

DANCE ROUND*WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.*
ROBERT FROST**PAAT: A PATH TO ATHEROSCLEROSIS?****Ivan S. Stankulov¹, Luigi Aloe², Peter I. Ghenev³, Luigi Manni², Pavel Pavlov¹, Marco Fiore², and George N. Chaldakov¹**¹Department of Forensic Medicine and Cell Biology, Medical University, Varna, Bulgaria, ²Institute of Neurobiology and Molecular Medicine, Consiglio Nazionale delle Ricerche (CNR), Rome, Italy, ³Department of General and Clinical Pathology, Medical University, Varna, Bulgaria

The artery wall consists of intima, media, and adventitia, the latter gradually transiting into the periadventitial adipose tissue (PAAT). Although many paths lead to atherosclerosis, the prevailing paradigm at present is Russell Ross's response-to-injury hypothesis, which states that atherosclerosis is a chronic inflammatory disease. This hypothesis considers the intimal smooth muscle cell proliferation as a key event in the generation, development and complication of atherosclerosis. Thus the potentially important role played by adventitial fibroblasts/myofibroblasts in atherosclerosis and postangioplasty restenosis, suggesting therapeutic perspectives targeted to these particular cells, has been neglected. Here we go further away from the intima, and focus on the potential involvement of PAAT in the process of atherogenesis and angioplasty-induced restenosis.

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An artery affected by atherosclerosis displays intimal and adventitial lesions associated with medial atrophy (1). Although many paths lead to atherosclerosis, the prevailing paradigm at present is Russell Ross's response-to-injury hypothesis, which states that atherosclerosis is a chronic inflammatory disease that involves several aspects of wound healing-like vascular remodeling (1,2). This hypothesis proposes endothelial dysfunction, lymphocyte and monocyte extravasation into the intima, and vascular smooth muscle cell (VSMC) proliferation and oversecretion of matrix molecules (1,3) as key events in the generation, development and complications of atherosclerotic plaques. Because advanced intimal lesions lead to erosion/rupture of plaque surface, resulting in acute coronary syndromes, the intima is considered by many authors the most important vascular area involved in the process of atherogenesis (reviewed in 1,2). However, growing evidence

raises the possibility of adventitial pathway of the vascular injury response (4-6), suggesting that atherogenesis is not just for intima and, respectively, intimal VSMC proliferation. For example, the otherwise excellent review (7), published in a special section on "Advances in Atherosclerosis" in the November 2002 issue of *Nature Medicine*, was a strongly VSMC-centric description and perspective in atherosclerosis, angioplasty-induced restenosis, and transplant vasculopathy. While recent findings demonstrate that lumen narrowing is caused in large part by a fibrocontractive adventitial scarring (4) and adventitial inflammation (8), no specific attention was paid by Dzau *et al* (7) (except their reference 13) to cells, signals and/or reactions that may derive from both the adventitia and the periadventitial adipose tissue (PAAT), as clearly illustrated in their Figs. 1 and 3. Meanwhile, in Fig. 3, the authors' legend "VSMC mediate proliferation, inflammation, matrix alterations and contraction"

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can be replaced by “Myofibroblasts mediate...”, without any substantial changes in the Figure. Thus the important role played by fibroblasts/myofibroblasts in vascular inflammatory-fibroproliferative diseases, suggesting therapeutic perspectives targeted to these particular cells, has been neglected.

Here, “bypassing” the adventitia, we will go further away from the intima, and focus on the potential involvement of PAAT in atherogenesis and angioplasty-induced restenosis.

The artery wall consists of intima, media, and adventitia, the latter gradually transiting into the PAAT. Adipose tissue is partitioned into few large depots, including subcutaneous and visceral location, and many small depots, associated with heart, large blood vessels, major lymph nodes, kidneys, adrenal glands, bone marrow, and even the brain (reviewed in 9). Indeed, the possibility that the endocrine secretory activity of large adipose depots may contribute to the altered plasma levels in various adipokine-associated diseases including cardiovascular disease (10-14) has recently gained considerable attention. However, the paracrine secretory activity of the small depots has until now attracted little attention in the adipobiology of disease. If signals can be targeted, *via* an endocrine pathway, from the large adipose depots through the blood circulation towards many organs in the body, then why not look for analogous paracrine influence from the organ-associated adipose tissue including PAAT?

The subepicardial adipose tissue surrounding the most proximal and atherosclerosis-prone portion of left anterior descending coronary artery is an excellent example of PAAT in atherosclerosis. The principle difference between coronary PAAT and adipose tissue elsewhere in the body is its greater capacity for free fatty acid release and uptake, thus acting as a local energy supply for the heart and/or as a buffer against toxic levels of free fatty acids (15) and toxic lipid-soluble substances that may accumulate in PAAT (16). This tissue therefore is “not a passive storehouse for fat”, as stated by Smith and Willius in 1933 (see 15). Neglected for nearly 70 years, the possible involvement of coronary PAAT in atherogenesis has been recently addressed (17-19). In PAAT surrounding human atherosclerotic coronary arteries, we have recently reported the first evidence for an elevated amount of nerve growth factor (NGF)(19), a prototypic member of the protein family of neurotrophins which, in addition to its action on differentiation and survival of sympathetic and sensory neurons, exerts multiple effects on immune and other non-neuronal cells (20). This was accompanied by a strong mesenchymal reaction by “atherosclerotic” PAAT, consisted of an increased number of mast cells, microvessels and connective tissue septa, compared to controls. Whether such an increased amount of both NGF and mast cells [the latter can produce NGF (20)] may represent an intrinsic effort of this tissue to compensate the reduced levels of NGF found in atherosclerotic coronary vascular tissue (19) and in the blood plasma of patients with

metabolic syndrome (21), remains to be evaluated. Also to be considered is whether the coronary PAAT’s mesenchymal reaction in atherosclerosis (17; and present study) may contribute to lumen narrowing, as suggested for the fibrocontractive adventitial scarring (4) and adventitial inflammation (8). Noteworthy, a recent work demonstrated that PAAT exerts an anti-contractile effect in aortic ring preparations, suggesting of PAAT-derived relaxing factor(s) (22). This finding reminds us the story of endothelium-derived relaxing factor, the now well-known nitric oxide.

Taken together, these data pressingly suggest that we should no longer consider PAAT separately from the respective artery, but keep PAAT attached and in place, and subject to thorough examination. Applying such an “adipoprotective” strategy, recent studies implicated major lymph node-associated adipose tissue in immune responses (23,24).

Another reason for subepicardial adipose tissue to serve as an excellent example of PAAT in atherogenesis is the close association of the coronary vasculogenesis with epicardial and subepicardial development, including epicardium-derived mesenchymal cells invading the subepicardial matrix and differentiating into coronary VSMC and perivascular fibroblasts, mediated by the transcription factor capsulin/epicardin (25-27). Thus coronary VSMC distinguish themselves ontologically, structurally and functionally as compared to VSMC in other blood vessels. The heterogeneity between these cells may subsequently involve an increased susceptibility of the coronary artery to atherosclerosis (28,29). Hence the question arises as to whether coronary PAAT may also contribute to that? Using capsulin/epicardin-based genetically modified mice fed with high-cholesterol diet may answer this and related questions. Accordingly, better understanding of the proepicardium-mediated coronary vasculogenesis may clarify mechanisms of cardiovascular disease and suggest new therapeutic strategies.

Overall, these findings support the hypothesis that PAAT may represent an important target for studying the adipobiology and therapy of atherosclerosis and postangioplasty restenosis.

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