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INSIDE LAB INVEST

ANGIOGENESIS FROM BONES—IMPLICATIONS FOR THE FUTURE: Angiogenesis, the formation of new vessels from those pre-existing, is critical for normal development, growth, and wound healing and is a requirement for tumor growth. Over the past few years several in vivo and in vitro models of angiogenesis have been developed, characterized, and used to elucidate specific aspects of this process and to evaluate pro- and anti-angiogenic agents. Endothelial monoculture in vitro models have as an advantage their ability to allow the study of the responses of one cell type to a variety of stimuli. The addition of other cell types (pericytes, smooth muscle cells, astrocytes, etc.) to these models allows for the study of specific heterotypic cell-cell interactions. A shortcoming of these models is the lack of preservation of the normal architecture of the microenvironment and interactions of several cell types with a native factor-rich extracellular matrix. This shortcoming has been addressed by investigators who have developed several organ culture models ranging from embryoid bodies to aortic, coronary artery, and thoracic duct rings to whole conceptus cultures. These culture models, although sometimes yielding complex data difficult to interpret, have revealed significant information regarding vascular bed specificity and potential. In this issue, **Deckers et al** (Lab Invest 2001, 81: 5–15) describe an organ culture angiogenesis model using murine E17 metatarsals. This model, although offering similar advantages and disadvantages compared with other established angiogenesis models, has the potential of enabling investigators to isolate and characterize a microvascular bed derived from bone. Such a culture model may well facilitate development of a better understanding of the roles of bone marrow endothelial and mural cells in the processes of stem cell engraftment during development and mature and stem cell gating from the marrow cavity during homeostasis and following responses to a variety of stimuli. In addition, this model may be of use in investigating tumor cell homing to bone.

NITRIC OXIDE—A MODULATOR OF BLOOD-BRAIN-BARRIER FUNCTION AND NEURAL RESPONSES TO INJURY: Nitric Oxide (NO) is known to be an important signaling molecule in a wide range of biological processes. It has been suggested that it plays a role in blood-brain barrier (BBB) breakdown. Indeed, NOS inhibitors have been shown to decrease BBB permeability following a variety of noxious stimuli. Other studies have demonstrated NO effects on angiogenesis ranging from modulation of endothelial cell survival, proliferation, migration, and differentiation to vascular remodeling. Thus, a more complete understanding of the complex roles and effects of NO and the expression of NOS isoforms in the neuropil is warranted and necessary if safe and effective therapeutics directed at NO metabolism are to be developed. In this issue, **Nag et al** (Lab Invest 2001, 81: 41–49) demonstrate up-regulation of endothelial (eNOS) and inducible (iNOS) nitric oxide synthases following cold-induced cortical brain injury and correlate this with BBB breakdown and nitrotyrosine formation in lesional tissue. The finding of up-regulation of selected NOS isoforms is not unexpected as eNOS expression is known to be enhanced by VEGF and is known to act downstream of VEGF. However, the prolonged up-regulation of iNOS following injury (during BBB breakdown and angiogenesis) suggests that NO may have specific roles in these processes over time.

The authors' observation of the appearance of nitrotyrosine in lesional areas raises interesting possibilities. In addition to being a marker of increased NO production and release (via interaction with superoxide anion and production of peroxynitrite), nitrosylation of proteins on tyrosine residues may elicit changes in protein conformation, in protein-protein and protein-cell interactions, and in the signaling functions of a variety of cell-surface proteins. These changes would have the potential of evoking both short-term and long-term effects on tissue architecture and cell behavior. Indeed, peroxynitrite has been implicated in the neurotoxicity noted in neurodegenerative diseases. Additional investigation into these and other potential effects of NO should lead to a more complete understanding of the importance of this structurally simple but functionally complex molecule.

THE LIGHT AT THE END OF THE TUNEL: After many years of study, the precise mechanism responsible for the destruction of exocrine glandular tissue observed in patients with Sjögren's syndrome (SS) remains elusive. Two cardinal features of SS are the presence of large foci of inflammatory mononuclear cells and the destruction of salivary gland parenchyma. It is thought that the chronic inflammatory infiltrate leads to the loss of secretory activity that eventually results in severe dryness of the mouth. The demonstration that apoptosis plays a role in autoimmune disease stimulated a flurry of studies designed to evaluate the frequency of apoptotic cells in the salivary glands of patients with SS. It is notable that disturbances in the apoptotic process have been invoked to explain the clinical and pathological findings in SS. Increased rates of apoptosis of epithelial cells, both ductal and acinar, could explain the loss of secretory function, whereas dysregulation of apoptosis could also play a role in the accumulation of infiltrating mononuclear cells. In this issue, **Ohlsson and collaborators** (Lab Invest 2001, 81: 95–105) present a carefully designed and executed study of the rate of apoptosis in SS as evaluated by the terminal deoxynucleotidyl transferase mediated dUTP-digoxigenin nick end labeling (TUNEL) method. The authors also determine the expression patterns of Fas and Fas-ligand and the phenotype of T-cells infiltrating the epithelium. By performing the TUNEL assay under controlled conditions, they find that only a sparse number of epithelial cells register as positive in SS. Furthermore, the prevalence of TUNEL-positive cells in patients with SS is not significantly different from that found in subjects suffering from atrophy and fibrosis of the salivary glands of nonautoimmune origin or from that found in normal controls. These findings stand in contrast to previously reported apoptotic rates as high as 37% of acinar epithelial cells and 68% in ductal cells! The results presented by **Ohlsson et al** also indicate that Fas-induced apoptosis is rare in SS salivary gland tissue, and they are compatible with the hypothesis that mononuclear cells can elude Fas/Fas-L–induced cell death.

TNF-α ENDOCYTOSIS IN MACROPHAGES: Tumor necrosis factor α (TNF-α) is a well-recognized pro-inflammatory and pro-apoptotic cytokine secreted by macrophages, monocytes, and to a lesser extent, other cells including tumors. Its actions underlie a variety of pathologic phenomena, including fever, septic shock, endothelial cell activation in hypersensitivity reactions, chronic inflammatory disease, and the cachexia of cancer. TNF-α comes in two forms: a pro-form of 26 kDa synthesized as an integral type II membrane protein that is moved to the plasma membrane via the secretory pathway and a mature, processed, and soluble 17 kDa form generated by the action of TNF-α–converting enzyme (TACE or ADAM17), a membrane-bound metaloproteinase. Both forms of TNF-α are biologically active. The soluble form can act locally and at a distance; the membrane-bound form functions as a paracrine and autocrine signal and can potentiate the direct cell-mediated killing of tumor cells. Yet, despite these understandings, insights into how the balance of surface versus soluble TNF-α is maintained have been limited. The key enzyme responsible for its release, TACE can be unregulated on the cell surface via the action of phorbol esters. TACE is also found on intracellular compartments, leading to the speculation that TNF-α may be cleaved not only on the cell surface, but also perhaps in the Golgi or other intracellular compartment before its release. Predictably, metaloproteinase inhibitors block the cleavage of TNF-α and its release from the cell, but paradoxically enhance the endocytosis of TACE. Such observations suggest that a negative feed-back regulatory loop might exist, in which the levels of plasma membrane TACE are reciprocally related to the levels of plasma membrane TNF-α. To more fully explore these questions, **Shurety and colleagues** in this month's *Laboratory Investigation* (Lab Invest 2001, 81: 107–117) have used a cultured macrophage cell line to study the fate of newly synthesized TNF-α. Their findings clearly establish several points: (a) greater than 90% of TNF-α is transported directly from the Golgi to the plasma membrane as the pro-protein (26 kDa); (b) once transported to the plasma membrane, its fate is determined by the balance of its proteolysis by TACE versus endocytosis as the intact pro-TNF-α with delivery to early endosomes; and (c) inhibitors of TACE do not modify the kinetics of pro-TNF-α transport out of the Golgi. Collectively these data demonstrate that two pathways regulate the postsynthetic display and secretion of TNF-α, membrane delivery/endocytosis and proteolysis. This study also shows that these pathways converge at the plasma membrane, but not in the Golgi. Although the full significance of these findings remains to be appreciated, they suggest that modulation of the secretory and endocytic pathways, in addition to pro-TNF-α proteolysis, may play a heretofore unappreciated role in controlling the balance of the endocrine versus autocrine and paracrine actions of TNF-α.