Angiogenesis Is Coming of Age

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Angiogenesis, the formation of blood vessels from preexisting vessels, occurs during embryonic and adult life. Although vascular endothelial cells in the normal adult organism have a very low turnover, they resume proliferation in diseases that are associated with neovascularization, such as solid tumor growth, wound healing, and retinopathy. A host of recent publications has indicated that the molecules and mechanisms involved in both embryonic and adult angiogenesis are similar. For example, neutralizing or deleting the function of vascular endothelial growth factor (VEGF) or its receptors (VEGF-R1/flt-1 and VEGF-R2/flk-1/KDR) abolished embryonic and adult blood vessel formation, including vasculogenesis, the formation of blood vessels from angioblasts in situ, and adult physiological (eg, in the corpus luteum) and pathological (eg, in tumors) angiogenesis. Therefore, one could propose that adult angiogenesis merely recapitulates embryonic angiogenesis. This view is now challenged by Lee et al., who, in this issue of Circulation Research, present the first evidence that adult angiogenesis may require additional factors and mechanisms. They found that Thy-1 was upregulated in newly formed blood vessels in four models of angiogenesis in adult rats, whereas it was not expressed during vasculogenesis or angiogenesis in the embryo. Notably, the four models included both pathological (tumor, balloon injury, and ligation of renal artery) and physiological (uterine vessels during pregnancy) angiogenesis. One of the conclusions from the work of Lee et al is therefore that embryonic vascular endothelium differs from adult vascular endothelium.

There is precedence for differences between “young” and “old” endothelium. Adult endothelium has a low turnover, is mature (ie, has complete vascular basal lamina in most vessels and “thin” endothelium with smooth luminal and abluminal surfaces), and expresses organ-specific characteristics. It is, in general, much less “plastic” than embryonic endothelium. For example, embryonic endothelium from the dorsal aorta can be transplanted into the brain and differentiate to blood-brain barrier endothelium. There is also massive remodeling, pruning, and angioblast migration occurring in the embryonic vascular system that is virtually absent in the adult. The tentative proposal from these observations is that the normal adult organism has adjusted its vascular system to its need, has established it to a certain degree, and may be refractory to new blood vessel formation.

This concept is supported by studies on angiogenesis inhibitors. Although no molecular mechanism of action is known for angiostatin, endostatin, and thrombospondin, they all have been reported by previous studies to inhibit angiogenesis in vitro and angiogenesis in vivo. Coincidentally, at present, none of these inhibitors is known to play a role in embryonic angiogenesis. Mice deficient for thrombospondin and plasminogen (of which angiostatin is a fragment) display an apparently normal embryonic circulation. The thrombospondin-2 knockout is particularly interesting because twice as many blood vessels were counted in mutant compared with wild-type tissues. This difference was reported to be significant for adult but not for embryonic or neonatal tissue. Therefore, angiogenesis inhibitors may play a role in shaping the postnatal but not the embryonic vascular system.

The second conclusion from the study of Lee et al is that as hypothesized earlier, adult angiogenesis is accompanied by, if not dependent on, inflammation. Inflammatory cytokines (eg, tumor necrosis factor [TNF], interleukin-1, and interleukin-8) are known to upregulate a number of molecules in endothelial cells, resulting in so-called activated endothelium. Furthermore, inflammatory cytokines, and also the molecules induced by them in endothelial cells (eg, soluble selectins), have been shown to stimulate endothelial cells in vitro and angiogenesis in vivo. None of these molecules has, so far, been shown to be involved in embryonic blood vessel formation, which is consistent with a difference between embryonic and adult angiogenesis. Inflammatory cells, eg, monocytes/macrophages and mast cells, have been shown to be associated with adult angiogenesis and have been detected by Lee et al in their models. Macrophage-derived TNF is believed to play a major role in wound-healing angiogenesis. TNF, shown by Lee et al and others to specifically induce Thy-1 in capillaries and cultured endothelial cells, is angiogenic in the cornea model of angiogenesis and stimulates endothelial cell chemotaxis but, paradoxically, inhibits endothelial cell proliferation. Provided that the latter activity also works in vivo, these results would suggest that this inhibitory activity can be overcome by counteracting stimulators released simultaneously during the inflammatory response noted in these corneas. These observations underline the importance of the inflammatory response in adult angiogenesis and suggest that inflammatory mediators, either alone or together with angiogenic factors, may be necessary to shift the balance of the fixed quiescent adult vessel to an activated proangiogenic endothelium.

The earliest event in activated adult endothelium is usually an increase in vascular permeability. Therefore, adult capillaries may react to pathological situations like ischemia, injury, inflammation, and neoplasia by a rapid increase in permeability in order to accelerate the delivery of nutrients and oxygen (and perhaps leukocytes) rather than by making...
new blood vessels. The potent biological activity of VEGF as an enhancer of both permeability and growth (with notable kinetic differences) is consistent with an early role of this factor. Unlike the counteracting activities of TNF and VEGF on endothelial cell growth, at least in vitro, TNF strongly synergizes with VEGF in the induction of vascular permeability (Matthias Clauss, PhD, oral communication, March 1998). These observations are compatible with a rapid compensatory action of adult blood vessels during tissue ischemia and hypoxia that is mediated by hypoxia-inducible VEGF by increasing the vascular permeability while, at least initially, suppressing the formation of new blood vessels. Additional factors (e.g., angiopoietins; see below) and/or sustained activation may then be required for angiogenesis to ensue.

Lee et al found a consistent upregulation of Thy-1 in four models of adult angiogenesis. Thy-1 is a 25-kD glycosylphosphatidylinositol-linked membrane glycoprotein, known since 1964 in mice and since 1980 in humans. There is not only this time difference between the identification in these species, but there is also a distinct species-specific tissue distribution of Thy-1. In mice, expression is seen on T lymphocytes and in the central nervous system, and there is evidence for inducibility of the molecule on a variety of hematopoietic cell lines. In contrast, Thy-1 expression in humans is found on only a small proportion of hematopoietic cells (see references in Lee et al). It is important to stress this point, because results in rodents may not be easily transferable to humans if one were to use Thy-1 as a target for antiangiogenic therapies. Thus, it is important that Lee et al point out that they have seen upregulation of Thy-1 not only in rats but also in mouse models of angiogenesis. Despite certain species differences, studies in a number of species suggest that Thy-1 distribution is particularly related to blood vessels (see Reference 28 and references therein). However, within blood vessels, Thy-1 has been found to be expressed in a somewhat heterogeneous pattern not only in endothelial cells but also in pericytes and smooth muscle cells. It is notable that von Willebrand factor, used by Lee et al as a marker for endothelial cells, appeared to be absent in the Thy-1–positive capillaries. It remains to be investigated whether this is due to a downregulation, a depletion from Weibel-Palade bodies and the subsequent inhibition of synthesis, or other mechanisms triggered by the inflammatory response. Lee et al show immunohistochemistry of high technical quality and have used it to demonstrate specificity by preparing a soluble dimeric Thy-1–immunoglobulin chimera that inhibits the specific staining. However, it is not easy to distinguish pericytes from endothelial cells using the methodology applied. Our own studies involving the immune function of blood-brain barrier endothelial cells in a rat model of experimental autoimmune encephalomyelitis have clearly revealed specific constitutive expression of Thy-1 in brain pericytes but not in endothelial cells. Nevertheless, the observation by Lee et al of Thy-1 upregulation in angiogenic capillaries would be no less important if Thy-1 were upregulated in pericytes rather than endothelial cells, given the recent discussions of the role of pericytes and angiopoietins in angiogenesis. Pericytes are believed to be the safeguards against uncontrolled endothelial proliferation. In some pathological situations, like diabetic retinopathy, they seem to be the first to migrate from the endothelium that is going to proliferate (for review, see Reference 34). Angiopoietins have been proposed to be involved in this process, but there is, as yet, no direct evidence of this. In any case, it is conceivable that pericytes and/or endothelial cells are involved in the integration of the capillary response to inflammatory and angiogenic factors.

Although Thy-1 has an impressive publication record, its function is not clear. Currently, it is best described as a multifunctional molecule possibly involved in cell adhesion, signaling, growth, and differentiation. No counterreceptor or ligand is known. In capillaries, it may be involved in adhesion (or antiadhesion?) of endothelial cells to one another, to pericytes, or to leukocytes. Glycosylphosphatidylinositol-linked membrane molecules seem to have the potential of high lateral mobility in membranes, consistent with a role in rapidly associating with other (trans)membrane proteins or in mediating the transmigration of leukocytes. The most consistent signaling response mediated by antibody cross-linking is the increase in intracellular calcium flux (see references in Lee et al). In endothelial cells, this is known to be associated with increases in vascular permeability, which would be consistent with the phenotype of wound or tumor capillaries. However, direct evidence for this function in endothelial cells is lacking, and in light of the absence of natural ligands or associated signaling molecules, the significance of the response is unclear. The knockout phenotype of Thy-1/−/− is complex and suggests instead that Thy-1 may be a negative regulator. A neuronal defect was correlated with regional impairment of long-term potentiation, and analysis of the immunological phenotype uncovered evidence of a signaling defect in T cells. No vascular phenotype has been described yet, but this may be due to the much larger number of Thy-1 molecules on T cells and/or to the lack of specific studies, e.g., studies involving wound healing and tumor growth. Obviously, these knockout mice would be ideal for testing the function of Thy-1 in pathological angiogenesis.

What function could Thy-1 perform in angiogenic capillaries? On the basis of the known properties of Thy-1 and the phenotype of Thy-1-deficient mice, one would suggest a modulation of the stimulating response for endothelial proliferation, similar to the enhanced mitogenic stimulation through the T-cell antigen receptor in Thy-1/−/− mice. An alternative intriguing possibility is that not only does Thy-1 serve as a marker of new adult blood vessels, but it may also mark newly formed blood vessels in the adult for regression after cessation of the angiogenic stimulus. It is known that new capillaries in wounds regress after wound closure, vessels regress in the corpus luteum in a cyclic fashion, and tumor blood vessels are prone to regression induced by inhibitors of angiogenesis. Despite the possibility that this system may be redundant and that Thy-1 may not be essential, this hypothesis may be subject to investigation by using the Thy-1–immunoglobulin chimera of Lee et al or by using knockout mice.

It is hoped that angiogenesis research will eventually cure diseases and ease and perhaps prolong life. Edgar Haber, in
whose laboratory the work of Lee et al.\textsuperscript{13} was performed, is deceased. Readers and colleagues who were not fortunate enough to know him, including myself, may be referred to Mark Fishman’s obituary in Circulation (1998;8:6–7). For scientists, death of a colleague may remind us that research is performed by human beings and that life as a scientist might sometimes be easier if we keep that in mind.

References

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