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INSIDE LAB INVEST

Angiogenesis is not angiogenesis is not angiogenesis: The regulation of angiogenesis has become a topic of intense interest during the past several years, due in part to the significant advances made in our understanding of the process of angiogenesis and its importance in development, growth, inflammation, repair, and tumor growth and metastasis. Since its discovery, vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) has been recognized as a major modulator of the vasculature, affecting not only endothelial cell proliferation and vessel permeability, but also endothelial cell survival. Because of its profound effects on the vasculature, VPF/VEGF has been proposed and is currently being tested as a potential therapeutic target in clinical scenarios that would benefit from either increased vascularization (such as peripheral and cardiac ischemia) or decreased vascularization (such as tumor vascularization). In light of its complex effects upon the vasculature, it is important to appreciate the range, intensity, and duration of the effects of VEGF on the vascular beds targeted for treatment. In this issue, **Pettersson et al** (Lab Invest 2000;80:99–115) report on the heterogeneity of the angiogenic response induced in different normal adult tissues by VPF/VEGF. In their analyses of nude mouse and rat ear skin, peri-muscular fat, myocardium, skeletal muscle, flank, and peritoneum, VPF/VEGF was delivered via injection of an adenoviral vector engineered to express murine VPF/VEGF under the control of a cytomegalovirus promoter. Initially, all of the injected tissues exhibited microvascular hyperpermeability, edema fibrin deposition and the formation of ectatic, thin-walled pericyte-poor vessels. This initial reaction, although expected given the properties of VPF/VEGF, may result in untoward side-effects in certain clinical settings, contributing to unwanted increased complement activation and/or coagulation. Additionally, although a dose dependent angiogenic response was observed in all tissues studied, it was noted to be more intense and persisted longer in skin and fat compared with myocardium and skeletal muscle. This tissue-dependent response may be a major determinant of long-term treatment efficacy. Lastly, this report documents the evolution of the VPF/VEGF induced ectatic, thin-walled pericyte-poor vessels into a variety of structures observed in a variety of benign and malignant conditions in which angiogenesis/arteriogenesis is a component. These findings support the concept that VPF/VEGF mediated responses are heterogeneous and tissue-specific and that these aspects of the response should be taken into account in the decision to treat particular organs/tissues.

Atherosclerosis and herpes virus: There are several lines of evidence, largely epidemiological, that link infections to atherosclerosis, implicating diverse microbes such as *Helicobacter pylori, Chlamydia pneumoniae*, and cytomegalovirus, a member of the human herpes virus family. Although most investigators remain skeptical that there is a causal relationship between chronic infection and atherogenesis, the lessons learned from the discovery of the infectious nature of peptic ulcer disease preclude a categorical dismissal of this idea. What is urgently required to test the infection hypothesis of atherogenesis more critically is a good animal model for examining possible mechanisms by which an infection can promote atherogenesis. The well known link between an avian herpes virus (Marek's disease) and vascular disease in chickens is not compelling because the chicken is otherwise not a good model for studying human atherosclerosis. On the other hand, the lipid-fed rabbit is perhaps the most widely used model in the atherosclerosis research community. In the current issue, **Lin and colleagues** (Lab Invest 2000;80:3–12) show that infection of rabbits with bovine herpes virus 4 (BHV-4) results in BHV-4 DNA being detectable in most tissues, including aortae, of infected but not control animals. A striking result is obtained when BHV-4 infection is combined with cholesterol feeding, namely that BHV-4 infection dramatically increases lipid deposition in the aortic wall of cholesterol-fed but not control animals without altering serum cholesterol levels. Furthermore, BHV-4 infection shifts the lipid composition of the atheromatous vessel to more closely resemble that of human atheromas, ie, cholesterol ester rich. This new model now opens the way to study how viruses contribute to atherogenesis, and more importantly, how we might prevent them from doing so.

ECV-304: Not an endothelial cell line? Advances in the study of endothelial cell function and pathology, which have exploded in the past 20 years, have been largely dependent upon well characterized cultured cells. Because there are species, tissue, and vessel-type differences among endothelia, it is dangerous to generalize results from one system to another. Nevertheless, the availability of a well behaved endothelial cell line, especially one of human origin, could be a great boon to endothelial cell researchers. The ECV304 cell line, which was reported to be a spontaneously transformant arising in a human umbilical vein endothelial cell culture in 1985, appeared to be just such a reagent. Indeed, ECV304 cells have been used in nearly 150 publications since the initial description was published in 1990. However, even from the outset, ECV304 was not a perfect model. For example, these cells did not express E-selectin (CD62E) or P-selectin (CD62P) nor did they express PECAM-1 (CD31), all characteristic of endothelium. In this issue, **Brown and colleagues** (Lab Invest 2000;80:37–46), who had been using ECV304 cells to study the hormonal responses of human endothelium, show that the pattern of responses (and receptor expression) observed in these cells are more suggestive of epithelium than endothelium. This prompted a more radical and definitive experiment: by means of DNA fingerprinting, these authors show that the ECV304 cell line is identical to the well studied bladder carcinoma cell line T24/83. This is true both for ECV304 cells maintained in the authors' laboratory as well as a fresh aliquot obtained from the European collection of animal cell cultures (ECACC). The inescapable conclusion is that the cells distributed by the ECACC, now provided with a warning, are not endothelial in origin. Was ECV304 always a bladder carcinoma or did an endothelial cell line become overgrown at some later time, much as HeLa cells had overgrown a significant number of different cell lines, including some deposited in the American Type Culture Collection in the early 1980s. This question may never be answered. However, the prudent response is that all results obtained in the past 10 years using ECV304 cells, although experimentally valid, should no longer be considered as indicative of endothelial cell behavior.

Pancreatic stellate cells and platelet factors in pancreatic fibrosis: Fibrosis of parenchymal organs is a complex process, partly dependent upon the responsiveness of resident parenchymal and stromal cell populations to a variety of locally produced and vascularly delivered soluble factors. Hepatic fibrosis has been shown to be due, in part, to the stimulation of its stellate (Ito cell, Vitamin A storage cell) cell population. During the past few years, stellate cells have been found in several parenchymal organs in addition to the liver, the pancreas being one such example. Recent rodent and human studies have demonstrated the presence of pancreatic stellate cells in fibrotic areas, suggesting that this cell type may be a participant in or responsible for the development of pancreatic fibrosis. In this issue, **Luttenberger et al** (Lab Invest 2000;80:47–56) present evidence implicating the platelet-derived factors transforming growth factor beta1 (TGFb1) and platelet derived growth factor (PDGF) as stimulators of pancreatic stellate cell extracellular matrix synthesis and proliferation during the development of pancreatic fibrosis. After observing that platelet aggregates were present in sections of human pancreas with acute pancreatitis, the authors hypothesized that platelet-derived factors may stimulate local, endogenous pancreatic stellate cells in a paracrine fashion, contributing to the development of pancreatic fibrosis. Indeed, the authors found that platelet lysates dramatically increased human stellate cell proliferation and extracellular matrix synthesis and that inhibition of PDGF abrogated the proliferative response, whereas inhibition of TGFb1 abrogated the extracellular matrix synthetic response. Thus, it appears that the pancreatic stellate cells are contributors to the development of pancreatic fibrosis and that platelet-derived factors contribute to stellate cell activation and pancreatic fibrosis via a paracrine pathway. Also of interest on the same subject area is the article by **Neuschwander–Tetri et al,** which will appear in the February issue.