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n this issue of *Circulation*, Baumgartner et al report a significant advance in angiogenic gene therapy. The authors induced collateral neovascularization in 10 critically ischemic limbs in 9 patients by the intramuscular gene transfer of naked plasmid DNA encoding the 165-amino-acid isoform of the human angiogenic protein, vascular endothelial growth factor (phVEGF<sub>165</sub>). The plasmid DNA was injected directly into the muscle of ischemic limbs. Anatomic and functional efficacy was demonstrated by increased serum levels of VEGF, improved hemodynamic measurements and angiographic evaluation, reduced pain, increased healing of ischemic ulcers, limb salvage, and immunohistochemical evidence of proliferating endothelial cells in tissue specimens. The authors emphasize that this is the first medical therapy to achieve an increase in limb perfusion that is equivalent to or greater than successful surgical or percutaneous intervention.

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Direct intramuscular gene transfer of plasmid DNA appears to effectively stimulate collateral vessel growth, despite the lower transfection efficiency that is usually associated with gene therapy in the absence of a viral vector. This result has implications for other clinical trials of gene therapy that use intramuscular naked DNA. The fact that Isner’s group could prepare the plasmid for human use in a university medical center laboratory dedicated to this purpose reveals why gene therapy has moved so rapidly from the laboratory to the clinic, in contrast to protein therapy, which requires expensive manufacturing facilities and years of scale-up effort.

Although the plasmid was injected into muscle of the ischemic limb, VEGF levels were apparently elevated in the whole circulation, as evidenced by transient peaks of VEGF in the serum and by edema of the ischemic limb and in some patients, in the opposite limb. The increased collateral vessels, however, are localized to the ischemic limb and do not develop in other areas of the body. This may reflect in part the short half-life of VEGF in the circulation (minutes) as well as the upregulation of receptors for VEGF in ischemic tissue. As the authors point out, endogenous upregulation of VEGF expression by hypoxic endothelial cells may provide an amplifying mechanism that tends to localize the action of exogenous VEGF to the ischemic limb.

Because VEGF is a mitogen for vascular endothelial cells and not for smooth muscle cells, the increased density of larger (200- to 800-μm) vessels observed by angiography may be secondary to neovascularization at the level of microvessels. This possibility is supported by the presence of foci of proliferating endothelial cells observed in an amputation specimen obtained 10 weeks after gene therapy. One would not expect to see endothelial proliferation in normal limb vasculature. However, the level of endothelial proliferation that might occur in an ischemic or a gangrenous limb before therapy is also unknown.

All gene therapy designed to potentiate local angiogenesis carries the theoretical risk that pathological angiogenesis at a remote site could be stimulated, ie, ocular angiogenesis or tumor angiogenesis. The authors cautioned readers about this. In one report, bFGF administered at very high doses over a prolonged period increased growth of a pre-existing mouse tumor. However, in our own studies of mice bearing dormant microscopic lung metastases that were not angiogenic, repeated systemic injections of either basic fibroblast growth factor (bFGF) or VEGF protein per se did not turn on angiogenesis in these lesions or stimulate tumor growth (M.S. O’Reilly, MD, PhD, J. Folkman, MD, unpublished data, 1997). We further found that corneal neovascularization induced in the mouse by implantation of a sustained-release pellet of bFGF was not enhanced by systemic administration of bFGF or VEGF (R. D’Amato, MD, unpublished data, 1997). The explanation for this result is unclear, except for the possibility that these sites were not hypoxic or that the VEGF exposure was too brief. Finally, there remains the question of whether atherosclerotic plaque neovascularization may be exacerbated by angiogenic therapy.

A fundamental and still unsolved biological question is, How are collateral blood vessels induced and sustained? Schaper stated, “Regional tissue ischemia is still the only situation which leads to collateral vessel formation in a predictable way.” No drug or growth factor has accomplished this in the absence of ischemia. In fact, chronic infusion of VEGF into the canine coronary system does not lead to endothelial proliferation. However, it is not clear whether tissue ischemia is a cause of collateral vessel formation or only correlates with it. VEGF expression is upregulated by hypoxia in normal tissues that become ischemic and in ischemic tumor cells. Because receptors for VEGF are also upregulated in hypoxic tissues, perhaps these two processes are accelerated by exogenous VEGF gene therapy. Nevertheless, in the ischemic limb, the growth of larger arterioles and arteries, including the
necessity of remodeling (without sprouting), differs from the growth of microvessels in a capillary bed, in which sprouting has been observed even in the muscle bed that is exercised.\textsuperscript{13} Arras et al\textsuperscript{13} distinguish between “angiogenesis” and “angiogenesis” in the ischemic limb. Three days after femoral artery ligation in rabbits, collateral arterioles in the thigh grew by proliferation of endothelial cells and smooth muscle cells (arteriogenesis), coincident with the adherence of monocytes that supplied growth factors, eg, bFGF and TNF-alpha, to the vessel wall. This process in the thigh appeared to be independent of perfusion deficiency or hypoxia. Capillary proliferation (angiogenesis) in the lower limb did not occur until 7 days after femoral artery ligation and also correlated with monocyte accumulation as well as with other sources of bFGF. Hypoxia was a major stimulus for angiogenic growth factors.

Why do collateral vessels form at a considerable distance from the ischemic capillary bed? No satisfactory mechanism has been proposed for retrograde diffusion of an angiogenic factor (such as VEGF) from the site of its overexpression in an ischemic vascular bed. Therefore, the conventional explanation is that collateral vessel growth is primarily a result of increased flow and lateral wall pressure in patent vessels that are carrying blood around an obstructed main artery to an ischemic vascular bed that is fully dilated. But this biomechanical hypothesis is inadequate to explain evidence of a humoral communication between a distal ischemic bed and its proximal feeding vessels. For example, after experimental coronary artery occlusion, increased endothelial thymidine labeling was found not only in the growing collaterals but also in the coronary venous system (see Reference 14 for review). In a model of renal artery occlusion, there was thymidine labeling in the renal collaterals but also in the renal vein. Furthermore, endothelial labeling in the collateral arteries spread from the ischemic zone in a time-related retrograde gradient. A similar retrograde spread of endothelial labeling along the spermatic artery was observed when tumor was implanted in the rat testis.\textsuperscript{14} Recent evidence suggests that vascular endothelial cells in the abdominal aorta of the rat are not stationary but migrate toward the heart.\textsuperscript{15} If they originated in a hypoxic area, could they transport growth factors in the way for angiogenic gene therapy of the ischemic human heart. When taken together with the seminal report by Schumacher et al\textsuperscript{15} in the February 24, 1998, issue of Circulation, in which the angiogenic protein acidic fibroblast growth factor was injected into the myocardium after coronary bypass graft surgery, the present article by Baumgartner et al\textsuperscript{15} suggests that in the future, VEGF gene therapy of the human heart may be administered by endoscopic thoracotomy or through a limited thoracotomy.

It will be of interest to learn whether the intramuscular injection of phVEGF\textsubscript{165} can be repeatedly administered to those patients who have recurrent pain or tissue loss due to ischemia. This pioneering study prepares the way for angiogenic gene therapy of the ischemic human heart. When taken together with the seminal report by Schumacher et al\textsuperscript{15} in the February 24, 1998, issue of Circulation, in which the angiogenic protein acidic fibroblast growth factor was injected into the myocardium after coronary bypass graft surgery, the present article by Baumgartner et al\textsuperscript{15} suggests that in the future, VEGF gene therapy of the human heart may be administered by endoscopic thoracotomy or through a limited thoracotomy.

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