Research News

Angiogenesis Research Comes of Age

The discovery of agents that stimulate angiogenesis—the growth of new blood vessels—has sparked interest in the research

A symposium early last month drew some 400 participants to the Bethesda campus of the National Institutes of Health to hear about the lastest developments in research on "angiogenesis," the formation of new blood vessels.* "What was remarkable," says symposium cochairman M. Judah Folkman of Harvard Medical School, "was that it attracted investigators working in embryology, in oncology, in cardiology, in molecular biology. A few years ago you would never have expected such a diverse group to come to a meeting on angiogenesis."

The current interest in the topic is partly due to the isolation over the past 2 to 3 years of several proteins that stimulate angiogenesis. Researchers had been looking for such agents at least since the early 1970s when Folkman proposed that the development of solid cancers was dependent on the growth of new blood vessels and that the cancers themselves produced a "tumor angiogenesis factor" that stimulates that growth. The inability for many years to purify any such factor helped to engender more than a little skepticism about the proposal, however.

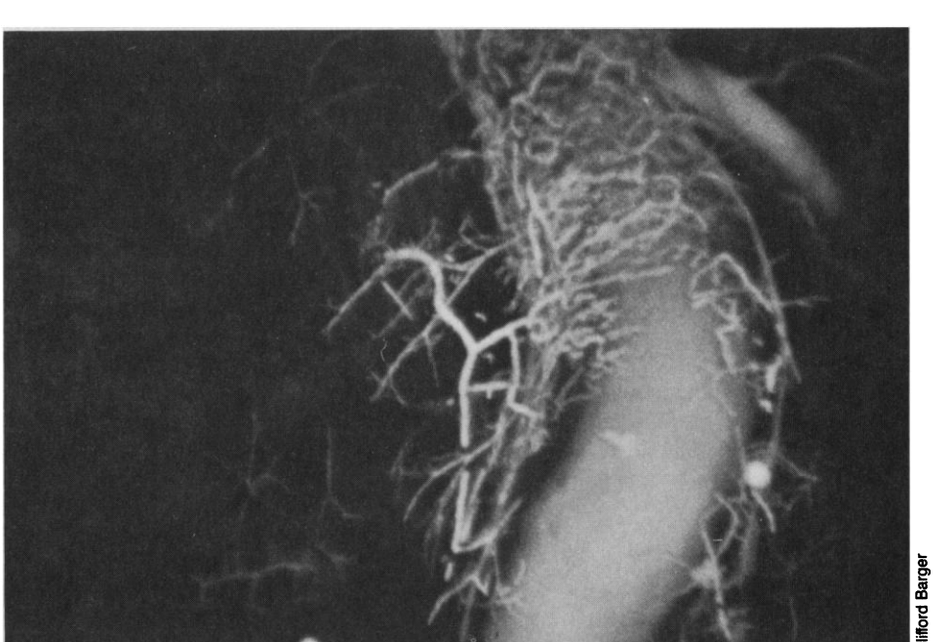
Researchers have now gone from having no angiogenesis factors to having several, thereby putting the research on a sounder footing, although, as it happens, data presented at the symposium have raised questions about whether one of the agents, namely angiogenin, is an important mediator of blood vessel development.

Also contributing to the interest in angiogenesis is the growing realization that aberrant blood vessel growth may play a role in the development of many diseases, not just solid cancers. Examples cited by Folkman include the joint degeneration occurring in rheumatoid arthritis and the eye damage frequently suffered by diabetes patients and by premature infants who are exposed to high concentrations of oxygen because of their immature lungs. It may even be a factor in the onset of heart attacks, according to A. Clifford Barger of Harvard Medical School.

Barger and his colleagues have found that the walls of the coronary arteries contain dense networks of small blood vessels, called vasa vasorum, in regions where atherosclerotic plaques are located, but not in healthy areas. Hemorrhage into the coronary arteries can initiate heart attacks by triggering clot formation and a consequent cutoff of blood flow to the heart muscle. The vessels of the vasa vasorum, which may be more fragile than normal blood vessels, are a potential source of such hemorrhages, Barger says. In addition, the coronary vasa vasorum and the nerves associated with it might cause heart attacks by releasing agents that constrict the smooth muscle surrounding the coronary arteries, thereby causing spasms in the arteries.

The many angiogenesis-stimulating agents discussed at the symposium included angiogenin, a protein produced by human colon cancer cells that was isolated and characterized about 18 months ago by Bert Vallee and his colleagues at Harvard Medical School. The Harvard work received a great deal of attention at the time, not just because of the interest inherent in a molecule that might elicit blood vessel development, both in normal organs and in tumors, but also because it was a product of a decade-long, $23-million effort at Harvard that was supported by the Monsanto Company of St. Louis. The agreement between Harvard and Monsanto, one of the first in which a major industry contracted to support biomedical research at a university, was highly controversial.

In an effort to determine whether the pattern of expression of the angiogenin gene is consistent with a physiological role in promoting blood vessel growth, Judith Swain and her colleagues at Duke University Medical School have now examined the distribution of the messenger RNA transcribed from the gene in tissues from fetal, newborn, and adult rats. Somewhat to their surprise they found that in adult rats the liver contains the highest concentrations of angiogenin messenger RNA, with other tissues having much lower amounts. Angiogenin messenger RNA is also present in tumor cell lines, indicating that the gene is



A. Clifford Barger

Vasa vasorum of the coronary arteries. *The vasa vasorum network are limited to atherosclerotic regions in coronary arteries. The healthy areas are almost free of the small vessels.*

*The symposium, which was held on 3 and 4 June, was sponsored by the National Heart, Lung, and Blood Institute. Roger Guillemin of the Salk Institute in San Diego cochaired the meeting with Folkman.

active there too, but the amounts are less than those in adult liver. "It looks like a predominantly liver protein," Swain says, rather than one characteristic of tumor cells.

The expresssion of the angiogenin gene changed during development, according to the Duke workers. It was lowest in fetal liver, somewhat higher in liver tissue from newborn mice, and highest of all in adult liver. In contrast, blood vessel growth is most active in the developing embryo and normally very inactive in adults, except in special circumstances such as during wound healing, placenta formation, and tumor growth. "If [angiogenin] protein production parallels transcription," Swain concludes, "it is backward from what is expected for an agent with a role in development and tumor growth. I think there is still a major question about what in vivo role this protein plays."

According to Vallee, Swain's conclusions are based on false expectations about angiogenin. He suggests that the early concentration on looking for a "tumor angiogenesis factor" led people to expect that such a molecule would be specifically made in tumors. "It doesn't surprise me if [angiogenin] isn't tumor-specific," Vallee says, noting that he and his colleagues have recently detected the protein in blood plasma.

He is unimpressed with the significance of the results concerning the distribution of angiogenin messenger RNA. He contends that they have no bearing on the mode of action of angiogenin, which is still unknown. "I don't see how questions can be raised when we have never proposed a mechanism of action. It [the Duke work] is negating a hypothesis that hasn't been made."

Whatever the role of angiogenin, it is certainly not the only protein that has been implicated as a stimulator of blood vessel growth. The others include the two forms of fibroblast growth factor (FGF), which are known as acidic FGF and basic FGF. According to Denis Gospodarowicz of the University of California School of Medicine in San Francisco, these two proteins are related, with about 55% of their amino acids being identical, although basic FGF has a much wider tissue distribution than acidic FGF. Both FGFs may stimulate blood vessel growth, at least partly, by stimulating the division of endothelial cells, which line the blood vessels and ordinarily divide slowly, if at all. Endothelial cell division is necessary at times of vessel growth, however.

Daniel Rifkin of New York University School of Medicine in New York City suggests another possible mode of action of basic FGF. He and his colleagues find that the growth factor sets in motion a chain of events that results in activation of the enzyme collagenase, which breaks down the protein collagen. Collagen is a major structural component of connective tissue and the basement membranes that underly the endothelial cells of blood vessels, and these structures must be dissolved so that new vessels can grow from parent vessels.

The transforming growth factors α and β (TFG-α and TGF-β) are proteins produced by tumor cells that were originally identified on the basis of their ability to confer certain malignant characteristics on some types of normal cells. Both TGFs have now also been found to stimulate angiogenesis. TGF-α does this directly, according to Rik Derynck of Genentech, Inc., in South San Francisco.

However, the angiogenic effects of TGF-β, which is structurally unrelated to TGF-α, are apparently indirect. Michael Sporn, Anita Roberts, and their colleagues at the National Cancer Institute find that TGF-β stimulates blood vessel growth in mice even though it inhibits the division of endothelial cells in culture. ("Transforming growth factor" has proved to be something of a misnomer for this protein as it is now known to inhibit, rather than stimulate, the division of most types of cells.) According to the NCI workers, TGF-β is extemely potent in attracting the cells known as macrophages, a finding that may account for its angiogenic effects in the live animals.

Aberrant blood vessel growth may play a role in the development of many diseases.

S. Joseph Leibovich of Northwestern University Dental School also reported at the meeting that TGF-β attracts macrophages. These cells are frequently found in tumors and their presence there may be at least partly due to TGF-β production by the tumor cells. Moreover, according to Leibovich, activated macrophages secrete angiogenic materials, one of which is closely related and perhaps identical to tumor necrosis factor-α.

This is a new function for the necrosis factor, a macrophage product that is better known for its role in killing tumor cells. In fact, the presence of macrophages in tumors is generally thought to be beneficial because the cells—literally named "big eaters"—can engulf and destroy tumor cells. However, macrophages may not be beneficial if they release angiogenic factors that can foster tumor growth. "We have the paradox that macrophages may inhibit tumor growth and stimulate tumor growth," Leibovich says.

A further indication of the possible enhancing effects of angiogenesis on cancer development comes from Douglas Hanahan and his colleagues at Cold Spring Harbor Laboratory. They produced a tumor-prone strain of mice by introducing into the germline of the animals a hybrid gene consisting of the control sequences of the insulin gene and the coding sequences of the gene for the T (tumor-inducing) antigen of simian virus 40. The beta cells of the pancreas, which synthesize insulin, also make the T antigen in animals that carry the hybrid gene. Moreover, Hanahan says, "The inevitable consequence of the inheritance of one of these genes is the development of beta cell tumors."

Simple expression of the T antigen is not sufficient for the tumor development, however. Only a few are produced in an animal even though the beta cells are generally making the T antigen and showing increased proliferation. The tumors, unlike the areas of simple, increased proliferation, are penetrated by blood vessels, and Hanahan postulates, angiogenesis may be a secondary event needed for tumor formation by beta cells that produce the T antigen.

The trigger for the angiogenesis is currently unknown, although the Cold Spring Harbor group has found that the tumors show signs of expression of the TGF-β and basic FGF genes. The researchers have not yet looked at the precancerous beta cells to see if they, too, are expressing the genes.

Finally, Patricia D'Amore of Children's Hospital in Boston is looking at the other side of the angiogenesis coin. "We are asking why endothelial cells stay so quiescent under normal conditions," she explains. One clue came from patients with diabetic retinopathy, a condition in which small blood vessels proliferate in the retina of the eye, eventually rupturing and causing blindness.

Before the vessels proliferate, a type of cell called the pericyte, which is intimately associated with the endothelial cells of blood vessels, disappears. D'Amore and her colleagues have shown in cell culture studies that pericytes suppress endothelial cell growth. The results indicate that the pericytes must be in physical contact with the endothelial cells for the suppression to occur. The exact nature of the inhibitory contacts is currently under investigation.

Although much still remains to be learned generally about blood vessel growth and how it works, the research of the past few years has finally provided tangible angiogenic factors that are amenable to further investigation. ■ JEAN L. MARX