Shedding Light on Blindness

Researchers may be closing in on the long-sought "factor X": a protein that could be responsible for vision loss in diabetics and premature infants

A devastating curtain threatens to fall across the vision of two disparate groups of human beings: diabetics and premature infants. Every year, about 60,000 diabetics and more than 10,000 premature infants in the U.S. develop a condition called proliferative retinopathy, in which abnormal blood vessels grow across the retina, damaging and sometimes permanently destroying vision.

In 1948, British ophthalmologist Isaac Michaelson hypothesized that the retina itself might be causing this condition by producing a factor that coaxes the new blood vessels to grow. For nearly 50 years, researchers have searched for Michaelson's postulated factor, which they dubbed "factor X," and have come up empty-handed. But work published over the past several months, and a paper appearing in next week's *Proceedings of the National Academy of Sciences*, has highlighted suspicious activity associated with one protein—vascular endothelial growth factor, or VEGF. And that's prompting a number of investigators to believe "factor X" may finally be in their grasp.

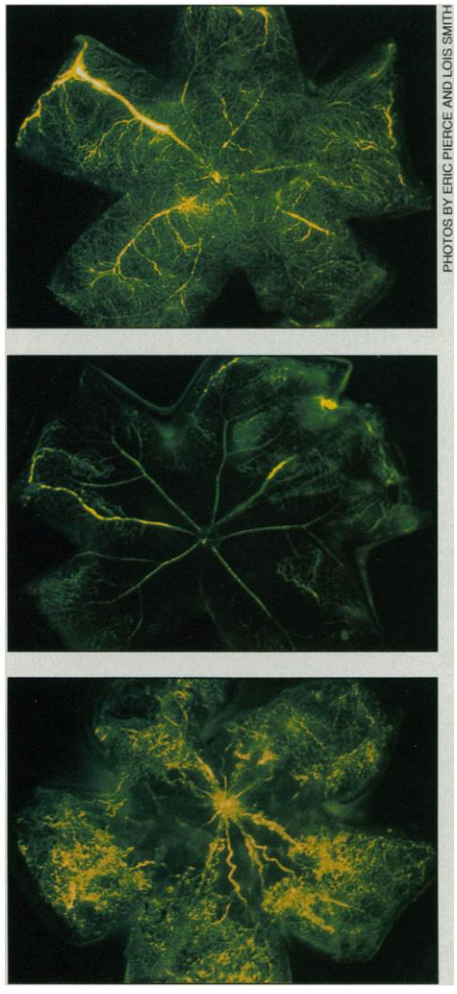
"VEGF is certainly a hot topic right now," says ophthalmologist Robert Frank of Wayne State University in Detroit. "Of all the angiogenic molecules that have been discovered, VEGF is the one that has best fit the factor X profile so far," adds Harvard University ophthalmologist Anthony Adamis, a member of Judah Folkman's laboratory, one of the groups that has zeroed in on VEGF. Some key features of that profile: Conditions that lead to retinopathy also boost production of VEGF by retinal cells, and VEGF is found in retinopathic eyes at the time when the abnormal blood vessels are growing.

But while the circumstantial evidence surrounding VEGF builds, no one has yet produced a biological smoking gun, such as evidence that blocking VEGF interrupts abnormal blood vessel proliferation. Some scientists believe that if they can find such evidence, they'll be on the way to preventing one of the leading causes of blindness.

An ill-fated alarm

While researchers have been mystified about the identity of factor X, there has been little doubt about the signal that calls the factor into action: the retina's need for oxygen. "The retina is gram for gram the most metabolically active tissue in the body," says Adamis. That activity endows the retina with a ravenous appetite for oxygen—and that can create problems for diabetics. Clogged capillaries are common in diabetes, and if capillaries in the retina get clogged, the retinal tissue quickly becomes oxygen-starved. It responds by issuing a distress signal—factor X—calling for new blood vessels to grow into the oxygen-poor area.

But these desperation measures—like the flailing of a drowning swimmer—turn out to be harmful. The new vessels don't "grow with the normal architecture," says Lloyd P. Aiello, an ophthalmologist at the Beetham Eye Institute in Boston. They leak fluid into the eye and in many cases break apart and form scar tissue that can contract and detach the retina, causing blindness. "It's a good attempt by the body," says Aiello, "but it ends up hurting more than it helps."



PHOTOS BY ERIC PIERCE AND LOIS SMITH

Mice without sight. A normal 17-day-old mouse *(top)* has blood vessels throughout the retina, here infused with green fluorescein. When a mouse is exposed to high oxygen levels following birth *(center)*, it halts blood vessel development. When returned to normal room air *(bottom)*, the mouse grows abnormal, leaky vessels *(bright spots)*.

Oxygen deprivation is likewise behind the threat to the eyes of premature infants. Premature babies are born before the blood vessels that normally supply the retina have had a chance to finish growing. The oxygen-deprived part of the retina puts out the call for new vessels, but those it gets are the leaky, breakable kind that can cause retinopathy and blindness.

The current treatment for these retinopathies makes use of lasers focused through the pupil of the eye or supercold probes applied to the outside of the eye to destroy the oxygen-starved parts of the retina that presumably are sending out the call for help. Those parts are usually outside the central area of the retina, and so can be destroyed without harming the center, which is essential for vision. But the treatments are successful only half the time, and even when they succeed in saving sight, they can cause loss of peripheral and night vision. These treatments "are the best we have," says University of California, Los Angeles, ophthalmologist Bradley Straatsma. "But I hope they are not the treatments we are using in the future."

That hope has fueled the search for factor X. Researchers began by looking at the half-dozen or so factors that trigger the growth of vascular endothelial cells (the cells that form blood vessels). They were looking for proteins that met two criteria: They had to be found in the eyes of patients or animals with retinopathy, and their production should be triggered by oxygen-poor conditions. Those criteria were hard to meet. "A lot of us spent a good deal of time looking at the role of other factors, such as FGF [fibroblast growth factor]," says Patricia D'Amore, a Harvard biologist who studies retinopathy. FGF and another factor, insulin-like growth factor-1 (IGF-1), both stimulate endothelial cells in culture, and many researchers think they play an accessory role in some types of retinopathy. But they fall short of meeting the first criterion: Neither is consistently found in the eyes of people with retinopathy.

Enter VEGF, a growth factor apparently specific for the vascular endothelium. VEGF

was discovered in the early 1980s by Harold Dvorak's group at Harvard Medical School in Boston, as a factor that made blood vessels leaky. Then in the late 1980s, several groups found that the same factor also boosts endothelial cell growth, and it was given the name VEGF. It was also found to play an important role in attracting blood vessels to tumors.

Do the eyes have it?

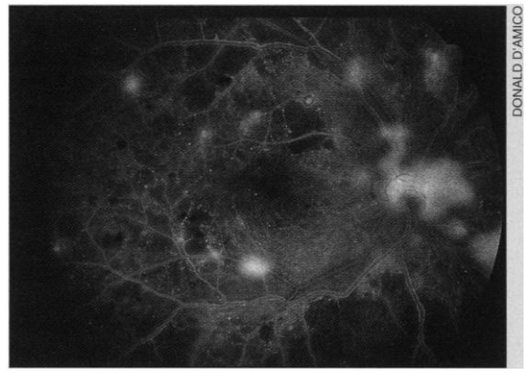
VEGF became a prime suspect in the factor X investigation in October 1992, when *Nature* published a pair of papers linking production of the protein to oxygen depletion. Both Eli Keshet's group at the Hadassah Medical School in Jerusalem and Werner Risau's team at the Max Planck Institute for Psychiatry in Martinsried, Germany, had found that VEGF was made specifically in the oxygen-deprived parts of tumors. "When it became obvious that there was a factor around that could be regulated by ambient oxygen concentrations, that [made it] an excellent candidate," says D'Amore.

Researchers fanned out to gather more evidence from cultured cells, animal models of retinopathy, and human patients that suffer from the condition. The result was a multiplicity of correlations: For starters, several teams found that VEGF was made by retinal cells and that starving the cells of oxygen boosted their production of VEGF.

The next step was to find out whether VEGF could be found in the vicinity of new blood vessels in experimental animals with retinopathy. To investigate this question, Adamis and David Shima, a graduate student with D'Amore, teamed up with Joan Miller's group at the Massachusetts Eye and Ear Infirmary. Miller had developed a way to cause abnormal blood vessel growth in the eyes of monkeys, using a laser to close off their retinal veins. The monkeys get a severe form of retinopathy, in which abnormal blood vessels also grow on their irises, as they do in the worst cases of diabetic retinopathy.

The group reported in the September 1994 issue of the *American Journal of Pathology* that levels of VEGF in the monkeys' eyes were high just when the blood vessels were growing on their irises. Later, when VEGF levels fell, the vessel growth stopped. VEGF, it turned out, was being made by the animals' oxygen-starved retinas and diffusing into the fluid that bathes the iris. That suggests, says Adamis, that VEGF is "arriving at the iris and triggering new vessel growth there."

Meanwhile, researchers in another Boston lab were linking VEGF to another form of retinopathy. Pediatric ophthalmologist Lois Smith at Children's Hospital had developed a way to produce retinopathy in newborn mice by giving them oxygen-rich air, which has the effect of obliterating some of the normal blood vessels in their retinas. When the mice are taken out of the oxygen, they proceed to develop a retinopathy that resembles the condition seen in premature children and diabetics. In next week's issue of the *Proceedings of the National Academy of Sciences*, Smith, postdoc Eric Pierce, and their co-workers report that the mouse retinas turn up production of VEGF just prior to the growth of the abnormal vessels. "If you look even more closely, you can see that it is being made by the parts of the retina that are [oxygen-starved]," says Pierce, and its production drops as new vessels enter those areas. That, he adds, means "VEGF is made at the right time, and in the right place, to be involved in [blood vessel growth]."



DONALD D'AMICO

Blood and blindness. In this human eye with diabetic retinopathy, blood flow is cut off in the dark areas. The white spots indicate vessels that are leaking abnormally.

A similar model of retinopathy in kittens has produced similar evidence. Margaret Donahue and Dale Phelps of the University of Rochester found that the retinal cells making VEGF in kittens are those just ahead of the advancing blood vessels, as if VEGF were a message coaxing the vessels to "come right this way," Phelps says.

From animal model to human disease

While the animal results were encouraging, they didn't guarantee similar findings in humans. "Clearly the big question at that point is: Does [VEGF] go up in humans that have proliferative diseases?" says Aiello. He answered his own question last month, with what seems to be a resounding "yes," in a study published in the 1 December issue of the *New England Journal of Medicine*. A group led by Aiello and George King looked at samples of ocular fluid from 164 patients undergoing eye surgery. Some had diabetic retinopathy, others had retinopathy caused by blockage of the retinal vein, and three were premature infants with retinopathy. The controls were patients who had surgery for conditions that didn't involve blood vessel growth.

Two other groups, Adamis's group and that of Jean Plouet at the University of Toulouse, France, had already published small studies showing that VEGF levels are elevated in eye fluid from patients with diabetic retinopathy; Plouet's group had found VEGF to be the only one of eight endothelial growth factors to show such a correlation. In agreement with those smaller studies, the Aiello team found high VEGF levels in patients with active blood vessel growth, whether it was caused by diabetes, prematurity, or vein blockage, compared with low or undetectable VEGF levels in the controls. In some patients they took samples before and after lasers had been used to destroy the oxygen-starved retinal cells. If the laser treatment stopped blood vessel growth, they found, the VEGF levels plummeted by an average of 75%.

Aiello's team also showed that VEGF was the main "active ingredient" in ocular fluid. Fluid from patients with active retinopathy stimulated the growth of cultured endothelial cells, they found, but that activity was curtailed by adding antibodies to VEGF into the culture. That, says Aiello, suggests that "the active state [of blood vessel growth] is probably due to the VEGF."

For many ophthalmologists, the *New England Journal* paper is the strongest evidence yet implicating VEGF. "They isolated this material from the vitreous fluid of patients with proliferative retinopathy," says UCLA's Straatsma. "That proves that it isn't just laboratory or animal material. It is present in the human eye, and that is very important."

But others caution that there is still not definitive proof that VEGF is factor X. For such proof, new blood vessel formation "has to be blocked by inhibitors of the factor's action" in an animal model, says Wayne State's Frank. The Aiello and Adamis groups are presently testing whether antibodies to VEGF slow new blood vessel growth in animal retinopathy models. The studies are expected to be complete within a few months.

If VEGF turns out to be the factor responsible for retinopathy, that will signal the start of yet another high-stakes hunt, this time by biotechnology companies eager to find a safe way to block the action of VEGF in humans. Smith, the pediatric ophthalmologist with the mouse model of retinopathy, is already collaborating with several companies to develop drugs to block VEGF production.

Such a strategy will be tricky, Smith cautions, especially in premature infants, whose eyes still need to develop normal blood vessels. VEGF is probably necessary for that normal development, albeit at lower levels than those that induce abnormal vessel growth. "Total inhibiton will not be the aim," says Smith. "What we are going to be looking for is something we can fine-tune." Despite uncertainty about how they will use all the evidence, researchers searching for a way to cure retinopathy-induced blindness are glad to finally have a quarry in sight.

–Marcia Barinaga