targeted at factors that promote neovascularization become more efficacious, vision loss from atrophic retinal changes associated with dry AMD are becoming more prevalent [6].

Formerly known as retrolental fibroplasia, ROP was originally described in the 1940s by Terry, who first connected disease symptoms with premature birth [7]. At that time, no treatment for ROP was available. Major advances in ROP treatment became available in the 1980s and 1990s, when cryotherapy and laser photocoagulation of the avascular retina were shown to be partially effective in preventing blindness in ROP infants [8]. Although these treatments can reduce the incidence of blindness by 25%, patients are often left with poor

visual acuity even after treatment. The pathogenesis of ROP has been associated with the use of high levels of oxygen on premature infants. The hyperoxia of the extra uterine environment, as well as the use of supplemented oxygen given to premature infants, slows or ceases retinal vascular growth that would normally occur *in utero*, and developed retinal vessels begin to regress [9]. As the infant matures, the non-vascularized retina becomes increasingly metabolically active, and in the absence of adequate vascularization, leads to tissue ischemia and hypoxia. This results in compensatory retinal neovascularization. New vessels form at the junction between the vascularized retina and the avascular zone of the retina. Over time, this pathological growth of vessels produces a fibrous scar extending from the retina to the vitreous gel and lens [9].

VEGF has been identified as one of the critical mediators of pathologic neovascularization. The role of VEGF in angiogenesis has been examined in great detail and is described in several review articles [10, 11]. In the eye, numerous studies have demonstrated the crucial role of VEGF during retinal vascular development [12, 13]. In this highly regulated process, VEGF is essential for the proliferation and migration of endothelial cells. It is also required for proper vascular patterning and tube formation (Figure 2). Additionally, the distinct vascular defects observed in experimental animal models depleted for alternative splice variants of VEGF confirmed the precise role of these isoforms in regulating retinal angiogenesis [14]. VEGF signaling has been attributed a key role in the maintenance of corneal transparency and the avascular state. Imbalance in VEGF related pathway is strongly associated with corneal neovascularization, a common clinical feature in diverse corneal diseases [15, 16]. More recent work demonstrated that VEGF also plays a prominent role in overall ocular development. Indeed, selective inactivation of VEGF expression in RPE resulted in a severe reduction of eyeball size associated with the loss of visual function [17]. RPE derived VEGF is also involved in the formation and maintenance of the choroidal vasculature that provides oxygen and nutrients to the outer retina [18].

The role of VEGF in ocular neovascular-related diseases has been characterized in experimental and translational studies [19]. Elevated intraocular VEGF is closely linked to the pathogenesis of several blinding eye diseases. In addition, early findings that the inhibition of VEGF signaling prevented neovascularization in ischemic retinal diseases highlighted the therapeutic relevance of anti-VEGF strategies in these conditions [20]. However, there is now substantial evidence showing that VEGF expression can also be induced through hypoxia-independent mechanisms [19].

Although VEGF clearly drives pathologic angiogenesis in disease, it also plays a crucial role in cellular homeostasis as a neurotrophic and cell survival factor [21-24]. Numerous cell types in the eye express and secrete VEGF, including endothelial cells, glial cells, retinal neurons, RPE, and immune cells such as monocytes/macrophages [25-28]. In disease states, intraocular VEGF expression is upregulated by hypoxia and inflammation [29]. VEGF-driven neovascularization and increased vascular permeability lead to complications including bleeding, retinal detachment and fibrovascular proliferation, all of which ultimately cause photoreceptor neuron death and blindness [1, 11]. As such, many treatment options for these complications are geared towards modifying VEGF levels within the eye in order to inhibit abnormal angiogenesis (Figure 1d,e). Numerous clinical trials have

investigated therapies targeting VEGF and have shown efficacy in controlling disease and preventing vision loss (Table 1) [30-41].

It is important to note that therapeutic VEGF inhibition leads to significant vision recovery in only a subset of patients with the retinal diseases highlighted above [42]. As such, VEGF-driven pathways are only a part of the complex machinery regulating angiogenesis in the eye [42]. This creates a large unmet need for developing novel strategies targeting pathways that regulate ocular angiogenesis that are independent of VEGF (Table 2). In addition, VEGF also plays a critical role in normal homeostasis in the eye in maintaining vascular integrity, as a neurotrophic and neuroprotective factor in the retina and the central nervous system, and indiscriminate or prolonged VEGF inhibition can lead to cell death and vision loss as demonstrated in animal models, cell culture and human randomized clinical trials [24, 42-44]. In this review, we will focus on VEGF-independent pathways involving the immune system that could provide additional insights into the regulation of normal and pathological angiogenesis in the eye.

Innate immunity and ocular angiogenesis

In both physiological and pathological conditions, the innate immune system has been shown to play a determinant role in the vascular remodeling process. The link between inflammation and angiogenesis has been defined in the pathophysiology of several diseases, including cancer, atherosclerosis and eye disorders [1, 5, 45]. In these conditions, activation of innate immunity is often an early event in the disease process that further guides the local tissue microenvironment and ultimately molds cellular and molecular responses. The crucial role of VEGF signaling in ocular angiogenesis has been extensively studied and the molecular response of endothelial cells to VEGF is well documented [1, 10, 11]. There is also a wealth of evidence implicating innate immunity in key processes during angiogenesis, including endothelial cell proliferation, migration and vessel anastomosis [46]. The concomitant activation of an innate immune response, associated with dysregulated blood vessel growth (as observed in many eye diseases), and the implication of inflammatory mediators in VEGF signaling have led to the belief that VEGF plays an omnipotent role in regulating ocular angiogenesis. Although recent translational studies targeting VEGF have confirmed its pivotal role in blinding eye diseases, there is emerging evidence highlighting VEGF-independent pathways in disease pathogenesis that suggest potentially novel approaches involving components of innate immunity that may modulate pathological angiogenesis in the eye.

Convergent complement pathways

The complement system is composed of three distinct pathways (classical, lectin, and alternative), the activation of which contributes to many aspects of immune responses. Defective complement components are causative in various pathologies, extending far beyond the autoimmune diseases [47]. For instance, polymorphisms in numerous complement pathway genes are strongly associated with neurodegenerative disorders such as AMD [48]. Recent studies have demonstrated that complement pathways tightly regulate pathological neovascularization in the retina, suggesting interplay between innate immunity and ocular angiogenic processes [49, 50].

Oxygen-induced retinopathy (OIR; Box 1) is widely used in animal models of pathological vascularization to mimic clinical features of proliferative retinopathies, including those observed in ROP, a leading cause of vision loss in children [51]. Specific targeting of C3 and C5, key components of the complement response, directly implicated this pathway in the biology of normal and pathological retinal blood vessel growth [49]. In the context of OIR, C3 deficiency induced an increase of pathological angiogenesis in the retina. In addition, selective depletion or functional blockade of the active component C5a also resulted in higher neovascularization, suggesting that activation of the complement system prevented retinal neovascularization [49]. Although the anti-angiogenic effects of C3 and C5 are mediated by macrophages, the effective pathways regulating retinal angiogenesis are slightly different. Macrophage depletion *in vivo* abolished the complement-dependent repression of neovascularization. Furthermore, complement activation polarized macrophages to an anti-angiogenic phenotype that directly inhibited endothelial cell proliferation [49].

The findings that complement inhibits the formation of pathological neovessels have been confirmed by recent studies specifically targeting the alternative pathway. Mice lacking the complement factor B (cfB) developed more neovessels in the OIR model [50]. In addition, deletion of cfB had no effect on VEGF signaling, suggesting that this complement component could directly target endothelial cells [50]. Early studies have demonstrated that cfB interaction with the membrane bound C3b is a key step that initiates the formation of the C3 convertase complex leading to death of the target cell after activation of the membrane attack complex [52, 53]. Interestingly, cfB was associated with retinal neovessels, whereas the convertase inhibitor (CD55 or decay-accelerating factor) was restricted to the normal vasculature [50]. Indeed, cfB deficiency altered the targeting of pathological endothelial cells by the complement alternative pathway, and resulted in an accumulation of neovessels. Given the involvement of complement system in diverse biological processes, the selective activation of a complement pathway, combined with specific cell targeting, provides a regulatory mechanism that prevents collateral tissue damage and harm to the host organism [50].

Macrophages: from effector to mediator

It is now well established that macrophages play a significant role in regulating ocular angiogenesis through multiple mechanisms [54-59]. As shown above, macrophages can also mediate the effect of activated complement system on retinal vasculature. Depending on the tissue context, macrophages can be classically (M1) or alternatively (M2) activated. These macrophage subpopulations are characterized by a variety of specific markers and exhibit differential cytokine production, receptor expression, and effector function [60-64]. A more recent macrophage classification has been proposed based on their activation conditions [64]. Pro-inflammatory M1 macrophages are anti-angiogenic, and express high levels of tumor necrosis factor alpha (TNF- α), interleukin-12 (IL-12), IL-6, IL-1 β , inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP9). In contrast, pro-angiogenic M2 macrophages mediate wound healing, and are characterized by low M1 signature markers but increased expression of IL-10, CD163, and transforming growth factor beta (TGF- β). In an animal model of neovascular AMD, it has been demonstrated that the switch

of macrophage polarization from M1 to M2 is responsible for the increase of choroidal neovascularization (CNV) in old mice [65]. A similar dynamic and functional switch was also observed in AMD patients with alternatively activated ocular macrophages in advanced stages of the disease [66, 67].

In early studies, it was suggested that macrophages inhibited CNV through Fas ligand (CD95) signaling. However, these findings did not rule out a possible contribution of VEGF pathways in this pathological angiogenesis [54, 68]. Subsequently it has been demonstrated that in addition to aging [65], dysregulation of cholesterol metabolism polarized macrophages to a pro-angiogenic phenotype [69]. Further characterization of mice lacking a key regulator of cholesterol homeostasis confirmed that impaired macrophage regulation of endothelial cells growth was VEGF-independent [69].

In the retina, resident macrophages (microglial cells) are essential for proper blood vessel formation [55]. During development or in a pathological context, remodeling of the retinal vasculature by microglial cells is mediated by secreted angiogenic factors, cytokines or regulators of the extracellular matrix (ECM). Recent identification of VEGF-independent angiogenesis through neuropilin-1 (NRP1) signaling provided new insights into the interplay between endothelial and surrounding retinal cells [70]. Activation of NRP1 by ECM signals induced a phosphorylation of integrin targets such as paxillin and subsequent actin remodeling which promoted endothelial cell motility [70]. Although NRP1 is a VEGF co-receptor, this study clearly demonstrated that selective targeting of ECM-stimulated NRP1 signaling altered the regulation of actin cytoskeleton and cell migration. These findings suggested that NRP1 signaling is involved in both physiological and pathological angiogenesis in the eye [70]. Similarly, previous studies showed that macrophage modulation of ECM proteins is critical for the retinal vascular remodeling [57]. During pathological conditions, macrophage metalloproteinases (MMP-2 and 9) promoted retinal neovascularization by directly targeting the ECM [57].

Despite the close interaction between macrophages and retinal blood vessels, the effect of macrophages on endothelial cells can be modulated through indirect mechanisms. Findings from a recent study showed that microglial cells regulated neovessel growth through inflammatory mediators that target adjacent neurons [71]. In the OIR model, activation of microglial cells resulted in overexpression of IL-1 β , which in turn induced Semaphorin-3a (Sema-3A) production by retinal ganglion cells through IL-1R signaling. The secreted pro-apoptotic factor Sema-3A then induced endothelial cell death. Furthermore, selective blockade of IL-1 β signaling prevents this cytotoxic cascade that leads to vascular degeneration [71]. The above findings demonstrate that although microglial cells contribute to retinal vascular development, they also play a pivotal role in modulating retinal neovascularization.

The role of adaptive immunity in ocular angiogenesis

Recent studies have suggested that the adaptive immune compartment may play a role in promoting pathologic angiogenesis in the eye [72-75]. A number of studies have suggested that Th17 cells play a role in advanced AMD [76-78]. Th17 cells are effector CD4⁺ T cells

that preferentially produce IL-17 upon specific surface markers stimulation or activation by various pathogens. IL-17 is a pro-inflammatory cytokine that mostly targets epithelial, endothelial and stromal cells and defective production of this cytokine is associated with several autoimmune diseases [79, 80]. Complement components such as C5a have been implicated in inducing CD4+ T cells to express IL-17 and IL-22. The level of these cytokines was also increased in serum from patients with AMD [76]. In addition, one study showed that the *IL-17RC* promoter, a component of the IL-17R complex, was significantly hypomethylated in patients with AMD [81]. This was associated with increased expression of IL-17RC in the peripheral blood, retina and choroid of AMD patients [81]. A subsequent study that carefully examined the methylation status of the *IL-17RC* promoter using global methylation arrays, bisulfite sequencing and PCR-based methylation assays found no significant difference between AMD patients and age-matched controls in two independent cohorts [82]. These findings were consistent for no difference either in the peripheral blood or in eye-derived tissues such as the retina or choroid [82]. Furthermore, it was demonstrated that IL-17 alone had no impact on vessel growth, although it can potentiate the effect of other angiogenic factors such as VEGF, basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) [72]. Recent findings have shown that combined production of IL-17 from $\gamma\delta$ T cells and innate lymphoid cells was responsible for the increased neovascularization observed in experimental model of CNV [74]. As such, the precise role of IL-17 in regulating angiogenesis in the eye remains unclear.

Complement components and lipid peroxidation can induce oxidation specific epitopes that may function as antigens for immune recognition [83]. Carboxyethyl pyrrole (CEP) is an example of such an epitope that is an oxidation fragment of docohexaenoic acid (DHA) [84]. CEP has previously been shown in animals to initiate an immune response that leads to damage to the RPE similar to that seen in non-neovascular AMD [85-87]. In addition, not only is CEP increased in AMD donor eyes, but autoantibodies to CEP and CEP-modified proteins can be identified in plasma from AMD patients [88, 89]. The mechanism by which CEP induces an immune response is thought to be via the activation of T lymphocytes by classically activated M1 macrophages [77]. In murine models, this leads to increased expression of IFN- γ and IL-17 [77]. Despite the association of CEP and similar epitopes with the disease phenotype in dry (non-neovascular) AMD, the link to pathologic angiogenesis and wet AMD remains elusive.

Autoantibody profiling of sera from AMD patients has identified retinal autoantibodies that have the ability to promote endothelial cell growth *in vitro*. Although underlying mechanisms that lead to endothelial cell proliferation are unknown, these findings suggest that autoantibodies could be involved in the pathogenesis of neovascular AMD and especially in CNV formation [73].

Concluding remarks and future perspectives

It is now apparent that chronic, intraocular anti-VEGF treatments could lead to permanent vision loss due to the pleiotropic effects of VEGF and, more importantly, its neurotrophic potential. Both experimental and translational studies have confirmed the deleterious effects of long-term VEGF inhibition. There are several lines of evidence for VEGF-independent

regulation of ocular angiogenesis. Different components of the immune system have been shown to modulate vascularization in the eye. Although some regulatory pathways are specific to the innate or adaptive compartment, the interplay between immune components and retinal cells is necessary for the control of ocular angiogenesis (Figure 3). For instance, macrophages/microglia can directly regulate endothelial cells growth upon activation, and also mediate the effects of the complement system. The impact of immune responses on retinal vasculature can be facilitated or enhanced by surrounding neurons. The latter shows the interconnection between the immune system, retinal vasculature and neurons. Future studies will determine the impact of selective inhibition of ocular angiogenesis on neuron survival and local immune responses (Box 2).

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Glossary

Angiogenesis	biological process of blood vessel formation
Adaptive immunity	specific antibodies or cell-mediated responses are developed after exposure to pathogens/antigens. This adaptive line of defense also produces an immunological memory
Complement	the complement system is a component of the innate immunity and comprises a network of proteins and receptors that recognizes pathogens
Choroidal neovascularization (CNV)	Pathological angiogenesis that develops underneath the retina. These new vessels are responsible for the hemorrhage and leakage of fluid causing photoreceptor death, neuroretinal degeneration and blindness
Immune system	is a network of specialized cells and structures that orchestrate the defense of the body against infections and diseases. It has innate and adaptive components
Inflammation	A cascade of events at the site of infection or tissue injury that lead to the recruitment of immune cells and secretion of soluble factors including cytokines and chemokines for the resolution and limitation of tissue damage
Innate immunity	this defense mechanism is not specific to pathogens, and recognizes common features of invading organisms
Ischemia	deficient blood supply to tissues. In vascular proliferative eye diseases such as retinopathy of prematurity and diabetic retinopathy, ischemia is a key signal that triggers neovascularization

Retina	is the light sensitive, transparent tissue lining the back of the eye. It is composed of different cells (neurons and glia) organized in well-structured layers. The photoreceptor neurons transform light photons into electrical signals for further processing in the brain
Vascular endothelial growth factor (VEGF)	an important regulator of developmental and pathological angiogenesis.

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Box1**Experimental models of ocular angiogenesis****Oxygen induced retinopathy (OIR)**

Ischemia induced neovascularization is a common feature of many retinal disorders including retinopathy of prematurity (ROP) and diabetic retinopathy (DR). In ROP, high oxygen exposure results in vaso-oblivation of central retinal vessels. When returned to normal oxygen conditions, hypoxic retinal areas develop new blood vessels [9]. This two-step neovascularization is also observed in patients with DR where insulin resistance and other metabolic factors cause hyperglycemia that induces vascular occlusion and the subsequent retinal ischemia promotes neovessel formation [90]. Multiple experimental procedures now recapitulate the sequential events that lead to pathological angiogenesis in animal models. Neonatal mice are exposed to high oxygen (75%) from postnatal days (P) 7 to 12, corresponding to the vaso-oblivation phase, and are then placed in normal room air (21% oxygen) for 5 additional days, where vessel proliferation occurs. At P17, there is spontaneous neovessel regression [91]. OIR is intensively used to study retinal neovascularization in a pathological context and to characterize mechanisms modulating blood vessel homeostasis [51].

Injury-induced choroidal neovascularization (CNV)

There are two forms of age related macular degeneration (AMD), a major cause of blindness in the elderly [4]. In advanced AMD the dry or non-neovascular form is characterized by retinal pigmented epithelial cell death and subsequent photoreceptor neuron loss associated with atrophic changes in the macula, whereas in the wet or neovascular form, there is a formation of new blood vessels underneath the retina called choroidal neovascularization (CNV). Although the dry form is more common, the majority of blindness occurs in patients with the neovascular form of AMD, due to macular edema and exudation of fluid and blood from CNV. To date, there is no animal model that recapitulates all the clinical features of AMD. In experimental models, to mimic CNV formation as seen in the wet form of AMD, neovessels are induced in the subretinal space after focal laser-induced disruption of the Bruch's membrane. A week after laser-induced injury, neovascularization is analyzed by immunohistochemistry [92].

Box2**Outstanding questions**

- What is the role of local neurons in mediating angiogenesis in the eye?
- How does immune privilege affect the regulation of ocular angiogenesis?
- Does blood retinal barrier integrity correlate with development of retinal autoantibodies?
- Is aging of the immune system causative in vascular proliferative diseases?

- VEGF in eye diseases
- Immune regulation of pathologic neovascularization
- VEGF-independent therapies in retinal vascular diseases

Figure1. Anti-VEGF treatment of neovascular eye diseases

Fluorescein angiogram of a control eye (a), a patient with wet AMD (b) or proliferative diabetic retinopathy (PDR) (c). Bright hyperfluorescence demonstrates leakage of dye from the choroidal neovascularization (CNV) under the macula (b, arrow) and retinal neovascularization (c, arrows). Anomalous circulation characterized by neovascularization, ischemia and collateral vessel formation can be noted in the retina of PDR patient (c). Optical coherence tomography (OCT) of the central retina of an AMD patient before (d) and after (e) anti-VEGF treatments. Intraocular injections of anti-VEGF neutralizing antibody (e) led to resolution of cystoid macular edema (d, stars) and significant improvement in the CNV (d, arrowheads). OCT of neovascular AMD patient (f) shows a pigment epithelial detachment (PED) overlying the CNV (f, arrow). After intravitreal anti-VEGF treatment (g), there is an increase in the size of the PED (including the CNV complex) as well as the amount of subretinal fluid indicating persistent CNV activity (g, arrows).

Figure2. Role of VEGF in retinal angiogenesis and homeostasis

Schematic cross-section through the human eye (a) and detailed representation of the retina (b). In the retina, VEGF is produced and secreted by neurons, glia and vascular endothelial cells. Additionally, different cell types also express VEGF receptor. During retinal vascular development, VEGF signaling plays an essential role in the formation and patterning of the vascular network (vascular plexus). The retinal pigmented epithelium (RPE)-derived VEGF is responsible for the development and the maintenance of the choriocapillaries. It also provides neurotrophic support to photoreceptors, retinal bipolar cells (RBC) and retinal ganglion cells (RGC). Retinal VEGF expression is upregulated in response to hypoxia and contributes to pathologic neovascularization. In these conditions, the majority of the VEGF is produced by Müller glial cells and astrocytes.

Figure3. Immune regulation of VEGF-independent angiogenesis

Depending on their activation state, macrophages/microglia can differentially modulate endothelial cell growth. M1 macrophages are anti-angiogenic, whereas M2 macrophages promote angiogenesis. The effect of macrophages on endothelial cells can be contact-dependent or through secretion of angiogenic factors. Complement factor B (cfB), a component of the alternative complement pathway, in combination with the complement inhibitor (CD55), selectively inhibits pathological neovessel formation. In hypoxic conditions, CD55 is downregulated in endothelial cells therefore allowing the specific targeting of neovessels by cfB. Complement components can also modulate macrophage activation. Complement components C3 and C5 polarize macrophages to an M1 anti-angiogenic phenotype. Activation of microglial cells upon exposure to ischemia induces the secretion of IL-1 β and subsequent production of Semaphorin-3a (Sema-3A) by retinal neurons. Sema-3A induces degeneration of the retinal vasculature. The adaptive immunity may promote angiogenesis through autoantibodies or secretion of IL-17 by T cells. Ocular angiogenesis can be further regulated through the remodeling of the extracellular matrix (ECM). Activation of ECM-stimulated pathways through neuropilin-1 (NRP1) signaling also promotes angiogenesis in the eye.

Table1Clinical trials targeting VEGF^a

Study	Disease	Summary	Anti-VEGF Therapy
VISTA/VIVID	Diabetes	Randomized controlled trial (RCT) of intravitreal aflibercept Injection for the treatment of diabetic macular edema (DME) [41]	Aflibercept: 97 kDa recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1
COPERNICUS/ GALILEO	Vein occlusion	RCT of intravitreal aflibercept injection in patients with macular edema secondary to retinal vein occlusions [37, 40]	
RISE/RIDE	Diabetes	RCT of 0.3 or 0.5 mg intravitreal ranibizumab for the treatment of DME [39]	
BRAVO	Vein occlusion	RCT of 0.5 mg of intravitreal ranibizumab injection in patients with macular edema secondary to branch retinal vein occlusion (BRVO) [34]	Ranibizumab: 48 kDa recombinant humanized IgG1 kappa isotype monoclonal antibody fragment that binds to VEGF-A
CRUISE	Vein occlusion	RCT evaluating 0.5 mg of intravitreal ranibizumab in patients with macular edema [33] secondary to central retinal vein occlusion (CRVO)	
ANCHOR/ MARINA	AMD	RCT of 0.3 or 0.5 mg of intravitreal ranibizumab for neovascular AMD [31, 32]	Bevacizumab: 149 kDa recombinant humanized monoclonal IgG1 antibody that binds to VEGF-A
BEAT-ROP	ROP	RCT comparing intravitreal bevacizumab monotherapy with conventional laser in retinopathy of prematurity (ROP) [36]	
VISION	AMD	RCT of intravitreal pegaptanib 0.3mg, 1.0mg and 3.0mg for neovascular AMD [30]	Pegaptanib Na: a pegylated aptamer that targets the 165 isoform of VEGF-A
CATT	AMD	A multicenter, noninferiority clinical trial comparing 1.25 mg bevacizumab to 0.5 mg ranibizumab in the treatment of neovascular AMD [35]	
VIEW1/2	AMD	RCT comparing safety and efficacy of intravitreal aflibercept to ranibizumab in neovascular AMD [38]	

^a Abbreviations: AMD, age-related macular degeneration; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; DME, diabetic macular edema; RCT, randomized controlled trial; ROP, retinopathy of prematurity; VEGF, vascular endothelial growth factor.

Table2Non-VEGF specific drugs that are being evaluated for inhibition of ocular angiogenesis^a

Drug	Disease	Summary	Mechanism
Pazopanib	AMD	5 mg/mL Pazopanib eye drops resulted in mean improvement in best-corrected visual acuity (BCVA) at Day 29 and improvements in vision [93]. Completed	Multitargeted tyrosine kinase inhibitor of VEGF receptors (VEGFRs)-1, VEGFRs-2, and VEGFRs-3, platelet-derived growth factor receptors (PDGFRs)- α and PDGFRs- β , and the stem cell growth factor c-Kit
Palomid 529	AMD	Inhibits the TORC1 and TORC2 complexes and shows both inhibition of Akt signaling and mTOR signaling similarly in tumor and vasculature. Shows that P529 inhibits tumor growth, angiogenesis, and vascular permeability [94]. Completed	Dual TORC1/2 inhibitor of the P13K/Akt/m TOR pathway
Squalamine Lactate	AMD	Systemically administered squalamine lactate partially reduced choroidal neovascular membrane development induced by laser trauma in experimental model [95]. Ongoing	An inhibitor of new blood vessel formation (angiogenesis) induced by VEGF, PDGF or bFGF.
AdGVPEDF.11D	AMD	Adenoviral vectored PEDF (Ad _{GV} PEDF.11D) administered by subtenon injection, inhibits choroidal neovascularization in experimental model [96]. Completed.	adenovirus vector containing the gene for the PEDF (pigment epithelium-derived factor) protein
Anecortave acetate	AMD	Anecortave acetate 15 mg has been shown to be efficacious at inhibiting vision loss and choroidal neovascularization lesion growth in a placebo-controlled, double-masked clinical trial in patients with exudative age-related macular degeneration [97]. Completed	Anecortave acetate blocks proteolytic cascade in the vascular endothelial cells
Fovista	AMD	Fovista has been administered in combination with an anti-VEGF agent (ranibizumab) for the treatment of patients newly diagnosed with wet AMD [98]. Recruiting	An anti-PDGF aptamer that prevent the binding of PDGF to its receptors on pericytes
Palomid 529	AMD	Subconjunctival injection in neovascular AMD patients that have not responded to standard anti-VEGF treatments [99]. Completed	An inhibitor of mTOR production
OC-10X	Diabetes	Evaluate the safety and tolerability of topical OC-10X Ophthalmic Suspension in healthy human subjects. OC-10X is under development for the treatment of Proliferative Diabetic Retinopathy (PDR). Completed	Oc-10X is a selective tubulin inhibitor
TG100801	AMD/ Diabetes	Topical treatment for inhibition of ocular angiogenesis and retinal edema [100]. Completed	Inhibitor of Src kinases and selected receptor tyrosine kinases

^a Abbreviations: AMD, age-related macular degeneration; BCVA, best-corrected visual acuity; bFGF, **basic fibroblast growth factor**; PEDF, pigment epithelium-derived factor; PDGF, platelet-derived growth factor; PDR, **proliferative diabetic retinopathy**; VEGF, **vascular endothelial growth factor**.