Adventitia and Perivascular Cells

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Introduction

The last few years have seen significant advances in our understanding of the multiple and dynamic roles of the adventitia and its companion perivascular tissues for vessel wall homeostasis and disease. It is now becoming clear that signals originating from within the adventitia and from perivascular cells play essential roles in regulation of vascular development, physiology, artery wall remodeling, immune surveillance and vascular disease.[1–4] The adventitia is in contact with and completely surrounds the media and is the interface between the vessel wall and its neighboring tissues. It contains many different interacting cell types including fibroblasts, microvascular endothelium, nerves, resident macrophages, T cells, B cells, mast cells and dendritic cells.[5–9] The adventitia is also home to resident vascular progenitor cells whose formation and maintenance depends, in part, on sonic hedgehog signaling.[1,10–12]

Perivascular cells are in close contact with the adventitia, particularly for the aorta and coronary arteries. Perivascular tissue includes adipocytes, lymphatic vessels, perivascular nerves, and stromal cells exhibiting mesenchymal stem cell-like properties.[3,13] The adventitia and periadventitial cells function in concert. They are linked by microvessels, nerves and migratory cells to regulate vascular physiology, homeostasis, structural remodeling and exert major influences on the progression or regression of vascular disease. Crosstalk between intima, media and adventitia further links the adventitia-periadventitial unit to the rest of the vessel wall. Much of the work advancing our concepts of the adventitia and periadventitial tissues has been published in *ATVB*, and some of those key studies are discussed in this highlights article. In the following summary, areas that have attracted the most interest over the past two years in *ATVB* are reviewed including the multiple roles of perivascular adipose tissue (PVAT) on control of vascular physiology and remodeling, adventitial progenitor cells and their contribution to neointimal formation, and the perivascular space as a target for local delivery of therapeutics.

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Disclosures
None.

Perivascular Control of Arterial Physiology

Control of peripheral resistance through contraction and relaxation of constituent vascular smooth muscle is a critical function of muscular arteries and arterioles *in vivo*. There are many known factors that stimulate or inhibit smooth muscle cell contraction in intact blood vessels.[14,15] A number of these factors are derived from endothelial flow-responsive pathways on the intimal side of the vessel wall and others arise from vasoactive nerves that penetrate the tunica media of these vessels from the adventitial side. Less well known, however, are the factors that originate from perivascular cell types surrounding the outside of the artery wall.[13,16] For example, one candidate linking PVAT to arterial contractile tone is the adipokine chemerin, a 14kDa protein that is secreted from adipocytes as prochemerin and then released by serine protease activity. Chemerin stimulates chemotaxis of macrophages and dendritic cells.[17] Chemerin receptors are also found on SMCs in the tunica media where they mediate smooth muscle contraction.[18] Obesity and hypertension are well-known comorbidities and arteries from obese animals and humans were found to exhibit amplified contractile responses to chemerin receptor agonists.[18]

On the other hand, production of vasorelaxing or anticontractile activity by perivascular adipose tissue has been described for many years.[19,20] One important component of this adipose tissue-derived anticontractile activity is adiponectin.[20,21] Adiponectin is an adipocyte-derived 244 amino acid long peptide hormone that regulates metabolic processes including fatty acid oxidation and also mediates vasorelaxation. Adiponectin receptors on vascular SMCs activate calcium-sensitive potassium channels (BK_{ca}) leading to stimulation of eNOS activity and production of nitric oxide (NO). A similar pathway exists in endothelial cells. Together, the production of NO from endothelial cells and SMCs mediates the anticontractile effects of PVAT-derived adiponectin.[20] In obesity, there is a loss of PVAT-mediated anticontractile activity that is correlated with reduced bioavailability of adiponectin.[22] Another feature characteristic of PVAT in obesity is local inflammation. Mammalian target of rapamycin complex 2 (mTORC2) is reported to control inflammation and is expressed in PVAT.[23] When rictor, an essential mTORC2 component, was deleted in mouse adipose tissue, gene expression and protein release of IL6, MIP-1α, and TNFα, were increased and dilations of aortic rings were impaired.[23] Moreover, a high fat diet alone was sufficient to induce downregulation of rictor gene expression in PVAT and epididymal adipose depots.[23] These studies suggest that controlling mTORC2 activity in perivascular adipose tissues may offer a therapeutic avenue for inflammation-driven cardiovascular damage frequently observed in obesity.

Adventitial/Perivascular Control of Wall Structure

Vascular SMCs are recruited from multiple types of progenitor cells in the embryo to form the tunica media.[24] The media is organized in layers of smooth muscle and cross-linked elastin called lamellae.[25] The number layers present in conduit arteries is established by mid-gestation and is characteristic of the position of the artery in the mechanical wall strain profile of the arterial circulation.[25–27] After birth, the overall geometry of artery wall changes substantially in response to pathophysiological demands. For example, pulmonary hypertension (PH) is characterized by significant increases in pulmonary vascular resistance

that result from excessive medial cell proliferation, inward remodeling and robust adventitial fibrosis.[28] Reactive oxygen species (ROS) have been proposed as major pathogenic stimuli for PH with the NADPH oxidase (Nox) family of enzymes, particularly Nox4, as a prominent source of ROS in the pulmonary artery wall.[29] Elevated expression of Nox4 was found in intima and adventitia in pulmonary arteries from three different rat models of PH and from PH patients.[29] Small molecule inhibitors of Nox4 were found to reduce adventitial ROS production, inhibit inward wall remodeling, and diminish indices of adventitial stiffness as indicated by morphometry and high-resolution ultrasound.[29] Adventitial fibrosis is a common response to sustained elevations of wall stress associated with hypertension. It is also associated with obesity where it is correlated with increased inflammation in PVAT and elastin fragmentation in the aortic wall.[30] These changes in wall structure were associated with decreases in lysyl oxidase (LOX) activity suggesting that elastin fragmentation and adventitial fibrosis may be linked to reductions in LOX activity and PVAT inflammation in settings of obesity.[30]

Progressive cardiac fibrosis is characteristic of the late stages of cardiomyopathy in Duchenne muscular dystrophy. Fibrotic tissue extends throughout the myocardium but conspicuously appears to radiate outward from the coronary adventitia. Indeed, using the mdx mouse model of Duchenne muscular dystrophy, cell types responsible for extensive perivascular fibrosis were found to express markers either of cardiac fibroblasts or of adventitial stem cell antigen-1 (Sca1)-positive vascular progenitor cells (AdvSca1 cells).[31] While the former are not surprising, the finding that major producers of type I and type III fibrillar collagens in the mdx heart were resident adventitial progenitor cells was unexpected and pointed to a pathogenic role for these cells in cardiomyopathy associated with muscular dystrophy.[31] Aortic stiffening is commonly found in hypertensive arteries and experimental hypertension is associated with inflammation of the adventitia and perivascular tissues. A link between aortic stiffening, adventitial inflammation and high blood pressure was uncovered by Guzik et al[8] who found that resident T-cells in the adventitia were activated by neoantigens formed in aortic adventitia of angiotensin II (AngII)-infused mice and were key mediators of AngII-induced hypertension. Activated adventitial T-cells produced locally acting cytokines that resulted in adventitial collagen production, aortic wall stiffening and the onset of hypertension.[8] Mice deficient in production of the T-cell-derived cytokine interleukin-17A were protected against AngII-induced aortic wall stiffening, adventitial fibrosis, and high blood pressure.[32] Another player in the production of perivascular fibrosis is the pericyte-like cell that has been shown to possess mesenchymal stem cell-like properties and is abundant in perivascular adipose tissue.[33] Adipose tissue fibrosis is found in obesity and is associated with metabolic dysfunction. Using a transgenic model of PDGF-Rα activated signaling in pericyte-like cells resident in white adipose tissue, it was observed that these pericyte-like cells can transition into matrix-producing profibrotic cells and are a major source of adipose tissue fibrosis *in vivo*.[33]

Adventitial/Perivascular Cells in Vascular Disease

The adventitia has long been known to accumulate inflammatory cells in atherosclerotic arteries.[7,34–36] More recent studies have shown that leukocytes are also present in the adventitia in normal, non-diseased artery wall.[6–8] With the development of atherosclerotic

plaques on the luminal side, adventitial leukocytes increase in number and organize into germinal center-like structures suggesting local antigen presentation and antibody production.[9,36,37] Perivascular cells in adipose tissue surrounding atherosclerotic human coronary arteries exhibit a heightened proinflammatory phenotype and contribute to leukocyte accumulation in the adventitia of these vessels.[3] Coronary perivascular adipose tissue may also produce factors that contribute to life-threatening coronary vasospasm in addition to the progression of atherosclerosis.[38]

To directly test the role of PVAT tissue on arterial responses to injury, Manka et al transplanted a small amount (2–3mg) of PVAT from donor mice fed a high fat diet to recipient LDL receptor-deficient mice also fed a high fat diet.[39] Inguinal subcutaneous adipose tissue was transplanted as controls.[40] Two weeks after transplant, mice were given wire injuries to the carotid artery and examined two weeks later. Transplanted PVAT tissue was associated with increased neointimal thickening, greater numbers of adventitial macrophages and pronounced increases in the density of vasa vasorum microvessels in the injured adventitia.[39] This effect of accelerated neointimal hyperplasia may be due, in part, to elevated perivascular leptin production in diet-induced obese PVAT tissue.[41] Studies in leptin-deficient (ob/ob) mice or in leptin receptor-deficient (db/db) mice showed that enhanced neointimal formation after carotid wire injury in HFD-induced obese mice depended upon leptin receptor signaling.[41] Thus current evidence suggests that adipokines, including leptin, produced by PVAT in obesity play important roles in the enhanced risk for cardiovascular disease in obese patients.[42] Adventitia and PVAT tissues also are involved in the pathogenesis of abdominal aortic aneurysms (AAAs).[43] For example, in transgenic mice overexpressing endothelin-1 (ET-1) selectively in the endothelium of $apoE^{-/-}$ mice (eET1/ $apoE^{-/-}$) development of AAAs correlated with increased ROS production, macrophage infiltration and CD4(+) T cell accumulation in PVAT compared to apoE−/− alone or eET mice alone.[43]

Adventitial Progenitor Cells

Not long ago, it was common to read descriptions of the adventitia as loosely organized connective tissue containing fibroblasts and perivascular nerves. In contrast, today we recognize that the adventitia is a hub of complex and dynamic interactions between many different types of leukocytes, microvessels, nerves, lymphatics and progenitor cells.[1–9] The known progenitor cells in the adventitia are heterogeneous in their potentials for cell differentiation, and current evidence suggests two types of progenitors coexist in the AdvSca1 population; one that differentiates to mural cells[10,11] and another that differentiates into macrophage-like cells.[4,12] AdvSca1 progenitor cells with smooth muscle differentiation protentials have been shown to migrate into the developing neointima in vein graft models and contribute to ~30% of intimal SMCs in atherosclerotic lesions from ApoE-deficient mice.[10,44] Similar findings were reported for a rat model of transplant arteriosclerosis. Grudzinska et al transplanted labeled adventitial tissues in rat aortic allograft experiments to show that the major source of intimal cells originated from the adventitia (79%±20.6 of intimal cells originated in the adventitia).[45] The movement of cells from the adventitia to the intima in this model was dependent on monocyte chemoattractant factor-1 (MCP1/CCL2). Moreover, despite the phenotypic conversion of medial SMCs from a contractile to a

synthetic phenotype very few, if any, medial SMCs migrated into the intima in this model.[45] Using ex vivo decellularized aortic segments in a bioreactor, Wong and colleagues showed that sirolimus stimulated adventitial progenitor cells that were seeded on the outside of the vessel scaffold to migrate in a CXCR4-dependent manner to the intimal side and form neointimal lesion-like accumulations of SM22α- and calponin-positive cells.[46] In addition, sirolimus stimulated differentiation of AdvSca1 progenitor cells into SMCs but not endothelial cells.[46] The above models are in contrast to the movement of labeled medial SMCs to the intima observed in a carotid wire injury model of arterial injury in the mouse.[47] It will be interesting to determine why the two models exhibit such differences in the origin of neointimal cells. It may be related to the degree of medial SMC death, which is usually robust in transplant models, or to differences in the extent of activation of innate immune responses in the two types of injury.

In the late fetal and early neonatal period, the adventitia exhibits strong expression of sonic hedgehog signaling reporters (patched-1, patched-2, gli-1) that are colocalized with AdvSca1 progenitor cells.[1,11] In a study investigating the role of hedgehog signaling in a transgenic mouse model of pancreatic cancer, Tian et al reported finding stromal tumors arising from the adventitia of blood vessels within the pancreas and suggested an origin from AdvSca1 progenitor cells.[48] These findings indicate that the adventitia-periadventitia unit is a site for pathological changes *in vivo* and that these tissues on the outer layer of artery walls can also communicate with organ-specific cell types surrounding the blood vessel in native tissues. While there is much to learn about the potentials for adventitial and periadventitial stem/progenitor cells for vascular therapy and vascular disease,[49] the possibility of harnessing their potential for vascular repair is an attractive therapeutic objective.[49,50]

Perivascular Drug Delivery

Introduction of therapeutics into the vascular lumen leads to rapid distribution throughout the body and systemic effects. To target therapy to specific vessels or vascular beds, more precise delivery methods have been developed that involve local administration to perivascular tissues. For example, Katare et al reported direct application of alginate microbeads encapsulated with mesenchymal stem cells (MSCs) into the perivascular space surrounding the femoral artery in CD1 mice following unilateral hindlimb ischemia.[51] In this particular example, the MSCs were engineered to express glucagon-like peptide-1 (GLP-MSCs), a factor with proangiogenic, anti-apoptotic and cardioprotective effects. Increased capillary and arteriole density in hindlimb muscles with increased foot salvage was observed after perivascular, but not intramuscular, administration of GLP-MSCs.[51] In another study, this time targeting SMC proliferation in a murine carotid artery ligation model, Redmond et al reported that administration of the hedgehog signaling inhibitor Ptc1 small interfering RNA markedly reduced smooth muscle proliferation and pathological vascular remodeling.[52] The Ptc1 siRNA was incorporated into a pluronic gel preparation and applied to the adventitial side of the carotid artery.[52] In addition to localizing delivery of a therapeutic compound directly to the site of arterial injury, the perivascular route of administration might recruit perivascular and adventitial cells to participate in signaling to the intima and media to achieve desired clinical outcomes.

Summary

The multiple roles of adventitia and its companion perivascular tissue in vascular homeostasis and disease are active areas of current interest as reflected in this *ATVB* highlights article. Interactions between the adventitia and perivascular cells with the rest of the artery wall are extensive.[53] These interactions extend to both resident and infiltrating leukocytes and further emphasize the dynamic interface that the adventitia and perivascular cells function within to regulate vessel wall growth, maintenance and disease.

Acknowledgments

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