American Journal of Pathology, Vol. 151, No. 1, July 1997

Commentary

Vascular Endothelial Growth Factor and Ocular Neovascularization

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In a paper in this issue, Okamoto et al[1] describe a transgenic mouse model of intraretinal and subretinal neovascularization driven by vascular endothelial growth factor (VEGF) overexpression in photoreceptors. Neovascularization within the eye is a complication of many eye diseases, resulting in blindness in patients from infants to the elderly. The authors are correct in noting that research in this area has been frustrated by the lack of a reliable, small animal model of retinal or choroidal neovascularization of long duration. The authors utilized a strategy of VEGF overexpression, as VEGF has been implicated in ocular neovascularization in animal models and clinical disease. They generated three transgenic lines expressing the rhodopsin promoter/VEGF fusion gene, one of which did not show increased expression of VEGF and did not have retinal changes, one that had increased expression of VEGF but developed retinal degeneration, and a third that showed increased VEGF expression and demonstrated neovascularization in the outer retina. The neovascularization that developed has characteristics of both retinal and subretinal neovascularization and may be useful in investigations of neovascularization secondary to diabetes and other retinal vascular diseases as well as age-related macular degeneration. The model will also be useful in assessing new treatments for these diseases, such as anti-angiogenic medications, photodynamic therapy, and radiation. The development of this model is a noteworthy contribution to the field.

Clinical Relevance

Neovascularization within the eye leads to fibrosis and disruption of delicate tissues required for vision and is the cause of blindness in many common eye diseases. Diabetic retinopathy is the leading cause of blindness in people age 25 and under, causing 12% of the new cases of blindness each year in the United States.[2] Diabetic retinopathy is characterized by capillary closure with retinal ischemia, followed by the growth of new blood vessels from the retina and optic nerve. Bleeding into the vitreous and retinal detachment from vitreoretinal traction can lead to vision loss. Retinopathy of prematurity (ROP) is a proliferative retinopathy in infants, the prevalence of which has increased with advances in neonatal care. In ROP, normal retinal vascular development is interrupted and a ridge of new blood vessels can extend into the vitreous, leading to retinal detachment and blindness. It is estimated that 3400 infants will suffer visual impairment from this condition each year in the United States and that 650 will be blinded.[3] Age-related macular degeneration is the leading cause of severe vision loss in people over 65, and the exudative form, characterized by neovascularization, is responsible for 80% of the cases with severe vision loss.[4,5] In this disease, new blood vessels arise from the choriocapillaris and invade the subretinal space, producing localized exudative detachments with hemorrhage, leading to fibrosis, loss of photoreceptors, and loss of central vision. Neovascularization of the iris and neovascular glaucoma occurs with diabetic retinopathy, retinal vein occlusion, and ocular tumors. A blind, painful eye secondary to neovascular glaucoma is the most common reason for enucleation in developed countries.[6,7]

Current treatments for ocular neovascularization vary depending on the site of neovascularization, but most are destructive in nature. Retinal neovascular-

Accepted for publication May 12, 1997.

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ization, such as in diabetic retinopathy, is the most successfully treated form of ocular neovascularization and relies on ablation of ischemic retina by laser photocoagulation or cryopexy. In panretinal laser photocoagulation, 1500 to 2000 burns of 200 to 500 μm are placed in the retina, from the arcade vessels to the equator, sparing the central retina. Although this form of treatment was demonstrated in the 1970s to decrease the rate of severe vision loss from proliferative diabetic retinopathy by 57%, it is accompanied by some vision loss (typically, one to two lines of central acuity) and loss of peripheral field and night vision.[8,9] Iris neovascularization is also treated with retinal ablation, but neovascular glaucoma is difficult to control and frequently leads to blindness.[6,7] Treatment of choroidal neovascularization relies on direct ablation of the subretinal vessels, accompanied by thermal destruction of the overlying neurosensory retina. If the blood vessels are not under the center of the macula, central vision is not lost, but neovascularization recurs in approximately 50% of cases.[10] Laser treatment of choroidal neovascularization of even a modest-sized area, located under the center of the macula, leads to immediate loss of vision, with a mean visual acuity of 20/320 at 5 years *versus* 20/400 in untreated eyes, making many retinal surgeons reluctant to proceed with therapy.[11] Indeed, most lesions in macular degeneration are not amenable to laser photocoagulation and lead to significant loss of vision.[12] Research is ongoing at the basic and preclinical level to develop new therapies for patients with neovascularization of the retina, choroid, and iris.

VEGF in Ocular Neovascularization

Okamoto et al[1] chose overexpression of VEGF as a strategy to produce retinal neovascularization as this growth factor has been strongly implicated in ocular neovascularization. In 1948, Michaelson[13] hypothesized that ischemic retina produced a diffusible angiogenic factor that stimulated the development of embryonic retinal vasculature. Ashton[14] proposed that oxygen deprivation stimulated the production of the angiogenic factor in development and in retinopathy of prematurity. In 1956, Wise expanded the idea of an angiogenic factor to explain retinal neovascularization in a number of adult eye diseases, and the clinical correlation of retinal ischemia and retinal neovascularization has since become well established.[8,15] Angiogenic activity has been identified from the retina and subsequently identified as acidic fibroblast growth factor.[16] However, attempts to correlate the role of a particular growth factor with neovascularization in the eye have remained inconclusive.

VEGF, also known as vascular permeability factor and vasculotropin, was identified in tumor and developmental systems as a vasopermeability factor and angiogenesis factor, which was up-regulated in hypoxia, a component of ischemia.[17–23] For a recent review, see Miller et al.[24] VEGF is a highly conserved protein existing in three isoforms in the mouse and four isoforms in the human, resulting from alternative splicing.[25] The smaller two species are secreted forms, whereas the larger species bind heparan avidly and are thought to be bound to the cell surface or basement membrane.[26] VEGF was found to be produced by retinal cells *in vitro* and to be up-regulated by hypoxia in these cells.[21,27] In our laboratory, we investigated the role of VEGF in a monkey model of iris neovascularization that develops after the production of retinal ischemia following laser vein occlusion. VEGF protein was undetectable in the normal eyes but rose quickly following the development of retinal ischemia, and increased VEGF protein in the ocular fluids correlated with the development and severity of iris neovascularization.[28] *In situ* hybridization identified the inner retina as the source of VEGF.[28,29] Other investigators confirmed the correlation of increased VEGF production with ocular neovascularization in ROP models.[30–32]

The role of VEGF in human disease has been substantiated in several studies. VEGF levels in vitreous obtained at surgery showed increased VEGF levels in patients with proliferative disease from diabetes or retinal vein occlusion.[33,34] Surgical specimens from eyes with proliferative diabetic retinopathy have also shown expression of VEGF,[35] and postmortem studies have demonstrated increased VEGF expression in eyes with retinal neovascularization.[36,37]

VEGF injections into normal monkey eyes can produce iris neovascularization and neovascular glaucoma mimicking human disease.[38] Intraretinal neovascularization developed after repeated injections of VEGF, although preretinal neovascularization with vessels crossing the internal limiting membrane was not seen.[39] Before the onset of neovascularization, many of the features of nonproliferative diabetic retinopathy were observed, including vessel dilation, tortuosity, microaneurysm formation, hemorrhage, edema, and capillary dropout, suggesting that VEGF may also play a role in the preproliferative changes. This role is also supported by findings in human postmortem specimens.[40]

Investigators in our laboratory and others have sought to show a causal role for VEGF in ocular neovascularization by using various strategies to inhibit VEGF to intervene in pathological neovascularization. Intravitreous injection of anti-VEGF antibodies completely prevents the development of iris neovascularization in the monkey model.[41] In the mouse ROP model, dominant-negative VEGF receptors and VEGF antisense oligonucleotides substantially decreased the neovascular response.[42,43]

Preliminary data suggest a role for VEGF in choroidal neovascularization secondary to age-related macular degeneration. There is less evidence for hypoxia as a factor in choroidal neovascularization, where the outer retina is supplied by the choroid, with a very high blood flow per tissue weight ratio. However, immunostaining of postmortem eyes has demonstrated increased VEGF expression in areas of choroidal neovascularization.[44,45] *In situ* hybridization for VEGF message in the laser injury model in the monkey has also demonstrated a correlation of VEGF expression with the development of choroidal neovascularization.[46]

VEGF appears to play a major role in ocular neovascularization in different capillary beds and in neovascularization due to varying etiologies. Therapeutic strategies directed against VEGF are being investigated as a potential new therapy for many ocular diseases. However, the development of anti-angiogenic therapies, including anti-VEGF therapies, would be facilitated by a reliable small animal model of persistent retinal or choroidal neovascularization, such as the model developed by Okamoto et al.[1] Many animal models of ocular neovascularization have been developed and used in previous studies, and it is worth reviewing the strengths and weaknesses of these models.

Models of Ocular Neovascularization

Cell culture experiments can provide some understanding of the mechanisms of angiogenesis. The effect of growth factors on endothelial cell proliferation, migration, and tube formation can provide insight into their role *in vivo*.[47] Co-culture systems can help to elucidate the role of cell-cell interaction and has demonstrated pericyte inhibition of endothelial cell proliferation in a contact-dependent manner via production of activated transforming growth factor-β.[48] A model closer to the *in vivo* situation is the chick chorioallantoic membrane bioassay, which has been used to test the effect of various growth factors and drugs on angiogenesis.[49–51] However, although it can provide a large-scale screening system for angiogenesis inhibitors, it is difficult to master and provides only a limited *in vivo* system.

Probably the most commonly used model of ocular neovascularization in animals remains the corneal micropocket model. In this model, pellets are inserted into a corneal stromal micropocket near the limbus, containing stimulators of angiogenesis. Reagents used in the micropocket include endotoxin and growth factors, such as basic fibroblast growth factor (bFGF), or VEGF.[49,52,53] Other methods to wound the cornea including chemical burning or suture placement and implantation of tumor have also been used. The most commonly used animal has been the rabbit, although smaller animals, including the rat and the mouse, have also been studied.[54,55]

The corneal neovascularization models have been criticized because they are either inflammatory (such as the use of endotoxin) or rely on the implantation of large quantities of growth factors and as such do not model a clinical disease. There has also been some difficulty in quantifying the neovascular response, although various methods have been used, including measuring the distance from the limbus that the blood vessels reach, assigning a vertical and horizontal quotient that estimates the length and circumferential extent of the vessels, and computerized image analysis of the vessels to include a measurement of their density.[56] The dose of the angiogenic agent and the distance from the micropocket to the limbus can have a marked effect on the degree of neovascularization produced.[57] Nevertheless, the corneal models do provide a relatively simple model in a small animal for screening anti-angiogenic substances and investigating ocular neovascularization.

A model of retinal neovascularization and vitreoretinopathy was developed in the rabbit using intravitreal injection of cultured fibroblasts.[58] A fibrous membrane grows along the path of injection into the midvitreous cavity, and when contact with the retina is made, capillaries grow along the strands, accompanied by traction retinal detachment. Vessels are often not evident clinically but could be identified histologically. This model was modified by Anne Hannekan to utilize fibroblasts overexpressing bFGF.[59] Antoszyk et al[60] further modified the model by utilizing intravitreal hyaluronidase to break down the vitreous and have shown that intravitreal triamcinolone reduces the rate and severity of neovascularization in this model. This is a useful model of retinal neovascularization and vitreoretinopathy in a medium-sized animal but resembles proliferative vitreoretinopathy more closely than ischemic retinopa-

thies such as diabetic retinopathy. The instigating event is traumatic and inflammatory, rather than retinal ischemia, and retinal detachment appears to be a prerequisite for neovascularization.

Animal models of ROP were investigated in the 1950s when the epidemic of ROP first appeared in newborns. The key elements of neovascularization simulating ROP are retinal vasoconstriction induced by relative hyperoxia in underdeveloped retinal vessels, followed by relative hypoxia and retinal vascular proliferation. Patz[61] described preretinal neovascularization in rats in a 1954 report. Ashton[14] and others found the rat somewhat unreliable as a model and developed a cat model of ROP. More recently, a number of investigators have established reliable models in the rat, using alternating hyperoxia and hypoxia,[3,62] or the newborn mouse.[63] Penn et al[3] placed newborn Sprague Dawley rats in an alternating oxygen environment, cycling from 40 to 80% O_2 every 12 hours for 7 to 14 days to simulate ischemia/reperfusion, followed by 2, 4, or 7 days in room air. Preretinal neovascularization, determined histologically as preretinal cellular proliferation the origin of which from existing retinal vessels could be demonstrated, occurred in 66% of all rats exposed for 7, 10, or 14 days, followed by 2, 4, or 7 days of room air. Neovascularization may have started to regress by 7 days. In the mouse model of ROP described by Smith et al,[63] 7-day-old mice were placed in hyperoxia (75%) for 5 days and then returned to room air. Preretinal neovascularization occurred in 100% of mice, when examined histologically (counting vascular cell nuclei anterior to the internal limiting membrane) between day 17 and day 21, followed by regression. ROP models have also been developed in the newborn cat and dog.[64,65] The ROP models in mice and rats offer the advantage of a reproducible model of retinal neovascularization in a small animal, resembling a human disease. They have the disadvantages of requiring histological grading, which is time-consuming, providing only one time point per animal, and not allowing one to follow neovascularization over time *in vivo*. Finally, pathological neovascularization occurs in the developing retina, which may confound the results and may limit the applicability to adult retinal disease.

Experimental studies of retinal vein occlusion created by diathermy or photocoagulation in pigs, cats, and monkeys were used in the 1960s and 1970s to model retinal vascular disease in humans.[66,67] Intraretinal neovascularization was found rarely following retinal vein occlusion in the monkey, although preretinal neovascularization was not seen.[68,69] Virdi and Hayreh[70] continued these studies investigating the development of iris and disc neovascularization. They described iris neovascularization lasting 13 to 60 days in four of six eyes following laser vein occlusion of the two major temporal retinal veins. They also noted neovascularization of the optic nerve in one eye. Packer et al[71] modified the model to include lensectomy and vitrectomy 1 to 6 months before laser vein occlusion of three of the four branch retinal veins; 12 of 12 eyes developed iris neovascularization, and 2 of the 12 developed neovascular glaucoma. The long interval between surgery and laser vein occlusion, and the complexity of this version of the model, limit its usefulness, although it reliably produced iris neovascularization. Nork demonstrated that endothelial cell proliferation occurred within the first 5 days after laser vein occlusion.[72]

In our laboratory, we modified the monkey model by using dye yellow laser, which is a more effective wavelength for occluding the retinal veins, and closing all branch retinal veins. Iris neovascularization develops in 75 to 90% of eyes within the first 5 to 7 days after laser vein occlusion, peaking in 10 to 14 days and regressing by 21 to 28 days, unless neovascular glaucoma develops, which occurs in a small percentage of cases. A grading system using standardized fluorescein iris angiograms was developed that permits semiquantitative analysis of neovascularization by masked readers.[28] Iris neovascularization in the monkey provides a model of ischemic retinopathy and ocular neovascularization that simulates human disease. The model can be used to assess treatments directed specifically at iris neovascularization and neovascular glaucoma[73–75] but more importantly can be used to investigate mechanisms of pathological angiogenesis in adult animals and to test potential anti-angiogenic therapies.[28,41,76] The disadvantages of the monkey model are primarily that it is a large animal model in a primate, which limits the experiments that can be performed, for economic and ethical reasons.

Laser induced retinal vein occlusion in miniature pigs has also been shown to cause retinal neovascularization. Pournaras[77] found retinal neovascularization in 9 of 19 eyes 3 weeks after argon laser occlusion of a single retinal vein. Danis et al[78] described neovascularization of the optic nerve or retina in 100% of 15 eyes of mixed-breed domestic pigs after argon laser occlusion of multiple branch retinal veins. However, in Danis's studies, fluorescein leakage during fundus angiography was not consistent, and grading of neovascularization required histological assessment. Thus, the porcine model offers the advantage of producing retinal and possibly optic nerve head neovascularization, but neovascularize-

tion may not always be consistently produced and may require a histological grading scheme rather than a photographic and angiographic one, limiting the ability to follow neovascularization in any dynamic fashion. The pig is still a large animal, creating economic constraints on the investigations pursued.

Choroidal neovascularization has been induced experimentally in several different species. Studies in the rabbit using laser injury or subretinal injection of autologous vitreous have resulted in microscopic evidence of neovascularization, but the new vessels do not demonstrate leakage on fluorescein angiography, characteristic of the clinical disease.[79,80] Kimura et al[81] implanted microspheres impregnated with bFGF into the subretinal space in rabbits, but again the fluorescein leakage was not convincing. Part of the difficulty in modeling choroidal neovascularization in the rabbit may be because unlike the primate eye, the choroidal circulation in the rabbit supplies and oxygenates the entire rabbit retina, which is otherwise avascular. This may alter the susceptibility of the choroidal vessels to neovascularization.

Archer and Gardiner[82,83] induced choroidal neovascularization in the monkey after laser retinal vein occlusion with photocoagulation burns intense enough to disrupt Bruch's membrane. Neovascularization was seen in 14 of 46 eyes, identified by fluorescein angiography and light microscopy. The model as described was limited by vitreous hemorrhage (which occurred in one-third of the cases) and the relatively low percentage of eyes with neovascularization in a large animal model. Ryan[84,85] modified this approach to eliminate any vascular occlusion, producing intense argon laser burns in the macula of monkeys, finding that the macula was much more susceptible to neovascularization than the retina outside the arcades. Choroidal neovascularization developed in 42% of lesions in the macula 4 weeks after laser and lasted 2 to 52 weeks (average, 16 weeks). The development of choroidal neovascularization seemed to occur more frequently in lesions that demonstrated subretinal hemorrhage immediately after laser, which were accompanied by more inflammatory cells, and seemed to prevent the retinal pigment epithelium (RPE) from walling off the injury site.[86] Similar to choroidal neovascularization in humans, the lesions demonstrated leakage of fluorescein into the subretinal space on fundus fluorescein angiography. Over time, this leakage gradually subsides, although the vessels themselves do not degenerate or disappear but are instead enveloped by proliferating RPE cells, which form tight junctions and prevent accumulation of fluid.[87]

The monkey model of choroidal neovascularization has been criticized as a model of human age-related macular degeneration, as it occurs secondary to laser injury. However, although the initiating step may differ, other characteristics may be shared, as evidenced by fluorescein angiography and light and electron microscopy. Recent evidence suggests that growth factors such as VEGF and integrins such as $\alpha_v\beta_3$ and $\alpha_v\beta5_3$, which have been implicated in human disease, are expressed by choroidal neovascular tissue in the monkey.[46,88] The model has been used to develop new treatments for choroidal neovascularization and to test potential anti-angiogenic agents.[89–93] The model is still in a large animal, and a primate, with the limitations described previously. Some attempt has been made to reproduce this model of choroidal neovascularization in the rat with some success.[94,95]

All of the previously described models have various limitations. The models of ROP by necessity complicate pathological neovascularization with abnormalities of development and may not be applicable to adult disease. Neovascularization in the mouse and rat ROP models is also short-lived, limiting the usefulness of the model. Models of neovascularization in the retina or choroid of the rabbit are limited by the difference in retinal blood supply of the rabbit, with its avascular retina. Retinal neovascularization in the pig occurs with limited frequency, in this large and still somewhat expensive model. The primate eye offers the advantage of resembling the human eye most closely, but has the obvious disadvantages of expense and ethical concerns. The ideal animal model would be a small animal with neovascularization of the retina and or/choroid that occurred in response to a meaningful stimulus, such as VEGF, a growth factor strongly implicated in many forms of ocular neovascularization. The neovascularization in the model should demonstrate many of the findings of clinical disease, such as leakage on fluorescein angiography and characteristic histological findings. Ideally, the model should permit a grading system that can be carried out *in vivo* and does not rely on histological grading of the neovascular response. Okamoto et al[1] have sought to do this by creating a transgenic mouse model of retinal neovascularization.[1]

Transgenic Model of Ocular Neovascularization

Transgenic animals refer to animals into which a foreign gene has been stably integrated into the genome by a method other than breeding (for a review see Pattengale et al[96]) Mice have been used most extensively, in part because there is a large

database of mouse genetics and in part because the technology is easier in this small animal. The most widely used method is micro-injection of DNA into the pronucleus of a fertilized oocyte that has been obtained from an animal mated after stimulation of ovulation. The oocytes are then reimplanted into a pseudopregnant female. Offspring are screened for the presence of the transgene by extracting DNA from a tissue sample, such as a tail biopsy. Once the transgenic founder is identified, it can be bred to produce future progeny carrying the transgene. Transgenic animal models offer the opportunity to study the expression and phenotypic changes induced by the transgene. Gene regulation can be studied by creating transgenes expressing constructs consisting of the regulatory portion of the gene linked to a reporter gene. Finally, as Okamoto et al[1] have described, one can create a whole animal model overexpressing a specific gene in the organism to produce a desired phenotype. This requires a heterologous promoter (in this case the bovine rhodopsin promoter) that is capable of high levels of induction of the gene to be linked to the cDNA in question, in this case, VEGF.

The production of tissue or organ specificity in transgenic systems depends on the choice of an appropriate tissue-specific promoter for the DNA construct. Okamoto et al[1] used the bovine rhodopsin promoter, which was previously shown to be expressed in photoreceptor cells.[97] Using a lacZ reporter, the 2.2-kb *Hin*dIII/*Nae*I fragment from the bovine rhodopsin promoter demonstrated an unusual topography of expression in mouse transgenic lines. Activity was first detected at 10 to 15 days of age and was localized to the posterior pole of the retina. Activity then increased linearly over the first 6 months of age but demonstrated a gradient with expression most prominent superotemporally, with decreasing expression inferonasally in the retina. Other organs did not demonstrate transgene expression, with the exception of selected areas of the brain, such as the Purkinje cells of the cerebellum. By using the rhodopsin promoter in the current study, Okamoto et al[1] were able to induce VEGF overexpression by a retinal cell, namely, the photoreceptor. This permits evaluation of VEGF overexpression in the tissue of interest without confounding systemic effects and without the drawbacks of exogenous administration. The authors chose this promoter because its characteristics and specificity were known and it drives expression in a retinal cell, and with it they were able to produce intra- and subretinal neovascularization. However, the photoreceptor would not be the first choice of retinal cell to create a model of retinal neovascularization by overexpression of VEGF, as the photoreceptor does not overexpress VEGF in situations of pathological neovascularization, either in animal models of ischemic retina or in clinical disease. In pathological neovascularization, the increased expression of VEGF is seen in ganglion cells, Mueller cells, and other cells in the inner nuclear layer and in the RPE.[28–31] The localization of VEGF expression to the photoreceptors, and the patchy expression previously noted with this promoter, may be responsible for some of the characteristics of the neovascularization in this transgenic model. Future studies may be able to use a promoter that directs expression to the ganglion cells, which might produce preretinal neovascularization, or to the RPE cells, which might produce choroidal neovascularization.

The authors produced three transgenic founders, which they backcrossed with normal mice to produce three lines of heterozygotes for the transgene. One line failed to produce VEGF and demonstrated a normal phenotype. One line showed overexpression of VEGF by the photoreceptors at postnatal days 7 to 14 and demonstrated widespread retinal degeneration by day 14. Although this phenotype was not the goal of the study, this line may provide an interesting model of retinal degeneration, and its molecular mechanism remains to be studied.

The third line demonstrated overexpression of VEGF by the photoreceptors, accompanied by neovascularization that originated in the inner nuclear layer and grew toward the subretinal space to become partially enveloped by proliferating RPE. The time course of VEGF expression in this line was somewhat more gradual and sustained than the line that exhibited retinal degeneration. In the line with neovascularization, VEGF expression began at 7 days but did not increase significantly until 1 month and remained constant for 3 to 4 months. Immunostaining to localize VEGF protein was performed in animals at age 3 months when subretinal neovascularization was well developed. VEGF immunostaining showed a patchy distribution in the photoreceptors, which one might expect with the rhodopsin promoter, but was demonstrated in photoreceptors overlying areas of subretinal neovascularization. It would be interesting to examine VEGF expression at earlier time points to determine whether the protein showed a different distribution earlier in the time course of the model. Another approach would be to use *in situ* hybridization to demonstrate expression of VEGF receptors, which may be increased in response to VEGF secretion by cells. It would be useful to show whether VEGF receptors are increased in

the vascular endothelium in the inner nuclear layer before the development of neovascularization and whether increased VEGF receptors are ever seen in the choroidal vessels.

Okamoto et al[1] examined the eyes using fluorescein angiography and were able to demonstrate multiple discrete spots of leakage in the retina beginning at 1 month. Fluorescein leakage on angiography is typical of retinal and choroidal neovascularization in humans but is not always reproduced in animal models. This feature substantiates the similarity to human disease of the neovascularization produced in this model and can also permit the development of an angiographic grading scheme to facilitate grading of neovascularization *in vivo* over time. This greatly strengthens the utility of the model, which will not need to rely on histological grading. Indocyanine green angiography, which demonstrates neovascularization with less leakage of dye, would be interesting to study in this model of deep retinal and subretinal neovascularization.

The histological appearance of the neovascularization in the transgenic line is interesting. The progression of neovascularization from the inner nuclear layer to the outer retina is unique and is not seen in clinical disease. This unusual location may be due to the fact that VEGF is produced by the photoreceptors, which is not a feature of pathological neovascularization. The endothelial cells of the inner nuclear layer are close to the cells producing VEGF, and as the authors postulate, there may also be differing abilities of endothelial cells in different capillary beds to respond to VEGF. One might elucidate this in part by examining the expression of VEGF receptors over time as suggested above. The new vessels grow into the outer nuclear layer and form networks in the outer segments by 1 month. At 3 months, the vessels have invaded the subretinal space and proliferating RPE partially envelopes the new vessel complex, and by 4 months, the complex is surrounded by RPE. The authors did not find any neovascularization originating from the choroid, although at 3 months, VEGF protein was most concentrated in the outer segments near the choriocapillaris. It is possible that VEGF cannot diffuse through the RPE to reach the choriocapillaris or that the endothelium of the choriocapillaris is unable to respond to VEGF in this model. Although choroidal neovascularization was not seen, one might look for endothelial cell proliferation in the choriocapillaris using proliferating cell nuclear antigen labeling and also examine VEGF receptor expression in the choroidal vessels. It would be interesting to study a model in which VEGF overexpression was produced in the RPE, which has been demonstrated to express VEGF in the monkey model of choroidal neovascularization.[46]

Although in this model the networks of vessels did not originate from choroidal vessels, they were located in the subretinal space that is typical of choroidal neovascularization in age-related macular degeneration. Because of this the model may prove to be useful for testing therapies for this disease. The response of the RPE to neovascularization in the model was also similar to experimental choroidal neovascularization in the monkey and to clinical disease, with proliferation of the RPE to surround the neovascular tissue as well as the production of spaces or acini within RPE cells. The RPE appears to have a dual role in subretinal and choroidal neovascularization: the ability to stimulate angiogenesis and perhaps also the ability to induce its involution.

Summary

Okamoto et al[1] have developed an extremely useful and interesting model of retinal and subretinal neovascularization. Using molecular techniques, they have developed a transgenic model driven by overexpression of VEGF, a growth factor demonstrated to play an important role in neovascularization in many ocular diseases. They have been able to demonstrate that VEGF overexpression is sufficient to cause intraretinal and subretinal neovascularization. The mouse model is relatively cheap and reliable, does not require any exogenous agent, and has many characteristics of clinical intraocular neovascularization. The new vessels develop in the outer retina and subretinal space and have a characteristic histological appearance. They leak fluorescein on angiography, demonstrating their similarity to human disease and allowing identification and grading of neovascularization *in vivo*. The model can be used to investigate molecular mechanisms of VEGF-dependent neovascularization, with applications beyond ocular eye disease. The model can also be used to study anti-angiogenic agents that have the potential to treat common blinding diseases such as age-related macular degeneration. Okamoto et al[1] have made a substantial contribution to the angiogenesis field with this work, and one looks forward to future investigations.

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