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Progress towards a unifying hypothesis for angiogenesis.

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Editorial

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Angiogenesis plays a central role in a number of physiologic and pathologic processes, including wound repair, placental development, diabetic retinopathy, inflammation, rheumatoid arthritis, and solid tumor growth. Endothelial cells in culture can mimic the formation of capillary beds, facilitating the understanding of this vitally important process. A number of in vitro model systems have been developed, involving endothelial cells grown on fibronectin (1), collagen (2), and fibrin (3), which have allowed investigators to make significant advances in this field in recent years. While most of these systems involve cellular differentiation with the necessity for transcription and protein synthesis, others, including the Matrigel system, require only posttranslational modifications to existing proteins, notably protein kinase C activity (4).

Proteolysis of extracellular matrix components has long been known to play an important role in both in vitro and in vivo angiogenesis (1). Provocative new information from experiments done in Judah Folkman's and David Cheresh's laboratories have potentially shed new light on the contributions of the proteolytic and the extracellular matrix systems to the formation of blood vessels. O'Reilly et al. isolated a peptide which they have named angiostatin from the serum and urine of mice bearing Lewis colon carcinoma tumors that showed a striking ability to inhibit tumor angiogenesis (5). This peptide was sequenced and proved to be a fragment of plasmin, containing several Kringle domains, which have been shown in the mature protein to be important in protein binding. In addition, experiments reported by Friedlander et al. suggest the existence of two pathways of angiogenesis. The angiogenic response initiated by fibroblast growth factor (FGF)-2 in the cornea of a rabbit was inhibited by a monoclonal antibody against the integrin $\alpha_v\beta_3$ but not by an antibody against $\alpha_v\beta_5$, while the angiogenic response initiated by vascular endothelial growth factor (VEGF) was inhibited by a monoclonal antibody against $\alpha_v\beta_5$, but not by an antibody against $\alpha_v\beta_3$ (6, 7). The protein kinase C inhibitor, calphostin C, blocked the angiogenesis induced by VEGF but had only a minimal effect on the angiogenesis initiated by FGF-2. Considering plasmin's potential role in fibrinolysis, metalloprotease activation, extracellular matrix degradation and growth factor release (8), and the ability of integrin receptors to trigger intracellular signaling, these findings raise intriguing possibilities.

While the absence of quantitative data in the angiostatin and integrin studies make it difficult to interpret rigorously their significance, a manuscript from J. Madri's laboratory, which appears in this issue of *The Journal*, presents quantitative evidence for the interplay of cytokines, the extracellular matrix, fibrinolytic enzymes and their inhibitors, and endothelial cell behavior. Sankar et al. (9) compare the response of microvascular endothelial cells from rat epididymal fat pads grown on either two dimensional (2D) collagen gels or three-dimensional (3D) collagen gels to transforming growth factor β (TGFβ). Based on previous observations, their hypothesis was that the cells in the 2D cultures behaved in a similar fashion to the cells at the tip of an angiogenic sprout, whereas the cells in the 3D culture mimicked the more differentiated cells distal to the sprout. They found that TGFβ inhibited proliferation of the endothelial cells in the 2D but not the 3D cultures, and increased the production of urokinase and plasminogen activator inhibitor 1 at 24 hours in the 3D culture but not in the 2D culture. An analysis of the TGFβ receptors revealed that cells in the 3D cultures had significantly decreased levels of types II and III receptors compared to cells in the 2D cultures, with a resulting increase in the ratio of type I receptors to type II and III receptors, which was not influenced by TGFβ. These findings suggest that one of the ways the extracellular matrix influences cellular behavior is by altering the distribution of cytokine receptors on the cell surface, thereby altering the response of the endothelial cell to the cytokine towards either a proliferative or a differentiated phenotype. Thus, these data present for the first time a convincing molecular mechanism to explain how an inhibitor of endothelial proliferation in vitro can modify angiogenesis in vivo. Also, it will be of interest to determine the potential role of angiostatin and integrins as modifiers of these processes.

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