

Review

## Angiogenesis and ophthalmic disease

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### Abstract

Ocular angiogenesis is responsible for the majority of irreversible blindness in the developed world [1]. This debilitating complication affects all age groups and characterizes such diverse and widespread diseases as trachoma, retinopathy of prematurity, diabetic retinopathy, neovascular glaucoma and age-related macular degeneration. Although numerous relatively rare conditions also exhibit ocular angiogenesis, the aim of this review will be to briefly summarize our current knowledge regarding the clinical and laboratory findings of the most epidemiologically significant diseases. We will also describe current concepts regarding the pathogenesis of ocular angiogenesis. In this review the term 'neovascularization', which is more prevalent in the ophthalmic literature, will be used to describe the development of pathological new vessels and should be considered synonymous with 'angiogenesis'.

### Corneal neovascularization

Neovascularization of the cornea causes vision loss through several different mechanisms (Figure 1). Corneal vessels are associated with an abnormal epithelium that resembles conjunctiva. This optically inferior epithelium produces a poor refractive surface, resulting in degradation of the visual image. The abnormal epithelium develops in association with blood vessels and appears to require them. The selective photothrombosis of corneal blood vessels induces the reversion of the epithelium to a more normal and optically superior phenotype, a process known as transdifferentiation [2, 3]. In addition to the epithelial changes, corneal vessels leak and deposit lipids in the underlying corneal stroma, obstructing the visual axis. Lastly, advanced corneal neovascularization can result in vessels growing into the center of the cornea and directly distorting incoming light. The leading cause of blindness in the world, trachoma, is an infectious disease manifesting dense conjunctivalization, keratinization and neovascularization of the ocular surface.

The major stimuli for corneal neovascularization are corneal inflammation, hypoxia and limbal stem cell destruction. Corneal inflammation resulting from various causes, whether sterile or infectious, is frequently accompanied by corneal neovascularization. Hypoxia, most commonly occurring with contact lens wear, is

another common precipitant. Studies have shown a direct correlation between the oxygen transmissibility of a contact lens polymer and the incidence of neovascularization [4]. The destruction of the limbal epithelial stem cells is another potent precipitant that often leads to the conjunctivalization and neovascularization of the cornea [5]. All three mechanisms can operate simultaneously. For example, a severe chemical burn that destroys the limbal stem cells is almost always associated with inflammation. The influx of inflammatory cells into the normally hypocellular cornea may then produce a hypoxic microenvironment.

The avascularity of the cornea has been utilized to test the angiogenic potential of numerous substances via the corneal micropocket assay [6]. Until recently, however, little was known about the endogenous angiogenic factors that trigger corneal neovascularization in disease. In a rodent model of limbal stem cell ablation, the expression of vascular endothelial growth factor (VEGF) was recently shown to be temporally and spatially correlated with corneal neovascularization. Moreover, the inhibition of VEGF with a specific antibody potently suppressed the process, making VEGF the first known endogenous mediator of corneal neovascularization [7]. A recent study has confirmed the increased expression of VEGF in human corneas with neovascularization [8].

### Iris neovascularization

Iris neovascularization is almost always secondary to retinal ischemia (Figure 2). This observation is in

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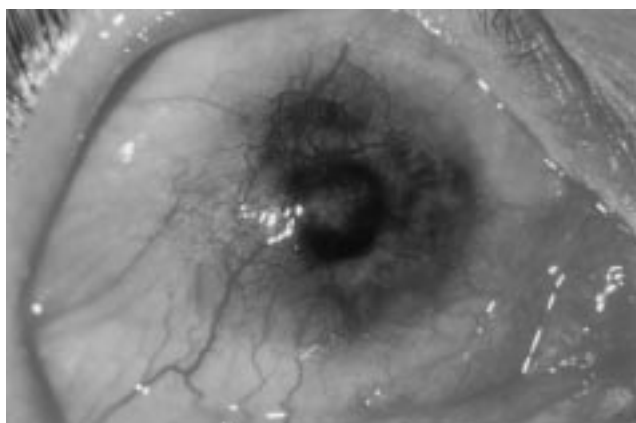


Figure 1. Human corneal neovascularization associated with an irregular 'conjunctivalized' epithelium. Note the irregular, broken light reflex indicating the poor refractive nature of the surface.

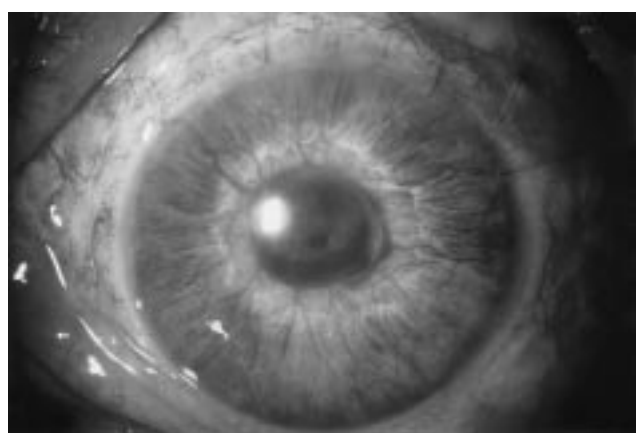


Figure 2. A human eye with circumferential iris neovascularization associated with glaucoma.

agreement with the hypotheses of Michaelson [9], Ashton [10] and Wise [11]. Michaelson's studies of the developing retinal vasculature led him to predict that the retina produces a soluble substance capable of triggering vascularization. Ashton implicated retinal hypoxia as the stimulus underlying the production of the angiogenic substance. George Wise proposed that the same substance mediated iris, retinal and optic nerve neovascularization in adult diseases manifesting retinal ischemia [11]. He named the hypothetical substance 'Factor X'.

Iris neovascularization, classically referred to as rubeosis iridis, occurs in association with retinal vein occlusions, advanced diabetic retinopathy, and angiogenic intraocular tumors such as retinoblastoma. Histopathologically, iris neovascularization in primates is easily identified by its location. Since the normal anterior iris is avascular, vessels in the anterior iris are, by definition, 'new'. In pigmented animals, these new vessels can be observed with fluorescein angiography. They appear as tortuous, leaky, randomly branching small diameter fluorescent lines. Pigmented cells block the fluorescence from the normal vessels in the posterior

iris. Labeling studies have documented that the new vessels are undergoing DNA synthesis [12]. In cases of profuse neovascularization, the new iris vessels can be seen with the slit-lamp biomicroscope. Left unchecked, the iris neovascularization leads to occlusion of the trabecular meshwork, producing an intractable and painful glaucoma (neovascular glaucoma) which is a leading cause for enucleation [13].

Animal experiments have shown that ischemia-associated iris neovascularization requires VEGF. In the monkey model of retinal vein occlusion, soluble VEGF isoforms have been shown to be produced in the ischemic retina [14] and high VEGF levels have been correlated with the development of iris neovascularization [15]. VEGF levels have also been shown to rise and fall prior to the onset and regression of iris neovascularization, respectively. Injections of specific anti-VEGF antibodies have potentially blocked the development of iris neovascularization in this model [16]. Moreover, VEGF injections into normal primate eyes have produced iris neovascularization and neovascular glaucoma [17]. These data demonstrate that VEGF is both necessary and sufficient for retinal-ischemia associated iris neovascularization in the monkey model. The relevance of these findings to the human condition has been established. High retinal and intraocular VEGF levels have been closely correlated with iris neovascularization in several human ocular disorders [18, 19]. Moreover, retinal re-oxygenation, as occurs with pan-retinal photocoagulation, has been shown to be associated with decreased retinal VEGF expression in monkeys [20, 21] and humans [19]. The decreased VEGF levels are followed by the regression of the new iris vessels [15, 19]. In many ways, VEGF appears to be 'Factor X'.

### Retinal neovascularization

Retinal neovascularization originates from the retinal circulation and can be either intraretinal or extraretinal (Figure 3). The latter is readily recognized clinically and is most commonly referred to as 'retinal neovascularization'. With this type of neovascularization, new vessels extend from the inner retina onto the face of the avascular vitreous gel. The new vessels lose the normal orderly, fractal-like branching pattern. Over a period of weeks to months, fibrous tissue becomes associated with the vessels and contributes to the development of traction retinal detachments and vitreous hemorrhages.

Except for their anatomical location, intraretinal new vessels can have the same appearance as extraretinal vessels. Alternatively, they can appear dilated and tortuous with focal sausage-like narrowings. Because these latter vessels do not breach an avascular region of the eye (i.e., vitreous) and may not leak fluorescein dye, the ophthalmic community has historically called them intraretinal microvascular abnormalities (IRMA).

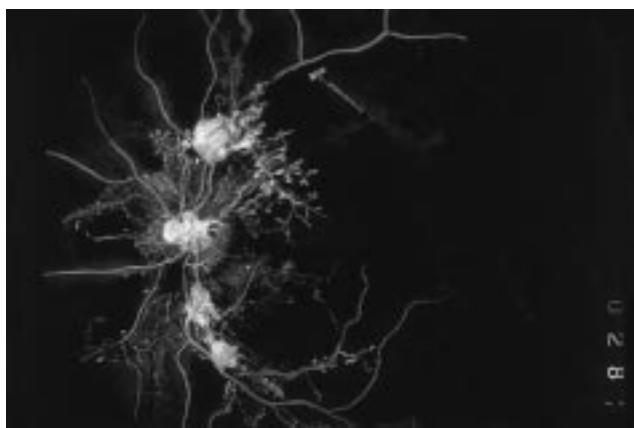


Figure 3. Fluorescein angiogram showing areas of iris and optic nerve head neovascularization (hyperfluorescent areas) juxtaposed with large zones of retinal non-perfusion and ischemia (hypofluorescent areas).

Recent human and animal studies now suggest that some IRMA may be comprised of proliferating endothelial cells [22, 23].

VEGF and hypoxia have been implicated in the pathogenesis of both types of retinal neovascularization. Retinal neovascularization most often originates in 'watershed' zones between ischemic and non-ischemic retina, suggesting the production of local, diffusible angiogenic factor [11]. Photocoagulation of ischemic retina restores oxygen levels [20] and is associated with regression of new vessels [24]. A vitreous scaffold upon which the vessels can grow also facilitates neovascularization. Therefore, surgical vitrectomy serves to prevent extraretinal neovascularization [25].

The cause(s) of diabetic retinal ischemia is still not known. In the more advanced stages of diabetic retinopathy, histopathologic death of the microvasculature is evident and is most prominent in the mid-periphery of the retina. However ischemia in the earlier stages of diabetic retinopathy may involve leukocytes. It is known that the number of leukocytes in the retinal vasculature is increased in diabetic animals and humans [26, 27]. Some of these leukocytes are trapped within the vasculature and may contribute to capillary non-perfusion [26, 28]. One theory states leukocyte stiffness is operative in development of capillary non-perfusion. Hatchell and colleagues have shown a decreased filterability and deformability of diabetic leukocytes [29, 30]. A second theory states that leukocyte entrapment occurs via adhesion molecules. Luty and coworkers have demonstrated increased immunoreactivity for intercellular adhesion molecule-1 (ICAM-1) in the diabetic retinal microvasculature [27]. A third theory implicates platelets in the generation of capillary nonperfusion. Increased numbers of platelets have been seen in the retinal vasculature [32]. All three mechanisms are not mutually exclusive and may operate simultaneously. Adherent activated leukocytes are stiffer and platelets have been shown to mediate leukocyte adhesion to vasculature elsewhere in the body [32]. As capillary ischemia progresses, areas of capillary death are pro-

duced. It is conceivable that activated and adherent leukocytes may participate in the progression to this later stage.

Because VEGF is a diffusible and oxygen-regulated angiogenic factor [33], it has been considered a prime candidate to mediate retinal neovascularization. The data supporting this role for VEGF are extensive. Cell culture experiments have demonstrated that VEGF is produced by many ocular cells [34] and that it induces retinal endothelial cells to proliferate [35–37]. Injections of VEGF into normal primate eyes have produced intraretinal neovascularization [23] and transgenic mice overexpressing VEGF in photoreceptors have developed neovascularization extending to the outer retina [38]. Most importantly from a therapeutic standpoint, VEGF inhibition appears to prevent retinal neovascularization. In a mouse model of retinopathy of prematurity, injections of a highly-specific, VEGF-neutralizing, soluble VEGF receptor chimera suppressed the extra-retinal neovascularization that otherwise universally occurs in these animals following the onset of retinal ischemia [39]. Tissue culture studies have documented the presence of VEGF receptors on retinal endothelial cells [37] and microvascular pericytes [40]. These studies appear relevant to human disorders since patients with ischemia-related retinal neovascularization have much higher intraocular VEGF levels than patients without new vessels [19, 41]. Among the several candidate ocular angiogenic factors assayed, VEGF alone appears to be consistently and specifically upregulated in hypoxic retinal tissue, both *in vitro* [42] and *in vivo* [43].

Many advances in our understanding of the signaling mechanisms of VEGF have recently been achieved both at the intracellular [36] and nuclear levels [44]. Many of these mechanisms implicate specific PKC isoforms in the mediation of the retinal angiogenic effects *in vivo*. Indeed, inhibition of certain PKC isoforms can prevent the neovascular [45, 46] and permeability [47] effects of VEGF in animals. Several VEGF inhibitory compounds are either in, or beginning, clinical trial.

Solid evidence has also implicated matrix components such as the integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  in ischemia-related retinal neovascularization [48, 49]. The integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  are thought to be involved in the migration of endothelial cells and to exert their action downstream from the effects of VEGF and/or basic fibroblast growth factor (bFGF). VEGF and bFGF have been shown to upregulate the gene expression for  $\alpha v \beta 5$  and  $\alpha v \beta 3$  in endothelial cells, respectively [50]. Significant inhibition of retinal neovascularization has been observed in rats when specific integrin small molecule antagonists are administered [48–40].

Growth hormone and its mediator, insulin-like growth factor, also appear to be operative in the development of retinal neovascularization in the mouse retinopathy of prematurity model. The specific inhibition of these two molecules produced a decrease in retinal neovascularization that was inversely proportional to their circulating levels [51]. Doubtless in the

future, other factors, both diffusible and non-diffusible, will be shown to be important in the complex processes of constructing and deconstructing retinal vessels.

### Choroidal neovascularization

Choroidal neovascularization occurs in age-related macular degeneration (AMD) and is the leading cause of severe and irreversible blindness in the elderly. Although fewer than 10% of AMD cases manifest neovascularization, the process produces most of the severe vision loss in this disease [1]. New vessels grow from the choroidal vasculature, through Bruch's membrane, and into the plane of the retinal pigment epithelium (RPE) and subretinal spaces. For reasons unknown, the neovascularization occurs almost exclusively in the central macular and perimacular regions of the retina where it produces the greatest damage. The vessels are leaky and friable, producing accumulations of fluid, lipid and blood that lead to the death of the central photoreceptors. Only 15% of patients are amenable to laser ablation of their choroidal neovascular membranes [52], and fully half of such treated membranes recur [53].

Compared to neovascularization elsewhere in the eye, little is known about the inciting stimulus for choroidal neovascularization. This is the result, in part, of relatively inadequate animal models. Although there is some indirect histopathologic and angiographic evidence to suggest local tissue hypoxia [54, 55], outer retinal hypoxia has not been conclusively proven. Likewise, the limited inflammation that characterizes the disease is considered by some to be of little significance in the pathogenesis of the neovascularization [56]. Although little is known about the underlying etiology of the disease, both genetic and environmental factors are thought to play a role [57, 58].

VEGF [59, 60] and the integrins  $\alpha v\beta 3$  and  $\alpha v\beta 5$  [61] have been shown to be temporally and spatially correlated with choroidal neovascularization in animals and humans, although definitive blocking experiments similar to those performed in models of iris and retinal neovascularization have not been completed. However, it is reasonable to assume that VEGF and the integrins  $\alpha v\beta 3$  and  $\alpha v\beta 5$  will be required for choroidal angiogenesis given their important role in angiogenesis elsewhere in the body [62]. Drugs aimed at inhibiting the bioactivity of these molecules are entering clinical trials.

### Normal vessel development

Many molecular and cellular mechanisms are shared between developmental and pathological angiogenesis. Because retinal vascular development occurs largely after birth in rodents, Stone and colleagues were able to show that hypoxia-induced VEGF expression by neuroglia mediates development of the retinal vasculature [63]. It

was also shown that the close association of retinal glia to the retinal vessels may control retinal vessel growth and maintain vessel integrity [64]. In separate studies, Benjamin and Keshet were able to show that pericyte coverage is important for the persistence of new vessels. Intravitreal injections of PDGF-BB were used to cause pericyte migration away from retinal vessels and triggered the regression of newly formed retinal vessels. The result demonstrated that before pericyte coverage, a window of plasticity exists in which vessels can be made to regress [65]. VEGF may be operative in the maintenance of new vessels. Withdrawal of VEGF from newly formed vessels was associated with their regression in an ROP model [66]. A similar phenomenon was recently seen in tumors where tumor vessels regressed following VEGF withdrawal but prior to periendothelial cell coverage [67].

### Summary

Ocular neovascularization represents a major public health problem, being responsible for more irreversible blindness in persons of middle and older age than any other pathology. The societal burden of ocular neovascularization has prompted extensive research into its mechanisms. From an investigational viewpoint, studies of ocular neovascularization provide important information, often permitting real-time, serial observations of neovascularization *in vivo*. A combination of new and old technologies allow investigators to visualize and quantify *in vivo* changes in the blood brain barrier [68], leukocyte dynamics [69], and vessel growth in ocular tissues. Thus, the eye may serve as a fundamental means for the development of new hypotheses, knowledge, and therapeutic approaches that will eventually save sight and alleviate human suffering.

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