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Milieu Intérieur – The Search for Myocardial Arteriogenic Signals

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Claude Bernard, the father of modern physiology, applied the scientific method to medicine and described the importance of context for physiological system functions. Bernard coined the term "*milieu intérieur*" (1). Today we recognize this physiological context and the intrinsic adaptive responses to maintain stable biological systems in response to myriad physiological and pathological challenges as "homeostasis".

An expert panel consensus report on arteriogenesis (2), acknowledging Bernardian principles, concluded that: (a) arteriogenesis is the preferred type of neovascularization for purposes of restoring myocardial perfusion; (b) combination growth factor therapy or use of "*master switch"* genes may be optimal for clinically beneficial therapeutic angiogenesis; (c) preclinical *and* clinical studies should be *preceded by tissue distribution* studies to define the myocardial uptake and retention or expression of growth factor(s); and (d) protein therapy is closer to practical use than is gene therapy.

Coronary artery syndromes present homeostatic challenges. Coronary arteriogenesis is considered to be temporally shaped by endogenous intramyocardial signaling molecules. For the coronary circulation to keep pace with pathological processes (e.g., plaque formation, vasospastic behavior, adrenergic vasoconstriction) within the native coronary arteries that eventually limit coronary flow and flow reserve under times of increased cardiac demand (i.e., exercise) new coronary arterial vessel growth must matched closely the loss of native coronary artery capacity and flow reserve. Failure to articulate the requisite "supply side" remodeling will result in myocardial ischemic injury and cell death.

The details of the myocardial "*milieu interieur"* that generates the expression of molecular signals for arteriogenesis in response to progressive coronary artery stenosis are incompletely understood. Heil *et al.* (3) distinguished between two important processes of coronary vascular growth: (a) arteriogenesis is growth of pre-existing arterio-arterial anastomoses induced by physical forces, most importantly shear stress, where as (b) angiogenesis is induced by hypoxia and results in new capillary growth. The potential for adaptive coronary collateral growth has

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been appreciated for many years. Progressive coronary artery stenosis in the porcine model amply illustrates the intrinsic capacity for coronary artery collateral artery growth sufficient to support viable myocardium (4). Native and adaptive coronary circulation in humans and myriad other animals are documented by Schaper (5) and Cohen (6).

With regard to coronary arteriogenesis, endothelial cells appear to orchestrate the response to ischemia (7) by sensing changes in fluid shear stress (FSS) translated by bio-signals into an integrated response. Integrins (8), tyrosine receptor kinases (9), G-protein coupled receptors (10), and ion channels (11) have each been proposed as endothelial cell membrane shear stress sensors. Signal cascades initiated by FSS changes activate endothelial cells. Adhesion molecule (ICAM-1) and vascular cell adhesion molecule-1 expression are up-regulated.(12) Several chemokines - TNF-α (13), GM-CSF) (14), G-CSF (15) - are increased and nitric oxide (NO) is released (16). These molecules establish a new "*milieu intérieur*" for coronary collateral growth.

In the current issue, Schirmer *et al.* (17) identify new bio-molecules correlated to human coronary collateral development. Their study implicates the chemokine (C-C motif) ligand 11 (CCL11; eotaxin-1) and macrophage migration inhibitory factor (MIF) in coronary arteriogenesis. These molecules, emanating from the "*milieu intérieur*" of human heart tissue are postulated to participated in the complex interplay of signals remodeling myocardium and coronary collaterals.

An important question arising from the outcomes and speculations of this study is "Can arteriogenic or angiogenic factor(s) improve cardiac pump function after scar is formed by promoting myocardial neovascularization?" This study provides no information on this. To date, clinical trials testing single arteriogenic bio-molecules have demonstrated insufficient efficacy or unacceptable side effects (18-20). Gene transfer from implanted cells has been shown to induce myocardial angiogenesis (21) as has combination proteins – fibroblast growth factor-2 (FGF-2) with platelet derived growth factor BB (PDGF-BB) (22). Enhancement of the intrinsic myocardial stromal cell-derived factor (SDF-1α) can recruit pluripotent mesenchymal stem cells to generate new cardiac myocytes and new blood vessels (23).

Clinical trails (24) are currently exploring safety and efficacy of: (a) bone marrow stem cells, (b) endothelial progenitor cells, (c) bi-cistronic VEGF-A 165/bFGF plasmid, (d) gene transfer of vascular endothelial growth factor combined with oral L-arginine supplementation, and (e) adenovirus serotype-5 mediated fibroblast growth factor-4 gene transfer, among others.

Clinical studies such as that reported here by Schirmer et al. (17), while presenting some surmountable limitations (coronary sampling rate and method, and fixed venous pressure in CFI calculations), have identified new molecular signals possibly involved in coronary arteriogenesis. Results from such studies may provide important clues for future coronary arteriogenic therapies. Experimental studies in models that reflect human coronary collateral biology will allow *in situ* tissue characterization of relevant signaling molecules. Meanwhile, Schirmer *et al.* (26) continue to expanding our understanding of arteriogenesis and its molecular signals.

Taken together, the search for myocardial signal molecules that support arteriogenesis remains an active area of investigation engaging basic research models, translational efforts, and clinical trials. It is likely that time will reinforce the Bernardian view that no one molecular entity or technological approach may be the "master switch" controlling the molecular signaling cascade establishing a new "*milieu intérieur*" for arteriogenesis.

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